

FOURTH EDITION

VETERINARY EPIDEMIOLOGY

MICHAEL THRUSFIELD

WITH ROBERT CHRISTLEY

AND HELEN BROWN, PETER J. DIGGLE, NIGEL FRENCH, KEITH HOWE, LOUISE KELLY,
ANNETTE O'CONNOR, JAN SARGEANT, AND HANNAH WOOD



WILEY Blackwell

Veterinary Epidemiology

Veterinary Epidemiology

Fourth Edition

Michael Thrusfield

*Veterinary Clinical Sciences
Royal (Dick) School of Veterinary Studies
University of Edinburgh*

With Robert Christley

*Epidemiology & Population Health
Institute of Infection & Global Health, and
Institute of Veterinary Science
University of Liverpool*

**And Helen Brown, Peter J. Diggle, Nigel French, Keith Howe, Louise Kelly, Annette O'Connor,
Jan Sargeant and Hannah Wood**

WILEY Blackwell

This edition first published 2018 © 2018 by John Wiley & Sons Ltd

Edition History

First edition © 1986 by Butterworth & Co. (Publishers) Ltd

Second edition © 1995 by Blackwell Science Ltd

Third edition © 2005, 2007 by Blackwell Science Ltd, a Blackwell Publishing company

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by law. Advice on how to obtain permission to reuse material from this title is available at <http://www.wiley.com/go/permissions>.

The right of Michael Thrusfield and Robert Christley to be identified as the authors of the editorial material in this work has been asserted in accordance with law.

Registered Offices

John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA

John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial Office

9600 Garsington Road, Oxford, OX4 2DQ, UK

For details of our global editorial offices, customer services, and more information about Wiley products visit us at www.wiley.com.

Wiley also publishes its books in a variety of electronic formats and by print-on-demand. Some content that appears in standard print versions of this book may not be available in other formats.

Limit of Liability/Disclaimer of Warranty

The contents of this work are intended to further general scientific research, understanding, and discussion only and are not intended and should not be relied upon as recommending or promoting scientific method, diagnosis, or treatment by physicians for any particular patient. In view of ongoing research, equipment modifications, changes in governmental regulations, and the constant flow of information relating to the use of medicines, equipment, and devices, the reader is urged to review and evaluate the information provided in the package insert or instructions for each medicine, equipment, or device for, among other things, any changes in the instructions or indication of usage and for added warnings and precautions. While the publisher and authors have used their best efforts in preparing this work, they make no representations or warranties with respect to the accuracy or completeness of the contents of this work and specifically disclaim all warranties, including without limitation any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives, written sales materials or promotional statements for this work. The fact that an organization, website, or product is referred to in this work as a citation and/or potential source of further information does not mean that the publisher and authors endorse the information or services the organization, website, or product may provide or recommendations it may make. This work is sold with the understanding that the publisher is not engaged in rendering professional services. The advice and strategies contained herein may not be suitable for your situation. You should consult with a specialist where appropriate. Further, readers should be aware that websites listed in this work may have changed or disappeared between when this work was written and when it is read. Neither the publisher nor authors shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

Library of Congress Cataloging-in-Publication Data

Names: Thrusfield, M. V., author. | Christley, Robert, 1968- author.

Title: Veterinary epidemiology / by Michael Thrusfield, Veterinary Clinical Sciences, Royal (Dick) School of Veterinary Studies, University of Edinburgh ; with Robert Christley and [8 others].

Description: Fourth edition. | Hoboken, NJ : Wiley, 2018. | Includes bibliographical references and index. |

Identifiers: LCCN 2017051658 (print) | LCCN 2017053201 (ebook) | ISBN 9781118280263 (pdf) | ISBN 9781118280270 (epub) | ISBN 9781118280287 (paperback)

Subjects: LCSH: Veterinary epidemiology. | MESH: Epidemiologic Methods--veterinary

Classification: LCC SF780.9 (ebook) | LCC SF780.9 .T48 2018 (print) | NLM SF 780.9 | DDC 636.089/44--dc23

LC record available at <https://lcn.loc.gov/2017051658>

Cover images: (Top: from left to right) © holbox/Shutterstock; © Paul Looyen/Shutterstock; © Lorado/Gettyimages; © Lisa Van Dyke/Gettyimages; (Map) © yukipon/Gettyimages; (Bottom: from left to right) © Palenque/Gettyimages; © l i g h t p o e t/Shutterstock; © Seiji/Shutterstock; © claire norman/Shutterstock

Cover design by Wiley

Set in 10/12 pt Warnock by SPi Global, Pondicherry, India

In memory of George

Contents

Contributors *xviii*

From the preface to the first edition *xix*

From the preface to the second edition *xx*

From the preface to the third edition *xxi*

Preface to the fourth edition *xxii*

About the companion website *xxiv*

1 The development of veterinary medicine *1*

Michael Thrusfield

Historical perspective *1*

Domestication of animals and early methods of healing *1*

Changing concepts of the cause of disease *2*

Impetus for change *5*

Quantification in medicine *10*

Contemporary veterinary medicine *12*

Current perspectives *12*

The fifth period *19*

Recent trends *20*

Further reading *25*

2 The scope of epidemiology *28*

Michael Thrusfield

Definition of epidemiology *28*

The uses of epidemiology *29*

Types of epidemiological investigation *32*

Epidemiological subdisciplines *33*

Components of epidemiology *35*

Qualitative investigations *35*

Quantitative investigations *36*

Epidemiology's locale *39*

The interplay between epidemiology and other sciences *39*

The relationship between epidemiology and other diagnostic disciplines *40*

Epidemiology within the veterinary profession *40*

Further reading *41*

3 Causality *42*

Michael Thrusfield

Philosophical background *42*

Causal inference *43*

Methods of acceptance of hypotheses *44*

Koch's postulates	45
Evans' rules	45
Variables	46
Types of association	46
<i>Non-statistical association</i>	46
<i>Statistical association</i>	46
<i>Confounding</i>	49
Causal models	50
Formulating a causal hypothesis	53
<i>Methods of deriving a hypothesis</i>	53
<i>Principles for establishing cause: Hill's criteria</i>	55
Further reading	56

4 Describing disease occurrence 58

Michael Thrusfield

<i>Some basic terms</i>	58
<i>Basic concepts of disease quantification</i>	61
The structure of animal populations	62
<i>Contiguous populations</i>	62
<i>Separated populations</i>	65
Measures of disease occurrence	67
<i>Prevalence</i>	67
<i>Incidence</i>	67
<i>The relationship between prevalence and incidence rate</i>	70
<i>Application of prevalence and incidence values</i>	72
<i>Mortality</i>	72
<i>Survival</i>	73
<i>Example of calculation of prevalence, incidence, mortality, case fatality and survival</i>	75
<i>Ratios, proportions and rates</i>	76
Mapping	80
<i>Geographic base maps</i>	80
Further reading	84

5 Determinants of disease 86

Michael Thrusfield

<i>Classification of determinants</i>	86
Host determinants	89
<i>Genotype</i>	89
<i>Age</i>	90
<i>Sex</i>	91
<i>Species and breed</i>	92
<i>Behaviour</i>	93
<i>Other host determinants</i>	93
Agent determinants	94
<i>Virulence and pathogenicity</i>	94
<i>Gradient of infection</i>	97
<i>Outcome of infection</i>	98
<i>Microbial colonization of hosts</i>	100
Environmental determinants	101
<i>Location</i>	101
<i>Climate</i>	101
<i>Husbandry</i>	104

Stress 105
 Interaction 106
Biological interaction 108
Statistical interaction 109
The cause of cancer 110
 Further reading 112

6 The transmission and maintenance of infection 115

Michael Thrusfield

Horizontal transmission 115
Types of host and vector 115
Factors associated with the spread of infection 118
Routes of infection 121
Methods of transmission 123
Long-distance transmission of infection 125
 Vertical transmission 129
Types and methods of vertical transmission 129
Immunological status and vertical transmission 129
Transovarial and trans-stadial transmission in arthropods 130
 Maintenance of infection 131
Hazards to infectious agents 131
Maintenance strategies 132
 Transboundary diseases 135
 Further reading 136

7 The ecology of disease 138

Michael Thrusfield

Basic ecological concepts 139
The distribution of populations 139
Regulation of population size 142
The niche 148
Some examples of niches relating to disease 150
The relationships between different types of animals and plants 152
 Ecosystems 155
Types of ecosystem 156
 Landscape epidemiology 158
Nidality 159
Objectives of landscape epidemiology 161
Landscape characteristics determining disease distribution 164
 Further reading 165

8 Patterns of disease 168

Michael Thrusfield

Epidemic curves 168
Kendall's Threshold Theorem 168
Basic reproductive number (R_0) 169
Dissemination rate 172
Common-source and propagating epidemics 172
The Reed–Frost model 173
Kendall's waves 175
 Trends in the temporal distribution of disease 177
Short-term trends 177

<i>Cyclical trends</i>	178
<i>Long-term (secular) trends</i>	179
<i>True and false changes in morbidity and mortality</i>	180
<i>Detecting temporal trends: time series analysis</i>	180
<i>Trends in the spatial and temporal distribution of disease</i>	186
<i>Spatial trends in disease occurrence</i>	186
<i>Space-time clustering</i>	186
<i>Further reading</i>	187

9 Comparative epidemiology 189

Michael Thrusfield

<i>Types of biological model</i>	189
<i>Cancer</i>	191
<i>Monitoring environmental carcinogens</i>	191
<i>Identifying causes</i>	192
<i>Comparing ages</i>	193
<i>Some other diseases</i>	196
<i>Diseases with a major genetic component</i>	196
<i>Some non-infectious diseases</i>	197
<i>Diseases associated with environmental pollution</i>	198
<i>Reasoning in comparative studies</i>	199
<i>Further reading</i>	199

10 The nature of data 201

Michael Thrusfield

<i>Classification of data</i>	201
<i>Scales (levels) of measurement</i>	201
<i>Composite measurement scales</i>	204
<i>Data elements</i>	205
<i>Nomenclature and classification of disease</i>	205
<i>Diagnostic criteria</i>	207
<i>Sensitivity and specificity</i>	208
<i>Accuracy, refinement, precision, reliability and validity</i>	209
<i>Bias</i>	210
<i>Representation of data: coding</i>	210
<i>Code structure</i>	211
<i>Numeric codes</i>	212
<i>Alpha codes</i>	213
<i>Alphanumeric codes</i>	214
<i>Symbols</i>	215
<i>Choosing a code</i>	215
<i>Error detection</i>	216
<i>Further reading</i>	217

11 Data collection and management 219

Michael Thrusfield

<i>Data collection</i>	219
<i>Questionnaires</i>	219
<i>Quality control of data</i>	228
<i>Data storage</i>	229
<i>Database models</i>	229
<i>Non-computerized recording techniques</i>	231

<i>Computerized recording techniques</i>	232
<i>Veterinary recording schemes</i>	232
<i>Scales of recording</i>	232
<i>Veterinary information systems</i>	234
<i>Some examples of veterinary databases and information systems</i>	237
<i>Geographical information systems</i>	244
<i>Further reading</i>	248

12 Presenting numerical data 251

Michael Thrusfield and Robert Christley

<i>Some basic definitions</i>	251
<i>Some descriptive statistics</i>	252
<i>Measures of position</i>	253
<i>Measures of spread</i>	254
<i>Statistical distributions</i>	254
<i>The Normal distribution</i>	254
<i>The binomial distribution</i>	255
<i>The Poisson distribution</i>	255
<i>Other distributions</i>	256
<i>Transformations</i>	256
<i>Normal approximations to the binomial and Poisson distributions</i>	257
<i>Estimation of confidence intervals</i>	257
<i>The mean</i>	257
<i>The median</i>	258
<i>A proportion</i>	258
<i>The Poisson distribution</i>	259
<i>Some epidemiological parameters</i>	260
<i>Other parameters</i>	261
<i>Bootstrap estimates</i>	261
<i>Displaying numerical data</i>	262
<i>Displaying qualitative data</i>	262
<i>Displaying quantitative data</i>	263
<i>Monitoring performance: control charts</i>	266
<i>Further reading</i>	269

13 Surveys 270

Michael Thrusfield and Helen Brown

<i>Sampling: some basic concepts</i>	270
<i>Types of sampling</i>	272
<i>Non-probability sampling methods</i>	272
<i>Probability sampling methods</i>	272
<i>What sample size should be selected?</i>	275
<i>Estimation of disease prevalence</i>	275
<i>Detecting the presence of disease</i>	284
<i>The cost of surveys</i>	290
<i>Calculation of confidence intervals</i>	290
<i>Further reading</i>	294

14 Demonstrating association 296

Michael Thrusfield

<i>Some basic principles</i>	296
<i>The principle of a significance test</i>	296
<i>The null hypothesis</i>	297

<i>Errors of inference</i>	297
<i>Multiple significance testing</i>	298
<i>One- and two-tailed tests</i>	298
<i>Independent and related samples</i>	299
<i>Parametric and non-parametric techniques</i>	299
<i>Hypothesis testing versus estimation</i>	300
<i>Sample-size determination</i>	300
<i>Statistical versus clinical (biological) significance</i>	300
<i>Interval and ratio data: comparing means</i>	302
<i>Hypothesis testing</i>	302
<i>Calculation of confidence intervals</i>	303
<i>What sample size should be selected?</i>	304
<i>Ordinal data: comparing medians</i>	304
<i>Hypothesis testing</i>	304
<i>Calculation of confidence intervals</i>	308
<i>What sample size should be selected?</i>	309
<i>Nominal data: comparing proportions</i>	309
<i>Hypothesis testing</i>	310
<i>Calculation of confidence intervals</i>	313
<i>What sample size should be selected?</i>	314
<i>χ^2 test for trend</i>	314
<i>Correlation</i>	316
<i>Multivariate analysis</i>	317
<i>Statistical packages</i>	318
<i>Further reading</i>	318

15 Observational studies 319

Michael Thrusfield

<i>Types of observational study</i>	319
<i>Cohort, case-control and cross-sectional studies</i>	319
<i>Measures of association</i>	321
<i>Relative risk</i>	321
<i>Odds ratio</i>	323
<i>Attributable risk</i>	325
<i>Attributable proportion</i>	327
<i>Interaction</i>	328
<i>The additive model</i>	328
<i>Bias</i>	330
<i>Controlling bias</i>	332
<i>What sample size should be selected?</i>	335
<i>Calculating the power of a study</i>	336
<i>Calculating upper confidence limits</i>	337
<i>Further reading</i>	338

16 Design considerations for observational studies 339

Robert Christley and Nigel French

<i>Descriptive observational studies</i>	339
<i>Analytical observational studies</i>	340
<i>Design of cohort studies</i>	340
<i>Design of case-control studies</i>	346
<i>Design of cross-sectional analytical studies</i>	352
<i>Overview of other study designs</i>	354
<i>Further reading</i>	359

17 Clinical trials 361*Michael Thrusfield*

- Definition of a clinical trial 361*
- Design, conduct and analysis 364*
- The trial protocol 364*
- The primary hypothesis 364*
- The experimental unit 367*
- The experimental population 368*
- Admission and exclusion criteria 368*
- Blinding 369*
- Randomization 369*
- Trial designs 370*
- What sample size should be selected? 372*
- Losses to follow-up 373*
- Compliance 373*
- Terminating a trial 374*
- Interpretation of results 374*
- Meta-analysis 375*
- Goals of meta-analysis 376*
- Components of meta-analysis 377*
- Sources of data 377*
- Data analysis 378*
- Further reading 380*

18 Validity in epidemiological studies 383*Robert Christley and Nigel French*

- Types of epidemiological error 383*
- Accuracy, precision and validity in epidemiological studies 384*
- Background factors 385*
- Interpretation bias 385*
- Selection bias 386*
- Examples of selection biases 387*
- Information bias 390*
- Examples of information biases 390*
- Statistical interaction and effect-measure modification 392*
- Confounding 392*
- Criteria for confounding 393*
- Confounding and causal diagrams 394*
- Controlling confounding 394*
- Errors in analysis 395*
- Communication bias 395*
- Further reading 396*

19 Systematic reviews 397*Annette O'Connor, Jan Sargeant and Hannah Wood*

- Evidence synthesis 397*
- Overview of systematic reviews 397*
- Differences between systematic reviews and narrative reviews 398*
- Questions that are suitable for systematic reviews 398*
- Types of review questions suitable for systematic reviews 399*
- Extensive search of the literature 399*
- Assessment of risk of bias in a systematic review 400*

Steps of a systematic review	400
<i>Step 1: Define the review question and the approach to conduct of the review (i.e., create a protocol)</i>	402
<i>Step 2: Comprehensive search for studies</i>	403
<i>Step 3: Select relevant studies from the search results</i>	406
<i>Step 4: Collect data from relevant studies</i>	407
<i>Step 5: Assess the risk of bias in relevant studies</i>	409
<i>Step 6: Synthesize the results</i>	412
<i>Step 7: Presenting the results</i>	416
<i>Step 8: Interpret the results and discussion</i>	419
Further reading	419

20 Diagnostic testing 421

Michael Thrusfield

<i>Serological epidemiology</i>	421
<i>Assaying antibodies</i>	421
<i>Methods of expressing amounts of antibody</i>	421
<i>Quantal assay</i>	423
<i>Serological estimations and comparisons in populations</i>	424
<i>Antibody prevalence</i>	424
<i>Rate of seroconversion</i>	425
<i>Comparison of antibody levels</i>	426
<i>Interpreting serological tests</i>	427
<i>Refinement</i>	427
<i>Accuracy</i>	429
<i>Evaluation and interpretation of diagnostic tests</i>	430
<i>Sensitivity and specificity</i>	430
<i>Youden's index</i>	433
<i>Diagnostic odds ratio</i>	434
<i>Predictive value</i>	434
<i>Likelihood ratios</i>	436
<i>ROC curves</i>	441
<i>Aggregate-level testing</i>	443
<i>Multiple testing</i>	444
<i>Diagnostic tests in import risk assessment</i>	446
<i>Guidelines for validating diagnostic tests</i>	447
<i>Validating diagnostic tests when there is no gold standard</i>	448
<i>Agreement between tests</i>	450
<i>Practical application of diagnostic tests</i>	456
<i>Further reading</i>	456

21 Surveillance 457

Michael Thrusfield

<i>Some basic definitions and principles</i>	457
<i>Definition of surveillance</i>	457
<i>Goals of surveillance</i>	458
<i>Types of surveillance</i>	459
<i>Some general considerations</i>	461
<i>Sources of data</i>	464
<i>Mechanisms of surveillance</i>	471
<i>Surveillance networks</i>	475
<i>Surveillance in less-economically-developed countries: participatory epidemiology</i>	475
<i>Principles of participatory epidemiology</i>	477
<i>Techniques of data collection</i>	478
<i>Strengths and weaknesses of participatory epidemiology</i>	481

<i>Some examples of participatory epidemiology</i>	483
<i>Companion-animal surveillance</i>	483
<i>Wildlife surveillance</i>	485
<i>Aquatic-animal surveillance</i>	485
<i>Assessing the performance of surveillance systems</i>	486
<i>Improving the performance of surveillance: risk-based surveillance</i>	486
<i>Further reading</i>	488

22 Statistical modelling 492

Robert Christley and Peter J. Diggle

<i>Simple linear regression models</i>	492
<i>Key assumptions of linear regression models</i>	495
<i>Modelling more than one input variable</i>	499
<i>Handling categorical input variables</i>	500
<i>Non-linear modelling of quantitative input variables</i>	502
<i>Additive models</i>	502
<i>Categorization of the input variable</i>	502
<i>Transformation of the input and/or output variable</i>	504
<i>Piece-wise regression</i>	504
<i>Modelling interactions</i>	505
<i>Model selection</i>	506
<i>Modelling binary outcomes</i>	509
<i>Generalized linear models</i>	511
<i>The multiple logistic regression model</i>	511
<i>Model selection for logistic regression models</i>	512
<i>Diagnostic checking of logistic regression models</i>	513
<i>Generalized additive models</i>	514
<i>Modelling clustered data</i>	514
<i>Further reading</i>	519

23 Mathematical modelling 520

Michael Thrusfield

<i>Types of model</i>	521
<i>Modelling approaches</i>	521
<i>Deterministic differential calculus modelling</i>	521
<i>Stochastic differential calculus modelling</i>	525
<i>Empirical simulation modelling</i>	526
<i>Process simulation modelling</i>	527
<i>Monte Carlo simulation modelling</i>	528
<i>Matrix population modelling</i>	530
<i>Network population modelling</i>	532
<i>Contact-network modelling</i>	533
<i>Systems modelling</i>	534
<i>The rational basis of modelling for active disease control</i>	534
<i>Available knowledge, and the functions of models</i>	534
<i>From theory to fact</i>	535
<i>Model building</i>	536
<i>Further reading</i>	538

24 Risk analysis 540

Michael Thrusfield and Louise Kelly

<i>Definition of risk</i>	540
<i>Risk analysis and the 'precautionary principle'</i>	543
<i>Risk analysis in veterinary medicine</i>	543

Components of risk analysis	545
<i>Hazard identification</i>	546
<i>Risk assessment</i>	546
<i>Risk management</i>	548
<i>Risk communication</i>	551
<i>Qualitative or quantitative assessment?</i>	551
<i>Semi-quantitative risk assessment</i>	551
Qualitative risk analysis	552
<i>Framework for qualitative risk assessment</i>	552
<i>Qualitative risk assessment during epidemics</i>	554
Quantitative risk analysis	556
<i>Framework for quantitative risk assessment</i>	556
<i>What level of risk is acceptable?</i>	560
Further reading	563

25 Economics and veterinary epidemiology 565

Keith Howe and Michael Thrusfield

General economic concepts	565
<i>Production functions</i>	565
<i>Disease and animal production functions</i>	566
<i>Value and money</i>	567
<i>Money and prices</i>	567
<i>Opportunity cost</i>	568
<i>Technical and economic efficiency</i>	568
<i>Positive and normative economics</i>	569
Levels of aggregation	569
<i>Disease contained at farm level</i>	569
<i>Disease not contained at farm level</i>	570
<i>Zoonotic disease</i>	570
<i>Disease at international level</i>	571
Evaluating disease-control policies	575
<i>Components of disease costs</i>	576
<i>Optimum control strategies</i>	577
<i>Partial budgets</i>	579
<i>Social cost-benefit analysis (CBA)</i>	579
<i>Summary of methods</i>	582
Further study	582
Further reading	584

26 Health schemes 586

Michael Thrusfield

Private health and productivity schemes	586
<i>Structure of private health and productivity schemes</i>	586
<i>Dairy health and productivity schemes</i>	588
<i>Pig health and productivity schemes</i>	591
<i>Sheep health and productivity schemes</i>	592
<i>Beef health and productivity schemes</i>	594
National schemes	597
<i>Accredited/attested herds</i>	597
<i>Health schemes</i>	598
Companion-animal schemes	599
Further reading	603

27 The control and eradication of disease 604*Michael Thrusfield**Definition of 'control' and 'eradication' 604**Strategies of control and eradication 605**Important factors in control and eradication programmes 616**Outbreak investigation 623**Cause known: foot-and-mouth disease 623**Cause unknown: chronic copper poisoning 625**The epidemiological approach to investigation of outbreaks 626**Veterinary medicine in the 21st century 628**Livestock medicine 628**Companion-animal medicine 629**Further reading 630***General reading 633****Appendices 635***Appendix I: Glossary of terms 636**Appendix II: Basic mathematical notation and terms 641**Appendix III: Some computer software 643**Appendix IV: Veterinary epidemiology on the Internet 648**Appendix V: Student's t -distribution 650**Appendix VI: Multipliers used in the construction of confidence intervals based on the Normal distribution, for selected levels of confidence 651**Appendix VII: Values of exact 95% confidence limits for proportions 652**Appendix VIII: Values from the Poisson distribution for calculating 90%, 95% and 99% confidence intervals for observed numbers from 0 to 100 658**Appendix IX: The χ^2 distribution 660**Appendix X: Technique for selecting a simple random sample 661**Appendix XI: Sample sizes 663**Appendix XII: The probability of detecting a small number of cases in a population 669**Appendix XIII: The probability of failure to detect cases in a population 671**Appendix XIV: Sample sizes required for detecting disease with probability, p_1 , and threshold number of positives 672**Appendix XV: Probabilities associated with the upper tail of the Normal distribution 676**Appendix XVI: Lower- and upper-tail probabilities for W_x , the Wilcoxon–Mann–Whitney rank-sum statistic 678**Appendix XVII: Critical values of T^+ for the Wilcoxon signed ranks test 683**Appendix XVIII: Values of K for calculating 95% confidence intervals for the difference between population medians for two independent samples 685**Appendix XIX: Values of K^* for calculating 95% confidence intervals for the difference between population medians for two related samples 688**Appendix XX: Common logarithms ($\log_{10}$) of factorials of the integers 1–999 689**Appendix XXI: The correlation coefficient 691**Appendix XXII: The variance-ratio (F) distribution 692***References 694****Index 841**

Contributors

Michael Thrusfield

Veterinary Clinical Sciences
Royal (Dick) School of Veterinary
Studies
University of Edinburgh
Edinburgh, UK

Robert Christley

Institute of Infection and Global Health
Institute of Veterinary Science
University of Liverpool
Liverpool, UK

Helen Brown

The Roslin Institute
University of Edinburgh
Edinburgh, UK

Peter J. Diggle

CHICAS
Lancaster Medical School
Lancaster University
Lancaster, UK

Nigel French

Institute of Veterinary Animal and
Biomedical Sciences
Massey University
Palmerston North, New Zealand

Keith Howe

Centre for Rural Policy Research
University of Exeter
Exeter, UK

Louise Kelly

University of Strathclyde
Glasgow, UK

Annette O'Connor

College of Veterinary Medicine
Iowa State University
Ames, Iowa, USA

Jan Sargeant

Ontario Veterinary College
University of Guelph
Guelph, Canada

Hannah Wood

York Health Economics Consortium
York, UK

From the preface to the first edition

The common aim of the many disciplines that comprise veterinary medicine is an increase in the health of animal populations, notably of domestic livestock and companion animals. This goal has traditionally been achieved by individual diagnosis and treatment: procedures that evolved contemporaneously in veterinary and human medicine, when infectious diseases, which had predominantly single causes and clearly identifiable signs, were commonplace.

Four major changes in the veterinarian's appreciation of and approach to disease problems have occurred over the past 20 years. First, despite traditional control techniques, for example slaughter and vaccination, some diseases remain at refractory levels and now require continuous scrutiny to detect changing levels of occurrence associated with ecological and management factors. An example is the detection of 'pockets' of bovine tuberculosis in England in areas where infection of badgers is recorded. Secondly, the control of infectious disease has freed animals from major causes of death, thereby facilitating the emergence of non-infectious diseases as major problems: examples are the cardiac, dermal and renal diseases of dogs. Many of these diseases have a poorly understood, often complex (i.e., multifactorial) cause. Thirdly, the intensification of animal industries has highlighted new 'diseases of production', often manifested as poor performance, rather than clinical

disease, and frequently with multifactorial causes. Fourthly, economic evaluation has become important: the economic advantages of disease control, which are obvious with the major animal plagues such as rinderpest, can be difficult to identify when overt disease and dramatic changes in levels of performance are not involved. These four changes in the approach to, and appreciation of, disease have added momentum to the emergence of veterinary epidemiology as a discipline concerned with the measurement of the amount of disease and its economic effects, the identification and quantification of the effects of factors associated with disease, and the assessment of the effects of prevention and treatment of disease in groups of animals.

A knowledge of elementary statistics is essential for an understanding of the full range of epidemiological techniques. Hitherto, most epidemiology books either have assumed a knowledge of statistics or have avoided a description of the mathematical manipulations that are commonly used in epidemiology. However, the extent of statistical teaching varies widely between veterinary schools. Two chapters therefore are included as an introduction to basic statistics, and are intended to make this book statistically self-sufficient (though not comprehensive). Similarly, a chapter includes an introduction to computers, which are now used widely in the recording and analysis of epidemiological data.

From the preface to the second edition

Since publication of the first edition, veterinary medicine has faced several new problems, and has been forced to evaluate established ones more critically. Bovine spongiform encephalopathy emerged as a serious problem in the United Kingdom. Rinderpest is still the subject of a global eradication campaign. There is an increasing demand for comprehensive, high-quality technical and economic information on animal disease and productivity at the national and international level; and information systems, such as the United States' National Animal Health Monitoring System, have been designed to suit these requirements. The moves towards an open market, both in the European Union and internationally following the Uruguay round of the General Agreement on Tariffs and Trade, highlight the need for information on animal disease status in trading nations. Multifactorial diseases continue to predominate in intensive production systems,

and many companion animal diseases are similarly complex. The solving of these problems and fulfilling of these tasks rely heavily on epidemiological principles and techniques.

All chapters of the first edition have been revised. Chapter 11 has been modified to take account of the increasing popularity of microcomputers, and the rapid development of veterinary information systems. New chapters on clinical trials and comparative epidemiology have been added in response to suggestions from colleagues. More statistical methods are included in Chapters 12–15 and 17. The goal of this edition nevertheless remains the same as that of the first: to provide an introduction to veterinary epidemiology for veterinary undergraduates, postgraduates who have received limited or no training in epidemiology, and practising veterinarians and members of other disciplines with an interest in the subject.

From the preface to the third edition

The ten years since publication of the second edition have witnessed further expansion in the application of veterinary epidemiology. Quantitative methods have increased dramatically, both in their development and use. Evidence-based clinical veterinary medicine is now widely appreciated, and relies heavily of the results of epidemiological analyses, including observational studies, clinical trials and the quantitative interpretation of diagnostic tests. The successful employment of epidemiological techniques has witnessed continued progress in rinderpest eradication, whereas the epidemic of foot-and-mouth disease in Europe (notably in the UK) in 2001 presented fresh challenges.

All chapters from the second edition have been revised. Numerous topics (e.g., causality, now exclusively considered in Chapter 3) have been expanded in response to questions raised by undergraduates, post-graduates and professional colleagues, with the aim of consolidating the more enduring principles and concepts of epidemiology. Surveillance is now addressed specifically in Chapter 10. Chapter 17 has been enlarged in response to increased interest in diagnostic-test validation and performance. The opportunity also has been taken to correct several typographical errors that were present in the second edition. The text continues to be an introduction to veterinary epidemiology, directed at all with an interest in the subject.

Preface to the fourth edition

Since the publication of the third edition, veterinary epidemiology has continued to contribute to the successful control of animal disease; notably, in the global eradication of rinderpest. However, some diseases remain refractory, both at the international level (e.g., rabies) and nationally (e.g., bovine tuberculosis in England). Additionally, new microbes, such as Schmallenberg virus, pose fresh challenges. Evidence-based veterinary medicine, which relies heavily on epidemiological techniques, has continued to expand in both livestock and companion-animal practice. Similarly, epidemiology is central to the practice of 'One Health', which recognizes the link between human, animal and ecosystem health, and which recently has gained increased attention.

All chapters of the third edition have been revised. Although the subject coverage remains essentially the same as earlier editions, the order of some chapters has been changed. The first part of the book now concerns mainly qualitative material. This is followed by largely quantitative topics. The last part addresses the application of epidemiology to disease control. Risk analysis, which was included as an appendix to the paperback re-issue of the third edition, is now incorporated into the main body of the text. An updated version of the bibliography of observational studies given in Appendix XXII of the third edition has been excluded from this edition on the grounds that, with the substantial increase in the number of such studies, it is unlikely to be comprehensive, whilst also adding considerably to the size of the book. Some old references have been retained, and some added, to give the reader a perspective on the historical development of knowledge and ideas. New, more advanced, chapters on the design of observational studies, validity in epidemiological studies, systematic reviews, and statistical modelling have been included, should the reader wish to pursue these topics in depth. However, the goal of this edition remains the same as that of the first three: to provide an introduction to veterinary epidemiology for veterinary undergraduates, post-graduates who have received little or no training in veterinary epidemiology, and practising veterinarians and

members of other disciplines with an interest in the subject.

My gratitude is again due to colleagues whose scholarship and comments during the writing of this fourth edition have been invaluable. Ian Handel was very generous with his time in engaging in discussions on test validation without a gold standard, and in providing data for the Bland–Altman analysis in Chapter 20. David Argyle supplied comments on the aetiology of cancer, discussed in Chapter 5. Alex Donaldson criticized aspects of disease control in the last chapter. Helen Brown, Louise Kelly and Keith Howe are recognized for their contributions to Chapters 13, 24 and 25, respectively. I am also indebted to Robert Christley, who collaborated with Nigel French in writing Chapters 16 and 18 and with Peter Diggle in writing Chapter 22, and who supplied additions to some of the other chapters. Annette O'Connor, Jan Sargeant and Hannah Wood wrote Chapter 19, and Marshall Dozier passed valuable comments on this chapter.

Eoghan Clarkson and Brian Mather skilfully converted rough diagrams into reproducible figures.

Pat Fairbairn, Doreen Graham, Bogusia McRoberts and Lisa Norman, librarians at the Royal (Dick) School of Veterinary Studies, University of Edinburgh, efficiently obtained many of the references cited in the text.

Justinia Wood at Wiley continued her earlier involvement with previous editions of the book, and, latterly, Erica Judisch and Purvi Patel took over her role. Jane Andrew, Nick Morgan, James Schultz and Kathy Syplwczak assisted with conversion of the manuscript to published text.

Finally, I am grateful to the Literary Executors of the late Sir Ronald A. Fisher FRS and the late Dr Frank Yates FRS, and to the Longman Group Limited, London, for permission to reprint Tables III, IV, V and VII from their book, *Statistical Tables for Biological, Agricultural and Medical Research* (6th edition, 1974).

Michael Thrusfield
Edinburgh

Over the years since publication of the third edition, veterinary science, veterinary epidemiology and animal health more generally have faced new challenges and opportunities. The eradication of rinderpest has seen the successful elimination of only the second disease and the first animal disease. However, the continued emergence of new animal diseases, the increased recognition of the role of zoonotic diseases in the emergence of diseases of people, and increasing appreciation of the impacts of global change, including climate change, global movement of people, animals and products has led to greater appreciation of the need to understand these phenomena and the changes in health with which they are associated. Simultaneously, new methods have been developed and older methods 'rediscovered'. From techniques for using 'big data' to participatory approaches to engaging with communities, veterinary epidemiology continues to develop across a broad front. It is an exciting time.

This fourth edition sees incorporation of additional chapters on epidemiological study design, validity of epidemiological studies, statistical modelling and systematic reviews. These new chapters provide up to date information on these key issues, complementing, it is hoped, the material in the revised and updated chapters from the third edition.

I am indebted to many people who have contributed to development of this book. In particular, Michael Thrusfield has provided essential guidance and scrutiny. At Wiley, Justinia Wood was a source of support and advice that has been seamlessly continued by Erica Judisch. I am indebted to Nigel French and Peter Diggle for agreeing to co-author

chapters with me, and to Annette O'Connor, Jan Sargeant and Hannah Wood who have authored the chapter on systematic reviews. Numerous people have provided guidance and critique on sections of the text, including, but not limited to, Gina Pinchbeck, Dan O'Neill, Dave Brodbelt, Dai Grove White, Cathy McGowan, Alan Radford, Navneet Dhand and Peter Cripps.

Many colleagues at the University of Liverpool have provided advice, including Sarah O'Brien, Matthew Baylis, Malcolm Bennett and Jo Turner. I have been (and continue to be) lucky to work with many talented postgraduate students who have challenged me to think again about things I might otherwise have taken for granted: David Sutton, Nick Malikides, Joel Hotchkiss, Emily Brook, Maz Behnke, Carri Westgarth, Chris Jewell, Susie Robinson, Jane Murray, Marnie Brennan, Lutfi Al Tunesi, Bryony Parsons, Richard Smith, John Mehers, Andy Stringer, Claire Scantlebury, Alex Berriman, Puteri Nohuddin, Judy Bettridge, Zelalem Gutu, Lisa Jameson, Kwankamon Dittakan, Gabby Laing, Elsa Sandoval Barron, Sophie Beale, Charlotte Rôbin, Margaux Mesle, Stephanie Begemann, Sara Owczarczak-Garstecka, Mohamed AL-Zeyadi, Arturo Hernandez Colina, Tamzin Furtado, John Tulloch, Catherine McLeonard and Hannah Brindle.

Finally, my heartfelt thanks and love go to Rosa and Jake, who have, more than is reasonable, ridden this rollercoaster with me.

*Robert Christley
Liverpool*

About the companion website

This book is accompanied by a companion website:

www.wiley.com/go/veterinaryepidemiology

The website material was produced by **Robert Christley** and includes:

- Interactive multiple-choice questions

1

The development of veterinary medicine

Veterinary epidemiology is concerned with disease in animal **populations**. Its evolution has spanned several centuries and has been central to the successful control of many animal diseases. This introductory chapter traces the development of veterinary medicine in general (including relevant aspects of human medicine), showing that it has been inseparably linked to that of veterinary epidemiology.

Although man's association with animals began in prehistoric times, the development of scientific veterinary medicine is comparatively recent. A milestone in this growth was the establishment of the first permanent veterinary school at Lyons, France, in 1762. Early developments were governed largely by economic rather than humanitarian motives, associated with the importance of domestic stock as a source of food and as working animals; and there are still important economic reasons for concern about disease in animal populations. Later, with the advent of the industrial revolution and the invention of the internal combustion engine, the importance of draft animals declined in the more-economically-developed countries. Although dogs and cats have been companion animals for several thousand years, it is only relatively recently that they and other pets have increased in importance as components of human society.

Until the last half of the 20th century, the emphasis of veterinary medicine had been on the treatment of individual animals with clearly identifiable diseases or defects. Apart from routine immunization and prophylactic treatment of internal parasites, restricted attention had been given to herd health and comprehensive preventive medicine, which give proper consideration to both infectious and non-infectious diseases.

Currently, the nature of traditional clinical practice is changing in the more-economically-developed countries. The stock owner is better educated, and, among livestock, the value of individual animals relative to veterinary fees has decreased. Therefore, contemporary large-animal practitioners, if they are to

meet modern requirements, must support herd health programmes designed to increase production by preventing disease, rather than just dispensing traditional treatment to clinically sick animals.

In the less-economically-developed countries, the infectious diseases still cause considerable loss of animal life and production. Traditional control techniques, based on identification of recognizable signs and pathological changes, cannot reduce the level of some diseases to an acceptable degree. Different techniques, based on the study of patterns of disease in groups of animals, are needed.

Similarly, contemporary companion-animal practitioners, like their medical counterparts, are becoming increasingly involved with chronic and refractory diseases which can be understood better by an investigation of the diseases' characteristics in populations.

This chapter outlines the changing techniques of veterinary medicine by tracing man's attempts at controlling disease in animals, and introduces some current animal disease problems that can be solved by an epidemiological approach.

Historical perspective

Domestication of animals and early methods of healing

The importance of animal healers has been acknowledged since animals were initially domesticated, when they were already likely to have been chronically affected by various infections (McNeill, 1977). The dog, naturally a hunter, was probably the first animal to be domesticated over 14 000 years ago when it became the companion of early hunters, with evidence of close proximity to humans as early as 31 000 years ago (Germonpré *et al.*, 2009); and differentiation from its ancestor, the wolf, was likely to have occurred at least 10 000 years ago, as hunter-gatherer societies gradually evolved into sedentary agricultural

populations (Vilà *et al.*, 1997). Sheep and goats were domesticated by 9000 BC in the fertile Nile valley and were the basis of early pastoral cultures. A few of these societies have lasted (e.g., Pfeffer and Behera, 1997), but many were superseded by cattle cultures; in some, the pig increased in importance (Murray, 1968). An Egyptian cattle culture evolved from 4000 BC, and farming spread from the Near East into Europe (Figure 1.1). There is archaeological evidence of cattle shrines in Anatolia dating back to 6000 BC (Mellaart, 1967). This record illustrates that animals had religious, as well as economic, significance in early civilizations. The aurochs was central to the religion of the Sumerians, who migrated throughout Asia, North Africa and Europe in the third millennium BC taking their animals and beliefs with them. India is the largest cattle culture that remains. Cattle cultures also persist in north-east Africa, the result of interaction between the Ancient Egyptians and early Nilotic tribes. Cattle still play important roles in these cultures: they are food, companionship, and status and religious symbols to the Suk (Beech, 1911) and Dinka (Lienhardt, 1961) of South Sudan.

The first extensive colonization of the Eurasian steppe and semi-arid areas occurred in the third millennium BC. The horse provided the key to successful exploitation of the area north of the Black Sea, the Caucasus, and the Taurus and Zagros mountains (Barracough, 1984), and a Eurasian horse culture, associated with warrior tribes, emerged (Simpson, 1951). Some of these tribes overran the older cattle cultures. The horse is represented in Iranian, Greek and Celtic pantheons. It has become a symbol of veterinary medicine in the form of a centaur, one of

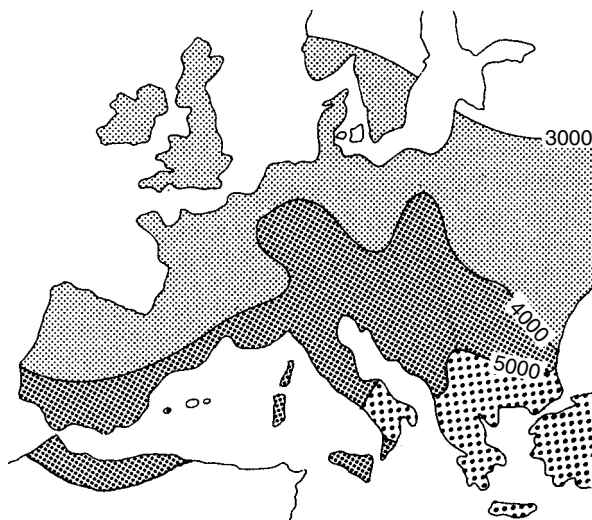


Figure 1.1 A generalized map to show the spread of farming from the Near East to Europe in years BC. (Adapted from Dyer, 1990.)

which, Chiron, was considered to be the mythological founder of Greek medicine.

There have been several movements of animals with concomitant social and agricultural modifications since the early changes. The camel was introduced into Saharan Africa in the first century BC, and into the Sub-Saharan region around AD 400 (Spencer and Thomas, 1978; Phillipson and Reynolds, 1996), the latter already having well established domestic cattle and goat populations (Cain, 1999; Tefera, 2004). The Spanish introduced cattle, sheep, pigs and goats to North America in the 16th century. Haired sheep were introduced to Africa by European slave traders. The Spanish brought turkeys to Europe from North America.

The early Egyptian healers combined religious and medical roles by being priest-healers, often associated with temples. Their therapeutic techniques are recorded in the veterinary *Papyrus of Kahun* (c. 1900 BC). Literary records of similar age, describing veterinary activities, are extant from other parts of the world, such as Indian Sanskrit texts from the Vedic period (1800–1200 BC).

Changing concepts of the cause of disease

Concepts of the cause of disease have changed and evolved¹. A method of treatment used by early Egyptians was incantation. This was partly ritual, but also reflected their belief in supernatural spirits as a possible cause of disease. Approaches to treatment and prevention are the direct result of theories of cause. There have been five main theories up to the middle of the last century². One theory was often superseded by another, but traces of each still can be seen in different parts of the world.

Demons

Early man attributed disease to supernatural powers, the product of animism, which imbued all moving things with a spirit. In this 'spirit-world', disease could be produced by witches³, superhuman entities and spirits of the dead (Valensin, 1972). Treatment therefore included: placation, for example by sacrifice; exorcism (forcible expulsion); evasion, for instance

¹ Causality is outlined in this chapter specifically in the context of disease. A more general discussion is presented in Chapter 3.

² Theories of the cause of disease also have similarities with theories of the origin of species, and both have rationalistic and theological dimensions (Bullock, 1992).

³ A witch was originally defined as 'one who by commerce with the Devil has a full intention of attaining his (or her) own ends' (Bodin, 1580). Witchcraft became widespread in Europe between the 12th and 18th centuries. In the depositions of witch trials, there are many examples of the supposed induction of disease and death in man and domestic animals by witches (L'Estrange Ewen, 1933).

Figure 1.2 Plague allegory: the fate of gamblers and lechers. Giovanni de Paolo: *Allegory of the Plague*, Sienese, 1436–1437. (Reproduced with permission of bpk/Kunstgewerbemuseum, Staatliche Museen zu Berlin/Saturia Linke.)



scattering millet seeds to avoid vampires (Summers, 1961); and transference, often to human and animal ‘scapegoats’⁴, probably the best known single example of which is the Gadarene swine (the Bible: *Mark* 5, i–xiii). The techniques included: ritual ceremonies; material objects that could be suspended (amulets and periapts), carried (talismans), hung in a building (fetishes and icons) or displayed in the community (totems); the use of special people such as witch doctors; and incantations⁵. The meaning of the Indian word ‘brahmin’ originally was ‘healer’ because the Brahmin were a class of healers. In the Neolithic period (4200–2100 BC), trepanation (the removal of a

bone disc from the skull) may have been practised to release evil spirits from sick people (Buckland, 1882; Wakefield and Dellinger, 1939). The practice also is recorded in veterinary texts as early as the 16th century, but may be much older (Binois, 2015); and the Greek physician, Galen, practised trepanation on apes in the second century AD (Arnott *et al.*, 2003).

During the 19th century, many European peasants still believed that diseases of cattle were caused by evil spirits, which could be kept at bay by fire (Frazer, 1890), and the African Nuer tribe occasionally still uses incantations during ritual sacrifice when cattle epidemics occur (Evans-Pritchard, 1956)⁶. Moreover, sacrifice was practised in England as late as the 19th century (Baker, 1974).

Divine wrath

The demonic theory involved many spirits; the next development, monotheistic in origin, argued that disease was the product of a displeased supreme being: disease was punishment for sinful behaviour (*Figure 1.2*). This belief is prominent in the Old Testament, for example, the animal plague of Egypt (the

⁴ The scapegoat had the dual purpose of averting and magically transferring guilt and evil, both generally and at a specific time of crisis, such as plague or failure of crops. It takes its name from the Hebrew rites of the Day of Atonement when a goat was driven into the wilderness after the High Priest had ritually confessed the sins of the people and transferred them to the goat. The custom occurs universally from Ancient Babylonian times to modern times, where human sacrificial scapegoats have been known in some tribal societies (Cooper, 1990).

⁵ Historical examples of the use of amulets, talismans and ‘white witches’ to prevent and control diseases of livestock in England and the British Colonies are given by Baker (1974) and, as late as the 19th century, ‘white witches’ (‘charmers’) were recommended by the Ministry of Agriculture’s veterinarians (St Leger-Gordon, 1994).

⁶ More recently, there has been a trend towards a contemporary understanding of disease (Hutchinson, 1996).

Bible: *Exodus* 9, iii) and is also evident in Persian and Aztec writings. The only effective treatment of disease induced in this way was placation because exorcism and evasion would not be effective against a supreme being. The English veterinary surgeon, William Youatt, writing in 1835, supported the practice of burning crosses on the heads of cattle to cure and prevent disease. In 1865, Queen Victoria, believing that the current British rinderpest (cattle plague) outbreak was the result of divine displeasure, ordered that a prayer should be used in each church in England while the epidemic continued. Beliefs in causation involving divine wrath are still evident today; for example, in some interpretations of the HIV/AIDs epidemic as punishment of the sinful (Kopelman, 2002).

Metaphysical medicine

The next development did not assume the existence of a supreme being, either demonic or divine, but assumed the presence of occult forces beyond the physical universe. This 'metaphysical' medicine embodied a theory of natural laws but excluded scientific principles such as observation and the repeatability of phenomena. The moon, stars and planets were considered to affect health (Whittaker, 1960), these concepts being obvious predecessors of astrology. Several outbreaks of rinderpest in Dark Age Europe were ascribed to earthquakes, floods and comets.

Treatment frequently included particularly foul medicines and practices that persisted for many centuries. A recommended 17th-century cure for broken wind in horses comprised toads, swallows and moles roasted alive and mixed with shoe soles⁷. Divination, practised by the Babylonians using sheep livers, and the 'Doctrine of Signatures' which suggested a similarity between the disease and its cure – for example, using toads to treat warts – were notable metaphysical developments.

The universe of natural law

A major intellectual revolution began in Greece in the sixth century BC in which the universe was rationalized without either demonic or metaphysical influences. The Greeks thought that disease was the result of derangement of four 'humours' of the body, which were associated with four properties (heat, moisture, dryness and cold) and with four elements (air, earth, water and fire) (*Figure 1.3*)⁸. Diseases were considered

		CHARACTERISTIC	
		Moisture	Dryness
CHARACTERISTIC	Heat	Humour = Blood Associated = Air element Source = Heart Excess → Sanguine temperament	Humour = Yellow bile Associated = Fire element Source = Liver Excess → Choleric (bilious) temperament
	Cold	Humour = Phlegm Associated = Water element Source = Pituitary gland Excess → Phlegmatic temperament	Humour = Black bile Associated = Earth element Source = Spleen Excess → Melancholic temperament

Figure 1.3 Components of humoral pathology.

to be caused by external forces, including climatic and geological changes that affected the population. Local outbreaks of disease were thought to be the result of local eruptions of noxious air: **miasmata (miasmas)**⁹. The word 'malaria' literally means 'bad air' and hints at the 19th-century belief that the disease was caused by stale air around swamps. This belief led to European colonists preferring to settle away from such areas, often choosing higher altitudes. This strategy was beneficial because it moved the colonists away from the breeding sites of the actual vectors of the disease, *Anopheles* spp. mosquitoes.

The concept of humoral derangement was reimported into mediæval Europe, via Sicily, during the Crusades, and food was imbued with the same properties as the humours (Tannahill, 1968). The concept persists in several cultures. In indigenous Indian Ayurvedic human and veterinary medicine, based on the Hindu Scriptures (Vedas), there are three humours (tridosha): vata (wind), pitta (bile) and kapha (phlegm); derangement of vata, for example, causing asthma and diarrhoea. This concept is also central to modern Mahayana Buddhist medicine. However, in Europe, the popularity of the miasmatic theory declined at the beginning of the 20th century, by which time the microbial theory of infectious disease was adequately supported.

⁷ Such mixtures were essentially witches' concoctions, which could be formulated for either good or evil (Fletcher, 1896).

⁸ These elements were originally posited by the Presocratic Greek philosopher, Empedocles, who termed them 'roots' and associated them with the mythical individuals, *Hera*, *Aidoneus* (*Hades*), *Nestis* (*Persephone*) and *Zeus* (Kirk *et al.*, 1983).

⁹ This explains why urban Victorians draped thick curtains in their windows in an attempt to keep out disease. Miasmata were documented as being responsible for the initial development of the European plague epidemics (the Black Death) throughout the sixth and seventh centuries (Maddicott, 1997) and during 1347–1350 (De Smet, 1856).

The Greek idea of disease was susceptible to scientific investigation. Careful observation and the identification of specific causes became the hallmarks of the fifth-century BC school of medicine at Cos, and were refined by Hippocrates whose text, *Discourse on Airs, Waters and Places* (Jones, 1923), dominated medicine for many centuries. Therapy was consistent with causal concepts, and included purges and alterations in diet.

Contagion

The idea that some diseases can be transmitted from one animal to another has its ubiquitous origins in antiquity, and ancient veterinary accounts of disease provide strong evidence of the concept of contagiousness (Bodson, 1994). Galen and the Roman, Lucretius, believed that disease could be spread by airborne **seeds** or **animacula** (not necessarily living), which were taken in through the nose and mouth¹⁰. The Jewish Talmud describes demons as hiding 'everywhere' – in water, crumbs and air – implying transmissibility. The primitive Hindus associated sick rats with human plague, the first suggestion of a zoonosis. In the 14th century, the Tartars besieging the Genoese settlement at Caffa (Feodosia) in Crimea lobbed the corpses of plague victims over the city walls in the hope that this would spread the disease in the city (Ziegler, 1969). The Veronan, Fracastorius, writing in the early 16th century, argued that diseases were transmitted by minute, invisible particles (Wright, 1930); and Giovanni Lancisi, physician to Pope Clement XI, freed Rome from rinderpest in the early 18th century by using a slaughter policy to prevent infection of unaffected animals (Mantovani and Zanetti, 1993). Thomas Lobb, writing in London in the same century, considered that human plague and rinderpest were caused by particles that multiplied in infected individuals and then infected others, either by contact or through the air (Lobb, 1745). The 18th-century American lexicographer and essayist, Noah Webster, classified diseases as either miasmatic (e.g., pneumonia) or contagious (e.g., smallpox), representing an intermediate stage in the evolution of the contagion theory (Winslow, 1934).

The main advances in the identification of microbes as causes of infectious diseases occurred in the 19th century, although the concept of a living contagious

agent, **contagium animatum**, was founded in the 17th century. Edward Jenner's development of a smallpox vaccine using cowpox-infective material (Fisher, 1991), and the proposed use of blankets belonging to smallpox victims as biological weapons against Native American Indians in the 18th century (Ewald, 2000; Brown, 2006), implicitly recognized contagion.

Louis Pasteur's investigation of anthrax and rabies (Walden, 2003), and Robert Koch's discovery of the bacteria causing tuberculosis¹¹ and cholera (Münch, 2003), firmly established microbiology and marked the downfall of the miasmatic theory. The set of postulates formulated by Koch to define causal agents has been used to identify many microbial diseases since those early days of bacteriology (see Chapter 3).

Viruses also were discovered in the late 19th century, although not actually 'seen' until the invention of the electron microscope in the 1930s. In 1892, Iwanowsky demonstrated that tobacco mosaic disease could be transmitted by sap that had been filtered through bacteria-proof filters (Witz, 1998). Beijerinck serially transmitted the disease using bacteria-free filtrates, and coined the term **contagium vivum fluidum** to describe the infectious 'living' agent. In 1898–1899, Loeffler and Frosch discovered the first animal virus, foot-and-mouth disease virus (Mahy, 2005), and in 1911 Rous reported the first virus-induced transmissible tumour (Weiss and Vogt, 2011).

Towards the end of the 19th century, the first arthropod carrier (a tick) of an infectious disease was identified by Smith and Kilbourne (1893) investigating Texas fever of cattle in the US.

Impetus for change

Changing attitudes towards the cause of disease and the concomitant alterations in techniques of treatment and prevention are a small part of shifts in overall scientific thought. These changes have not taken place gradually, but have occurred as distinct 'revolutions', which terminate periods of stable science (Kuhn, 1970¹²). Each period has its paradigm (model), which serves to guide research. As time passes, anomalies accumulate. Initially, scientists accommodate these by modifying the paradigm, but a time comes when the pressures on the old framework become so great that a crisis occurs, and there is a revolutionary shift

¹⁰ Galen also was an early exponent of preventive medicine, arguing that a good health regimen involved regulation of six 'non-naturals' (air; motion and rest; sleep and waking; food and drink; things excreted; and passions and emotions) (Curth, 2003). The idea was imported into medieval Europe from Arabian medicine in the 11th century (Porter, 1997).

¹¹ Anthrax and tuberculosis are diseases of great antiquity, dating back at least to Ancient Egypt, where the former bacterium was most likely the cause of some of the biblical plagues (Manchester, 1984; Blaisdell, 1994; Willcox, 2002; Witowski and Parish, 2002; Sternbach, 2003; Kyriacou *et al.*, 2006).

¹² For a critical assessment of Kuhn's philosophy, see Hoyningen-Huene (1993).

in paradigms. For example, in astronomy, the old Ptolemaic model of the universe had to be modified and remodified by adding new planetary epicycles to account for the observed motion of the heavenly bodies, but eventually the critical point was reached when the old model was falling apart under the strain and was ceasing to be credible. Thus, the time was ripe for the dramatic shift in models called the Copernican revolution. Kuhn's thesis also has been applied to political, social and theological 'revolutions' (Macquarrie, 1978) and to the applied sciences (Nordenstam and Tornebohm, 1979) of which veterinary medicine is a part¹³.

Veterinary medicine has experienced five stable periods and revolutions up to the middle of the 20th century relating to disease control (Schwabe, 1982), which stimulated the changes in the causal concepts already described. The major problem that persisted during these periods, precipitating crises, was large-scale outbreaks of infectious disease: the classical animal plagues¹⁴ (Table 1.1). Military campaigns frequently assisted the spread of these infections (Table 1.2 and Karasszon, 1988).

The first period: until the first century AD

The initial domestication of animals brought man into close contact with animals and therefore with their diseases. The demonic theory was prevalent. However, despite the use of control techniques consistent with the theory, draft animals continued to die, and a crisis arose when urbanization increased the importance of animals as food resources. This resulted in the development of the first stable phase of veterinary medicine. This was characterized by the emergence of veterinary specialists such as the early Egyptian priest-healers and the Vedic *Salihotriya* who founded the first veterinary hospitals. Humoral pathology developed and the miasmatic theory of cause evolved. Techniques of treatment required careful recognition of clinical signs following the Greek Coan tradition. Quarantine

(derived from the Italian word meaning 'forty' – the traditional length, in days, of isolation in the Middle Ages) and slaughter became preventive strategies. These local actions, which lasted until the first century AD, were incapable of solving major problems in the horse, which was becoming an important military animal. This crisis resulted in the second phase: that of military healers.

The second period: the first century AD until 1762

Veterinarians specialized in equine medicine and surgery, reflecting the importance and value of horses (e.g., Richards, 1954). A major veterinary text, the *Hippiatrica*, comprising letters between veterinarians, cavalry officers and castrators, dates from early Byzantine times. The major contributor to this work was Apsyrtus, chief *hippiatros* to the army of Constantine the Great. This phase lasted until the mid-18th century and was marked by a continuing interest in equine matters. Several important texts were written, including Vegetius Renatus' *Ars Veterinaria* (published in 1528) and Carlo Ruini's *Anatomy of the Horse* (published in 1598). Interest was taken in other species, too. The 15th-century *Boke of Saint Albans* described diseases of falcons (Comben, 1969); and John Fitzherbert's *Boke of Husbandrie* (published in 1523) included diseases of cattle and sheep. The horse, however, was pre-eminent. This bias survived in Europe until early in the 20th century, when equine veterinary medicine was still considered to be a more respectable occupation than the care of other species.

During this period, varying emphasis was placed on the miasmatic and metaphysical theories of cause and on humoral pathology. The Arabians, for example, based their medicine largely on the metaphysical theory.

The third period: 1762–1884

The animal plagues, especially those of cattle, became particularly common in Europe in the mid-18th century with the introduction of rinderpest from Asia (Scott, 1996). They provided the next major crisis involving civilian animals. The miasmatic theory persisted but the miasmata were thought to originate from filth generated by man, rather than from natural sources. A third stable phase developed, characterized by improvement of farm hygiene, slaughter and treatment as control techniques. When rinderpest entered England from Holland in 1714, Thomas Bates, surgeon to George I, advocated fumigation of buildings, slaughter and burning of affected animals, and resting of contaminated pasture as typical tactics (Bates, 1717–1719). Cattle owners also were compensated for loss. By the mid-19th century, disinfection (notably

¹³ The concept of dramatic paradigm shifts, however, may not be applicable to all areas of thought and progress. The 17th-century German philosopher, Leibnitz, argued that change (e.g., in ethics and aesthetics) is gradual.

¹⁴ A plague (Greek: *plege* = 'a stroke', 'a blow'; Latin: *plangere* = 'to strike') traditionally is any widespread infectious disease with a high fatality rate among clinically affected individuals. In veterinary medicine, the term is extended to any widespread infectious disease causing major economic disruption, although the fatality rate may not be high (e.g., foot-and-mouth disease). In human medicine, the term is now commonly restricted to infection with the bacterium, *Yersinia (Pasteurella) pestis*: the cause of plague, historically famed as the mediaeval Black Death (Bos *et al.*, 2011).

Table 1.1 Some dates of occurrence of animal plagues. (Most dates before 1960 are extracted from Smithcors, 1957.)

Date	Animal plagues					
	Rinderpest	Pleuropneumonia	Canine distemper	Anthrax	Foot-and-mouth disease	Equine influenza
500 BC						Egypt 500 BC to time of Christ Egypt 278 BC abortion
AD				Rome AD 500		Rome fourth century AD (cattle) France sixth century AD (cattle) Ireland eighth century AD France 820, 850, 940–943 (cattle) England 1314 (cattle)
AD 1400	England 1490*, 1551			Italy 1514		England 1688
AD 1700	France 1710–1714 Rome 1713 England 1714, 1745–1746 France 1750	Europe 18th century				England 1727 Ireland 1728 England 1733, 1737, 1750, 1760, 1771, 1788
AD 1800		England 1841–1898			England 1839	England 1837
	England 1865				England 1870–1872, 1877–1885	North America 1872 England 1889–1890
	Africa 1890–1900					
AD 1900						

(Continued)

Table 1.1 (Continued)

<i>Animal plagues</i>						
<i>Date</i>	<i>Rinderpest</i>	<i>Pleuropneumonia</i>	<i>Canine distemper</i>	<i>Anthrax</i>	<i>Foot-and-mouth disease</i>	<i>Equine influenza</i>
	Belgium 1920				England 1922–1925, 1942, 1952, 1967–1968	Czechoslovakia 1957 Britain 1963 US 1963
					Canada 1951–52	Europe 1965 Poland 1969 USSR 1976
	Middle East 1969–1970					France, The Netherlands,
	Africa 1979–1984				Switzerland 1980	Sweden 1978–1979
	India 1983–1985				Channel Islands, France, Isle of Wight, Italy, Spain 1981	England 1989
	Turkey 1991–1992				Denmark, Spain 1983 Germany (W.), Greece, The Netherlands, Portugal 1984 Italy 1985, 1988, 1993 India, S. Arabia 1990 Nepal 1993–1994 Turkey 1995 Albania, Bulgaria, Greece, Macedonia, Yugoslavia 1996 Taiwan 1997 Bhutan 1998 China 1999	
AD 2000					Greece, Japan, Mongolia, S. Africa 2000 France, Ireland, The Netherlands, UK 2001 UK 2007 Japan 2010 S. Korea 2010–2011	

* Named as 'steppe murrain' (derivative of Latin *morí* = 'to die').

Table 1.2 Military campaigns that disseminated rinderpest.

Century	Campaign
5th	Fall of Rome
8–9th	Charlemagne's conquest of Europe
12th	Genghis Kahn's invasion of Europe
13th	Kublai Kahn's invasion of Europe and China
15–16th	Spanish–Hapsburg conquest of Italy
17th	War of the League of Augsburg
18th	War of the Spanish Succession
18th	War of the Austrian Succession
18th	Seven Years' War
18–19th	Napoleonic Wars
19–20th	US invasion of the Philippines
19–20th	Italian conquest of Eritrea
20th	World War I
20th	World War II
20th	Vietnam War
20th	Lebanese War
20th	Sri Lankan conflict
20th	Azerbaijan conflict
20th	Gulf War

using carbolic and cresylic acids) also was being applied to control the disease (Brook, 2002)¹⁵.

Rinderpest came to particular prominence in Europe in the 18th century (*Figure 1.4*). Half of the cattle in France were destroyed by rinderpest between 1710 and 1714. The disease occurred irregularly until 1750, when it again became a serious problem. Little was known about the disease. This provided impetus for the establishment of the first permanent veterinary

school at Lyons in 1762, and others were subsequently founded to combat the disease (*Table 1.3*)¹⁶.

The lifting of animal importation restrictions in England in 1842 increased the risk of disease occurring in Britain. Sheep pox entered Britain in 1847 from Germany, and pleuropneumonia became a serious problem. Public concern, highlighted by the rinderpest outbreak of 1865 (Scott, 1997), was responsible for the establishment of the British State Veterinary Service in the same year. Similar services were founded in other countries. The legislature continued to strengthen the power of the veterinary services by passing Acts relating to the control of animal diseases.

The fourth period: 1884–1960

The animal plagues continued despite sanitary campaigns. This crisis coincided with the inception and acceptance of the microbial theory which, epitomized by Koch's postulates, defined a specific, single cause of an infectious disease and therefore implied a suitable control strategy directed against the causal agent.

This fourth stable phase of campaigns or mass actions began in the 1880s. Treatment of disease was based on laboratory diagnosis involving isolation of agents and identification of lesions followed by therapy. Control of disease by prevention and, subsequently, eradication involved mass testing of animals and immunization when an increasing number of vaccines became available (e.g., canine distemper vaccine: Bresalier and Worboys, 2014)¹⁷. The discovery of disease vectors facilitated prevention by vector control. An improved understanding of infectious agents' life histories enabled their life-cycles to be broken by manipulating the environment; the draining of land to prevent fascioliasis is a good example. Bacterial diseases remained as major clinical problems until the discovery and synthesis of antibiotics in the 20th century, when, in human medicine, the effectiveness of control measures was starkly reflected in changes in

¹⁵ A notable example of disinfection from human medicine was Ignaz Semmelweis' implementation of a schedule of hand-washing and use of chlorinated lime solutions to reduce the incidence of puerperal fever (Semmelweis, 1861; Carter, 1983). He had noted a clear difference in the frequency of puerperal fever ('childbed fever') in two maternity clinics at the Vienna General Hospital. One clinic (with a low mortality due to the disease) trained midwives, whereas the other (with a higher mortality) trained physicians. He ascribed the difference to the fact that the medical students also conducted post-mortem examinations, after which they transmitted toxic 'decaying animal-organic matter' from the cadavers to the clinic. However, his views were treated with disdain by the medical establishment of the time and led to the term 'Semmelweis reflex' or 'Semmelweis effect' as a metaphor for the reflex-like tendency to reject new evidence or new knowledge because it contradicts established norms, beliefs or paradigms.

¹⁶ There were other pressures, too, fostering the establishment of European veterinary schools. These included the need for military authorities to improve the effectiveness of horse-dependent armies, and the requirement for increased agricultural productivity in the face of population growth (Mathijsen, 1997).

¹⁷ There had been earlier attempts to immunize animals against rinderpest, mimicking the practice of 'variola' in humans, in which smallpox virus was inoculated to prevent the disease (Weiss and Esparza, 2015). The first such rinderpest trial was undertaken in 1711 by Bernardino Ramazzini at the University of Padua (Koch, 1891). Some authorities believe that the practice of immunization began in China; see Parish (1965), Bazin (2003) and Stern and Markel (2005) for brief histories.



Figure 1.4 Rinderpest in Holland, 1745, Jacobus Eussen, Amsterdam. (Reproduced with permission of Atlas Van Stolk, Rotterdam.)

Table 1.3 Veterinary schools founded to combat rinderpest.

<i>Year of foundation</i>	<i>City</i>	<i>Country</i>
1762	Lyon	France
1765	Vienna	Austria
1766	Alfort	France
1769	Turin	Piedmont
1773	Copenhagen	Denmark
1777	Giessen	Hesse
1778	Hannover	Hannover

the 'league table' of causes of deaths (*Figure 1.5*). The veterinarian also experienced a similar increase in therapeutic power.

Many infectious diseases were either effectively controlled or eradicated between the latter part of the 19th century and the middle of the 20th century in the more-economically-developed countries using the new techniques of the microbial revolution and older techniques including quarantine, importation restrictions, slaughter and hygiene. In 1892, pleuropneumonia in the US was the first disease to be regionally eradicated after a campaign lasting only five years. Notable British successes included rinderpest,

eradicated in 1877, pleuropneumonia in 1898, and glanders and equine parasitic mange in 1928.

Quantification in medicine

The evolution of understanding of the cause of disease purely qualitatively was accompanied by increased interest in disease in quantitative terms. This began primarily as a descriptive exercise. The ancient Japanese reported outbreaks of animal diseases. John Graunt (1662) published quantitative observations on London parish registers and 'Bills of Mortality'. An outbreak of rinderpest in France in the late 18th century was responsible for the establishment of a commission on epidemics, headed by Felix Vicq d'Azyr, Marie Antoinette's personal physician. This evolved into the Royal Society of Medicine, which pioneered the collection of statistical data on animal and human epidemics and the weather (Matthews, 1995).

Post-Renaissance thinking and 'The Enlightenment'

The scientific revolution that began during the 16th century posited that the physical universe was orderly and could be explained mathematically (Dampier, 1948). This argument was extended to the biological world, where it was considered that 'laws of mortality' must exist. Graunt's mortality studies included

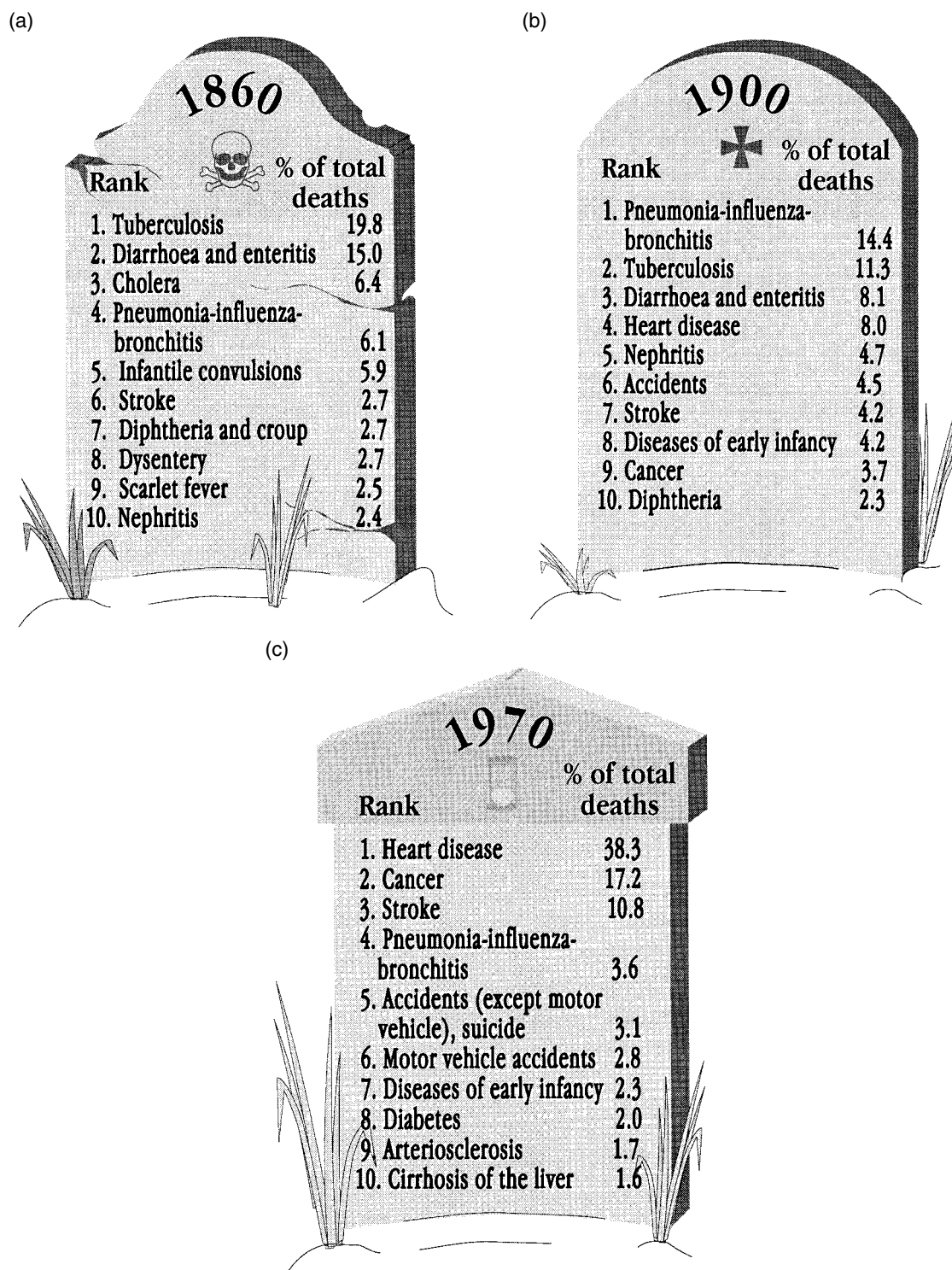


Figure 1.5 League table of causes of human mortality in the UK: (a) 1860, (b) 1900, (c) 1970. (Data from Thrusfield, 2001.)

attempts to formulate such laws by constructing life tables (see Chapter 4); Edmund Halley (1656–1742) constructed life tables for Breslau (Benjamin, 1959); and Daniel Bernoulli (1700–1782) applied life-table

methods to smallpox data, thereby demonstrating that inoculation was efficacious in conferring lifelong immunity (Speiser, 1982). A hundred years later, William Farr, the Collator of Abstracts at the Office

of the Registrar General in England in the mid-19th century (Halliday, 2000), produced a simple mathematical model of the 1865 rinderpest epidemic in the UK (see Chapter 23).

Quantitative analysis of biological (including medical) phenomena evolved in the 18th century, when the *Age of Enlightenment* saw a growth in literature dealing with the relationship between probability and the need for objectivity in science and society (the 'Probabilistic Revolution'). The mathematical foundation of probability was laid by Jakob Bernoulli in his *Ars Conjectandi*, which was published posthumously in 1713. He developed a theory of 'inverse probability', which stated that the frequency of an event would approach its probability of occurrence if the number of observations was large enough. This theory was mathematically refined by Siméon-Denis Poisson, who proposed a 'law of large numbers', which stated that, if an event was observed a large number of times, one could assume that the probability of its future occurrence would correspond to its observed frequency (Poisson, 1837). The logical consequence of this conclusion is that, if there are sufficient observations, sound predictions can be made. Thus, in relation to therapy, Pierre-Simon Laplace (1814) suggested that a preferred method of treatment '*will manifest itself more and more in the measure that the number (of observations) is increased*'.

A pivotal move towards comparative statistical techniques occurred when Pierre-Charles-Alexander Louis developed his 'numerical method', requiring systematic record keeping and rigorous analysis of multiple cases (Bollett, 1973). He documented typhoid in Paris, showing that the disease occurred predominantly in young adults, and that the average age of fatal cases was higher than that of survivors, suggesting that the younger patients had the best prognosis (Louis, 1836). He subsequently demonstrated that blood-letting was of no benefit to typhoid cases, and his calculation of average values was adopted by other early protagonists of clinical trials (e.g., Joseph Lister; see Chapter 17)¹⁸. Average values were also applied to provide a quantitative definition of a 'normal' individual; Adolphe Quetelet (1835), for example, recorded the range of the human cardiac and respiratory rates.

Application of probability theory to medicine was cautiously and tendentiously accepted by British and French medical statisticians, who were largely

concerned with the descriptive statistics of the major public health issues (e.g., *Figure 1.5*), rather than with statistical inference. Nevertheless, during the 19th century, strong links were forged between epidemiologists, mathematicians and statisticians through the common influence of Louis (Lilienfeld, 1979)¹⁹, and, by the 20th century, rigorous methods of statistical inference were developing (Stigler, 1986) and were being applied in medicine and agriculture. These methods necessitate observation of events in populations, rather than in the individual, and are thus central to the development of quantitative epidemiology (see Chapter 2).

The formulation of physical and biological events, however, now, as then, needs to be very carefully assessed, and may convey an illusion of certainty and security that is not warranted (Gupta, 2001). Moreover, it is not always considered to be socially beneficial²⁰, and is not a substitute for rigorous, albeit sometimes onerous, analysis of field data (*The Economist*, 2002). Additionally, there may be a tendency to use whatever numerical data are available, regardless of their relevance and quality (Gill, 1993; Wang *et al.*, 2013)²¹.

Contemporary veterinary medicine

Current perspectives

Infectious diseases

Although there have been notable successes in the control of the infectious diseases, some still pose problems in both more-economically-developed and less-economically-developed countries (*Table 1.4*), and some continue to recur; for example, foot-and-mouth disease (*Table 1.5*), which erupted in Western Europe with devastating consequences (notably in the UK) in

¹⁸ The application of statistical methods in medicine by Louis, however, was not without its contemporary critics (Fernández-Guerrero *et al.*, 2014).

¹⁹ An interesting 'family tree', showing the links between 18th–20th-century statisticians, public health physicians and epidemiologists, is depicted by Lilienfeld and Lilienfeld (1980).

²⁰ See, for example, Gregory (2002) for a brief theological discussion.

²¹ Chambers (1997) amalgamates Gupta's and Gill's points, with sights set particularly on economists: '*Quantification and statistics can mislead, distract, be wasteful, simply not make sense or conflict with common values...Yet professionals, especially economists and consultants tight for time, have a strong felt need for statistics. At worst they grub around and grab what numbers they can, feed them into their computers and print out not just numbers, but more and more elegant graphs, bar charts, pie charts and three-dimensional wonders...Numbers can also reassure by appearing to extend control, precision and knowledge beyond their real limits...wrong numbers, one might add, are worst of all because all numbers pose as true.*' Porter (1995) provides a philosophical discussion of quantification in general.

Table 1.4 Current trends in the distribution of some infectious diseases of animals. (Extracted mainly from Mulhern *et al.*, 1962; Knight, 1972; Blaha, 1989; West, 1995; and Radostits *et al.*, 1999.)

<i>Disease</i>	<i>Host</i>	<i>Trends</i>
Anthrax	All animals, particularly devastating in cattle	Worldwide range, now contracting to mainly tropics and sub-tropics
Aujeszky's disease	Pigs	Spreading
Bluetongue	Sheep and other ruminants	Spreading for past 100 years
Bovine brucellosis	Cattle	Eradicated from many of the more-economically-developed countries
Contagious bovine pleuropneumonia	Cattle	Eradicated from Europe
Glanders	Horses	Mostly eradicated from the more-economically-developed countries
Johne's disease	Cattle, sheep, goats	Worldwide distribution with increasing prevalence in some countries, and spreading in Europe
Lumpy skin disease	Cattle	Extending from Africa to the Middle East
Rabies	All mammals, some birds	Eradication is problematic. Geographically isolated areas (including some island masses) are generally free, although most countries experience rabies to some extent
Rift Valley fever	Cattle, sheep, goats, man	Extending from Africa to the Middle East
Rinderpest	Artiodactyls	Declared eradicated globally in 2011
Sheep pox	Sheep	Eradicated from Europe in 1951. Present in Africa, the Middle East and India
Swine vesicular disease	Pigs	Decreased significance since 1982
Tuberculosis	Many species, especially serious in cattle	Eradication has proved problematic but some success has been achieved

Table 1.5 Number of outbreaks of foot-and-mouth disease in the UK, 1892–2016.

<i>Year</i>	<i>Outbreaks</i>	<i>Year</i>	<i>Outbreaks</i>	<i>Year</i>	<i>Outbreaks</i>	<i>Year</i>	<i>Outbreaks</i>
1892	95	1919	75	1939	99	1959	45
1893	2	1920	13	1940	100	1960	298
1894	3	1921	44	1941	264	1961	103
1895	0	1922	1140	1942	670	1962	5
1896–1899	0	1923	1929	1943	27	1963–1964	0
1900	21	1924	1	1944	181	1965	1
1901	12	1925	260	1945	129	1966	34
1902	1	1926	204	1946	64	1967	2210
1903–1907	0	1927	143	1947	104	1968	187
1908	3	1928	138	1948	15	1969–1980	0
1909	0	1929	38	1949	15	1981	1
1910	2	1930	8	1950	20	1982–2000	0
1911	19	1931	97	1951	116	2001	2030
1912	83	1932	25	1952	495	2002–2006	0
1913	2	1933	87	1953	40	2007	8
1914	27	1934	79	1954	12	2008–2016	0
1915	56	1935	56	1955	9		
1916	1	1936	67	1956	162		
1917	0	1937	187	1957	184		
1918	3	1938	190	1958	116		

Table 1.6 Some emergent infectious diseases and plagues of animals in the 20th century.

Year	Country	Infection	Source
1907	Kenya	African swine fever	Montgomery (1921)
1910	Kenya	Nairobi sheep disease	Montgomery (1917)
1918	US	Swine influenza	Shope (1931)
1929	South Africa	Lumpy skin disease	Thomas and Maré (1945)
1929	US	Swine pox	McNutt <i>et al.</i> (1929)
1930	US	Eastern equine encephalomyelitis	Kissling <i>et al.</i> (1954)
1930	US	Western equine encephalomyelitis	Kissling (1958)
1932	US	Vesicular exanthema of pigs	Traum (1936)
1933	Iceland	Maedi-visna	Sigurdsson (1954)
1939	Colombia	Venezuelan equine encephalomyelitis	Kubes and Rios (1939)
1946	Canada	Mink enteritis	Schofield (1949)
1947	US	Transmissible mink encephalopathy	Hartsough and Burger (1965)
1953	US	Bovine mucosal disease	Ramsey and Chivers (1953)
1955	US	Infectious bovine rhinotracheitis	Miller (1955)
1956	Czechoslovakia	Equine influenza A (H7N7)	Bryans (1964)
1962	France	West Nile equine encephalomyelitis	Panthier (1968)
1963	US	Equine influenza A (H3N8)	Bryans (1964)
1966	UK	Bovine ulcerative mammillitis	Martin <i>et al.</i> (1966)
1972	Iran	Camel pox	Baxby (1972)
1972	US	Lyme disease	Steere <i>et al.</i> (1977)
1974	Kenya	Horsepox	Kaminjolo <i>et al.</i> (1974)
1975	South Africa	Haemorrhagic Rift Valley fever	Van Velden <i>et al.</i> (1977)
1977	Worldwide	Canine parvovirus	Eugster <i>et al.</i> (1978)
1977	USSR	Cat pox	Marennikova <i>et al.</i> (1977)
1980	UK	Infectious bursitis-2	McFerran <i>et al.</i> (1980)
1981	Zimbabwe	Mokola virus infection	Foggin (1983)
1983	US	Fulminating avian influenza (H5N2)	Buisch <i>et al.</i> (1984)
1985	Denmark	Danish bat rabies (Duvenhage)	Grauballe <i>et al.</i> (1987)
1986	UK	Bovine spongiform encephalopathy	Wells <i>et al.</i> (1987)
1986	US	Cache Valley teratogenesis	Chung <i>et al.</i> (1991)

2001 (*Table 1.1*). Some have emerged as major problems this century, although there is circumstantial evidence that the infectious agents have existed for some time (*Table 1.6*). Others are apparently novel (*Table 1.7*)²². Military conflict continues to be

responsible for spreading these diseases (*Table 1.2*); for example, at the end of the Second World War, retreating Japanese soldiers brought rinderpest from Myanmar (Burma) to north-eastern Thailand.

²² Emergent and novel infectious diseases may be classified together as 'emerging diseases': '... infections that have newly appeared in a population or have existed but are rapidly increasing in incidence or geographic range' (Morse, 1995). Alternatively, emerging diseases can also be categorized as either 'newly emerging' or 're-emerging', the former being recognized in a host for the first time, and the latter comprising historical infections that continue to appear in new locations or in drug-resistant forms, or that reappear after apparent control (Fauci and Morens, 2012); but the complicated nature of many emerging infections often leaves their classification debatable

(Morens and Fauci, 2013). Infectious diseases may emerge either as a result of genetic changes in infectious agents or their hosts (see Chapter 5) or following ecological changes (see Chapter 7). Schrag and Wiener (1995) conclude that both ecological and genetic evolutionary changes can contribute to the emergence of infectious diseases, but that ecological change is probably the more general explanation for new epidemics (probably because ecological changes are less constrained than evolutionary changes in hosts and pathogens). The emergence of exclusively human pathogens also may be the last stage in transformation from animal pathogens (Wolfe *et al.*, 2007).

Table 1.7 Some novel infectious diseases and plagues of animals in the 20th and 21st centuries.

<i>Year</i>	<i>Country</i>	<i>Infection</i>	<i>Source</i>
1907	Hungary	Marek's disease	Marek (1907)
1912	Kenya	Rift Valley fever	Daubney <i>et al.</i> (1931)
1923	The Netherlands	Duck plague	Baudet (1923)
1925	US	Avian laryngotracheitis	May and Tittsler (1925)
1926	Java	Newcastle disease	Doyle (1927)
1928	France	Feline panleukopenia	Verge and Cristoforoni (1928)
1930	US	Avian encephalomyelitis	Jones (1932)
1930	US	Avian infectious bronchitis	Schalk and Hawn (1931)
1932	US	Equine virus abortion	Dimock and Edwards (1932)
1937	US	Turkey haemorrhagic enteritis	Pomeroy and Fenstermacher (1937)
1942	Ivory Coast	Goat plague (peste des petits ruminants)	Gargadennec and Lalanne (1942)
1945	US	Duck virus hepatitis-1	Levine and Fabricant (1950)
1945	US	Transmissible gastroenteritis of pigs	Doyle and Hutchings (1946)
1946	US	Bovine virus diarrhoea	Olafson <i>et al.</i> (1946)
1946	US	Aleutian disease of mink	Hartsough and Gorham (1956)
1947	Sweden	Infectious canine hepatitis	Rubarth (1947)
1950	US	Avian adenovirus-1	Olson (1950)
1951	US	Turkey bluecomb disease	Peterson and Hymas (1951)
1953	US	Feline infectious anaemia	Flint and Moss (1953)
1954	Japan	Akabane disease	Miura <i>et al.</i> (1974)
1954	Canada	Avian reovirus	Fahey and Crawley (1954)
1956	China	Goose plague	Fang and Wang (1981)
1957	US	Feline calicivirus	Fastier (1957)
1958	US	Feline viral rhinotracheitis	Crandell and Maurer (1958)
1959	Canada	Turkey viral hepatitis	Mongeau <i>et al.</i> (1959)
1959	Japan	Ibaraki disease	Omori <i>et al.</i> (1969)
1960	Israel	Turkey meningoencephalitis	Komarov and Kalmar (1960)
1962	US	Bovine adenovirus-1,2	Klein (1962)
1962	US	Avian infectious bursitis-1	Cosgrove (1962)
1964	UK	Feline leukaemia	Jarrett <i>et al.</i> (1964)
1964	UK	Porcine adenovirus	Haig <i>et al.</i> (1964)
1965	UK	Duck virus hepatitis-2	Asplin (1965)
1965	US	Canine herpes	Carmichael <i>et al.</i> (1965)
1965	US	Porcine enteroviruses	Dunne <i>et al.</i> (1965)
1966	Italy	Swine vesicular disease	Nardelli <i>et al.</i> (1968)
1967	UK	Porcine parvovirus	Cartwright and Huck (1967)
1967	UK	Border disease in sheep	Dickinson and Barlow (1967)
1967	US	Chronic wasting disease of deer	Williams and Young (1980)
1968	Canada	Bovine adenovirus-3	Darbyshire (1968)
1969	US	Duck virus hepatitis-3	Toth (1969)
1972	UK	Lymphoproliferative disease of turkeys	Biggs <i>et al.</i> (1974)
1973	US	Equine adenovirus	McChesney <i>et al.</i> (1973)

(Continued)

Table 1.7 (Continued)

Year	Country	Infection	Source
1974	US	Feline herpes urolithiasis	Fabricant and Gillespie (1974)
1974	US	Caprine arthritis-encephalitis	Cork <i>et al.</i> (1974)
1974	Japan	Kunitachi virus	Yoshida <i>et al.</i> (1977)
1976	The Netherlands	Egg-drop syndrome	Van Eck <i>et al.</i> (1976)
1976	Japan	Avian infectious nephritis	Yamaguchi <i>et al.</i> (1979)
1977	US	Chicken parvovirus	Parker <i>et al.</i> (1977)
1977	Ireland	Contagious equine metritis	O'Driscoll <i>et al.</i> (1977)
1978	Iraq	Pigeon paramyxovirus-1	Kaleta <i>et al.</i> (1985)
1979	Japan	Chick anaemia agent	Yuasa <i>et al.</i> (1979)
1981	US	Canine calicivirus	Evermann <i>et al.</i> (1981)
1984	China	Rabbit haemorrhagic disease	Liu <i>et al.</i> (1984)
1985	US	Potomac horse fever	Rikihisa and Perry (1985)
1985	UK	Rhinotracheitis of turkeys	Anon. (1985)
1985	Japan	Chuzon disease of cattle	Miura <i>et al.</i> (1990)
1987	USSR	Phocid distemper-2 (Baikal)	Grachev <i>et al.</i> (1989)
1987	US	Porcine reproductive and respiratory syndrome	Keffaber (1989)
1987	US	Morbillivirus of dolphins	Lipscomb <i>et al.</i> (1994)
1988	The Netherlands	Phocid distemper-1 (North Sea)	Osterhaus and Vedder (1988)
1990	The Netherlands	Bovine birnavirus	Vanopdenbosch and Wellemans (1990)
1994	Australia	Hendra virus (formerly equine morbillivirus)	Murray <i>et al.</i> (1995)
1995	New Zealand	Wobbly possum disease virus	Anon. (1997)
1996	US	Porcine wasting disease syndrome	Daft <i>et al.</i> (1996)
2003	Taiwan	Herpes-like virus of abalone	Chang <i>et al.</i> (2005a)
2011	Germany	Schmallenberg virus	Hoffman <i>et al.</i> (2012)
2012	US	Canine circovirus	Kapoor <i>et al.</i> (2012)

The infectious diseases are particularly disruptive in the less-economically-developed countries, where more than half of the world's livestock are located (Table 1.8), accounting for over 80% of power and traction (Pritchard, 1986) and, in pastoral communities, at least 50% of food and income (Swift, 1988) (with milk, alone, accounting for up to 75% of human daily energy requirements: Field and Simkin, 1985). There have been some successes, for example, the JP15 campaign against rinderpest in Africa (Lepissier and MacFarlane, 1966) and, despite setbacks to this campaign caused by civil strife and complacency (Roeder and Taylor, 2002), the disease was declared globally eradicated in 2011 (FAO/OIE, 2011). Several vector-transmitted diseases with complex life-cycles, including haemoprotozoan infections such as trypanosomiasis, have not been controlled

satisfactorily²³. The techniques of the microbial revolution have enabled these diseases to be identified, but accurate means of assessing their extent and distribution also are necessary in order to plan control programmes.

²³ The reasons for lack of progress in the control of animal diseases in less-economically-developed countries are complex, including more than lack of technical feasibility. Insufficient applied research to solve field problems, poor information, and neglect of farmers' needs and the requirement for farmer participation in disease control, all contribute to the problem (Huhn and Baumann, 1996; Bourn and Blench, 1999). With the demand for livestock products in less-economically-developed countries estimated to double by 2020 (Delgado *et al.*, 1999), the supply of livestock services, including veterinary services, is likely to increase in importance, and issues such as privatization (Holden *et al.*, 1996; Ahuja, 2004) and delivery to the poor (Ahuja and Redmond, 2004) will require close scrutiny.

Table 1.8 World livestock populations, 2011 (1000s of animals). (From FAO: faostat.fao.org/site/573/default.aspx#ancor; accessed 16 August 2013.)

	<i>Cattle</i>	<i>Sheep</i>	<i>Goats</i>	<i>Pigs</i>	<i>Horses</i>	<i>Chickens</i>	<i>Buffaloes</i>	<i>Camels</i>
North America	104 838	6 379	3 026	79 146	10 556	2 244 690	–	–
Central America	46 940	8 875	9 196	20 530	7 273	659 892	–	–
South America	350 960	73 149	22 155	62 383	15 051	2 190 251	1 279	–
Europe	121 263	127 532	17 281	187 536	5 829	2 039 218	390	7
Africa	276 361	304 671	321 353	32 205	6 104	1 732 180	3 800	22 684
Asia	477 541	466 059	542 366	576 632	13 755	11 513 988	189 922	3 944
Oceania	39 261	104 247	4 917	5 178	395	116 094	210	–
All parts of the world	1 426 389	1 093 566	924 146	967 165	58 472	20 708 000	195 397	26 635

Some infectious diseases, for example, brucellosis and tuberculosis, persist at low levels in the more-economically-developed countries, despite the application of traditional control methods. This problem can result from inadequate survey techniques and insensitive diagnostic tests (Martin, 1977). In some cases, an infectious agent may have a more complex natural history than initially suspected. For example, continued outbreaks of bovine tuberculosis in problem herds in England (Wilesmith *et al.*, 1982) have been shown to be associated with pockets of infection in wild badgers (Little *et al.*, 1982; Krebs, 1997), which has resulted in a somewhat contentious control strategy of badger culling (Donnelly *et al.*, 2003; DEFRA, 2004; Grant, 2009).

The effective control of the major infectious diseases has allowed an increase in both animal numbers (Table 1.9) and productivity (Table 1.10) in the more-economically-developed countries (mechanization making draft horses the exception)²⁴. There generally has been an increase in the size of herds and flocks, notably in dairy, pig (Table 1.11) and poultry enterprises. Intensification of animal industries is accompanied by changes in animal health problems.

²⁴ Some earlier improvements in productivity had occurred as a consequence of improved nutrition. For example, in England in the 18th century, more land was planted with high-yielding roots (e.g., turnips) and new types of grass, enabling animals to be fed adequately throughout the year. The average weight of an ox at London's Smithfield Market increased from 370 lb in 1710 to 800 lb in 1795 (Paston-Williams, 1993). Additionally, in the 19th century, crop yields were increased further by the development of fertilizers (Sneddon, 2014), and livestock feed conversion and weight gain were boosted in the 20th century by the use of growth promoters (Lawrence, 1998). The demand for increased livestock production is historically linked to human population growth, rising incomes and urbanization, although future trends are likely to be affected by competition for natural resources (notably, land and water), socio-economic factors (e.g., environmental and animal-welfare legislation) and carbon constraints (Thornton, 2010).

Complex infectious diseases

The animal plagues are caused by 'simple' agents, that is, their predominant causes can be identified as single infectious agents. Diseases caused by single agents still constitute problems in the more-economically-developed countries. Examples include salmonellosis, leptospirosis, babesiosis and coccidiosis. However, diseases have been identified that are produced by simultaneous infection with more than one agent (mixed infections), and by interaction between infectious agents and non-infectious factors. These are common in intensive production enterprises. Diseases of the internal body surfaces – enteric and respiratory diseases – are particular problems. Single agents alone cannot account for the pathogenesis of these complex diseases.

Subclinical diseases

Some diseases do not produce overt clinical signs although they often affect production. These are called **subclinical** diseases. Helminthiasis and marginal mineral deficiencies, for example, decrease rates of live-weight gain. Porcine adenomatosis decreases growth in piglets, although there may be no clinical signs (Roberts *et al.*, 1979). Infection of pregnant sows with porcine parvovirus in early pregnancy destroys fetuses, the only sign being small numbers of piglets in litters (Mengeling, 2006). These diseases are major causes of production loss; their identification often requires laboratory investigations.

Non-infectious diseases

Non-infectious diseases have increased in importance following control of the major infectious ones. They can be predominantly genetic (e.g., canine hip dysplasia), metabolic (e.g., bovine ketosis) or neoplastic (e.g., canine mammary cancer). Their cause may be

Table 1.9 The livestock population of Great Britain, 1866–2012 (1000s of animals). (From HMSO, 1968, 1982, 1991, 1998; DEFRA, 2012c.)

Year	Cattle	Sheep	Pigs	Horses (agricultural use)	Fowls	Turkeys
1866	4 786	22 048	2 478	–	–	–
1900	6 805	26 592	2 382	1 078	–	–
1925	7 368	23 094	2 799	910	39 036	730
1950	9 630	19 714	2 463	347	71 176	855
1965	10 826	28 837	6 731	21	101 956	4 323
1980	11 919	30 385	7 124	–	115 895	6 335
1989	10 510	38 869	7 391	–	121 279	–
1997	9 902	39 943	7 375	–	111 566*	–
2012 [†]	9 900	32 215	4 281	–	102 558	3 747

– Data not available.

* 1995 figure. (A new approach to collecting poultry information began in 1997, preventing direct comparisons with previous years.)

[†] Data for 2012 collected in a different manner to previous years, preventing direct comparison.

Table 1.10 World cattle productivity, 2011. (From FAO: faostat.fao.org/site/569/default.aspx#ancor, accessed 16 August 2013.)

	Number of animals slaughtered (1000s of animals)	Carcass weight (kg/animal)	Milk yield (kg/animal)	Milk production (1000s metric tonnes)
US	35 108	341	9 678	89 015
South America	64 492	225	1 780	65 597
Asia	89 089	154	1 631	168 396
Africa	35 345	152	533	31 171
Europe	43 517	247	5 520	208 986
Oceania	12 135	227	4 192	27 065
All parts of the world	295 828	212	2 363	614 579

associated with several factors; for example, feline urolithiasis is associated with breed, sex, age and diet (Willeberg, 1977).

Some conditions, such as ketosis, are particularly related to increased levels of production; ketosis is more likely in cows with high milk yields than in those with low yields. Intensive production systems may also be directly responsible for some conditions; for example, foot lesions in individually-caged broilers (Pearson, 1983).

Diseases of unknown cause

The cause of some diseases has not been fully elucidated, despite intensive experimental and field investigations over many years. Examples include the related diseases feline dysautonomia (Edney *et al.*, 1987; Nunn *et al.*, 2004) and equine grass sickness (Hunter *et al.*, 1999; McCarthy *et al.*, 2001; Wlaschitz, 2004).

In some situations, infectious agents have been isolated from cases of a disease but cannot be unequivocally associated with the disease. An example is *Mannheimia haemolytica* (previously named *Pasteurella haemolytica*) in relation to 'shipping fever' (Martin *et al.*, 1982). This syndrome occurs in cattle soon after their arrival at feedlots. Post-mortem examination of fatal cases has revealed that fibrinous pneumonia is a common cause of death. Although *M. haemolytica* frequently is isolated from lungs, it is not invariably present. Attempts to reproduce the disease experimentally are fraught with problems, not the least of which is the difficulty of establishing colonization of the nasal tract with the bacterium (Whiteley *et al.*, 1992). Other factors also seem to be involved (Radostits *et al.*, 2007). These include mixing animals and then penning them in large groups, the feeding of corn silage, dehorning, and,

Table 1.11 Pig herd structure in England and Wales (June)*. (Data supplied by the Meat and Livestock Commission and BPEX.)

	1965	1971	1975	1980	1991	1999	2005 [†]	2012 [†]
Number of farms with pigs	94 639	56 900	32 291	22 973	13 738	10 460	7 560	7 860
Total sows (1000s)	756.3	791.0	686.0	701.1	672.4	580.5	384.2	351.2
Average herd size (sows)	10.4	18.5	27.6	41.4	70.4	86.3	81.4	79.3
Number of herds by size (sows):								
1–49	56 560 (75.4%)	39 000 (90.9%)	20 873 (84.0%)	12 900 (76.3%)	6 471 (67.8%)	4 714 (70.1%)	1 972 (41.8%)	2 203 (49.8%)
50–99	10 445 (13.9%)	2 700 (6.3%)	2 401 (9.7%)	2 000 (11.8%)	1 050 (11.0%)	522 (7.8%)	1 201 (25.5%)	1 072 (24.2%)
100–199	8 034 (10.7%)	1 000 (2.3%)	1 141 (4.6%)	1 300 (7.7%)	1 115 (11.7%)	581 (8.6%)	700 (14.8%)	458 (10.3%)
200 and over	–	200 (0.5%)	426 (1.7%)	700 (4.1%)	914 (9.5%)	911 (13.5%)	845 (17.9%)	693 (15.7%)
Total number of herds with sows	75 039	42 900	24 841	16 900	9 550	6 728	4 718	4 426

* The methodology for the June survey has changed. Figures are now only collected from 'commercial' holdings. Commercial holdings are those with significant levels of farming activity. These significant levels are classified as any holding with one or more of the following: more than five hectares of agricultural land, one hectare of orchards, 0.5 hectares of vegetables or 0.1 hectares of protected crops, or more than 10 cows, 50 pigs, 20 sheep, 20 goats or 1000 poultry. Therefore, figures for 2005 and 2012 are not directly comparable with those for previous years, which would have included non-commercial holdings.

[†] Figures are for England only. Of the numbers quoted, published figures for Wales now only give the total number of farms with pigs (810 in 2005 and 1410 in 2012) and the total number of sows (3900 in 2005 and 4900 in 2012).

paradoxically, vaccination against agents that cause pneumonia, including *M. haemolytica* – factors associated with adrenal stress (see *Figure 3.6b* and Chapter 5).

Management and environment also appear to play significant, although often not clearly defined, roles in other diseases. Examples include enzootic pneumonia and enteritis in calves (Roy, 1980), enteric disease in suckling pigs, porcine pneumonia, bovine mastitis associated with *Escherichia coli* and *Streptococcus uberis* (Blowey and Edmondson, 2000) and mastitis in intensively housed sows (Muirhead, 1976).

In some instances, the infectious agents that are isolated are ubiquitous and also can be isolated from healthy animals, for example, enteric organisms (Isaacson *et al.*, 1978). These are 'opportunistic' pathogens, which cause disease only when other detrimental factors are also present.

In all of these cases, attempts to identify a causal agent fulfilling Koch's postulates frequently fail, unless unnatural techniques, such as abnormal routes of infection and the use of gnotobiotic animals, are applied.

The fifth period

The animal health problems and anomalies that emerged in the 20th century stimulated a change, which began in the 1960s, in attitude towards disease causality and control.

Causality

The inappropriateness of Koch's postulates as criteria for defining the cause of many syndromes suggested that more than one factor may sometimes operate in producing disease. A **multifactorial** theory of disease has developed, equally applicable to non-infectious and infectious diseases. Interest in human diseases of complex and poorly understood cause grew in the early years of the 20th century (Lane-Clayton, 1926) and was responsible for the development of new methods for analysing risk factors, for example smoking in relation to lung cancer (Doll, 1959), and these epidemiological techniques are now firmly established in veterinary medicine, too (e.g., in the investigation of risk factors for respiratory disease in pigs: Stärk, 2000).

There is also now awareness that the causes of disease include social, geographical, economic and

political factors, as well as biological and physical ones (Hueston, 2001). For instance, although bovine spongiform encephalopathy subsequently spread to mainland Europe, its initial emergence in the UK (*Table 1.6*) was the result of recycling infective meat and bone meal to cattle, and this practice was extensive because meat and bone meal was an inexpensive source of high-quality protein in cattle rations in a country that had heavily intensified and in which plant proteins were limited. Likewise, bovine tuberculosis is a manifest and expanding problem in white-tailed deer in north-eastern Michigan in the US because the deer populations have increased as a result of feeding programmes established to serve the hunting industry that has replaced cattle farming in this economically deprived area.

New control strategies

Two major strategies have been added to the earlier techniques (Schwabe, 1980a,b):

1. the structured recording of information on disease;
2. the analysis of disease in populations.

These methods involve two complementary approaches: the continuous collection of data on disease – termed **surveillance** and **monitoring** – and the intensive investigation of particular diseases. A further technique, used at the individual farm level, is the recording of information on both the health and productivity of each animal in a herd, as a means of improving production by improving herd health.

Recent trends

Several recent trends have occurred in relation to the services that the veterinarian supplies to his or her clients, and to national and international disease reporting.

Veterinary services

Veterinarians practising in the livestock sector continue to control and treat disease in individual animals. Developments in molecular biology are improving diagnostic procedures (Buckingham, 2011), and offer new opportunities for vaccine production (Report, 1990). Additionally, in intensive production systems, the multifactorial nature of many diseases necessitates modification of the environment of the animal and management practices, rather than concentrating exclusively on infectious agents.

Diseases of food animals also are being considered directly in relation to their effect on **production**. Reduced levels of production can be used as 'diagnostic indicators'; for example, small litter size as an

indicator of infection with porcine parvovirus. More significantly, veterinary emphasis has shifted from disease as a clinical entity in the individual animal to disease assessed in terms of suboptimal health, manifested by decreased herd performance: disease is being defined as the unacceptable performance of **groups** of animals. There is thus a need to identify all factors that contribute to the occurrence of disease, to select suitable 'performance indicators' (e.g., 'calving to conception interval'), and to define targets for these indicators in herds under a particular system of husbandry. It is then possible to identify those herds that miss the targets. This is called **performance-related diagnosis** (Morris, 1982), and includes not only the measurement of overt indicators, such as live-weight gain, but also estimation of covert biochemical values, such as metabolite levels in serum. Thus, clinical disease, subclinical disease and production need to be monitored in the context of anticipated ('normal') levels for a particular production system (Dohoo, 1993).

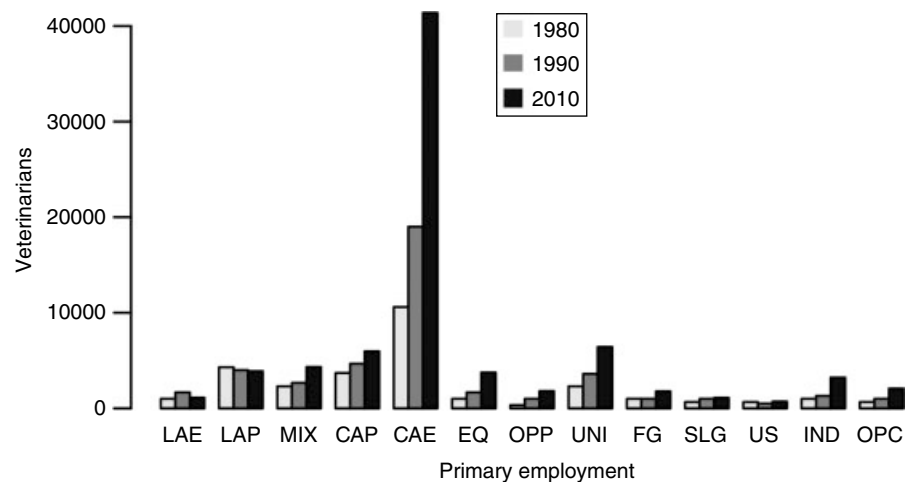
The veterinarian therefore has become more involved in husbandry, management and nutrition than previously, and less involved in traditional 'fire brigade' treatment of clinically sick animals. However, the livestock owner frequently still regards the veterinarian solely as a dispenser of treatment (Goodger and Ruppner, 1982), relying on feed representatives, dairy experts and nutritionists for advice on breeding, nutrition and management. The extent of this problem varies from one country to another, but indicates that the veterinarian's evolving role in animal production requires a change not only in veterinary attitudes but also sometimes in those of animal owners²⁵.

Government veterinary services, too, are becoming increasingly concerned with investigations of specific animal health problems of complex cause, such as mastitis, thereby extending their role beyond the traditional control of mass infectious diseases.

As the mass infectious diseases are controlled, and animal production becomes more intensive, other diseases become relatively more important. They are currently major problems in the more-economically-developed countries and in some less-economically-developed countries that have intensive enterprises, such as poultry and pig units in Malaysia, the Philippines and Taiwan. These diseases will become increasingly significant in the less-economically-developed countries when the mass infectious diseases are controlled.

²⁵ Inertia in veterinary attitudes towards preventive medicine is well-documented (Woods, 2013a; Ruston *et al.*, 2016).

Figure 1.6 Areas of employment of veterinarians in the US, 1980, 1990 and 2010. LAE: exclusively large animal; LAP: predominantly large animal; MIX: mixed practice; CAP: predominantly companion animal; CAE: exclusively companion animal; EQ: equine; OPP: other private practice; UNI: university; FG: federal government; SLG: state or local government; US: uniformed services; IND: industry; OPC: other public and corporate. (Source: Wise and Yang, 1992 and AVMA, 2013. Reproduced with permission of the American Veterinary Medical Association.)



The 20th century saw a turn towards companion-animal practice, particularly in the more-economically-developed countries (e.g., Heath, 1998)²⁶. In the UK, for example, this trend began in the inter-war years, influenced by increasing wealth and the rise of lay animal-welfare charities (Gardiner, 2014). This continues to be reflected in the employment trends in the veterinary profession (Figure 1.6). Many health problems of companion animals are complex too, and a full understanding of their cause and control is possible only when the contribution of genetic and environmental factors is appreciated. Examples include urinary tract infections in bitches, in which concurrent disease and recent chemotherapy are important factors (Freshman *et al.*, 1989), and equine colic, which is related to age and breed (Morris *et al.*, 1989; Reeves *et al.*, 1989). Problems of veterinary interest now extend beyond clinical conditions to wider social issues, such as the biting of children by dogs (Gershman *et al.*, 1994) and animal welfare (see later in this chapter).

Additionally, veterinary services have an enlarging responsibility for human health, including preventing and controlling emerging zoonotic diseases, and addressing antibiotic resistance (an area of endeavour that has been traditionally labelled **veterinary public health**), and protecting the environment and ecosystems (Chomel, 1998; Marabelli, 2003; Pappaioanou, 2004).

Food quality

A particular area of concern in veterinary public health is food quality. The public's concern about what it consumes is not new (Figure 1.7). However, during the last

two decades of the 20th century, concern increased because of major outbreaks of foodborne infections of animal origin (Cohen, 2000). Examples include an outbreak of salmonellosis affecting over 200 000 people in the US in 1994, and *Escherichia coli* O157:H7 infection affecting over 6000 schoolchildren in Japan in 1996 (WHO, 1996). Other emerging foodborne pathogens include *Cryptosporidium* and *Campylobacter* spp. (Reilly, 1996) and *Listeria monocytogenes* (WHO, 1996). Additionally, the emergence of bovine spongiform encephalopathy, with its putative role as the cause of the fatal human disease, variant Creutzfeldt–Jakob disease (Will, 1997), served to heighten public concern over food safety. In several Western countries, this has led to the establishment of Food Standards Agencies whose remit is to oversee food quality.

The veterinarian's role is now extended beyond guaranteeing wholesomeness of food at the abattoir, and addresses all levels of the production chain, from the farm to the table ('from paddock to plate') (Smulders and Collins, 2002, 2004). This necessitates the establishment of quality assurance programmes both on the farm and throughout the food chain, using techniques such as HACCP (Hazard Analysis Critical Control Points) (Noordhuizen, 2000; Arvanitoyannis, 2009), thus marking a shift in focus from herd health, alone, to quality control of food throughout the production chain (Figure 1.8). This approach is strengthened by quantitative evaluation of the risk of transmission of infection throughout the chain (see Chapters 2 and 24).

Animal welfare

The attitude of the public to animals, notably in the more-economically-developed countries, is reflected

²⁶ In many less-economically-developed countries, private veterinary practice remains a minority employer of veterinarians, with companion animals of little significance (e.g., Turkson, 2003).



Figure 1.7 A Victorian satire: *Microcosm dedicated to the London Water Companies 'Monster soup commonly called Thames Water'* by George Cruikshank (1792–1878). (Courtesy of British Museum, London, UK/Bridgeman Images.)

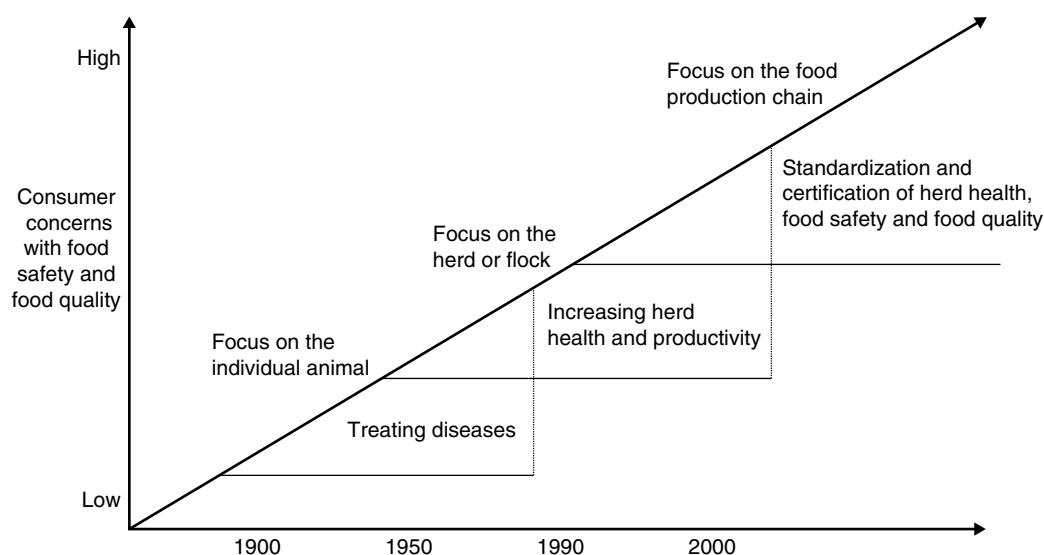


Figure 1.8 The changing focus of veterinary medicine practised in livestock, in relation to consumer concerns with food quality. (Source: *Preventive Veterinary Medicine*, 39, Blaha, Th., Epidemiology and quality assurance application to food safety, Copyright © (1999), with permission from Elsevier Science.)

in contemporary concern for animal welfare, both among the scientific community (Moss, 1994; Appleby and Hughes, 1997; Broom and Fraser, 2015) and the general public (Bennett, 1996), and its importance is now being appreciated globally (Mellor and Bayvel, 2014)²⁷. It encompasses health and 'well-being' (Ewbank, 1986; Webster, 2001)²⁸. The latter term is difficult to define²⁹, and is also included in the *World Health Organization's* definition of human health (Old English: *hal* = 'whole') as '*a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity*' (WHO, 2014). Although this definition was not designed to be a framework for formulating goals of health policy (Noack, 1987), it illustrates that health is more than just absence of disease.

Obvious aspects of animal welfare are deliberate physical abuse (non-accidental injury) and neglect; contentious topics are surgical mutilation, such as tail docking of dogs (Morton, 1992), horses (Cregier, 1990) and pigs (Day and Webster, 1998), and velvet antler removal from deer (Pollard *et al.*, 1992). Companion animals are known to suffer a wide range of types of physical abuse (Munro and Thrusfield, 2001a,b,c, 2015) and sexual abuse (Munro and Thrusfield, 2001d, 2005), which are remarkably similar to those found in child abuse, and a link between abuse of animals and abuse of children is now recognized (Ascione and Arkow, 1999). Other animal-welfare issues may be more subtle; for example, the relationship between bovine mastitis and use of bovine somatotropin to increase milk production (Willeberg, 1993, 1997).

Welfare in livestock production systems is often evaluated in the context of the 'five freedoms' (Spedding, 2000):

1. freedom from hunger and thirst;
2. freedom from discomfort;
3. freedom from pain, injury and disease;

4. freedom to express normal behaviours;
5. freedom from fear and distress.

Behavioural problems may be associated with intensive husbandry systems, for example, cannibalism in laying hens (Gunnarsson *et al.*, 1998); but there may be less tangible issues (Ewbank, 1986) such as behavioural deprivation in sows tethered in stalls. The move towards organic farming in some Western countries is justified, in part, by improved animal welfare (Sundrum, 2001), and the veterinarian therefore is concerned with disease, productivity and well-being, all of which can be interrelated, in all types of production system (Webster, 2011).

Ethical aspects of animal welfare also are now receiving increasing attention (Algers, 2012).

National and international disease reporting

There is a requirement for improved disease reporting systems at the national and international level to identify problems, define research and control priorities and assist in the prevention of spread of infectious agents from one country to another. Additionally, residues need to be identified and eliminated (WHO, 1993). These include contamination of meat by pesticides (Corrigan and Seneviratna, 1989) and hormones (McCaughey, 1992), as well as the more long-standing issue of antibiotic residues, with the attendant problem of antibiotic resistance (Hugoson and Wallén, 2000; Teal, 2002), which can be pronounced in the less-economically-developed countries (Ibrahim *et al.*, 2009).

Global goals to liberalize international trade through the *World Trade Organization* (WTO) (Hoekman and Kostecki, 2011) are highlighting the requirement for comprehensive disease reporting, and governmental veterinary services are responding to this need (DEFRA, 2002c). If liberalization is achieved, it will have marked effects on world trade, including that in livestock commodities (Page *et al.*, 1991). An important component of free trade therefore is assessment of the **risk** of disease and related events (e.g., carcass contamination) associated with the importation of animals and animal products (Morley, 1993a,b). Established organizations, such as the *Office International des Epizooties/World Organisation for Animal Health* (OIE), are modifying their goals and reporting techniques, taking account of these new requirements (Blajan and Chillaud, 1991; Thiermann, 2005; Slorach, 2013).

The advent of low-cost computing following the microelectronic revolution offers powerful means of storing, analysing and distributing data. Information can be transported rapidly using modern communications systems. These developments increase the scope

²⁷ Concern for the welfare of animals is not just recent, though. In 1790, for example, there was public outrage over the wastage of horses in the British Army, which was losing more animals from disease and lack of care than from enemy action. This resulted, in 1795, in the Army directing that a veterinarian should be attached to each regiment. (The first to enlist as an Officer was John Shipp in 1796.) The shortage of trained veterinarians was so acute that London's Royal Veterinary College temporarily reduced its period of training from three years to three months.

²⁸ Animal welfare is therefore assessed primarily biologically, and is distinct from 'animal rights', which is an ethical and philosophical issue (Singer, 2000; Sandøe *et al.*, 2016).

²⁹ For discussions of the relationships between 'welfare', 'well-being' and 'quality of life', see Appleby and Sandøe (2002) and Nordenfelt (2006).

for efficient disease reporting and analysis of the many factors that contribute to clinical disease and suboptimal production, both of which require increased statistical acumen among veterinarians.

One Health

The term 'One Health' (also sometimes termed 'Global Health': Kappas *et al.*, 2012) has come to encapsulate the recognition of the link between the health of humans and animals (domestic and wild), and the environment (Atlas and Malloy, 2014; Barrett and Bouley, 2014; Grace, 2014; Bardosh, 2016). Formal descriptions of One Health include:

'One Health involves recognizing the essential link between human, domestic animal and wildlife health and the threat disease poses to people, their food supplies and economies, and the biodiversity essential to maintaining the healthy environments and functioning ecosystems we all require.'

(From *The Manhattan Principles*: Anon., 2004)

'One Health is the collaborative effort of multiple disciplines – working locally, nationally, and globally – to attain optimal health for people, animals and the environment.'

(One Health Initiative Task Force, 2008)

Despite recent increases in popularity and adoption of the label 'One Health', the idea has much older origins (Woods and Bresalier, 2014; Bresalier *et al.*, 2015). Rudolf Virchow (1821–1902), a German physician (Saunders, 2000; Schultz, 2008), noted the intimate link between human and animal diseases in 1856: '*Between animal and human medicine, there is no dividing line – nor should there be*' (Klauder, 1958). William Ostler (1849–1919), a Canadian physician influenced by Virchow and regarded as a 'Father of Modern Medicine' and the founder of the discipline of veterinary pathology, is credited with introducing the phrase 'One Medicine' into the English-speaking world (Dukes, 2000). Within the recent history of veterinary medicine, One Health was developed by Calvin Schwabe (1984), although he also used the term 'One Medicine'. In 1979, a One Health approach was adopted in identifying the cause of a human anthrax outbreak in Russia as being accidental release of the bacterium from a military microbiology establishment, rather than ingestion of contaminated meat or contact with contaminated carcasses (Meselson *et al.*, 1994).

One Health often is associated with the study and prevention of zoonotic diseases (e.g., Okello *et al.*,

Table 1.12 The scope of One Health. (From One Health Initiative Task Force, 2008.)

Agro-and bio-terrorism
Animal agriculture and animal sciences
Antimicrobial resistance
Basic and translational research
Biomedical research
Clinical medicine
Combating existing and emerging diseases and zoonoses
Comparative medicine
Conservation medicine
Consumer support
Diagnosis, surveillance, control, response and recovery directed at natural or intentional threats that are chemical, toxicological or radiological in nature
Entomology
Ethics
Food safety and security
Global food and water systems
Global trade and commerce
Health communications
Health of the environment and environmental preservation
Implications of climate change
Infectious disease ecology
Integrated systems for detection
Land use and production systems and practice
Mental health
Microbiology education
Occupational health
Public awareness and public communications
Public health and public policy
Regulatory enforcement
Scientific discovery and knowledge creation
Support of biodiversity
Training
Veterinary and environment health professionals and organizations
Wildlife promotion and protection

2011; Yamada *et al.*, 2014), but it is now recognized to have a much broader scope (*Table 1.12*). Numerous groups advocating One Health have developed, including the One Health Initiative (2006) and the One Health Global Network (2011); and the *Food and Agriculture Organization of the United Nations* (FAO), *OIE* and the *World Health Organization* (WHO) have released a tripartite 'concept note'

highlighting the need for collaboration among these institutions and with other stakeholders in order to advance the implementation of One Health approaches (Anon., 2010). The 'tools' of One Health are beginning to be described (Zinsstag *et al.*, 2009, 2011, 2015) and the successes of several interventions have been attributed to the One Health approach; for example, Abbas and others (2011), Häslar and co-workers (2014) and Hall and others (2016) describe a One Health approach to rabies control in Tamil Nadu, India, Colombo, Sri Lanka, and Bali, Indonesia, respectively, while Zinsstag and his colleagues (2005) report on combined human and animal health programmes in Chad to deliver childhood and livestock vaccines. A series of One Health case studies is presented by Cork and others (2016). There also is potential for the approach in controlling future disease incidents (e.g., Ebola virus outbreaks: Mwangi *et al.*, 2016).

However, despite growing interest in the concept, implementation of One Health initiatives is hampered by defects in current forms of health governance (Lee and Brumme, 2013) and currently remains somewhat sidelined, compared with the traditional discrete disciplines of human and veterinary medicine (Kahn *et al.*, 2007, 2008), where it is also appreciated less by the former than by the latter (Järhult, 2015). Moreover, the benefits of a One Health approach have yet to be fully evaluated (Rüegg *et al.*, 2017).

Translational medicine

'Translational medicine' ('translational research') is a recent neologism, initially emerging in human medicine (Woolf, 2008; Rubio *et al.*, 2010), which describes both the need to 'translate' basic scientific research into practical diagnostic procedures and therapies, and to ensure that new treatments actually reach the patients or populations for whom they are intended; that is, to bridge the gap between 'bench

and field' (Tugwell and Knottnerus, 2015). It does not reflect new concepts: it merely packages, under a single name, the established idea that the results of medical and veterinary research are employed in active disease control, necessitating the breaking down of artificial boundaries between basic scientists and clinicians.

In human medicine, there has been an emphasis on translational medicine in the context of new chemotherapeutic agents to combat, for example, cancer (Chabner *et al.*, 1998) and cardiovascular disease (Adams *et al.*, 2009). However, it also legitimately encompasses the translation of medical research in a broad context; for instance, including epidemiological research such as that which identified obesity as a risk factor for coronary heart disease, which should then lead to the uptake of healthier diets by the human population (Lavie *et al.*, 2009).

In veterinary medicine, translational medicine has a similarly broad remit, including not only drug therapies but also management practices. For many years, for instance, the veterinary profession has applied the results of epidemiological research (e.g., the identification of poor hygiene as a risk factor for bovine mastitis: Neave *et al.*, 1969) to improve animal health.

Translational medicine also has a One Health dimension, where, for example, the benefit of translational research involving infectious diseases relevant to both humans and animals (e.g., the development of influenza diagnostic methods) can be maximized when both medical and veterinary research are integrated (Tang, 2013).

The success of translational veterinary medicine requires that the results of pertinent research should reach the veterinary practitioner during primary veterinary training, via journals, and by continuing professional development; and the following chapters in this book present many of the methods that are used in relevant epidemiological research.

Further reading

Ainsworth, G.C. (1986) *Introduction to the History of Medical and Veterinary Mycology*. Cambridge University Press, Cambridge

Bayry, J. (Ed.) (2017) *Emerging and Re-emerging Diseases of Livestock*. Springer International Publishing AG, Cham

Blancou, J. (2002) History of the control of foot and mouth disease. *Comparative Immunology, Microbiology and Infectious Diseases*, **25**, 283–296

Blancou, J. (2003) *History of the Surveillance and Control of Transmissible Animal Diseases*. Office International

des Epizooties, Paris. (Includes a global bibliography of veterinary writings from ancient times to the 15th century)

British Veterinary Association Trust Project (1982) *Future of Animal Health Control – The Control of Infectious Diseases in Farm Animals*. Report of a symposium, University of Reading, 14–16 December 1982. British Veterinary Association, London

Broom, D.M. (2011) A history of animal welfare science. *Acta Biotheoretica*, **59**, 121–137

- Brown, C. and Bolin, C.A. (2000) *Emerging Diseases of Animals*. ASM Press, Washington DC
- Burroughs, T., Knobler, S. and Lederberg, J. (Eds) (2002) *The Emergence of Zoonotic Diseases: Understanding the Impact on Animal and Human Health*. National Academy Press, Washington DC
- Carter, K.C. (2003) *The Rise of Causal Concepts of Disease*. Ashgate Publishing Ltd, Aldershot
- Cohen, M.L. (2000) Changing patterns of infectious disease. *Nature*, **406**, 762–767
- Cornelius, S.T. (2003) Emerging African animal diseases. *Commonwealth Veterinary Association News*, **19** (2), 44–50
- Deem, S.L. (2015) Conservation medicine to One Health: the role of zoologic veterinarians. In: *Fowler's Zoo and Wild Animal Medicine*, Vol. 8. Eds Miller, E. and Fowler, M.E., pp. 698–703. Elsevier-Saunders, St Louis
- Dorn, C.R. (1992) Veterinary epidemiology and its economic importance in AD 2000. *Preventive Veterinary Medicine*, **13**, 129–136
- Dunlop, R.H. (2004) Bourgelat's vision for veterinary education and the remarkable spread of the 'meme'. *Journal of Veterinary Medical Education*, **31**, 310–322. (A brief history of veterinary education)
- Dunlop, R.H. and Williams, D.J. (1995) *Veterinary Medicine: an Illustrated History*. Mosby Year-Book, St Louis
- Faber, K. (1930) *Nosography: The Evolution of Clinical Medicine in Modern Times*, 2nd edn. Paul B. Hoeber, New York. Reprinted by AMS Press, New York, 1978. (A history of concepts of pathogenesis)
- Fleming, G. (1871) *Animal Plagues: Their History, Nature and Prevention*. Chapman and Hall, London
- Fleming, G. (1882) *Animal Plagues: Their History, Nature and Prevention, Volume 2 (from AD 1800 to 1844)*. Baillière, Tindall and Cox, London
- Gibbs, E.P.J. (2014) The evolution of One Health: a decade of progress and challenges for the future. *Veterinary Record*, **174**, 85–91
- Heath, S.E. (2006) Challenges and options for animal and public health services in the next two decades. *Revue Scientifique et Technique, Office International des Epizooties*, **25**, 403–419
- International Atomic Energy Agency (1998) *Towards Livestock Disease Diagnosis and Control in the 21st Century*. Proceedings of an international symposium on the diagnosis and control of livestock diseases using nuclear and related techniques jointly organized by the International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations and held in Vienna, 7–11 April 1997. International Atomic Energy Agency, Vienna
- Jackson, R. (2002) Veterinary epidemiology in New Zealand: a 50-year perspective. *New Zealand Veterinary Journal*, **50**, Suppl. 3, 13–16
- Kappas, M., Groß, U. and Kelleher, D. (Eds) (2012) *Global Health: A Challenge for Interdisciplinary Research*. Universitätsverlag Göttingen, Göttingen
- Karasszon, D. (1988) *A Concise History of Veterinary Medicine*. Akadémiai Kiadó, Budapest
- Karesh, W.B. (Ed.) (2014) One Health. *Revue Scientifique et Technique, Office International des Epizooties*, **33**, 363–668
- Lancaster, H.O. (1994) *Quantitative Methods in Biological and Medical Sciences: A Historical Essay*. Springer-Verlag, New York
- Lombard, M., Pastoret, P.-P. and Moulin, A.-M. (2011) A brief history of vaccines and vaccination. *Revue Scientifique et Technique, Office International des Epizooties*, **26**, 29–48
- Lueddeke, G.R. (2016) *Global Population Health and Well-Being in the 21st Century*. Springer Publishing Company, New York. (Includes a discussion of One Health and ecosystem health in the context of public health)
- MacIntyre, D.K. and Breitschwerdt, E.B. (Eds) (2003) Emerging and re-emerging infectious diseases. *The Veterinary Clinics of North America, Small Animal Practice*, **33**, 677–943
- Mackenzie, J.S., Jeggo, M., Daszak, P. and Richt, J.A. (Eds) (2013) *One Health: The Human–Animal–Environment Interfaces in Emerging Infectious Diseases: Food Safety and Security, and International and National Plans for Implementation of One Health Activities*. Springer, Heidelberg
- Matthews, J.R. (1995) *Quantification and the Quest for Medical Certainty*. Princeton University Press, Princeton. (A history of the application of statistical methods in medicine)
- Melby, E.C. (1985) The veterinary profession: changes and challenges. *Cornell Veterinarian*, **75**, 16–26
- Michell, A.R. (Ed.) (1993) *The Advancement of Veterinary Science. The Bicentenary Symposium Series. Volume 1: Veterinary Medicine Beyond 2000*. CAB International, Wallingford
- Michell, A.R. (Ed.) (1993) *The Advancement of Veterinary Science. The Bicentenary Symposium Series. Volume 3: History of the Healing Professions, Parallels Between Veterinary and Medical History*. CAB International, Wallingford
- Ministry of Agriculture, Fisheries and Food (1965) *Animal Health: A Centenary 1865–1965*. Her Majesty's Stationery Office, London. (A history of the government veterinary service in the UK)
- Morens, D.M. and Fauci, A.S. (2013) Emerging infectious diseases: threats to human health and global stability. *PLoS Pathogens*, **9** (7): e1003467; doi 10.1371/journal.ppat.1003467
- OIE (1994) Early methods of animal disease control. *Revue Scientifique et Technique, Office International des Epizooties*, **13**, 332–614

- PAHO (1975) *A Competency-Based Curriculum for Veterinary Public Health and Preventive Medicine*. Scientific Publication No. 313. Pan American Health Organization, Washington. (A detailed discussion of the teaching of veterinary public health and preventive medicine – including epidemiology – from the North-American perspective in the mid-20th century)
- Pattison, I. (1984) *The British Veterinary Profession 1791–1948*. J.A. Allen, London
- Phalen, D. (Ed.) (2004) Emerging diseases. *Seminars in Avian and Exotic Pet Medicine*, **13**, 49–107. (Describes some emerging diseases in birds, exotic pets and wild animals)
- Prescott, L.M., Harley, J.P. and Klein, D.A. (2002) *Microbiology*, 5th edn. McGraw Hill, Boston. (Includes a concise description of the development of microbiology)
- Pritchard, W.R. (1986) Veterinary education for the 21st century. *Journal of the American Veterinary Medical Association*, **189**, 172–177. (A general review of contemporary veterinary requirements in the more-economically-developed countries)
- Pugh, L.P. (1962) *From Farriery to Veterinary Medicine, 1785–1795*. W. Heffer, Cambridge
- Richards, R.J. and Daston, L. (Eds) (2016) *Kuhn's Structure of Scientific Revolutions at Fifty: Reflections on a Science Classic*. University of Chicago Press, Chicago and London. (Essays on the historical context of Kuhn's work)
- Saunders, L.Z. (1996) *A Biographical History of Veterinary Pathology*. Allen Press, Lawrence. (An international history of veterinary pathology, including microbiology and parasitology, described through the lives of individuals)
- Schwabe, C.W. (1978) *Cattle, Priests, and Progress in Medicine. The Wesley W. Spink Lectures on Comparative Medicine*, Vol. 4. University of Minnesota Press, Minneapolis. (A comparative history of veterinary and human medicine)
- Schwabe, C.W. (1993) The current epidemiological revolution in veterinary medicine. Part II. *Preventive Veterinary Medicine*, **18**, 3–16
- Sherman, D.M. (2002) *Tending Animals in the Global Village*. Lippincott Williams and Wilkins, Baltimore. (Includes a discussion of recent global trends in veterinary medicine)
- Shryock, R.H. (1961) Quantification. The history of quantification in medical science. In: *A History of the Meaning of Measurement in the Natural and Social Sciences*. Ed. Woolfe, H., pp. 85–107. The Bobbs-Merrill Company, Indianapolis
- Smith, F. (1976) *The Early History of Veterinary Literature*. 4 vols. J.A. Allen and Co., London
- Smith, G. and Kelly, A.M. (Eds) (2008) *Food Security in a Global Economy*. University of Pennsylvania Press, Philadelphia. (A broad discussion of the challenges facing veterinary medicine)
- Smithcors, J.F. (1957) *Evolution of the Veterinary Art: A Narrative Account to 1850*. Veterinary Medicine Publishing Company, Kansas City.
- Spinage, C.A. (2003) *Cattle Plague: A History*. Kluwer Academic/Plenum Publishers, New York (An encyclopaedic history of rinderpest)
- Stalheim, O.V.H. (1994) *The Winning of Animal Health: 100 Years of Veterinary Medicine*. Iowa State University Press, Ames. (A history of veterinary medicine in the US)
- Súilleabháin, B.Ó. (1994) The evolution of the State Veterinary Services. *Irish Veterinary Journal*, **47**, 21–27
- Swabe, J. (1999) *Animals, Disease and Human Society. Human–Animal Relations and the Rise of Veterinary Medicine*. Routledge, London and New York
- Thrusfield, M. (1988) The application of epidemiological techniques to contemporary veterinary problems. *British Veterinary Journal*, **144**, 455–469
- Thrusfield, M. (1990) Trends in epidemiology. *Society for General Microbiology Quarterly*, **17**, 82–84
- Tizard, I. (1999) Grease, anthraxgate, and kennel cough: a revisionist history of early veterinary vaccines. *Advances in Veterinary Medicine*, **41**, 7–24
- US Department of Health and Human Resources (1998) *Preventing Emerging Infectious Diseases. A Strategy for the 21st Century*. US Department of Health and Human Resources, Centers for Disease Control and Prevention, Atlanta. (A comprehensive discussion of the control of emerging human infections in the US, but also including some general principles and details of zoonotic and foodborne infections)
- Waltner-Toews, D. and Lang, T. (2000) A new conceptual basis for food and agriculture policy: the emerging model of links between agriculture, food, health, environment and society. *Global Change and Human Health*, **1**, 116–130. (A debate of the future of food and farming)
- Waterson, A.P. and Wilkinson, L. (1978) *An Introduction to the History of Virology*. Cambridge University Press, Cambridge
- Wilkinson, L. (1992) *Animals and Disease: An Introduction to the History of Comparative Medicine*. Cambridge University Press, Cambridge
- Winslow, C.E.A. (1944) *The Conquest of Epidemic Disease*. Princeton University Press, Princeton. (An historical account of the control of infectious diseases)
- Worboys, M. (1991) Germ theories of disease and British veterinary medicine, 1860–1890. *Medical History*, **35**, 308–327
- Zinsstag, J., Schelling, E., Waltner-Towes, D., Whittaker, M. and Tanner, M. (Eds) (2015) *One Health: The Theory and Practice of Integrated Health Approaches*. CABI, Wallingford

2

The scope of epidemiology

Many contemporary disease problems can be best solved by an investigation of animal populations rather than the individual. The natural history of infectious diseases can be understood by studying their distribution in different populations. The measurement of the amount of infectious and non-infectious diseases in a population assists in determining their importance and the efficacy of control campaigns. Complex and unknown causes of diseases can be elucidated by studying the diseases in various groups of animals. The effects of diseases on production can be realistically estimated only in relation to decreased production in the herd or flock, rather than in a single animal. The economic impact of disease and of attempts to control it similarly are evaluated best in groups of animals, ranging from the individual farm to the national level. The investigation of disease in **populations** is the basis of epidemiology.

Definition of epidemiology

Epidemiology is the **study of disease in populations and of factors that determine its occurrence**; the key word being **populations**. Veterinary epidemiology additionally includes investigation and assessment of other health-related events, notably **productivity**. All of these investigations involve **observing** animal populations and making **inferences** from the observations.

A literal translation of the word 'epidemiology', based on its Greek roots *επι-* (*epi-*) = 'upon', *δημο-* (*demo-*) = 'people' and *λογο-* (*logo-*) = 'discoursing', is 'the study of that which is upon the people' or, in

modern parlance, 'the study of disease in populations'¹. Traditionally, 'epidemiology' related to studies of human populations, and 'epizootiology', from the Greek *ζωο-* (*zoo-*) = 'animal', to the studies of animal (excluding human) populations (e.g., Karstad, 1962). Outbreaks of disease in human populations were called 'epidemics', in animal populations were called 'epizootics', and in avian populations were called 'epornitics', from the Greek *ορνιθ-* (*ornith-*) = 'bird'

¹ Two adjectives, 'epidemiologic' and 'epidemiological' are in use, the former being more common in North American publications, whereas the latter is usual in British literature. The shorter version probably has arisen because of the general human tendency to go for simpler forms wherever possible. In the case of '-ic' and '-ical' this tendency is helped by the fact that they are virtually interchangeable. '-ic-' (pedantically, '-ik-') is one of the commonest Greek suffixes used to turn a noun into an adjective, whereas '-al-' performs the same function in Latin. Very many adjectives of these types were borrowed into English, and native speakers became so familiar with both suffixes that they used them without any conscious reference to their linguistic origin (e.g., 'anecdote' is a Greek borrowing, and should really have 'anecdotic' as its adjective, instead of the hybrid 'anecdotal'). Moreover, it is a feature of both Latin and Greek to use adjectives syntactically as nouns, and this has been carried over when words have been borrowed into English, resulting in 'logic' (properly an adjective) being used as a noun. This was then converted to the common adjective, 'logical', using the Latin suffix. However, since many of the '-ic' forms also remained as adjectives (e.g., 'comic', 'classic'), doublets such as 'comic(al)' and 'classic(al)' arose. The existence of these doublets explains why 'epidemiologic' and 'epidemiological' are essentially identical, one not being 'more correct' than the other.

(e.g., Montgomery *et al.*, 1979)². Other derivatives, such as ‘epidemein’ (‘to visit a community’), give hints of the early association between epidemiology and infections that periodically entered a community, in contrast to other diseases that were usually present in the population.

The various derivatives can be used in different contexts. A study of a disease that is present only in an animal population, such as *Brucella ovis* infection of sheep, would not involve a simultaneous study of disease in humans; the term ‘epizootiology’ might then be used by some to indicate that the study was confined to animals other than man. Many diseases, called **zoonoses**, may be shared by man and lower animals³. Thus, when studying diseases such as bovine brucellosis and leptospirosis, both of which are zoonoses, mechanisms of transfer of disease between human and non-human populations have to be considered. An important factor that determines the occurrence of such occupationally acquired zoonoses (in veterinarians, abattoir workers and farmers in these examples) is the amount of disease in domestic animals. The ‘epidemiology’ of brucellosis and leptospirosis in dairy farmers is therefore closely associated with the ‘epizootiology’ of these diseases in cattle. The semantic differentiation between studies involving human diseases and those concerned with animal diseases therefore is considered neither warranted nor logical (Dohoo *et al.*, 1994); accordingly, there is a strong case for simplifying terminology by universally using ‘epidemiology’ and its derivatives. Therefore, throughout this book, the word ‘epidemiological’ is used to describe any investigation relating to disease in a population, whether or not the population consists of humans, domestic animals or wildlife.

The uses of epidemiology

There are five broad objectives of epidemiology:

1. determination of the origin of a disease whose cause is known;
2. investigation and control of a disease whose cause is either unknown or poorly understood;

3. acquisition of information on the ecology and natural history of a disease;
4. planning, monitoring and assessment of disease-control programmes;
5. assessment of the economic and other social effects of a disease, and analysis of the costs and benefits of alternative control programmes.

Determination of the origin of a disease whose cause is known

Many diseases with a known cause can be diagnosed precisely by the signs exhibited by the affected animals, by appropriate laboratory tests and by other clinical procedures such as diagnostic imaging. For instance, the diagnosis of foot-and-mouth disease is relatively straightforward: the infection produces distinct clinical signs in most species (sheep can be an exception), and can be readily diagnosed in the laboratory. However, determining why an outbreak occurred is important in limiting its spread and eradicating the disease. For example, the first reported case of the UK epidemic in 2001 was in an abattoir in south-east England. However, epidemiological investigations revealed that the disease had originated on a pig farm several hundred miles north (Gibbens *et al.*, 2001b), and it was only by careful tracing of movements of animals that had been exposed to infection at this source that the widespread dissemination of the virus through sheep marketing was identified, and appropriate national control measures therefore instituted (Mansley *et al.*, 2003).

There are many examples of the investigation of diseases with known causes that involve answering the questions ‘Why has an outbreak occurred?’ or ‘Why has the number of cases increased?’. For instance, an increased number of actinobacillosis cases in a group of cattle might be associated with grazing a particular pasture of ‘burnt-off’ stubble. Such an occurrence could be associated with an increase in abrasions of the buccal mucosae, caused by abrasive ash, which could increase the animals’ susceptibility to infection with *Actinobacillus lignieresii* (Radostits *et al.*, 2007). Similarly, consumption of prickly pears (*Opuntia* spp.) may be associated with an increased frequency of the disease in sheep, for a similar reason. An increased number of cases of bone defects in puppies might be due to local publicity being given to the use of vitamin supplements, resulting in their administration to animals that were already fed a balanced diet, with consequent hypervitaminosis D, inducing osteosclerosis and bone rarefaction (Maxie, 2007). An increase in the number of lamb carcasses with high ultimate pH values could be associated with excessive washing of the animals prior to slaughter (Petersen, 1983). These

² The term ‘epidemic’ also is generally applied to disease outbreaks in plants (e.g., Madden and Campbell, 1987), although the etymological purist might prefer the term ‘epiphytotic’ (Greek: *φυτόν* = ‘plant’).

³ Transmission can occur in both directions: from animals to humans, and from humans to animals (Messenger *et al.*, 2014). The term **humanosis** sometimes is used to describe an infection normally present in humans that is spread to other animals; for example, methicillin-resistant *Staphylococcus aureus* in companion animals (Morgan, 2008).

possible explanations can be verified only by epidemiological investigations.

Investigation and control of a disease whose cause is either unknown or poorly understood

There are many instances of disease control based on epidemiological observations before a cause was identified. Contagious bovine pleuropneumonia was eradicated from the US by an appreciation of the infectious nature of the disease before the causal agent, *Mycoplasma mycoides*, was isolated (Schwabe, 1984). Giovanni Lancisi's slaughter policy to control rinderpest (see Chapter 1) was based on the assumption that the disease was infectious, even though the causal agent had not been discovered. Similarly, Edward Jenner's classical observations on the protective effects of cowpox virus against human smallpox infection in the 18th century (see Chapter 1), before viruses were isolated, laid the foundations for the global eradication of smallpox.

More recently, epidemiological studies in the UK suggested that cattle developed bovine spongiform encephalopathy following consumption of feedstuffs containing meat and bone meal contaminated with a scrapie-like agent (Wilesmith *et al.*, 1988). This was sufficient to introduce legislation prohibiting the feeding of ruminant-derived protein, although the causal agent had not been identified at the time.

Although the exact cause of 'blood splashing' (haemorrhages in muscle) in carcasses is not known, observations have shown that there is a correlation between this defect and electrical stunning by a 'head only' method (Blackmore, 1983); and the occurrence of this condition can be reduced by adopting a short 'stun-to-stick' interval, stunning animals with a captive bolt, or by using a method of electrical stunning that ensures that an animal is killed outright by cardiac arrest (Collins and Huey, 2015). Similarly, there is a strong correlation between grass sickness and grazing, and the disease can be almost totally prevented by stabling horses continuously during spring and summer, although the cause of the disease remains uncertain (Gilmour, 1989)⁴.

The cause of squamous cell carcinoma of the eye in Hereford cattle ('cancer eye') is not known. Epidemiological studies have shown that animals with unpigmented eyelids are much more likely to develop the condition than those with pigment (Anderson *et al.*, 1957). This information can be utilized by cattle breeders to select animals with a low susceptibility to this neoplasm.

Epidemiological studies are also used to identify causes of disease (many of which are multifactorial and initially poorly understood) so that the most

appropriate disease-control techniques can be applied. Thus, the identification of low levels of water intake as an important component of the cause of feline urolithiasis (Willeberg, 1981) facilitated control of this syndrome by dietary modification. Investigations also can be used to identify characteristics of animals that increase the risk of disease. For example, entire, nulliparous bitches with a history of oestrus irregularity, pseudopregnancy and use of oestrus-suppression drugs are particularly at risk of developing pyometra (Fidler *et al.*, 1966; Niskanen and Thrusfield, 1998); this information is of diagnostic value to the clinician, and is of assistance when advising owners on breeding policy.

Acquisition of information on the ecology and natural history of a disease

An animal that can become infected with an infectious agent is a **host** of that agent. Hosts and agents exist in communities that include other organisms, all of which live in particular environments. The aggregate of all facts relating to animals and plants is their **natural history**. Related communities and their environments are termed **ecosystems**. The study of ecosystems is **ecology**.

A comprehensive understanding of the natural history of infectious agents is possible only when they are studied in the context of their hosts' ecosystems. Similarly, an improved knowledge of non-infectious diseases can be obtained by studying the ecosystems and the associated physical features with which affected animals are related. The geological structure of an ecosystem, for example, can affect the mineral content of plants and therefore can be an important factor in the occurrence of mineral deficiencies and excesses in animals.

The environment of an ecosystem affects the survival rate of infectious agents and of their hosts. Thus, infection with the helminth *Fasciola hepatica* is a serious problem only in poorly drained areas, because the parasite spends part of its life-cycle in a snail that requires moist surroundings.

Each of the 200 antigenic types (serovars) of *Leptospira interrogans* is maintained in one or more species of hosts. Serovar Copenhageni, for instance, is maintained primarily in rats (Babudieri, 1958). Thus, if this serovar is associated with leptospirosis in either man or domestic stock, then part of a disease-control programme must involve an ecological study of rat populations and control of infected rats. Similarly, in Africa, a herpesvirus that produces infections without signs in wildebeest is responsible for malignant catarrhal fever of cattle (Plowright *et al.*, 1960). Wildebeest populations, therefore, must be investigated when attempting to control the disease in cattle.

An ecosystem's climate also is important because it limits the geographical distribution of infectious

⁴ *Clostridium botulinum* has been implicated, and is the subject of a pilot vaccinal trial (AHT, 2015).

agents that are transmitted by arthropods by limiting the distribution of the arthropods. For example, the tsetse fly, which transmits trypanosomiasis, is restricted to the humid parts of Sub-Saharan Africa (Ford, 1971).

The importance of the social environment in which disease occurs is becoming increasingly recognized. For example, military campaigns and conflict throughout history have led to the dissemination of rinderpest (see Table 1.2). Furthermore, social factors, such as poverty, have major effects on the ability of individuals and nations to prevent and control animal diseases, as well as affecting the health of individuals themselves (Adler *et al.*, 1994). Moreover, a 'vicious circle' can occur, in which animal disease increases levels of poverty (Perry and Grace, 2009; Rich and Perry, 2011).

Infectious agents may extend beyond the ecosystems of their traditional hosts. This has occurred in bovine tuberculosis in the UK, where the badger population is an alternative host for *Mycobacterium tuberculosis* (Little *et al.*, 1982; Wilesmith *et al.*, 1982) in which the disease has been refractory (Report, 2000, 2007; Macdonald *et al.*, 2006; Reynolds, 2006). Similarly, in certain areas of New Zealand, wild opossums are infected with this bacterium and can therefore be a source of infection to cattle (Thorns and Morris, 1983; Ramsey and Efford, 2010). Purposeful routine observation of such infections provides valuable information on changes in the amount of disease and relevant ecological factors and may therefore indicate necessary changes in control strategies.

Infectious diseases that are transmitted by insects, ticks and other arthropods, and which may be maintained in wildlife, present complex ecological and climatic relationships, and even more complex problems relating to their control (Miles, 1964; Yuill, 1986; Lemon *et al.*, 2008; Tabachnick, 2010). Comprehensive epidemiological studies of these diseases help to unravel their life-cycles, and can indicate suitable methods of control.

In addition to studying the ecology of a *specific* disease in a *specific* host, the 'health' of ecosystems, *per se*, also can be investigated. This is termed **ecosystem health**⁵, concern for which has arisen because of the observed deterioration of particular ecosystems (e.g., degradation of coral reefs: Bellwood *et al.*, 2004)⁶. Such

studies require definition of a healthy ecosystem, and this is often characterized in terms of an ecosystem's 'sustainability'; that is, its ability to maintain its structure and function in the face of external stress (Rapport *et al.*, 1998; Costanza and Mageau, 1999), and veterinary epidemiology has a role in studying component causes of ecosystem degradation (Work *et al.*, 2008).

Study of the complex interactions between human health, animal health and ecosystem health recently has been termed **conservation medicine** (Aguirre *et al.*, 2002). This therefore shares a similar philosophy with One Health and Global Health (see Chapter 1) and **planetary health**, which relates improvements in human health to environmental change and exploitation of natural resources (Whitmee *et al.*, 2015).

Planning, monitoring and assessment of disease-control programmes

The institution of a programme to either control or eradicate a disease in an animal population must be based on a knowledge of the amount of the disease in that population, the factors associated with its occurrence, the facilities required to control the disease, and the costs and benefits involved. This information is equally important for a mastitis control programme on a single dairy farm and for a national brucellosis eradication scheme involving all the herds in a country. The epidemiological techniques that are employed include the routine collection of data on disease in populations (**monitoring** and **surveillance**) to decide if the various strategies are being successful.

Surveillance also is required to determine whether the occurrence of a disease is being affected by new factors. For example, during the eradication scheme for bovine tuberculosis in New Zealand, opossums became infected in certain areas. New strategies had to be introduced to control this problem (Julian, 1981). During the foot-and-mouth disease epidemic in the UK in 1967 and 1968, surveillance programmes indicated the importance of windborne virus particles in the transmission of the disease (Smith and Hugh-Jones, 1969). This additional knowledge was relevant to the establishment of areas within which there was a restriction of animal movement, thus facilitating eradication of the disease.

Assessing the economic effects of a disease and of its control

The cost of the control of disease in the livestock industry must be balanced against the economic loss attributable to the disease. Economic analysis

⁵ This should not be confused with **ecohealth**, which is generally understood to apply to investigation of environmental influences, such as pollution, on human health (Charron, 2012), although its scope is widening (Mi *et al.*, 2016). The two terms are sometimes confusingly conflated into **ecological health**.

⁶ The idea that the term 'health' can be applied to the environment, however, is not new (Anon., 1816) and is arguably consonant with the *Gaia hypothesis/theory*, which posits the notion that the earth is a single, self-regulating system (Lovelock, 1979; Wallace and Norton, 1992).

therefore is required. This is an essential part of most modern planned animal health programmes (Marsh, 1999; Morris, 1999). Although it may be economic to reduce a high level of disease in a herd or flock, it may be uneconomic to reduce even further the level of a disease that is present at only a very low level. If 15% of the cows in a herd were affected by mastitis, productivity would be severely affected and a control programme would be likely to reap financial benefit. On the other hand, if less than 1% of the herd were affected, the cost of further reduction of the disease might not result in a sufficient increase in productivity to pay for the control programme.

This introduction to the uses of epidemiology indicates that the subject is relevant to many areas of veterinary science. The general agricultural practitioner is now primarily concerned with herd health. The companion-animal practitioner is faced with chronic refractory diseases, such as the idiopathic dermatoses, which may be understood better by an investigation of the factors that are common to all cases. The state veterinarian cannot perform his routine duties without reference to disease in the national animal population. The diagnostic pathologist investigates the associations between causes and effects (i.e., lesions); this approach is epidemiological when inferences are made from groups of animals. The veterinarian in abattoirs and meat-processing plants attempts to reduce the occurrence of defects and contamination by understanding and eliminating their causes. Similarly, industrial veterinarians, concerned with the design of clinical trials, compare disease rates and response to treatment in groups of animals to which different prophylactic and therapeutic compounds are administered.

Types of epidemiological investigation

There are four approaches to epidemiological investigation that traditionally have been called 'types' of epidemiology. These types are **descriptive**, **analytical**, **experimental** and **theoretical** epidemiology.

Descriptive epidemiology

Descriptive epidemiology involves observing and recording diseases and possible causal factors. It is usually the first part of an investigation. The observations are sometimes partially subjective, but, in common with observations in other scientific disciplines, may generate hypotheses that can be tested more rigorously later. Darwin's theory of evolution, for

example, was derived mainly from subjective observations, but with slight modification it has withstood rigorous testing by plant and animal scientists.

Analytical epidemiology

Analytical epidemiology is the analysis of observations using suitable diagnostic and statistical procedures.

Experimental epidemiology

Experimental epidemiologists observe and analyse data from groups of animals from which they can select, and in which they can alter, the factors associated with the groups. An important component of the experimental approach is the control of the groups. A common application is phase III clinical trials (see Chapter 17).

Experimental epidemiology developed in the 1920s and 1930s (Cushing, 1928), and utilized laboratory animals whose short lifespans enabled events to be observed more rapidly than in humans (see Chapter 9). A notable example is the work of Topley (1942) who infected colonies of mice with ectromelia virus and *Mannheimia* (*Pasteurella*) spp. The effects of varying the rate of exposure of mice maintained in groups of various sizes provided insights into the behaviour of human epidemic diseases such as measles, scarlet fever, whooping cough and diphtheria, which followed similar patterns to the experimental infections (MRC, 1938). This work demonstrated the importance of the proportion of susceptible individuals in the population in determining the progress of epidemics (see Chapter 8). Hitherto, changes in the virulence of a microorganism were thought to be the most important factor affecting epidemic patterns; for example, the decline in the occurrence of rinderpest in the UK in the 18th century was ascribed to its passing into a mild form of the disease (Spinage, 2003).

Natural experiments Rarely, a 'natural' experiment can be conducted when the naturally-occurring disease or other fortuitous circumstance approximates closely to the ideally designed experiment. For instance, a six-month ban on smoking in public areas and the workplace in Helena (an isolated community in Montana, US) resulted in a significant reduction in the the number of hospital admissions for acute myocardial infarction during the ban, which was reversed after the ban was lifted (Sargent *et al.*, 2004). This supported the hypothesis that passive smoking is a risk factor for heart disease. Similarly, when bovine spongiform encephalopathy occurred in the UK, outbreaks of the disease on the Channel Islands (Jersey and Guernsey), which maintain isolated

populations of cattle, provided an ideal situation in which to study the disease, uncomplicated by the possibility of transmission by contact with infected animals (Wilesmith, 1993). This added credence to the hypothesis that the disease was transmitted in contaminated feedstuffs. Other ideal 'experimental' populations include nuns (Gagnon, 1950) and prostitutes (Keighley, 1968) for the study of sexual activity as a risk factor for cervical cancer.

Theoretical epidemiology

Theoretical epidemiology consists of the representation of disease using mathematical 'models' that attempt to simulate natural patterns of disease occurrence.

Epidemiological subdisciplines

Various epidemiological subdisciplines⁷ are now recognized. These generally reflect different areas of interest, rather than fundamentally different techniques. They all apply the four types of epidemiology described above, and can overlap, but their separate identities are considered by some to be justifiable⁸.

Clinical epidemiology

Clinical epidemiology is the use of epidemiological knowledge, reasoning and methods to study clinical issues and improve patient care (Porta, 2014), and therefore brings a numerate approach to traditional clinical medicine, which has tended to be anecdotal and subjective (Grufferman and Kimm, 1984). It is concerned with the frequency and cause of disease, the factors that affect prognosis, the validity of diagnostic tests, and the effectiveness of therapeutic and preventive techniques (Haynes *et al.*, 2006; Fletcher *et al.*, 2014; Villarroel, 2015), and therefore is an important component of **evidence-based medicine** (Polzin *et al.*, 2000; Cockcroft and Holmes, 2003; Marr *et al.*, 2003; Rosenthal, 2004; Strauss *et al.*,

2011), which is concerned with patient care based on evidence from the best available studies.

Computational epidemiology

Computational epidemiology involves the application of computer science to epidemiological studies (Marathe and Vullikanti, 2013). This includes the representation of disease by **mathematical and simulation models** (see 'Quantitative investigations', later in this chapter) and the use of **expert systems**. These systems are commonly applied to disease diagnosis where they incorporate a set of rules for solving problems, details of clinical signs, lesions, laboratory results, and the opinions of experts; examples are the identification of the cause of coughing in dogs (Roudebush, 1984) and the diagnosis of bovine mastitis (Hogeveen *et al.*, 1993). Expert systems also are employed in formulating disease-control strategies (e.g., for East Coast fever: Gettinby and Byrom, 1989), predicting animal productivity (e.g., reproductive performance in dairy herds: McKay *et al.*, 1988) and supporting management decisions (e.g., decisions on replacing sows: Huirne *et al.*, 1991).

Genetic epidemiology

Genetic epidemiology is the study of the cause, distribution and control of disease in related individuals, and of inherited defects in populations (Morton, 1982; Roberts, 1985; Khoury *et al.*, 1993; Palmer *et al.*, 2011). It indicates that the disciplinary boundary between genetics and epidemiology is blurred. Many diseases involve both genetic and non-genetic factors (see Chapter 5), and genes are increasingly incriminated in diseases of all organ systems (*Figure 2.1*). Thus, the geneticist and epidemiologist are both concerned with interactions between genetic and non-genetic factors – only the frequently indistinct time of interaction may be used to classify an investigation as genetic or epidemiological.

Field epidemiology

Field epidemiology is the practice of epidemiology in response to problems of a magnitude significant enough to require a rapid or immediate action (Goodman and Buehler, 2008). For example, when outbreaks of foot-and-mouth disease occur, field epidemiologists promptly trace potential sources of infection in an attempt to limit spread of the disease (see Chapters 21 and 27). Field epidemiology is a timely, judgemental process based on description, analysis, common sense and the need to design practical control policies. It is sometimes termed '**shoe-leather epidemiology**' or '**intervention epidemiology**' because

⁷ The term 'subdiscipline' implies that epidemiology is a discipline. That it is, is generally accepted (Howe and Christiansen, 2004), following the criterion of Hirst (1965); namely, that a discipline involves certain central concepts that are peculiar in character and form. The central concept of epidemiology is measuring, and drawing inferences from, disease in populations.

⁸ The author's preference is not to compartmentalize epidemiology, which implies an insular, specialists' *modus operandi* rather than a broad approach to problems. However, listing of subdisciplines is vindicated on the grounds that they are widely cited.

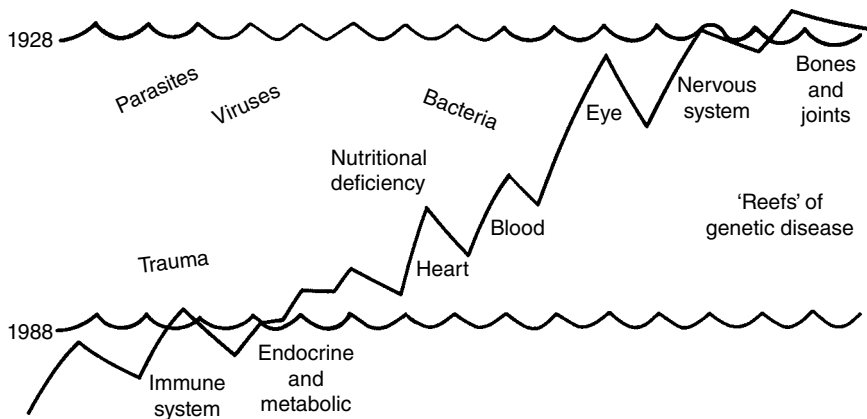


Fig. 2.1 The discovery of the role of genes in the pathogenesis of disease. An analogy is made using the sea and reefs. The sea represents environmental factors (infectious and non-infectious); the reefs represent genetic factors. Only the reefs above the water level are known. As time passes, the water level falls, and more genetic factors are identified. (Source: Thrusfield, 1993, pp.121–142; after Patterson, 1993.)

the investigator is often required to visit the field to study and control disease (Koo and Thacker, 2010)⁹.

Applied epidemiology

Applied epidemiology is a wider term than field epidemiology, including: priority setting; the evaluation of health policies, programmes, technologies and services; the monitoring of disease; and evaluating disease intervention strategies (Porta, 2014). Field epidemiology and applied epidemiology therefore share the common goal of actively controlling disease and improving health.

Participatory epidemiology

Awareness, in the 1980s, of the rudimentary development of veterinary services in some less-economically-developed parts of the world, where animals were both economically and socially important, prompted the use of local knowledge to gain information, with the main goal of improving animal health (Catley *et al.*, 2002a). However, participatory epidemiology goes beyond the acquisition of data. Catley and his colleagues (2012) emphasize the essential role of communities in defining and prioritizing veterinary-related problems and in the development of solutions. A key concept behind the participatory approach is the need for professionals to change their behaviour and attitudes when working with poor people in less-economically-developed countries in recognition of the ability of people, despite limited formal education, to conduct their own investigations and to design, develop and implement plans to resolve local problems. The techniques that are employed evolved in the social sciences, and consist

of simple visual methods and interviews to generate qualitative and semi-quantitative data. This approach became known as 'participatory appraisal' and its application in veterinary medicine is now termed 'participatory epidemiology'. It is a tool for the field epidemiologist and is increasingly used in less-economically-developed countries. Participatory epidemiological approaches and methods also have been applied in more-economically-developed countries, but remain under-utilized in this setting (Catley *et al.*, 2012).

This area of interest is closely related to **ethnoveterinary medicine** (McCorkle *et al.*, 1996; Martin *et al.*, 2001; Fielding, 2004), which is concerned with local knowledge of, and practices relating to, the health of animals.

An introduction to participatory epidemiology is given in Chapter 21.

Molecular epidemiology

New biochemical techniques now enable microbiologists and molecular biologists to study small genetic and antigenic differences between viruses and other microorganisms at a higher level of discrimination than has been possible using conventional serological techniques. The methods include peptide mapping, nucleic acid 'fingerprinting' and hybridization (Keller and Manak, 1989; Kricka, 1992), restriction enzyme analysis, monoclonal antibodies (Oxford, 1985; Goldspink and Gerlach, 1990; Goldspink, 1993), the polymerase chain reaction (Belák and Ballagi-Pordány, 1993) and whole genome sequencing (Zadoks and Schukken, 2006). For example, nucleotide sequencing of European foot-and-mouth disease virus has indicated that some outbreaks of the disease involved vaccinal strains, suggesting that improper inactivation or escape of virus from vaccine production plants may have been responsible for the outbreaks (Beck

⁹ This contrasts with 'armchair epidemiology': a term (sometimes used cynically) referring to the analysis of data within the confines of one's office.

and Strohmaier, 1987) and has identified likely farm-to-farm transmission patterns during outbreaks (Cottam *et al.*, 2008). Sequencing also has indicated that unrestricted animal movement is a major factor in dissemination of the disease in West Africa (Sangaré *et al.*, 2004).

Additionally, infections that hitherto have been difficult to identify are now readily distinguished using these new molecular techniques; examples are infection with *Mycobacterium paratuberculosis* (the cause of Johne's disease: Murray *et al.*, 1989) and latent infection with Aujeszky's disease virus (Belák *et al.*, 1989). The application of these new diagnostic techniques constitutes **molecular epidemiology** (Schulte and Perera, 1993). A general description of the methods is given by Persing and others (1993), Riley (2004) and Rothman and others (2008).

Molecular epidemiology is part of the wider use of **biological markers** (Hulka *et al.*, 1990). These are cellular, biochemical or molecular alterations that are measurable in biological media such as tissues, cells or fluids. They may indicate susceptibility to a causal factor, or a biological response, suggesting a sequence of events from exposure to disease (Perera and Weinstein, 1982). Some have been used by veterinarians for many years, for instance serum magnesium levels as indicators of susceptibility to clinical hypocalcaemia (Whitaker and Kelly, 1982; van de Braak *et al.*, 1987), serum transaminase levels as markers for liver disease, and antibodies as indicators of exposure to infectious agents (see Chapter 20).

Other subdisciplines

Several other epidemiological subdisciplines have also been defined. **Chronic disease epidemiology** is involved with diseases of long duration (e.g., cancers), many of which are non-infectious. **Environmental epidemiology** is concerned with the relationship between disease and environmental factors such as industrial pollution and, in human medicine, occupational hazards. Domestic animals can act as monitors of environmental hazards and can provide early warning of disease in man (see Chapter 9). **Micro-epidemiology** is the study of disease in a small group of individuals with respect to factors that influence its occurrence in larger segments of the population. For example, studies of feline acquired immunodeficiency syndrome (FAIDS) in groups of kittens have provided insights into the widespread human disease, AIDS (Torres-Anjel and Tshikuka, 1988; Bendinelli *et al.*, 1993). Micro-epidemiology, which frequently uses animal biological models of disease, therefore is closely

related to **comparative epidemiology** (see Chapter 9). In contrast, **macro-epidemiology** is the study of national patterns of disease, and the social, economic and political factors that influence them (Hueston and Walker, 1993; Hueston, 2001). Other subdisciplines, such as **nutritional epidemiology** (Slater 1996b; Willett, 2013), **subclinical epidemiology** (Evans, 1987), **forensic epidemiology** (Goodman *et al.*, 2003), **socio-psychological veterinary epidemiology** (Wauters and Rojo-Gimeno, 2014) and, specifically in human medicine, **social epidemiology** (Berkman and Kawachi, 2000; Kasl and Jones, 2002) and **psychosocial epidemiology** (Martikainen *et al.*, 2002), also can be identified to reflect particular areas of interest.

Components of epidemiology

The components of epidemiology are summarized in *Figure 2.2*. The first stage in any investigation is the collection of relevant data. The main sources of information are outlined in Chapter 21. Investigations can be either **qualitative** or **quantitative** or a combination of these two approaches.

Qualitative investigations

The natural history of disease

The ecology of diseases, including the distribution, mode of transmission and maintenance of infectious diseases, is investigated by field observation. Ecological principles are outlined in Chapter 7. Methods of transmission and maintenance are described in Chapter 6, and patterns of disease occurrence are described in Chapter 8. Field observations also may reveal information about factors that may directly or indirectly cause disease. The various factors that act to produce disease are described in Chapter 5.

Causal hypothesis testing

If field observations suggest that certain factors may be causally associated with a disease, then the association must be assessed by formulating a causal hypothesis. Causality (the relating of causes to effects) and hypothesis formulation are described in Chapter 3.

Qualitative investigations were the mainstay of epidemiologists before the Second World War. These epidemiologists were concerned largely with the identification of unknown causes of infectious disease and sources of infection. Some interesting examples of the epidemiologist acting as a medical 'detective' are described by Roueché (1991) and Ashton (1994);

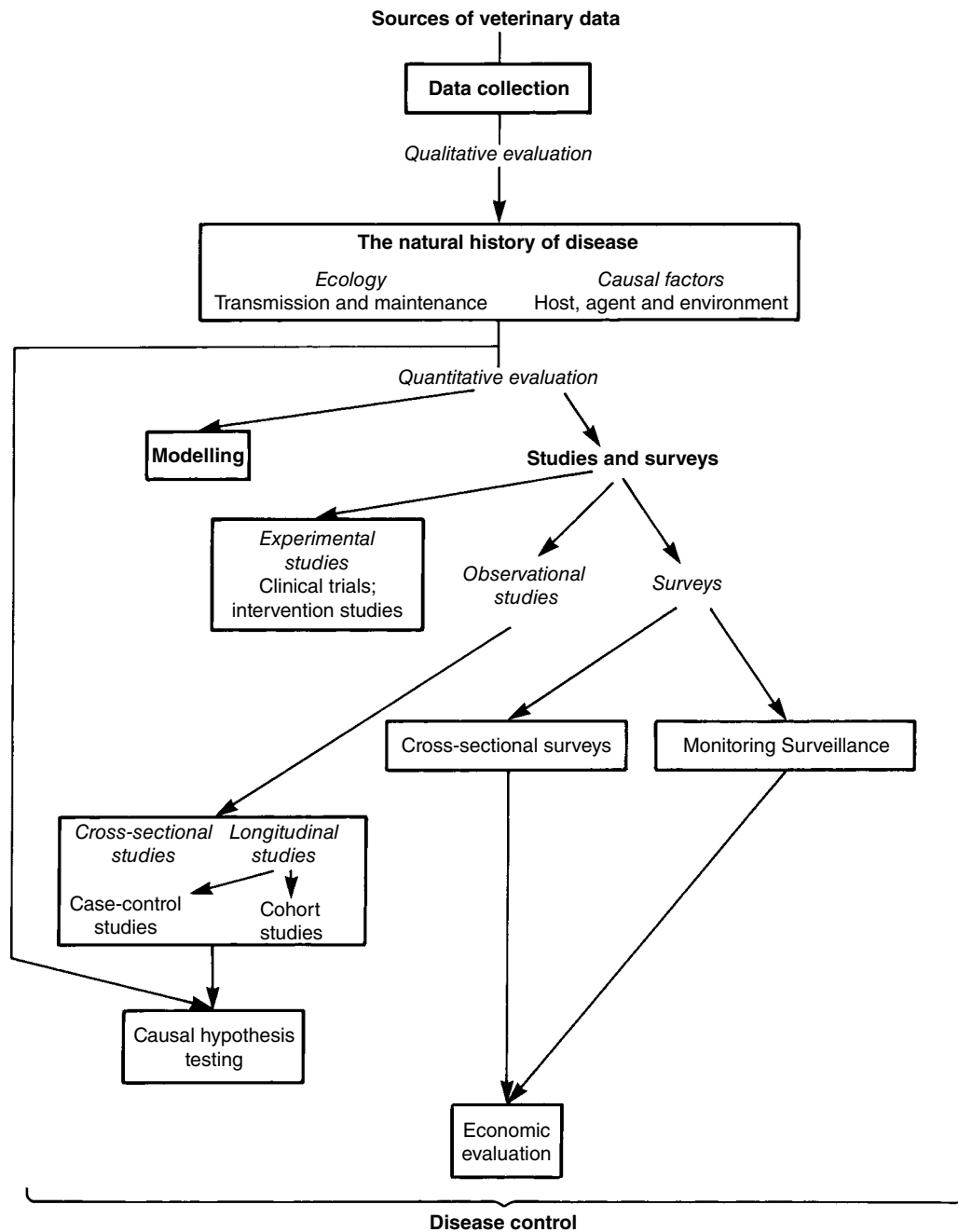


Fig. 2.2 Components of veterinary epidemiology. (Adapted from Thrusfield, 1985a.)

and Hempel (2006) relates the classical investigation of the cause of cholera in London by the Victorian physician, John Snow (see Chapter 3).

Quantitative investigations

Quantitative investigations involve measurement (e.g., the number of cases of disease), and therefore expression and analysis of numerical values. Basic

methods of expressing these values are outlined in Chapters 4 and 12. The types of measurement that are encountered in veterinary medicine are described in Chapter 10. Quantitative investigations include **surveys, monitoring and surveillance, studies, modelling** and the biological and economic **evaluation of disease control**. Some of these may be confined within the walls of the research organization (i.e., 'armchair epidemiology').

Surveys

A survey is an examination of an aggregate of units (Kendall and Buckland, 1982). A group of animals is an example of an aggregate. The examination usually involves counting members of the aggregate and characteristics of the members. In epidemiological surveys, characteristics might include the presence of particular diseases, or production parameters such as milk yield. Surveys can be undertaken on a **sample** of the population. Less commonly, a **census**, which examines the total animal population, can be undertaken (e.g., tuberculin testing). A **cross-sectional** survey records events occurring at a particular point in time. A **longitudinal** survey records events over a period of time. These latter events may be recorded **prospectively** from the present into the future; or may be a **retrospective** record of past events.

A particular type of diagnostic survey is **screening**. This is the identification of undiagnosed cases of disease using rapid tests or examinations. The aim is to separate apparently healthy individuals that probably have a disease from those that probably do not. Screening tests are not intended to be diagnostic; individuals with positive test results (i.e., that are classified as diseased by the screening test) usually require further investigation for definite diagnosis. They therefore differ from diagnostic tests, which are applied to animals showing suspicion of disease.

Screening frequently involves investigation of the total population (**mass screening**); for example, the screening of cattle populations for tuberculosis. It also may be targeted at animals only in areas where there have been cases of disease (**strategic screening**); for example, the serological sampling of sheep within a 3-km radius of premises on which foot-and-mouth disease has been diagnosed (Donaldson, 2000). **Pre-scriptive screening** aims at early identification of diseases that can be controlled better if they are detected early in their pathogenesis (e.g., mammography and other techniques to detect breast cancer in women: Lee *et al.*, 2010).

Screening also may be applied more generally to include the measurement of any characteristic or health problem which may not be apparent in a population (e.g., measurement of heavy metal levels in wild and domesticated animals) (Toma *et al.*, 1999).

Diagnostic tests and screening are considered in Chapter 20. The design of surveys in general is described in Chapter 13.

Monitoring and surveillance

Monitoring is the making of routine observations on health, productivity and environmental factors and the recording and transmission of these observations.

Thus, the regular recording of milk yields is monitoring, as is the routine recording of meat inspection findings at abattoirs. The identity of individual diseased animals usually is not recorded.

Surveillance is a more intensive form of data recording than monitoring. Originally, surveillance was used to describe the tracing and observation of people who were in contact with cases of infectious disease. It is now used in a much wider sense (Langmuir, 1965) to include all types of disease – infectious and non-infectious – and involves the collation and interpretation of data collected during monitoring programmes, usually with the recording of the identity of diseased individuals, with a view to detecting changes in a population's health. It is normally part of control programmes for specific diseases. The recording of tuberculosis lesions at an abattoir, followed by tracing of infected animals from the abattoir back to their farms of origin, is an example of surveillance. The terms 'monitoring' and 'surveillance' have previously been used synonymously, but the distinction between them is now generally accepted.

Surveillance is discussed in detail in Chapter 21.

Studies

'Study' is a general term, which refers to any type of investigation. However, in epidemiology, a study usually involves **comparison** of groups of animals; for example, a comparison of the weights of animals that are fed different diets. Thus, although a survey generally could be classified as a study, it is excluded from epidemiological studies because it involves only description rather than comparison and the analysis that the comparison requires. There are four main types of epidemiological study:

1. experimental studies;
2. cross-sectional studies;
3. case-control studies;
4. cohort studies.

In an **experimental study** the investigator has the ability to allocate animals to various groups, according to factors that the investigator can randomly assign to animals (e.g., treatment regimen, preventive technique); such studies are therefore part of experimental epidemiology. An important example is the **clinical trial**. In a clinical trial, the investigator assigns animals either to a group to which a prophylactic or therapeutic procedure is applied, or to a control group. It is then possible to evaluate the efficacy of the procedure by comparing the two groups. Clinical trials are discussed in Chapter 17.

The other types of study – cross-sectional, case-control and cohort – are **observational**. An

observational study is similar to an experimental study: animals are allocated to groups with respect to certain characteristics that they possess (trait, disease or other health-related factors). However, observational studies are conducted on naturally-occurring cases of disease in the field, and so it is not possible to assign animals to groups randomly because the investigator has little control over the factors that are being studied¹⁰. For instance, a study of the relationship between bovine mastitis, type of housing and management practices would involve investigation of cases of the disease on farms under different systems of husbandry.

A **cross-sectional study** investigates relationships between disease and hypothesized causal factors in a specified population. Animals are categorized according to the presence and absence of disease and hypothesized causal factors. Inferences then can be made about associations between disease and the hypothesized causal factors; for example, between heart valve incompetence (the disease) and breed (the hypothesized causal factor).

A **case-control study** compares a group of diseased animals with a group of healthy animals with respect to exposure to hypothesized causal factors. For example, a group of cats with urolithiasis (the disease) can be compared with a group of cats without urolithiasis with respect to consumption of dry cat food (the factor) to determine whether that type of food has an effect on the pathogenesis of the disease.

In a **cohort study**, a group exposed to factors is compared with a group not exposed to the factors with respect to the development of a disease. It is then possible to calculate a level of risk of developing the disease in relation to exposure to the hypothesized causal factors. For instance, a group of young neutered bitches can be compared with a group of young entire bitches with respect to the development of urinary incontinence, to ascertain if neutering is a risk factor for the condition.

Case-control and cohort studies often have been applied in human medicine in which experimental investigations of cause are usually unethical. For example, it would not be possible to investigate

the suspected toxicity of a drug by intentionally administering the drug to a group of people in order to study possible side-effects. However, if symptoms of toxicity have occurred, then a case-control study could be used to evaluate the association between the symptoms and the drug suspected of causing the toxicity. Some argue that there are fewer ethical restraints on experimental investigation in veterinary medicine than in human medicine and so experimental investigation of serious conditions is more tenable. However, observational studies have a role in veterinary epidemiology; for example, when investigating diseases in farm and companion-animal populations. Moreover, the increasing concern for animal welfare (see Chapter 1) is making these techniques even more attractive and useful than previously.

Methods of assessing the association between disease and hypothesized causal factors in observational studies are described in Chapters 14, 15 and 22.

Observational studies form the majority of epidemiological studies. Observational and experimental science have their own strengths and weaknesses, which are discussed in detail by Trotter (1930). A major advantage of an observational investigation is that it studies the natural occurrence of disease. Experimentation may separate factors associated with disease from other factors that may have important interactions with them in natural outbreaks.

Modelling

Disease dynamics and the effects of different control strategies can be represented using mathematical equations. This representation is **mathematical modelling**. Most modern methods rely heavily on computers. Another type of quantitative modelling is **statistical modelling**, which evaluates theoretical models of natural processes using observed data. Statistical hypothesis tests (see Chapter 14) are derived from statistical models, and form the basis of statistical inference; for example, in observational studies.

Additionally, **biological models**, using experimental animals (frequently laboratory animals), can be used to simulate the pathogenesis of diseases that occur naturally in animals and man. Additionally, the spontaneous occurrence of disease in animals can be studied in the field (e.g., using observational studies) to increase understanding of human diseases.

Mathematical modelling is outlined in Chapter 23; statistical modelling is introduced in Chapter 22; and spontaneous disease models are described in Chapter 9.

¹⁰ The distinction between experimental and observational inquiry was made in the early 19th century by the English scientific philosopher, John Herschel: experiment involving 'putting in action causes and agents over which we have control and purposely varying their combinations, and noticing what effects take place', whereas observation involves 'noticing facts as they occur, without any attempt to influence the frequency of their occurrence, or to vary the circumstances under which they occur' (Cobb, 2012). Herschel was also a protagonist of inductive reasoning in science (see Chapter 3).

Risk analysis

There is increasing and widespread interest in evaluation of the **risk** of the occurrence of adverse events, such as accidents and disasters (Report, 1983, 1992). The analysis, perception and management of risk therefore have been the focus for the development of formal methods of qualitative and quantitative **risk analysis** (Stewart, 1992; Vose, 2008).

In veterinary medicine, disease is an adverse event, and observational studies provide a framework for identifying risk factors for disease occurrence. However, veterinary risk analysis has a much broader remit than identifying risks to the individual animal. For example, although diseases may occur at low levels and be adequately controlled, there may be a risk of importing them from other countries. Such a risk can only be removed completely if importation is totally prohibited. However, current political pressures in the world favour movement towards free trade, and the unquantified risk of introduction of a disease can now no longer be presented as a trade barrier. There is therefore a need to assess objectively the risks associated with the importation of livestock and their products. Examples include the risk of disease transmission by bovine embryo transfer (Sutmoller and Wrathall, 1995) and the risk of introduction of bovine spongiform encephalopathy (Wahlström *et al.*, 2002). Similarly, the risk of disease transmission between animals (e.g., transmission of *Mycobacterium tuberculosis* from badgers and other wild animals to cattle: Gallagher *et al.*, 2003; Delahay *et al.*, 2007) can be assessed.

Microbiological risk analysis (Kelly *et al.*, 2003) commonly is concerned with food-safety risks (WHO/FAO, 2006), and involves estimation of the magnitude of microbial exposure at various stages in the production chain (rearing on the farm; transport and processing; retail and storage; preparation), so that the risk of foodborne infection can be estimated. It has been applied notably to *Campylobacter* spp. (e.g., Rosenquist *et al.*, 2003) and *Salmonella* spp. (e.g., Oscar, 1998) infections. The approach also has been used to assess the contribution of animal growth promoters to the transfer of antibiotic resistance to pathogens in humans (Kelly *et al.*, 2003).

Risk analysis is discussed in Chapter 24. Some aspects of import risk assessment are presented in Chapter 20.

Disease control

The goal of epidemiology is to improve the veterinarian's knowledge so that diseases can be controlled effectively, and productivity thereby

optimized. This can be fulfilled by treatment, prevention or eradication. The economic evaluation of disease and its control is discussed in Chapter 25. Herd health schemes are described in Chapter 26. Finally, the principles of disease control are outlined in Chapter 27.

The different components of epidemiology apply the four epidemiological approaches to varying degrees. Surveys and studies, for example, consist of a descriptive and an analytical part. Modelling additionally may include a theoretical approach.

Epidemiology's locale

The interplay between epidemiology and other sciences

During the first half of the 20th century most epidemiologists were trained initially as bacteriologists, reflecting epidemiologists' early involvement in the qualitative investigation of outbreaks of infectious disease. As the century proceeded, epidemiology became established in the context of the ecology of infectious diseases, and was addressed as such in the standard veterinary and infectious-disease textbooks (e.g., Blood and Henderson, 1960; Andrewes and Pereira, 1964). The epidemiological approach, however, is now practised by veterinarians from many disciplines: the geneticist concerned with an hereditary defect in a population, the nutritionist investigating a deficiency or toxicity, and the clinician concerned with risk factors for non-infectious diseases such as cancer.

Today, members of a variety of other sciences also take part in epidemiological studies: statisticians analysing data from groups of animals, mathematicians modelling diseases, economists assessing the economic impact of disease, and ecologists studying the natural history of disease. Each of these sciences is concerned with different facets of epidemiology, ranging from the purely descriptive, qualitative approach to the quantitative analytical approach. There have been many definitions of epidemiology (Lilienfeld, 1978), which reflect these facets. These definitions vary from the ecological, relating only to infectious diseases ('the study of the ecology of infectious diseases': Cockburn, 1963), to the mathematical, referring only to human populations ('the study of the distribution and dynamics of diseases in human populations': Sartwell, 1973). However, they all have the study of populations in common, and so are encompassed by the broad definition that was given at the beginning of this chapter. Moreover, the most profitable approach to

epidemiology lies in a balance between these qualitative and quantitative facets, with neither dominating the other¹¹, and in an appreciation that the validity of qualitative and quantitative research may be judged differently (Park, 1989; Maxwell, 1992; Creswell, 2003).

The relationship between epidemiology and other diagnostic disciplines

The biological sciences form a hierarchy, ranging from the study of non-replicating molecules to nucleic acids, organelles, cells, tissues, organs, systems, individuals, groups and, finally, whole communities and ecosystems (Wright, 1959; Ahl and Allen, 1996). The various disciplines in veterinary medicine operate at different levels in this hierarchy. Histologists and physiologists study the structure and dynamics of the individual. Clinicians and pathologists are concerned with disease processes in the individual: clinicians diagnose disease using signs displayed by the patient; pathologists interpret lesions to produce a diagnosis. Epidemiologists investigate populations, using the frequency and distribution of disease to produce a diagnosis. These three diagnostic disciplines, operating at different levels in the hierarchy, are complementary (Schwabe *et al.*, 1977). Epidemiologists, dealing with the higher levels¹², must have a knowledge of those disciplines 'lower' in the hierarchy – they must be able to see both the 'wood' and 'trees'. This means that they must adopt a broad rather than a specialist approach, avoiding the dangers of the specialist; dangers that have been described (somewhat cynically) by Konrad Lorenz (1977) in his book on the natural history of human knowledge:

‘The specialist comes to know more and more about less and less, until finally he knows everything about a mere nothing. There is a serious

danger that the specialist, forced to compete with his colleagues in acquiring more and more specialised knowledge, will become more and more ignorant about other branches of knowledge, until he is utterly incapable of forming any judgement on the role and importance of his own sphere within the context of human knowledge as a whole.’

Moreover, the specialist may be inclined to a 'positivist' approach (see Chapter 3), which requires strict separation of the object of inquiry from the investigating subject (and therefore, sometimes, the investigator) and thus may be somewhat divorced from the consequences of the knowledge that he or she generates, which may have profound social and economic effects.

Thus, the major attributes required to become a competent veterinary epidemiologist are a natural curiosity, a logical approach, a general interest in, and knowledge of, veterinary medicine, and experience of the realities of animal disease. In spite of the preceding remarks on specialists, a special interest and expertise in a particular sphere of veterinary science may, however, be useful in some investigations; for example, a knowledge of economics when undertaking an evaluation of the economic effects of disease.

Epidemiology within the veterinary profession

Brandeis (1971) proposed three 'peculiar characteristics' of a profession, as distinguished from other occupations:

‘First. A profession is an occupation for which the necessary training is intellectual in character, involving knowledge and to some extent learning, as distinguished from mere skill. Second. It is an occupation which is pursued largely for others and not merely for one's self. Third. It is an occupation in which the amount of financial return is not the accepted measure of success.’

The practice of clinical veterinary medicine is entirely congruous with these characteristics, and there is a similar consistency in the five objectives of veterinary epidemiology, outlined earlier in this chapter, which all focus on the control of animal disease, to the benefit of animals, their owners and society in general.

¹¹ The need for balance between different disciplines with a common interest is acknowledged in several areas of endeavour. For example, a healthy relationship between philosophy and theology is beneficial, with the philosopher providing the theologian with useful methods of argument; this contrasts with periods when the relationship between these disciplines has been marked by acute controversy, with little benefit accruing (Macquarrie, 1998).

¹² The epidemiologist – operating at the higher levels – has always been concerned with the characteristics and effects of disease in populations and ecosystems: a concept reinvented in One Health, Global Health, ecosystem health and conservation medicine.

Further reading

- Ashton, J. (Ed.) (1994) *The Epidemiological Imagination: a Reader*. Open University Press, Buckingham
- Butler, L.M., Bonnett, B.N. and Page, R.L. (2013) Epidemiology and the evidence-based medicine approach. In: *Withrow & MacEwen's Small Animal Clinical Oncology*, 5th edn. Eds Withrow, S.J., Vail, D. M. and Page, R.L., pp. 68–82. Elsevier-Saunders, St Louis. (A discussion of the application of epidemiology to small-animal oncology)
- Catley, A., Alders, R.G. and Wood, J.L.N. (2011) Participatory epidemiology: approaches, methods and experiences. *The Veterinary Journal*, **191**, 151–160
- Costanza, R., Norton, B.G. and Haskell, B.D. (1992) *Ecosystem Health: New Goals for Environmental Management*. Island Press, Washington DC
- Davies, G. (1983) Development of veterinary epidemiology. *Veterinary Record*, **112**, 51–53
- Davies, G. (1985) Art, science and mathematics: new approaches to animal health problems in the agricultural industry. *Veterinary Record*, **117**, 263–267
- Ferris, D.H. (1967) Epizootiology. *Advances in Veterinary Science*, **11**, 261–320. (An early description of veterinary epidemiology)
- Hungerford, L.L. and Austin, C.C. (1994) Epidemiological investigation of disease and health in feline populations. In: *Consultations in Feline Internal Medicine* 2. Ed. August, J.R., pp. 593–606. W.B. Saunders, Philadelphia
- James, A. (2005) The state of veterinary epidemiology and economics. *Preventive Veterinary Medicine*, **67**, 91–99
- Jenicek, M. (2006) The hard art of soft science: Evidence-Based Medicine, Reasoned Medicine or both? *Journal of Evaluation in Clinical Practice*, **12**, 410–419. (A critique of evidence-based medicine and its future direction)
- Martin, S.W. (1998) Art, science and mathematics revisited: the role of epidemiology in promoting animal health. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Ennis, 25–28 March 1998. Eds Thrusfield, M.V. and Goodall, E.A., pp. xi–xxii
- McCallum, H.I., Kuris, A., Harvell, C.D., Lafferty, K.D., G. W. Smith, G.W. *et al.* (2004) Does terrestrial epidemiology apply to marine systems? *Trends in Ecology and Evolution*, **19**, 585–591
- Morabia, A. (Ed.) (2004) *A History of Epidemiologic Methods and Concepts*. Birkhäuser Verlag, Basel
- Nutter, F.W. (1999) Understanding the interrelationships between botanical, human, and veterinary epidemiology: the Ys and Rs of it all. *Ecosystem Health*, **5**, 131–140
- Peeler, E.J. and Taylor, N.G.H. (2011) The application of epidemiology in aquatic animal health – opportunities and challenges. *Veterinary Research*, **42**, 94; doi 10.1186/1297-9716-42-94
- Ramey, D.W. (Ed.) (2007) Evidence-based veterinary medicine. *Veterinary Clinics of North America: Equine Practice*, **23**, 191–532
- Rapport, D.G. (Ed.) (1998) *Ecosystem Health*. Blackwell Science, Oxford
- Rapport, D.J. (1999) Epidemiology and ecosystem health: natural bridges. *Ecosystem Health*, **5**, 174–180
- Riemann, H. (1982) Launching the new international journal 'Preventive Veterinary Medicine'. *Preventive Veterinary Medicine*, **1**, 1–4
- Scarlett, J.M. (1995) Companion animal epidemiology. *Preventive Veterinary Medicine*, **25**, 151–159
- Schmidt, P.L. (Ed.) (2007) Evidence-based veterinary medicine. *Veterinary Clinics of North America: Small Animal Practice*, **37**, 409–616
- Smith, G.D. (2001) Reflections on the limitations to epidemiology. *Journal of Clinical Epidemiology*, **54**, 325–331
- Smith, G.D. and Ebrahim, S. (2001) Epidemiology – is it time to call it a day? *International Journal of Epidemiology*, **30**, 1–11
- Thacker, S.B. and Buffington, J. (2001) Applied epidemiology for the 21st century. *International Journal of Epidemiology*, **30**, 320–325
- Thrusfield, M. (1992) Quantitative approaches to veterinary epidemiology. In: *The Royal Veterinary College Bicentenary Symposium Series: The Advancement of Veterinary Science, London 1991. Volume 1: Veterinary Medicine Beyond 2000*. Ed. Michell, A.R., pp. 121–142. Commonwealth Agricultural Bureaux, Farnham Royal
- Thrusfield, M. (2001) Changing perspectives in veterinary epidemiology. *Polish Journal of Veterinary Sciences*, **4**, 19–25
- Thrusfield, M. (2016) Epidemiology. In: *Diseases of Coral*. Eds. Woodley, C.M., Downs, C.A., Bruckner, A.W., Porter, J.W. and Galloway, S.B., pp. 28–51. John Wiley and Sons, Hoboken
- Wilson, G. (1974) The use of epidemiology in animal disease. *British Veterinary Journal*, **130**, 207–213
- Wing, S. (1994) Limits of epidemiology. *Medicine and Global Survival*, **1**, 74–86

3

Causality

Chapter 1 included a brief historical description of changing concepts of the cause of disease. This chapter initially discusses the cause of events more generally, as a necessary background to the investigation of the cause of disease, which follows.

Philosophical background

Causality (causation) deals with the relationship between cause and effect¹, and is addressed in both science and philosophy. The scientist is primarily concerned with identification of causes to explain natural phenomena, whereas the philosopher attempts to understand the nature of causality, including its role in human actions (Vollmer, 1999)². The philosopher provides an insight into the theory of the nature and scope of knowledge, including its presuppositions, foundations, extent and validity (formally termed 'epistemology'); and so a basic knowledge of the philosophy of causality contributes to the scientist's ability to assess the validity and limitations of his or her inferences in the broader context of human knowledge as a whole. Concepts of causality have progressed historically, as new areas of interest emerge (e.g., the recent interest in causality in relation to artificial intelligence: Shafer, 1996).

The Classical period

In Classical times, Aristotle defined a doctrine of four causes (Barnes, 1984). These four 'causes' are really four different explanations or reasons for how and why a thing is as it is: (1) the **material cause** – what

'stuff' (matter) a thing comprises (some combination of the four elements: earth, air, fire and water: see *Figure 1.3*); (2) the **formal cause** – the 'form and pattern' of a thing, or those properties without which a thing would not exist as it does; (3) the **efficient cause** – which is the maker of a thing (and by which the formal cause is therefore explained); and (4) the **final cause** – which is the purpose of the thing (and therefore, in the case of natural things, is usually synonymous with the formal cause). The notion of the 'universe of natural law' as a cause of disease (see Chapter 1) was consistent with this philosophy. Aristotle's emphasis lay on *purpose* as the only satisfactory explanation of why a thing is; and his doctrine failed to account for certain natural phenomena (e.g., why bodies accelerate while falling).

The Scholastics

The Christian mediæval philosophers (termed the 'Scholastics') generally endorsed Aristotle's ideas³, but focussed on God as the efficient cause of all things (a view posited by St Thomas Aquinas in his *Summa Theologica*)⁴. The notion that disease was induced by divine wrath (see Chapter 1) was consistent with this philosophy. The extent to which individuals were secondary efficient causes of things, with God as the primary cause, was a subject of debate during this period, which was tempered by the view of some monastic scholars, grounded in Classical ideas, that

¹ Generically, a causal relationship is one that has a *mechanism* that, by its operation, *makes a difference* (Joffe, 2011).

² For broad, contrasting views on explanation in science, see Hempel (1965), Kitcher and Salmon (1989) and Weber and others (2013).

³ The origins of Scholasticism can be traced back to the sixth-century Roman philosopher, Boethius (Rand, 1928). Aristotle's influence was pre-eminent in mediæval universities, only being tempered by a broader philosophical curriculum in the late 14th century, possibly as a result of the teachings of the Byzantine scholar, Manuel Chrysoloras, who was brought to Florence in 1397 by the Chancellor of the city, Coluccio Salutati.

⁴ This idea had a wide influence; for example, on the concept that right and wrong are absolute – a notion that subsequently was tempered by one that they are determined by circumstances and conscience (termed 'casuistry': Bretzke, 2011).

nature was designed by God but operated independently (Fara, 2009).

The 'Modern' period

Debate on causality expanded substantially during the so-called 'Modern' period of philosophy. Beginning with the French Philosopher, René Descartes (1596–1650), the debate (which was an integral part of 'The Enlightenment': see Chapter 1) was complex, but essentially aimed to simplify and secularize the Classical and Scholastic concepts of causality (Clatterbaugh, 1999).

In science, the erosion of the Classical and Scholastic view had begun with Galileo. In his book, *Discorsi e Dimostrazioni Matematiche* (published in 1638), he emphasized the need to explain events mathematically in terms of 'how' (description) and 'why' (explanation). This was prompted, in part, by increasing mechanization and the consequent need for engineers to predict the effects of different designs (e.g., of ships, as navigation extended throughout the world). Increasingly, in science, a physical and mathematical explanation for natural phenomena was sought, and the techniques of causal inference were debated.

Causal inference

Scientific conclusions are derived by two methods of reasoning: **deduction** and **induction** (Medawar, 1969)⁵. Deduction is arguing from the general to the particular; that is, a general case is established, from which all dependent events are argued to be true. For example, in Euclidean geometry, axioms are established, from which specific theorems (e.g., Pythagoras' Theorem) are then reasoned. Thus, if one posits the truth of the general proposition 'all dogs are mammals', it follows by deduction that any particular example of a dog will be a mammal. If the premisses of a deductively valid argument are true, it follows that the conclusion must also be true.

Induction, in contrast, is arguing from the particular to the general. For instance, a dog may be vaccinated against distemper virus, and shown to be immune to challenge with the agent, from which the conclusion is drawn that the vaccine prevents distemper in all

dogs. It is important to note that, unlike the deductive example, the premiss in this example could be true and yet the conclusion false.

Induction has generally driven modern scientific investigation, requiring detached observation of events, and is frequently associated with 'positivism', a philosophical term describing scientific study based on the objective analysis of data, which excludes unverifiable speculation (*Britannica*, 1992)⁶. The English theologian and philosopher, Thomas Bayes (1702–1761), was the first to apply statistical probability inductively (Bayes, 1763). His method, eponymously named 'Bayesian inference', involves calculating, from the frequency with which an event had occurred previously (the 'prior probability'), the probability that it will occur in the future (the 'posterior probability'). The Bayesian view of probability is a way of registering degrees of belief, which may be strengthened or weakened by numerical data. A notable example of the Bayesian method is calculation of the probability of disease in an individual, depending on results of diagnostic tests (see Chapter 20).

Induction was later addressed in some detail by the 19th-century English philosopher, John Stuart Mill (1868), whose 'canons' of inductive reasoning are still widely used in epidemiology, and are outlined and exemplified later in this chapter.⁷ However, the validity of induction as a demonstration of proof had already been challenged by the scepticism of the 18th-century Scottish philosopher, David Hume (1739–1740), whose ideas are still the subject of debate (Strawson, 1989). Hume argued that the mere observation that one event preceded another (e.g., that a vaccine against canine distemper is observed to confer immunity to distemper virus when administered to a dog) was not

⁵ This is a simplification, which is inevitable in a brief introduction. Additionally, a third type of reasoning – **abduction** (reasoning by hypothesis) – was described in the mid-19th century by Charles Peirce (Fisch, 1982). This typically begins with an incomplete set of observations and proceeds to the likeliest possible explanation for the set (e.g., a diagnosis is based on finding the best explanation for a given collection of signs). See Plutynski (2011) for a detailed discussion of abduction.

⁶ This approach, which generates 'instrumental knowledge', has become synonymous with contemporary scientific method, but represents only one way of acquiring knowledge (Feyerabend, 1975; Giddens, 1987; Uphoff, 1992; Liamputton and Ezzy, 2005), and may not present the 'full picture'. For example, during the 2001 epidemic of foot-and-mouth disease in the UK, field investigations revealed uncorroborated suspicion of transmission of the disease by undisclosed movements of livestock, thereby providing a fuller explanation of the disease's spread (Gibbens *et al.*, 2001b) – an approach with which positivists would be unsympathetic. In the social sciences, the need to locate oneself 'within' the area of research is accepted as fundamental to the analytical procedure, and is formally termed 'experiential analysis' (Reinharz, 1983). This is similar to the acquisition of 'interactive knowledge', which is obtained by direct involvement with, and experience of, society (Habermas, 1972). Moreover, positivism excludes moral and ethical judgements (e.g., on the social and psychological effects of a particular disease-control campaign: Mephram, 2001; Convery *et al.*, 2008; Hibi *et al.*, 2015; Makita *et al.*, 2015), which are made possible only by reflection – so-called 'critical knowledge' (Habermas, 1972).

⁷ Other 19th-century protagonists of induction included John Herschel and William Whewell (Snyder, 2011).

proof that the former was the cause of the latter; because, first, even if the observation were repeated many times, coincidence could not be excluded, and, secondly, previous patterns cannot be guaranteed to continue in the future. Logically, then, inductive proof of a hypothesis⁸ is impossible. It is, however, possible to refute a hypothesis by observing events that conflict with it on a single occasion⁹. This led the philosopher, Karl Popper (1959), to conclude that science advances only by elimination of hypotheses¹⁰.

In epidemiology, studies are generally undertaken to identify causes of disease so that preventive measures can be developed and implemented, and their subsequent effectiveness identified (Wynder, 1985). Such investigations of cause are usually based on inductive reasoning, which, despite its philosophical deficiencies, still forms the pragmatic basis on which most conclusions need to be drawn in epidemiology and other sciences, and on which a workable definition of 'proof' is based¹¹.

Methods of acceptance of hypotheses

One may accept (or reject) a causal hypothesis by four methods (Cohen and Nagel, 1934):

1. tenacity;
2. authority;
3. intuition;
4. scientific inquiry.

⁸ A hypothesis is a theory that is not well tested. This contrasts with laws and facts. (See Chapter 23 for a fuller discussion.)

⁹ Probably the commonest example quoted in the literature is of the cockerel and the sunrise. The crowing of the cockerel always precedes the rising of the sun. However, the hypothesis that the cockerel causes the sun to rise can be refuted by strangling it before dawn. The 20th-century philosopher, Bertrand Russell, gives another poultry example: an 'inductivist turkey' who reasons to the effect that, since every day in the past it has been fed at nine o'clock by the farmer, it will today again be fed as the farmer approaches at nine o'clock, as usual – but today is Christmas Eve!

¹⁰ Popper's philosophy contrasts with Kuhn's concept of paradigm shifts in science (see Chapter 1). Between the rare crises that stimulate the latter, conservatism and conformity do not favour serious criticism of prevailing scientific assumptions; whereas Popper urges constant challenging of current scientific beliefs (Fuller, 2003). For a critical treatment of Popper's writings, see Notturmo (1999) and, for an interpretation of Popperian principles in the context of epidemiology, see Maclure (1985).

¹¹ Philosophers, too, have been aware of the utility of induction. Thus, although Mill and Hulme are both sceptical about induction, because induction itself cannot be established empirically (i.e., as being reliable merely by observation), they both appear to conclude that induction – although philosophically unjustifiable – is, none the less, indispensable.

Tenacity

Habit makes it easy to continue to believe a proposition and to offer a closed mind either to the opinions of others or to evidence that contradicts the proposition. Thus, some people continued to believe that smoking was beneficial because it 'cleared the chest', even after Doll (1959) provided evidence that it induced lung cancer. The method of tenacity is unsatisfactory because it disregards the opinions of others, and, if they are considered, provides no framework for choosing between them.

Authority

Sometimes, appeal is made to a highly respected source to substantiate views. First, this may involve an appeal to an allegedly infallible source. This is, for instance, the basis of religious beliefs. However, this cannot lead to unanimity or stability of belief because there may be a variety of conflicting sources¹².

Secondly, appeal may be made to the opinions of experts, whose authority is generally acknowledged. This is a reasonable and widely-used approach. However, experts' opinions may vary, and such authority is only relatively final because opinions may be modified in the light of new knowledge or more convincing arguments.

Intuition

Some propositions may be considered to be self-evident, without being sustained by evidence. Thus, many veterinarians judged speed of slaughter of animals on infected premises to be crucial in the control of foot-and-mouth disease before firm evidence in support of this proposition was presented (Honhold *et al.*, 2004a; Thrusfield *et al.*, 2005b). Intuition may be moulded by training, experience and fashion. However, intuitive notions (e.g., that the Earth is flat) may subsequently be shown to be false. Therefore, intuitions need to be tested.

Tenacity, authority and intuition may all contribute, to varying degrees, to an individual's belief in the veracity of a hypothesis, and such belief can be insidious, colouring scientific investigation – a danger highlighted by Sir Peter Medawar, former Director of the National Institute for Medical Research, London (1979): '*... the intensity of the conviction that a hypothesis is true has no bearing on whether it is true or not.*'

¹² These may not only vary between faiths, but also within faiths, leading, for example, to shifting definitions of orthodoxy and heresy (George, 1995).

Scientific inquiry

Clarity, order and consistency in fixing beliefs, independent of the idiosyncrasies of a few individuals, are required. This is the basis of scientific inquiry, which involves making objective observations that can be repeated by many investigators. Scientific method encourages doubt (and the scientist's prudent distrust of himself: Baker, 1973) and, as either new evidence or new doubts arise, they are incorporated in the accumulating body of knowledge. Thus, science is progressive and is never too certain about its results¹³. It therefore differs radically from tenacity, authority and intuition, which generally exclude the possibility of errors and have no provision for correcting them.

The remainder of this chapter now focusses on causality specifically in the context of disease.

Koch's postulates

Chapter 1 indicated that there has been a transition from the idea that disease has a predominantly single cause to one of multiple causes¹⁴. The former idea is epitomized by the postulates, formulated by Robert Koch in the late 19th century, to determine the cause of infectious disease (Koch, 1892). These postulates¹⁵, which are based on inductive reasoning, state that an organism is causal if:

- it is present in all cases of the disease;
- it does not occur in another disease as a fortuitous and non-pathogenic parasite;
- it is isolated in pure culture from an animal, is repeatedly passaged, and induces the same disease in other animals.

Koch's postulates brought a necessary degree of order and discipline to the study of infectious disease. Few would argue that an organism fulfilling the above criteria does not cause the disease in question; but is it

¹³ The 19th-century biologist, T.H. Huxley, concisely summarized a necessary consequence of scientific inquiry: *'The great tragedy of Science – the slaying of a beautiful hypothesis by an ugly fact'*.

¹⁴ Complexity, however, should not be sought when it is not justified. This guideline is encapsulated in the 'principle of parsimony' (Hamilton, 1852), whose frequent and thorough use by the mediaeval English philosopher, William of Occam, gained it the name of 'Occam's razor': *'Pluralitas non est ponenda sine necessitate'*: 'multiplicity ought not to be posited without necessity'. More generally, one should choose the simplest hypothesis that will fit the facts (Edwards, 1967).

¹⁵ The postulates are more fully termed the **Henle–Koch postulates**. Koch empirically validated postulates deductively reasoned 40 years earlier by his teacher, Jacob Henle (Rosen, 1938). The postulates have been updated by Thomas Rivers (1937) to include viruses.

the *sole* and *complete* cause? Koch provided a rigid framework for testing the causal importance of a microorganism but ignored the influence of environmental factors, which were relatively unimportant in relation to the diseases that were being studied. Microbiologists found it difficult enough to satisfy the postulates without concerning themselves with interactions between complex environmental factors. Therefore the microorganisms were assumed to be the sole causes of the diseases that the microbiologists were investigating.

Dissatisfaction became evident in two groups (Stewart, 1968). Some microbiologists thought that the postulates were too difficult to satisfy because there can be obstacles to fulfilling Koch's postulates with some infectious agents that are causes of disease (e.g., some pathogens can be isolated in pure culture from cases, but do not readily induce disease in other animals; see, for example, Chapter 5: 'Diseases caused by mixed agents'). Others thought that the postulates were insufficient because they did not specify the environmental conditions that turned vague associations into specific causes of disease. Furthermore, the postulates were not applicable to non-infectious diseases. A more cosmopolitan theory of cause was needed.

Evans' rules

Alfred Evans (1976) produced a set of 10 rules that are consistent with modern concepts of causality:

1. the proportion of individuals with the disease should be significantly higher in those exposed to the supposed cause than in those who are not;
2. exposure to the supposed cause should be present more commonly in those with than those without the disease, when all other risk factors are held constant;
3. the number of new cases of disease should be significantly higher in those exposed to the supposed cause than in those not so exposed, as shown in prospective studies;
4. temporally, the disease should follow exposure to the supposed cause with a distribution of incubation periods on a bell-shaped curve;¹⁶

¹⁶ The bell shape is often obtained only when the horizontal 'time' axis is mathematically transformed (Sartwell, 1950, 1966; Armenian and Lilienfeld, 1974; Armenian, 1987); if a linear time scale is used, then the curve is usually positively skewed; that is, there are few long incubation periods relative to the number of short incubation periods. Mathematical transformation is described in Chapter 12.

5. a spectrum of host responses, from mild to severe, should follow exposure to the supposed cause along a logical biological gradient;
6. a measurable host response (e.g., antibody, cancer cells) should appear regularly following exposure to the supposed cause in those lacking this response before exposure, or should increase in magnitude if present before exposure; this pattern should not occur in individuals not so exposed;
7. experimental reproduction of the disease should occur with greater frequency in animals or people appropriately exposed to the supposed cause than in those not so exposed; this exposure may be deliberate in volunteers, experimentally induced in the laboratory, or demonstrated in a controlled regulation of natural exposure;
8. elimination (e.g., removal of a specific infectious agent) or modification (e.g., alteration of a deficient diet) of the supposed cause should decrease the frequency of occurrence of the disease;
9. prevention or modification of the host's response (e.g., by immunization or use of specific lymphocyte transfer factor in cancer) should decrease or eliminate the disease that normally occurs on exposure to the supposed cause;
10. all relationships and associations should be biologically and epidemiologically credible.

An important characteristic of Evans' rules, which unified principles of establishing causality for both infectious and non-infectious diseases¹⁷, is that some require the association between a hypothesized causal factor and the disease in question to be **statistically significant**. This involves comparing **groups** of animals, rather than investigating associations in the individual. Some of the statistical techniques of demonstrating association are described in Chapters 14, 15 and 22.

Variables

The object of detailed statistical analysis is to identify those factors that cause disease. Disease and causal factors are examples of **variables**.

¹⁷ In so doing, Evans built on the Henle–Koch postulates, the criteria for causality for non-infectious diseases established by Yerushalmy and Palmer (1959) and Huebner's (1957) argument that prevention should be counted among the criteria. Using a similar set of rules, Donham and Thorne (1994) have constructed a matrix for assessing the strength of causal evidence. For a detailed discussion of the evolution of causal criteria, see Lower and Kanarek (1983) and Blackburn and Labarthe (2012).

Variable

A variable is any observable event that can vary. Examples of variables are the weight and age of an animal and the number of cases of disease.

Study variable

A study variable is any variable that is being considered in an investigation.

Response and explanatory variables

A **response** variable is one that is affected by another (**explanatory**) variable. In epidemiological investigations, disease is often the response variable. For example, when studying the effects of dry cat food on the occurrence of urolithiasis, cat food is the explanatory variable and urolithiasis is the response variable.

Types of association

Association is the degree of dependence or independence between two variables. There are two main types of association (*Figure 3.1*):

1. non-statistical association;
2. statistical association.

Non-statistical association

A non-statistical association between a disease and a hypothesized causal factor is an association that arises by chance; that is, the frequency of joint occurrence of the disease and factor is no greater than would be expected by chance. For example, *Mycoplasma felis* has been isolated from the eyes of some cats with conjunctivitis. This represents an association between the mycoplasma and conjunctivitis in these cats. However, surveys have shown that *M. felis* also can be recovered from the conjunctivae of 80% of apparently normal cats (Blackmore *et al.*, 1971). Analysis of these findings revealed that the association between conjunctivitis and the presence of *M. felis* arose by chance: the mycoplasma could be present in healthy cats as well in those with conjunctivitis. In such circumstances, where a chance, non-statistical association occurs, a factor cannot be inferred to be causal.

Statistical association

Variables are positively statistically associated when they occur together more frequently than would be

Fig. 3.1 Types of association between disease and hypothesized causal factors.

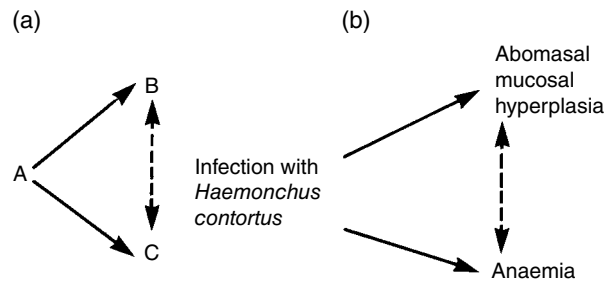
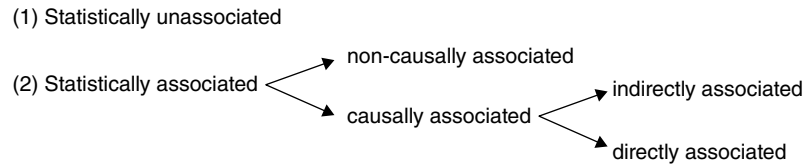


Fig. 3.2 Path diagrams indicating the paradigm (a) and an example (b) of causal and non-causal statistical associations. A = cause of disease (explanatory variable); B and C = manifestations of disease (response variables); → causal association; ↔ non-causal association.

expected by chance. They are negatively statistically associated when they occur together less frequently than would be expected by chance.

Positive statistical associations may therefore indicate a causal relationship. However, not all factors that are positively statistically associated with a disease are necessarily causal. This can be understood with the aid of a simple path diagram (Figure 3.2a). The explanatory variable, A, is the cause of a disease. The response variables, B and C, are two manifestations of the disease. In these circumstances, there is a statistical causal association between A and B, and between A and C. There is also a positive statistical association between the two response variables, B and C, arising from their separate associations with A, but this is a non-causal association.

An example of these associations is given in Figure 3.2b. If infection of cattle with *Haemonchus contortus* were being investigated, then the following positive statistical associations could be found:

- between the presence of the parasite and abomasal mucosal hyperplasia;
- between the presence of the parasite and anaemia;
- between abomasal mucosal hyperplasia and anaemia.

The first two associations are causal and the third non-causal.

Abomasal mucosal hyperplasia and infection with *H. contortus* are **risk factors** for anaemia, that is, their presence increases the risk of anaemia. Similarly, in cats, lack of skin pigmentation results in white fur and also increased ultraviolet irradiation of the skin. The latter is associated with cutaneous squamous cell carcinoma (Dorn *et al.*, 1971), and white fur is a risk factor for this condition.

Risk factors therefore may be either causal or non-causal. (Some authors reserve 'risk factor' exclusively for causal factors, and use 'risk marker' to describe non-causally-associated factors, with 'risk indicator' applying to both terms: Porta, 2014.) A knowledge of risk factors is useful in identifying populations at which veterinary attention should be directed. Thus, high milk yield is a risk factor for ketosis in dairy cattle. When developing preventive measures it is important to identify those risk factors that are causal, against which control should be directed, and those that are non-causal and which will not therefore affect the development of disease.

Explanatory and response variables can be causally associated either **directly** or **indirectly** (Figure 3.3). Path diagrams 1 and 2 illustrate direct causal associations. Indirect associations are characterized by an intervening variable. Path diagram 3 illustrates an indirect causal association between A and C where the effect of A is entirely through the intervening variable B, whose effect is direct. This is equivalent to saying that A and B operate at different levels, therefore either A or B can be described as the cause of C. Leptospirosis, for example, causes haemoglobinuria by haemolysing red blood cells; a clinician would say that leptospirosis causes the haemoglobinuria, whereas a pathologist might attribute it to intravascular haemolysis.

Path diagram 4 in Figure 3.3 illustrates the situation where one explanatory variable, A, has not only a direct causal association with a response variable, C, but also an indirect effect on C by influencing another variable, B. For example, in the US people have contracted rabies by inhalation on entering caves where rabies-infected bats roost. They can also contract rabies from foxes that are infected by living in bat-infested caves.

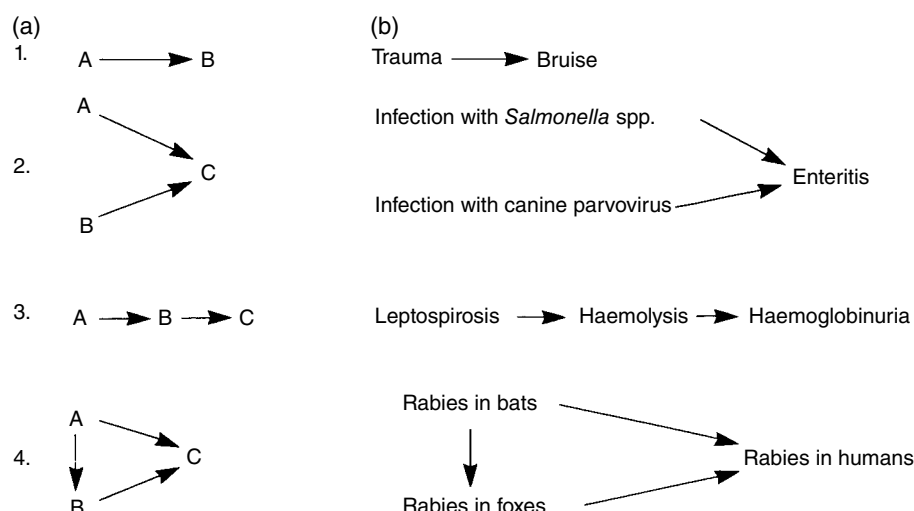


Fig. 3.3 Path diagrams indicating the paradigm (a) and examples (b) of direct and indirect causal associations: 1 and 2 = direct causal associations; 3 = indirect causal association (A with C) and direct causal association (B with C); 4 = direct and indirect causal association (A with C).

Utility of statistical association

Demonstration of a statistically significant association does not *prove* that a factor is causal¹⁸. The logical reduction of proof requires that the mechanism of induction of a disease by a cause needs to be explained by describing the chain of events, from cause to effect, at the molecular level (ultimately to mechanisms of adverse changes at the nucleic-acid level), corresponding to a 'deeper' level of understanding than that offered by statistical association (Lower, 1983). However, in the absence of such evidence, identification of an association can be of considerable preventive value because it can indicate factors, the reduction or removal of which is empirically shown to reduce the occurrence of disease, although a specific cause has not been identified.

Is an investigator remiss in not explaining (or not attempting to explain) all disease/factor causal relationships in intimate biological detail? Such a line of action represents a 'black-box' approach, which does not address internal structures and workings, but which nevertheless permeates many branches of science (Latour, 1999). It is sceptical of the possibility of fully describing mechanisms, and relies on the pragmatic success of research. For example, eschewing smoking is known to reduce the risk of lung cancer. Similarly, the *Framingham Heart Study* has identified several risk factors for heart disease and stroke (e.g., age, sex, systolic blood pressure and diabetes), some

of which can be manipulated, without fully elucidating their mechanisms (Pencina *et al.*, 2009). Likewise, untreated sewage discharged onto coral reefs induces disease in corals (Pastorok and Bilyard, 1985) and subsequent diversion of sewage outfalls away from the reefs results in the return of healthy corals (Baker *et al.*, 2007), thus resolving a problem without precise knowledge of the causal mechanism.

The merits and deficiencies of incomplete explanations are debatable (Weed, 1998). Epidemiology can be approached either 'mechanistically', emphasizing molecular-based causal processes (notably in relation to carcinogenesis) or via 'black-box' methods (Thrusfield, 2016a). Mechanistic proponents discredit the 'black-box' strategy as being of low scientific merit (Peto, 1984), whereas defenders of the 'black box' justify its approach on the grounds that it facilitates disease prevention in the absence of detailed causal mechanisms (Savitz, 1994)¹⁹. Moreover, there are also limitations on what mechanistic exploration at the molecular level can achieve (Van Regenmortel, 2004). This assumes that dissection of biological systems into their constituent parts, and study of such individual parts, facilitates a complete description of the systems (i.e., disease, in the current context): so called 'reductionism'. However, there is evidence that biological systems contain features that cannot wholly be described by all of their individual components (Alm and Arkin, 2003) leading to the conclusion that a mechanistic/reductionist explanation may not be complete.

¹⁸ Statisticians' views on the relationship between statistical association and causality, and the extent to which statistical associations demonstrated in either a single study or several studies indicate cause, vary widely (Pearson, 1911; Cox and Wermuth, 1996); Petitti (1991), for example, arguing that only intervention studies can be used to infer a causal effect. Pearl (2009a) discusses this issue in detail.

¹⁹ Breslow (2003) convincingly argues that the two approaches – identified over 100 years ago by Sir Ronald Ross, discoverer of the life-cycle of the malaria parasite (Nye and Gibson, 1997) – are complementary.

A balanced judgement, however, requires extension of the box analogy to that of one of nested Chinese boxes (or Russian dolls) (Susser and Susser, 1996), reflecting the biological hierarchy, from molecules to ecosystems (see Chapter 2). The epidemiologist is concerned with populations, and therefore occupies a larger box than the molecular biologist, who deals with components of an animal's individual cells. The latter may challenge the former to supply detailed molecular causal mechanisms. However, the molecular biologist should be reminded that he or she does not occupy the smallest box: the particle physicist, dealing with atoms and sub-atomic particles, occupies a smaller box (Griffiths, 2008). The molecular biologist, therefore, equally could be challenged to explain mechanisms at a lower level than the one at which he or she operates (noting the deficiencies of reductionism).

Dangers of statistical association

Statistical relationships can be used to justify erroneous biological hypotheses. This is elegantly demonstrated by the work of William Farr (see Chapter 1). He accumulated statistics during cholera years, showing that more cases occurred in low-lying areas of London (e.g., near to the River Thames) than in higher places, such as Hampstead. He then put forward his 'Theory of Elevation', arguing that cholera was caused by miasmata (see Chapter 1) that emanated from the Thames, and that these were logically diluted as one rose above it, resulting in fewer cases at higher altitudes (Burke, 1985; Eyler, 2001)²⁰. This explanation failed to account for differences in the level of water pollution due to sewage in different areas, which were eventually identified by the medical epidemiologist, John Snow (1855), who mapped cholera cases in relation to water supply. He noted that the cases were linked to consumption of water from a contaminated pump in Broad Street (now Broadwick Street), Soho (Figure 3.4), thereby demonstrating that the disease was transmitted in water²¹.

²⁰ The simple formula that Farr applied was: $C = C' (e' + a)/(e + a)$, where C and C' are cholera mortality rates per 100 000 in two districts having mean elevations in feet of e and e' , respectively, and a is a constant (12.8) (Farr, 1852). His formulaic predictions agreed remarkably well with observed mortalities at various heights. Farr may, or may not, have known that his Theory of Elevation did not hold for other disease outbreaks in London; notably, the Great Plague in 1665 (Boghurst, 1665).

²¹ However, the precise connection between the pump's disengagement and the end of the outbreak is debatable (McLeod, 2000).

Confounding

Confounding (Latin: *confundere* = 'to mix together') is the effect of an extraneous variable that can wholly or partly account for an apparent association between variables. Confounding can produce a spurious association between study variables, or can mask a real association. A variable that confounds is called a **confounding variable** or **confounder**.

A confounding variable is distributed non-randomly (i.e., is positively or negatively correlated with the explanatory and response variables that are being studied: Hernán, 2008). A confounding variable generally must²²:

- be a risk factor for the disease that is being studied;
- be associated with the explanatory variable;
- not be affected by exposure to the disease or the explanatory variable.

Examples to illustrate the concept

An investigation of leptospirosis in dairy farmers in New Zealand (Mackintosh *et al.*, 1980) revealed that wearing an apron during milking was associated with an increased risk of contracting leptospirosis. Further work showed that the larger the herd being milked, the greater the chance of contracting leptospirosis. It also was found that farmers with large herds tended to wear aprons more frequently for milking than farmers with small herds. The association between the wearing of aprons and leptospirosis was not causal but was produced spuriously by the confounding effect of large herd size (Figure 3.5a), because large herd size was associated with leptospirosis, and also with the wearing of aprons. Figure 3.5b illustrates a similar confounding effect in relation to respiratory disease in pigs (Willeberg, 1980b). A statistical association was demonstrated between fan ventilation and respiratory disease. This was not because fan ventilation caused respiratory disease. The association resulted from the confounding effect of herd size: large herds are more likely to develop respiratory disease than small herds, and are also more likely to have fan ventilation rather than natural ventilation.

These two examples have been selected to illustrate confounding in situations where the spurious association is obviously rather implausible. However, in many situations, confounding is less obvious, but must be

²² Criteria for confounding are given in standard texts (e.g., Rothman *et al.*, 2008), but there is controversy over conflicting definitions of confounding, and therefore over the conditions required (Kass and Greenland, 1991; Weinberg, 1993; Shapiro, 1997; McNamee, 2003).



Fig 3.4 Detail from John Snow's London cholera map of 1854. Black dots indicate deaths from cholera. (Source: Stamp, 1964.)

considered, for example, in observational studies that test causal hypotheses (see Chapters 15 and 18).

Causal models

The associations and interactions between direct and indirect causes can be viewed in two ways, producing two causal 'models'.

Causal model 1

The relationship of causes to their effects allows classification of causes into two types: 'sufficient' and 'necessary' (Rothman, 1976).

A cause is **sufficient** if it inevitably produces an effect (assuming that nothing happens that interrupts the development of the effect, such as death or prophylaxis).

A sufficient cause virtually always comprises a range of component causes; disease therefore is *multifactorial*. Frequently, however, one component is commonly described, in general parlance, as *the cause*²³. For example, distemper virus is referred to as the cause of

²³ Philosophers are uneasy with such a definition. Note, for instance, Mill's 'argument from caprice': 'Nothing can better show the absence of any scientific ground for the distinction between the cause of a phenomenon and its conditions, than the capricious manner in which we select from among the conditions that which we choose to denominate the cause.' (Mill, 1868). Mill's argument has won the philosophical field, and is echoed by contemporary authors such as Lewis (1986): 'We sometimes single out one among all the causes of some event and call it "the" cause, as if there were no others. Or we single out a few as the "causes", calling the rest mere "causal factors" or "causal conditions"... We may select the abnormal or extraordinary causes, or those under human control, or those we deem good or bad, or just those we want to talk about. I have nothing to say about these principles of invidious discrimination.'

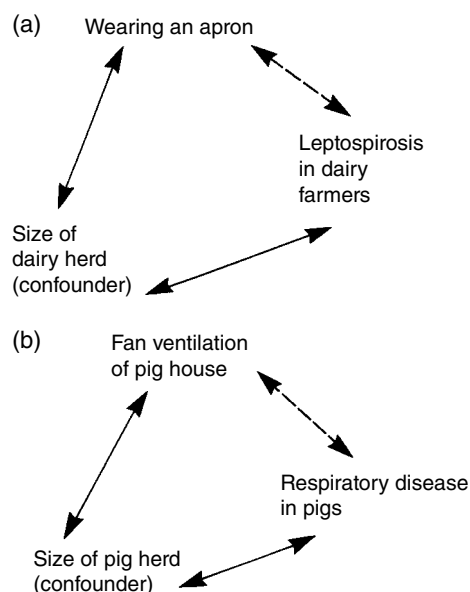


Fig. 3.5 Examples of confounding: (a) large dairy herds in relation to leptospirosis in dairy farmers and the wearing of milking aprons; (b) large pig herds in relation to respiratory disease in pigs and fan ventilation. $\leftrightarrow$ 'Real' association; $\dashrightarrow$ spurious association.

distemper, although the sufficient cause actually involves exposure to the virus, lack of immunity and, possibly, other components. It is not necessary to identify all components of a sufficient cause to prevent disease because removal of one component may render the cause insufficient. For example, an improvement in floor design can prevent foot abscesses in pigs even though the main pyogenic bacteria are not identified.

A particular disease may be produced by different sufficient causes. The different sufficient causes may have certain component causes in common, or they may not. If a cause is a component of every sufficient cause, then it is **necessary**²⁴. Therefore, a necessary cause must always be present to produce an effect.

In Figure 3.6a, A is the only (potentially) necessary cause, because it is the only component appearing in all of the sufficient causes. The remaining causes (B–J) are definitely not *necessary* because there are some sufficient causes without them. This concept is exemplified in Figure 3.6b, which depicts hypothesized sufficient causes of pneumonic pasteurellosis in cattle. Infection with *Mannheimia (Pasteurella)* spp. is the necessary cause, but other component causes,

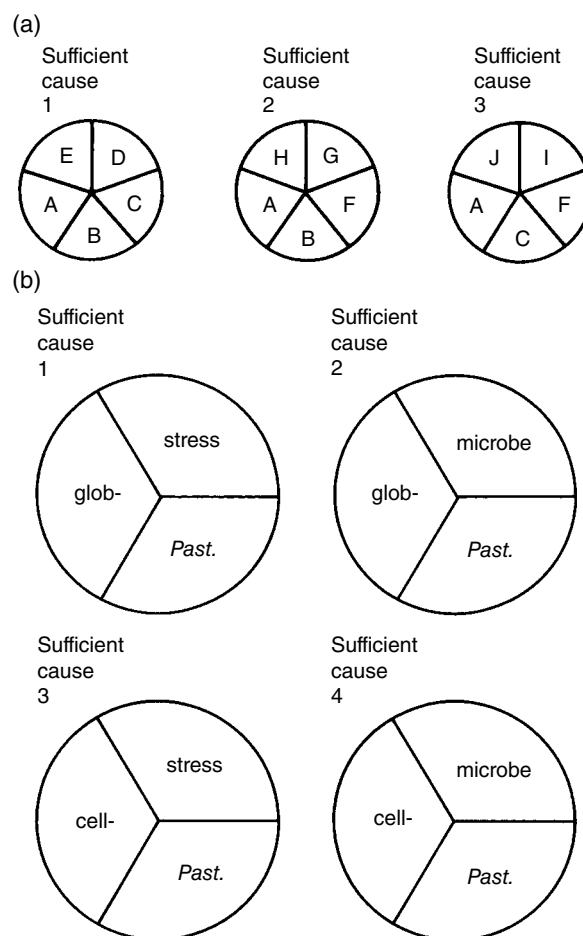


Fig. 3.6 Scheme for the causes of disease (causal model 1): (a) paradigm; (b) hypothetical example for bovine pneumonic pasteurellosis. cell- = lack of cellular immunity; glob- = lack of specific globulins; microbe = presence of viruses or mycoplasmas; Past. = presence of *Mannheimia (Pasteurella)* spp.; stress = adrenal stress of environmental origin (e.g., weather). ((a) Source: Rothman, 1976. Reproduced with permission of Oxford University Press. (b) Adapted from Martin *et al.*, 1987.)

including lack of immunity, are required for induction of the disease.

Another example of a cause that is necessary but not sufficient is infection with *Actinobacillus ligneresi*, which must occur before actinobacillosis ('wooden tongue') can develop. However, other factors that damage the buccal mucosae (e.g., sharp, abrasive vegetation) must be present before the disease develops. In the absence of these factors, the bacterium can be present without disease developing.

It is obvious that necessary causes are frequently related to the definition of a disease; for example, lead is a necessary cause of lead poisoning, and *Mannheimia multocida* is a necessary cause of pneumonic pasteurellosis.

²⁴ Again, the philosopher would be uneasy. Just because one component has always been present in all cases, it does not follow that it is the causally operative/necessary component, or that it is a necessary component at all – what if the next case lacks this component?

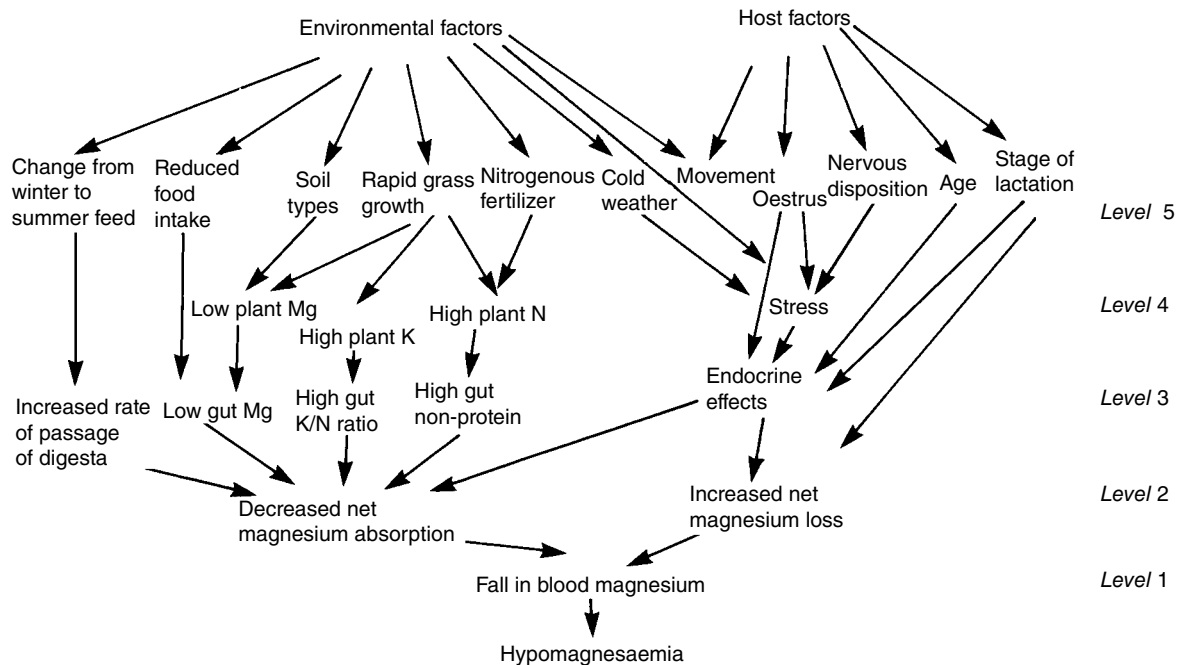


Fig. 3.7 Causal web of bovine hypomagnesaemia (causal model 2).

A cause may be necessary, sufficient, neither, or both, but it is unusual for a single component cause to be both necessary and sufficient. One example is exposure to large doses of gamma radiation with the subsequent development of radiation sickness.

Component causes therefore include factors that have been classified as:

- **predisposing factors**, which increase the level of susceptibility in the host (e.g., age and immune status);
- **enabling factors**, which facilitate manifestation of a disease (e.g., housing and nutrition);
- **precipitating factors**, which are associated with the definitive onset of disease (e.g., many toxic and infectious agents);
- **reinforcing factors**, which tend to aggravate the presence of a disease (e.g., repeated exposure to an infectious agent in the absence of an immune response).

Pneumonia is an example of a disease that has sufficient causes, none of which has a necessary component. Pneumonia may have been produced in one case by heat stress where a dry, dusty environment allowed microscopic particulate matter to reach the alveoli. Cold stress could produce a clinically similar result.

Multifactorial syndromes such as pneumonia can have many sufficient causes, although no single component cause is necessary. Part of the reason is

taxonomic: pneumonia is a loosely-connected group of diseases whose classification (see Chapter 10) is based on lesions (inflammation of the lungs) rather than specific causes; the lesions can be produced by many different causes. When a disease is classified according to aetiology, there is, by definition, usually only one major cause, which therefore is likely to be necessary. Examples include lead poisoning, actinobacillosis and pasteurellosis, mentioned earlier, and many 'simple' infectious diseases, such as tuberculosis and brucellosis.

The object of epidemiological investigations of cause is the identification of sufficient causes and their component causes. Removal of one or more components from a sufficient cause will then prevent disease produced by that sufficient cause.

Causal model 2

Direct and indirect causes represent a chain of actions, with the indirect causes activating the direct causes (e.g., Figure 3.3, path diagram 3). When many such relationships occur, a number of factors can act at the same level (but not necessarily at the same intensity), and there may be several levels, producing a 'web of causation'²⁵. Again, disease is *multifactorial*. Figure 3.7 illustrates the causal web of bovine hypomagnesaemia.

²⁵ For a critique of this model, see Krieger (1994) and Joffe and others (2012).

Formulating a causal hypothesis

The first step in any epidemiological investigation of cause is descriptive. A description of time, place, and population is useful initially.

Time

Associations with year, season, month, day, or even hour in the case of food-poisoning investigations, should be considered. Such details may provide information on climatic influences, incubation periods and sources of infection. For example, an outbreak of salmonellosis in a group of cattle may be associated with the introduction of infected cattle feed.

Place

The geographical distribution of a disease may indicate an association with local geological, management or ecological factors, for example, nutritionally-deficient soil or arthropod transmitters of infection. Epidemiological maps (see Chapter 4) are a valuable aid to identifying geographical associations. For example, mapping of the location of cattle fatalities in South Africa, linked to meteorological data, revealed that the fatalities were due to ingestion of grass contaminated with copper from a nearby copper mine (Gummow *et al.*, 1991: addressed more fully in Chapter 27).

Population

The type of animal that is affected often is of considerable importance. Hereford cattle are more susceptible to squamous cell carcinoma of the eye than other breeds, suggesting that the cause may be partly genetic. In many parts of the world, meat workers are affected more often by Q fever than are other people, implying a source of infection in meat-processing plants.

When the major facts have been established, alternative causal hypotheses can be formulated. An epidemiological investigation is similar to any detective novel that unfolds a list of 'suspects' (possible causal factors), some of which may be non-statistically associated with a disease, and some statistically associated with the disease, either causally or non-causally.

Methods of deriving a hypothesis

There are four major methods of arriving at a hypothesis:

1. method of difference;
2. method of agreement;
3. method of concomitant variations;
4. method of analogy.

Method of difference

The method of difference (Mill's canon 1) argues that, if the frequency of a disease is different in two different circumstances, and a factor is present in one circumstance but is absent from the other, then the factor may be suspected of being causal. For instance, Semmelweis noted a difference in mortality due to puerperal fever between two hospital clinics in 19th-century Vienna (see Chapter 1). After ruling out overcrowding and climate as possible causes of increased mortality (mortality was lower in the more crowded clinic; the climate was the same in both clinics), he concluded that contamination by noxious post-mortem material was the culprit because the clinic with the higher mortality was attended by medical students who had conducted post-mortem examinations, whereas the clinic with the lower mortality was attended by student midwives, who did not conduct autopsies.

Likewise, Wood (1978) noted an increased occurrence of stillbirths in pigs in one of three farrowing houses. The only difference between this house and the other two was a different type of burner on its gas heaters. A hypothesis was formulated: that the different type of burner caused the stillbirths. Subsequently, the burners were shown to be defective and producing large amounts of carbon monoxide; the carbon monoxide was assumed to cause the stillbirths. The occurrence of stillbirths decreased when the faulty burners were removed, thus supporting the hypothesis.

Similarly, bovine spongiform encephalopathy occurred to a different extent on the Channel Islands (Jersey and Guernsey), and meat and bone meal was used more frequently in feedstuffs on the island with the greater frequency of bovine spongiform encephalopathy (Wilesmith, 1993). This added credence to the hypothesis that the causal agent was transmitted in meat and bone meal in concentrate rations.

Mill considered that it is hard to fulfil the requirements for the method of difference in nature, but that it is philosophically a more resilient test than the method of agreement. A clear defect of a hypothesis based on the method of difference is that several different factors may be incriminated as possible causes. For example, coronary heart disease in humans may be ascribed to high levels of cholesterol (Chen *et al.*, 1991) and to an imbalance of $\omega 3/\omega 6$ fatty acids (Okuyama, 2007). Regional differences in bleaching of coral may be explained by regional variation in temperature and irradiance stress (Kleypas *et al.*, 2008), variation in tolerance of different regional coral species to increased temperature (Loya *et al.*, 2001), contrasting degrees of eutrophication (Szmant, 2002) and

Table 3.1 The relationship between frequency of milking and serological evidence of exposure to leptospirosis in dairy farm personnel in the Manawatu region of New Zealand.

Frequency of milking of cows by personnel	Serological leptospirosis		Total number of personnel	Percentage of personnel with serological leptospirosis
	Present	Absent		
9 times/week	61	116	177	34.5
1–8 times/week	4	11	15	26.7
Rarely or never	0	20	20	0.0

sedimentation (Wesseling *et al.*, 1999), and regional variation in adjustment to thermal stress (Berkelmans and van Oppen, 2006). In contrast, the marked occurrence of mannosidosis in Angus cattle (Jolly and Townsley, 1980), compared with the absence of this disease in other breeds, strongly suggests that a genetic factor is the cause. The value of a hypothesis generated by the method of difference therefore is reduced if many alternative hypotheses can be formulated.

Method of agreement

The method of agreement (Mill's canon 2) reasons that, if a factor is common to a number of different circumstances in which a disease is present, then the factor may be the cause of the disease²⁶. Thus, if a batch of meat and bone meal was associated with salmonellosis on widely different types of pig farms, and this was the only circumstance in common, then the causal hypothesis – the disease was caused by contamination of that batch – is strengthened.

A second example relates to bovine hyperkeratosis which was identified in cattle in the US (Schwabe *et al.*, 1977). The disease was called 'X disease' because initially the cause was unknown. It occurred in different circumstances:

- in cattle that were fed sliced bread;
- in calves that had been licking lubricating oil;
- in cattle that were in contact with wood preservative.

The bread-slicing machine was lubricated with a similar oil to that which had been licked by the calves. The lubricating oil and the wood preservative both contained chlorinated naphthalene. This chemical was common to the different circumstances and subsequently was shown to cause hyperkeratosis.

Method of concomitant variations

The method of concomitant variations (Mill's canon 5) involves a search for a factor, the frequency or strength

Table 3.2 The relationship between number of cigarettes smoked per day and deaths from lung cancer in British doctors, 1951–1961. (From Doll and Hill, 1964a.)

Cigarettes/day in 1951	Annual lung cancer death rate/1000 (1951–1961)
None	0.07
1–14	0.54
15–24	1.39
≥25	2.27

of which varies continuously with the frequency of the disease in different situations. Thus, the distance over which cattle are transported before slaughter appears to be related to the occurrence of bruises in their carcasses (Meischke *et al.*, 1974). Similarly, there appear to be relationships between the occurrence of squamous cell carcinoma of the skin of animals and the intensity of ultraviolet radiation, between the occurrence of bovine hypomagnesaemia and pasture levels of magnesium, and between infection of dairy personnel with leptospires and the frequency with which the personnel milk cows (*Table 3.1*). The classical medical epidemiological investigation of the association between smoking and lung cancer (Doll and Hill, 1964a,b) also illustrates this method of reasoning (*Table 3.2*): the number of deaths due to lung cancer is proportional to the number of cigarettes smoked per day²⁷.

Method of analogy

This method of reasoning involves comparison of the pattern of the disease under study with that of a disease that already is understood, because the cause of a disease that is understood may also be the cause of another poorly understood disease with a similar pattern. For example, some mammary tumours of mice

²⁶ For intrication of this method, see van Heuveln (2000).

²⁷ This seminal investigation eventually became a 50-year study (Doll *et al.*, 2004).

are known to be caused by a virus, therefore some mammary tumours of dogs may have a viral cause. The climatic conditions associated with outbreaks of Kikuyu grass poisoning of cattle may suggest a mycotoxin as the cause because the circumstance is similar to those circumstances present in other mycotoxicoses (Bourke, 2007). Bovine petechial fever, caused by *Cytoecetes ondiri*, is present in a limited area of Kenya (Snodgrass, 1974). The mode of transmission of this infectious agent is unknown. However, other members of the genus *Cytoecetes* are known to be transmitted by arthropods, and geographical limitation is a feature of arthropod-transmitted diseases. Therefore, using the method of analogy, it has been suggested that *C. ondiri* may be transmitted by arthropods.

Evidence by analogy is not, in the strictest sense, evidence of fact. It can point to probabilities, and can confirm conclusions that may be reached by other means; but it can be dangerously misleading (and was not considered sound enough by Mill to be included as a canon). A classical example is the inference, made by John Snow, that yellow fever was transmitted by sewage (Snow, 1855). He had already demonstrated that cholera was transmitted by sewage (see 'Dangers of statistical association' earlier in this chapter) and then observed that cholera and yellow fever were both associated with overcrowding. He then inferred that cholera and yellow fever had similar modes of transmission, whereas the latter actually is transmitted by an arthropod rather than by contaminated sewage.

Principles for establishing cause: Hill's criteria

The British medical statistician, Austin Bradford Hill, proposed several criteria for establishing a causal association (Hill, 1965), including:

- the time sequence of the events;
- the strength of the association;
- biological gradient;
- consistency;
- compatibility with existing knowledge.

Time sequence

Cause must precede effect. In a bacteriological survey, Millar and Francis (1974) found an increased occurrence of various infections in barren mares compared with others whose reproductive function was normal. However, unless the bacterial infections were present before the mares became infertile, it would be incorrect to infer that the bacterial infections caused infertility. The causal pathway may have been in the other direction: absence of normal reproductive cyclic

activity may allow previously harmless infections to flourish.

Strength of association

If a factor is causal, then there will be a strong positive statistical association between the factor and the disease.

Biological gradient

If a dose–response relationship can be found between a factor and a disease, the plausibility of a factor being causal is increased. This is the basis of reasoning by the method of concomitant variations. Examples have already been cited: frequency of milking in relation to leptospirosis (see *Table 3.1*), and smoking in relation to lung cancer (see *Table 3.2*).

Consistency

If an association exists in a number of different circumstances, then a causal relationship is probable. This is the basis of reasoning by the method of agreement. An example is bovine hyperkeratosis, mentioned earlier.

Compatibility with existing knowledge

It is more reasonable to infer that a factor causes a disease if a plausible biological mechanism has been identified than if such a mechanism is not known. Thus, smoking can be suggested as a likely cause of lung cancer because other chemical and environmental pollutants are known to have a carcinogenic effect on laboratory animals. Similarly, if a mycotoxin were present in animal foodstuffs, then it might be expected to produce characteristic liver damage. On the other hand, the survey of leptospirosis of dairy farmers, mentioned earlier, showed a positive association between wearing an apron and having a leptospiral titre. This finding was not compatible with either existing knowledge or common sense, and a factor that might have confounded the result was sought and found.

Hill also included specificity of response (a single cause leads to a single effect) and analogy in his set of criteria²⁸. However, the former is faulted (Susser, 1977): some causes are known to induce more than one syndrome (see *Figure 10.3d*), and, earlier in this chapter, the latter has been shown to be a suspect method of reasoning²⁹. However, application of

²⁸ Specificity of response, having its origins in Koch's postulates, had already been argued as being central to causal inference (Yerushalmy and Palmer, 1959), and was cited in the debate on the relationship between smoking and lung cancer (Berkson, 1960, 1962).

²⁹ For a summary of the extent to which John Snow's investigation of cholera fulfilled Hill's criteria, see Sergeant and Perkins (2015).

Hill's criteria has achieved notable successes (e.g., allowing the US Surgeon General's Advisory Committee to sense a causal link between smoking and lung cancer: Advisory Committee, 1964), although the philosophical origin of the criteria (particularly their relationship to the thinking of Hume and Mill) remains unclear (Thygesen *et al.*, 2005; Morabia, 2013).

A fuller discussion of reasoning and causal inference in general is given by Taylor (1967) and Pinto and Blair (1993), and, in the context of epidemiology, by Buck (1975), Weed (1986), Evans (1993) and Rothman and others (2008). The testing of hypotheses using observational studies is described in Chapter 15.

Further reading

- Broadbent, A. (2013) *Philosophy of Epidemiology*. Palgrave Macmillan, New York
- Carter, K.C. (2003) *The Rise of Causal Concepts of Disease*. Ashgate Publishing, Aldershot
- Cochran, G.M., Ewald, P.W. and Cochran, K.D. (2000) Infectious causation of disease: an evolutionary perspective. *Perspectives in Biology and Medicine*, **43**, 406–448
- Doll, R. (2002) Proof of causality: deduction from epidemiological observation. *Perspectives in Biology and Medicine*, **45**, 499–515
- Evans, A.S. (1993) *Causation and Disease: A Chronological Journey*. Plenum Publishing Corporation, New York
- Falkow, S. (1988) Molecular Koch's postulates applied to microbial pathogenicity. *Reviews of Infectious Diseases*, **10**, Suppl. 2, S274–S276. (*Modification of Koch's postulates to suit the genetic basis of microbial pathogenicity*)
- Fedak, K.M., Bernal, A., Capshaw, Z.A. and Gross, C. (2015) Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology. *Emerging Themes in Epidemiology*, **12**, 14; doi 10.1186/s12982-015-0037-4
- Franco, E.L., Correa, P., Santella, R.M., Wu, X., Goodman, S.N. *et al.* (2004) Role and limitations of epidemiology in establishing a causal association. *Seminars in Cancer Biology*, **14**, 413–426. (*A discussion of the limitations of epidemiological methods in identifying carcinogens*)
- Goodstein, D. (2011) How science works. In: *Reference Manual on Scientific Evidence*, 3rd edn. National Research Council, pp. 37–54. The National Academies Press, Washington DC. (*A concise, selective discussion of scientific method*)
- Greenland, S., Pearl, J. and Robins, J.M. (1999) Causal diagrams for epidemiologic research. *Epidemiology*, **11**, 313–320
- Howick, J. (2011) *The Philosophy of Evidence-Based Medicine*. Wiley Blackwell, Oxford. (*Includes a discussion of causal reasoning specifically in the context of evidence-based medicine*)
- Illari, P.M., Russo, F. and Williamson, J. (Eds) (2011) *Causality in the Sciences*. Oxford University Press, Oxford and New York
- Jeffreys, H. (1973) *Scientific Inference*, 3rd edn. Cambridge University Press, Cambridge
- Joffe, M. (2011) The gap between evidence discovery and actual causal relationships. *Preventive Medicine*, **53**, 246–249
- Kundi, M. (2006) Causality and the interpretation of epidemiologic evidence. *Environmental Health Perspectives*, **114**, 969–974
- Lower, G.M. (1983) Systematic epidemiologic theory: conceptual foundations and axiomatic elements. *Medical Hypotheses*, **11**, 195–215. (*A concise discussion of causality, and causal criteria*)
- Pearl, J. (2001) Causal inference in the health sciences: a conceptual introduction. *Health Services and Outcomes Research Methodology*, **2**, 189–220
- Rothman, K.J. and Greenland, S. (2005) Causation and causal inference in epidemiology. *American Journal of Public Health*, **95**, S144–S150
- Rothman, K.J., Greenland, S. and Lash, L.T. (2008) *Modern Epidemiology*, 3rd edn. Lippincott, Williams and Wilkins, Philadelphia
- Russo, F. and Williamson, J. (2011) Epistemic causality and evidence-based medicine. *History and Philosophy of the Life Sciences*, **33**, 563–581
- Schwartz, S., Susser, E. and Susser, M. (1999) A future for epidemiology? *Annual Review of Public Health*, **20**, 15–33. (*A critique of causal investigation*)
- Susser, M. (1973) *Causal Thinking in the Health Sciences. Concepts and Strategies of Epidemiology*. Oxford University Press, New York, London and Toronto
- Susser, M. (1977) Judgment and causal inference: criteria in epidemiologic studies. *American Journal of Epidemiology*, **105**, 1–15
- Sutter, M.C. (1996) Assigning causation in disease: beyond Koch's postulates. *Perspectives in Biology and Medicine*, **39**, 581–592
- Tarski, A. (1995) *Introduction to Logic and to the Methodology of Deductive Sciences*. Dover Publications, New York

- Taylor, R. (1967) Causation. In: *The Encyclopedia of Philosophy*, Vol. 2. Ed. Edwards, P., pp. 56–66. The Macmillan Company and The Free Press, New York
- Thrusfield, M. (2016) Etiology. In: *Diseases of Coral*. Eds Woodley, C.M., Downs, C.A., Bruckner, A.W., Porter, J.W. and Galloway, S.B., pp. 16–27. John Wiley and Sons, Hoboken. (*A general discussion of causality, including examples from diseases of corals*)
- Vineis, P. (2004) Causality in epidemiology. In: *A History of Epidemiologic Methods and Concepts*. Ed. Morabia, A., pp. 337–349. Birkhäuser, Basel

4

Describing disease occurrence

This chapter discusses the types of animal population that are encountered in veterinary medicine, and describes the methods of expressing the amount and temporal and spatial distribution of disease in these populations.

Some basic terms

Endemic occurrence

'Endemic' is used in two senses to describe:

1. the **usual frequency** of occurrence of a disease in a population;
2. the **constant presence** of a disease in a population.

Thus, the term implies a stable state; if a disease is well understood, then its endemic level is often *predictable*. The term endemic can be applied not only to overt disease but also to disease in the absence of clinical signs and to levels of circulating antibodies. Therefore, the exact context in which the term is used should always be defined. For example, laboratory mice kept under conventional systems of 'non-barrier maintenance' (i.e., with no special precautions being taken to prevent entry and spread of infection into the population) are invariably infected with the nematode *Syphacia obvelata*. Infection of 100% of the mice would be considered the usual level of occurrence; that is, the endemic level of infection. When a disease is continuously present to a high level, affecting all age groups equally, it is **hyper-endemic**. In contrast, the endemic level of actinobacillosis in a dairy herd is likely to be less than 1%.

Endemic is applied not only to infectious diseases but also to non-infectious ones: the veterinary meat hygienist is just as concerned with the endemic level of carcass bruising as is the veterinary practitioner with the endemic level of pneumonia in pigs.

When endemic disease is described, the affected population and its location should be specified. Thus, although bovine tuberculosis is endemic in badgers in south-west England, the infection apparently is not

endemic in all badger populations in the UK (Atkins and Robinson, 2013).

Epidemic occurrence

'Epidemic' originally was used only to describe a sudden, usually unpredictable, increase in the number of cases of an infectious disease in a population. In modern epidemiology, an epidemic is an occurrence of an infectious or non-infectious disease to a level *in excess of the expected (i.e., endemic) level*. Thus, infection with *S. obvelata* should be absent from specific-pathogen-free (SPF) mice kept under strict barrier conditions where precautions are taken to prevent entry and spread of infectious agents in the colony. If an infected mouse gained entry to the colony, the infection would be transmitted throughout the resident population and an epidemic of the nematode infection would occur. Such an infection in SPF mice colonies would be *unusually frequent*; that is, epidemic. Similarly, if cattle grazed on rough pasture, which could abrade their mouths, there might be an increase in the number of cases of actinobacillosis. Although only 2% of the animals might become infected, this would be an unusually high (epidemic) level compared with the endemic level of 1% in the herd. Thus, an epidemic need not involve a large number of individuals.

When an epidemic occurs, the population must have been subjected to one or more factors that were not present previously. In the example of the SPF mouse colony that became infected with *S. obvelata*, the factor was a breakdown in barrier maintenance and the entry of an infected mouse. In the case of the herd with actinobacillosis, the new factor was an increased consumption of vegetation that could cause buccal abrasions.

The popular conception of an epidemic frequently is an outbreak of disease that is noticed immediately. However, some epidemics may go undetected for some time after their occurrence. Thus, in London, in 1952, the deaths of 4000 people were associated

with a particularly severe smog (fog intensified by smoke). The deaths occurred at the same time as the Smithfield fat stock show (HMSO, 1954). Although an epidemic of severe respiratory disease in the cattle was recognized immediately, and was associated with the air pollution caused by the smog, the epidemic of human respiratory disease was not appreciated until statistics recording human deaths were published more than a year later.

In contrast, some epidemics may be exaggerated. An increased number of deaths in foxes occurred in the UK in the late 1950s. This apparent epidemic of a 'new' fatal disease received considerable publicity and every dead fox was assumed to have died from the disease. Subsequent laboratory analyses identified chlorinated hydrocarbon poisoning as the cause of the increased fox fatality, but only 40% of foxes submitted for post-mortem examination had died from the poisoning. The other 60% had died of endemic diseases that had not previously stimulated general interest (Blackmore, 1964). This example illustrates that the endemic level of disease in a population has to be known before an epidemic can be recognized.

Pandemic occurrence

A pandemic is a widespread epidemic that usually affects a large proportion of the population. Many countries may be affected. Pandemics of rinderpest (see *Table 1.1*), foot-and-mouth disease and African swine fever have been the cause of considerable financial loss. By the 1970s, rinderpest was found only in north-west Africa and the Indian subcontinent, but the disease became pandemic in Africa and the Middle East during the early 1980s (Sasaki, 1991), and became the target of a global eradication campaign (Wojciechowski, 1991), which has reached a successful climax (see *Table 1.4*). In the late 1970s, a pandemic of parvovirus infection occurred in dogs in many parts of the world (Carmichael and Binn, 1981). Serious human pandemics have included plague (the Black Death: see *Figure 1.2*), which spread throughout Europe in 1348¹, cholera in the 19th century (see

Figure 3.4), influenza soon after the First World War, and, currently, HIV/AIDS (particularly in Africa).

Sporadic occurrence

A sporadic outbreak of disease is one that occurs *irregularly and haphazardly*. This implies that appropriate circumstances have occurred locally, producing small localized outbreaks.

Foot-and-mouth disease is not endemic in the UK. A sporadic outbreak, thought to be associated with the importation of infected Argentine lamb that entered the animal food chain, occurred initially in Oswestry in October 1967 (Hugh-Jones, 1972). Unfortunately, this incident, and several subsequent ones associated with the imported lamb, resulted in an epidemic, which was not eliminated until the middle of 1968 (*Figure 4.1a*). Similarly, the more widespread epidemic of foot-and-mouth disease that occurred in the UK in 2001 (*Figure 4.1b*) began as a sporadic outbreak in a pig herd at Heddon-on-the-Wall in Northumbria (DEFRA, 2002b; Mansley *et al.*, 2003), and was most likely caused by contamination of swill by illegally imported meat. However, in both epidemics, the disease did not become endemic because of veterinary intervention. Conversely, in 1969, a single sporadic case of rabies occurred in a dog in the UK after it had completed the statutory six-month quarantine period (Haig, 1977). No other animal was infected and so this sporadic outbreak was confined to the original case.

Thus, 'sporadic' can indicate either a single case or a cluster of cases of a disease or infection (without obvious disease) that is not normally present in an area.

Infection with *Leptospira interrogans* serovar Pomona, is endemic in domestic pigs in New Zealand. The bacterium also is frequently the cause of sporadic epidemics of abortion in cattle. Infected cattle excrete the bacterium in their urine only for approximately three months. The bacterium therefore cannot usually be maintained, and so become endemic, in the herd. If a cow becomes infected with the bacterium by direct or indirect contact with pigs, this constitutes sporadic infection. This animal may now become a short-term source of infection to other pregnant cattle in the herd, and a sporadic epidemic of abortion, of three or four months' duration, is likely to occur. Post-infection leptospiral antibodies persist for many years in cattle, and sporadic infection with the bacterium is not uncommon;

¹ The pandemic eliminated nearly a third of the population, but population decline continued long after. In 1450, Europe's population was probably nearly half of what it had been in the early 14th century. Thus, agricultural labour became scarce, there were continent-wide crop and livestock deficiencies, labourers became more powerful, and landlords became weaker (Herlihy, 1997). Consequently, wealth was sought in commerce, and the great cities of Amsterdam, Florence, London, Paris, Vienna and Venice developed. In Italy, this produced the urban cultural climate in which *The Renaissance* could be born and flourish (Holmes, 1996) and, in literature, plague was responsible for the ubiquitous mediaeval

allegory, *The Dance of Death* (*Danse Macabré*) (Warren, 1931), illustrating that disease can have consequences far beyond the bounds of health. Plague continued to recur in Western Europe for nearly 500 years (Cohn, 2008), notably in London in 1665 (Bell, 2001), and outbreaks still occur in various parts of the world (Bertherat *et al.*, 2011).

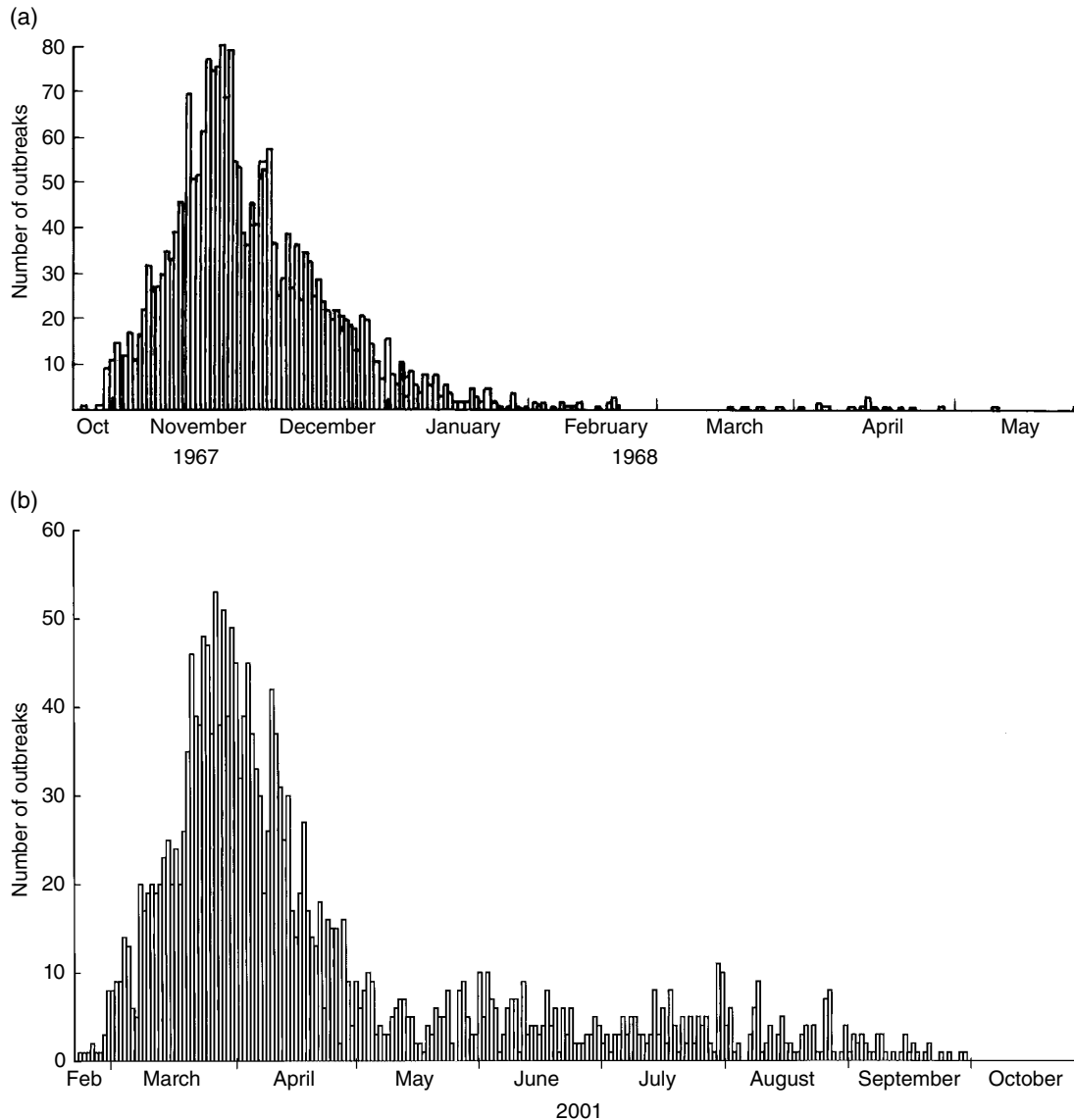


Fig. 4.1 Epidemics of foot-and-mouth disease in the UK: number of outbreaks per day: (a) 1967–1968; (b) 2001. ((a) Source: HMSO, 1969. (b) Source: DEFRA, 2002b. © Crown copyright; reproduced by kind permission of DEFRA.)

18% of New Zealand cattle have been reported to have detectable antibodies to this organism. Thus, although infection and the abortion that may ensue are *sporadic*, there is an *endemic* level of antibody in the bovine population (Hathaway, 1981).

Outbreaks

The *Office International des Epizooties/World Organisation for Animal Health* defines an outbreak as ‘*the occurrence of one or more cases in an epidemiological unit*’ (OIE, 2014), the term generally implying that several animals are affected. An **epidemiological unit** is a group of animals with a defined epidemiological relationship that share approximately the same likelihood

of exposure to a pathogen because they share a common environment (e.g., animals in a pen; fish in a pond), or because of common management practices that make it likely that a pathogen in one group of animals would quickly spread to other animals. Usually, an epidemiological unit is a herd or a flock. However, it also may refer to groups such as animals belonging to residents of a village, or animals sharing a communal animal-handling facility. The epidemiological relationship may differ from disease to disease, or even from strain to strain of pathogen.

Livestock in developed countries are usually kept as separated populations (see later in this chapter) and so ‘outbreak’ can be applied unambiguously to an

occurrence of disease on an individual farm. For example, the epidemic of foot-and-mouth disease in the UK in 2001 comprised 2030 infected premises (i.e., outbreaks), which all originated from a single infected pig farm. In contrast, the term sometimes also is used in the context of a single source, irrespective of the number or premises involved. Thus, in the US in 2002–2003, exotic Newcastle disease occurred in 21 commercial flocks in California, and over 1000 'backyard flocks', including some in the neighbouring states of Arizona and Nevada. This was documented as only one outbreak because it was considered to have arisen from a single introduction of the disease. Subsequently, a second outbreak, involving only one premises, was recorded in Texas (some distance from the first outbreak); this was determined to be a separate introduction, based on DNA-sequencing differences between the two virus strains that were isolated from each outbreak. Moreover, the definition of outbreak may include criteria other than the presence of clinical cases, and may be tailored to specific infections (*Table 4.1*).

In less-economically-developed countries, animal populations are frequently contiguous (see later in this chapter) and so it may be difficult to define the limits of one outbreak. An outbreak is then considered as occurring in part of a territory in which, taking local conditions into account, it cannot be guaranteed that both

susceptible and unsusceptible animals have not had direct contact with affected or susceptible animals. For example, in certain areas of Africa, an outbreak means the occurrence of disease within a sixteenth square degree; the occurrence is still referred to as an outbreak, even though the disease may occur in several places within the same sixteenth square degree. (Note that the area within a sixteenth square degree is not constant: it varies with latitude.)

Basic concepts of disease quantification

A necessary part of the investigation of disease in a population is the counting of affected animals so that the **amount** of disease can be described. Furthermore, it is usually desirable to describe **when** and **where** disease occurs, and to relate the number of diseased animals to the size of the **population at risk** of developing disease so that a disease's importance can be assessed. A report of 10 cases of infectious enteritis in a cattery, for example, does not indicate the true extent of the problem unless the report is considered in terms of the number of cats in the cattery: there may be only 10 cats present, in which case all of the cats are affected, or there may be 100 cats, in which case only a small proportion of the cats is affected.

Table 4.1 Criteria for declaring an outbreak of foot-and-mouth disease. (From European Commission, 2002.)

An outbreak shall be declared where a holding meets one or more of the following criteria:	
1.	foot-and-mouth disease virus has been isolated from an animal, any product derived from that animal, or its environment;
2.	clinical signs consistent with foot-and-mouth disease are observed in an animal of a susceptible species, and the virus antigen or virus ribonucleic acid (RNA) specific to one or more of the serotypes of foot-and-mouth disease virus has been detected and identified in samples collected from the animal or animals of the same epidemiological group;
3.	clinical signs consistent with foot-and-mouth disease are observed in an animal of a susceptible species and the animal or its cohorts are positive for antibody to foot-and-mouth disease virus structural or non-structural proteins, provided that previous vaccination, residual maternal antibodies or non-specific reactions can be excluded as possible causes of seropositivity;
4.	virus antigen or virus RNA specific to one or more of the serotypes of foot-and-mouth disease virus has been detected and identified in samples collected from animals of susceptible species and the animals are positive for antibody to foot-and-mouth disease virus structural or non-structural proteins, provided that in the case of antibodies to structural proteins previous vaccination, residual maternal antibodies or non-specific reactions can be excluded as possible causes of seropositivity;
5.	an epidemiological link has been established to a confirmed foot-and-mouth disease outbreak and at least one of the following conditions applies:
(a)	one or more animals are positive for antibody to foot-and-mouth disease virus structural or non-structural proteins, provided that previous vaccination, residual maternal antibodies or non-specific reactions can be excluded as possible causes of seropositivity;
(b)	virus antigen or virus RNA specific to one or more of the serotypes of foot-and-mouth disease virus has been detected and identified in samples collected from one or more animals of susceptible species;
(c)	serological evidence of active infection with foot-and-mouth disease by detection of seroconversion from negative to positive for antibody to foot-and-mouth disease virus structural or non-structural proteins has been established in one or more animals of susceptible species, and previous vaccination, residual maternal antibodies or non-specific reactions can be excluded as possible causes of seropositivity.

Where a previously seronegative status cannot be reasonably expected, this detection of seroconversion is to be carried out in paired samples collected from the same animals on two or more occasions at least five days apart, in the case of structural proteins, and at least 21 days apart, in the case of non-structural proteins.

The amount of disease is the **morbidity** (Latin: *morbus* = 'disease'); the number of deaths is the **mortality**. The times of occurrence of cases of a disease constitute its **temporal** distribution, whereas places of occurrence comprise its **spatial** distribution. The measurement and description of the size of populations and their characteristics constitute **demography**² (Greek: *demo*- = 'people'; *-graphia* = 'writing', 'description').

The structure of animal populations

The structure of populations influences the extent to which the size of the population at risk can be assessed, as well as affecting the ways in which disease occurs and persists in animals. The organization of animal populations can usually be described as either **contiguous** or **separated**.

Contiguous populations

A contiguous population is one in which there is much contact between individuals in the population and members of other populations. Contiguous populations therefore predispose to transfer and persistence of infectious diseases over large areas because of the inherent mixing and movement of animals.

Most human populations are contiguous because there is mixing of individuals by travel. Populations of small domestic animals also are usually contiguous. Dogs and cats that are not confined to houses move freely within cities, coming into contact with other urban, suburban and rural animals of their own and different species³. African nomadic tribes similarly own animals that comprise contiguous groups. Many wild animals belong to this category, too.

Assessing the size of contiguous populations

It is often difficult to assess the size of contiguous animal populations. Only limited routine demographic data about small domestic animals are available; for example, from Kennel Club registers (Tedor and Reif, 1978; Wong and Lee, 1985), and so it may be necessary to undertake specific demographic surveys (e.g., Gsell *et al.*, 2012). In some developed countries, dogs must be legally registered, but this is a difficult law to enforce and so many dogs may not be recorded. There

are pet registries that record and identify animals, for example, by ear or leg tattooing (Anon., 1984), but these records are voluntary and so exclude the majority of animals. An increased uptake of identification of companion animals by 'microchips', designed to an international standard (Anon., 1995; Ingwersen, 2000), should facilitate improved enumeration and tracing of these animals⁴. Over half a million animals were implanted with microchips in the UK within the 10 years up to 2000 (Anon., 2000) and, since 2012, all dogs in Northern Ireland (and, from 2016, in England and Wales) are required to have a microchip.

Some studies have been undertaken to establish the size and other characteristics of small-animal populations (Table 4.2). However, such animals frequently are kept in small numbers – often only one animal per household. It is therefore necessary to contact many owners to gain information about relatively few animals (i.e., the animal:owner ratio is low). This can be a difficult and costly exercise. The results also may be distorted by the lack of information on undetectable segments of the population such as stray, semi-domesticated and feral animals. Limited demographic data can be obtained from animal-cemetery records (Hayashidani *et al.*, 1988, 1989), animal shelters (New *et al.*, 2000), dog-rescue organizations (Stavisky *et al.*, 2012), veterinary clinics (O'Neill *et al.*, 2013) and information held by pet insurance companies (e.g., Bonnett *et al.*, 1997; Egenvall *et al.*, 2000; Sallander *et al.*, 2001; Dobson *et al.*, 2002; Bonnett and Egenvall, 2010; Inoue *et al.*, 2015a,b,c). Periodic commercial household surveys also are undertaken (e.g., the *Pet Food Manufacturers' Association's* regular surveys in the UK: PFMA, 2015; and the *American Pet Products Association's* biennial surveys in the US: APPA, 2015), but are frequently unrepresentative and may overestimate population sizes (Scarlett, 2008).

Non-thoroughbred horses and ponies kept as leisure animals similarly are difficult to count (Robin *et al.*, 2011). Periodic surveys of the characteristics of horse ownership may be undertaken (e.g., BETA, 2011) and information also may be available from insurance companies (e.g., Egenvall *et al.*, 2005). Enumeration of horse numbers also may be indirect. In the UK, for example, estimation of population size has been based on the number of farriers (McClintock, 1988), the amount of shoeing steel produced (*Horse and*

² A distinction between zoography and demography is not made in this book, for the reasons given in Chapter 2 in relation to epizootiology and epidemiology.

³ Such animals are classified as 'free-roaming' (Slater, 2001), a category that includes strays (i.e., recently lost or abandoned animals) as well as unrestrained, but owned, animals.

⁴ Microchips are an accurate means of identification of animals, provided they are properly implanted (Sorensen *et al.*, 1995; Fry and Green, 1999). The use of different standards in the US and Europe has hampered international identification of all 'chipped' animals (Fearon, 2004) but there is now a world-standard 15-digit microchip that meets ISO standards 11784/11785.

Table 4.2 Some demographic studies of dog and cat populations.

Country/region	Characteristics recorded	Source
Argentina: General Pico	Population size, age, mortality, fecundity	Larrieu <i>et al.</i> (1992)
Argentina: General San Martín	Population size, sex, age, owner characteristics	Agostini <i>et al.</i> (1986, 2000); Degregorio <i>et al.</i> (1995)
Australia	Population size	Baldock <i>et al.</i> (2003); ACAC (2010)
Australia: Indigenous communities	Population size, veterinary services	Burleigh <i>et al.</i> (2015)
Australia: Sydney	Sex, age, breed, reproductive status, husbandry	Toribio <i>et al.</i> (2009)
Bali	Type and characteristics of urban pet ownership*	Margawani and Robertson (1995)
Brazil: Curitiba	Household characteristics	Serafini <i>et al.</i> (2008)
Brazil: São Paulo	Population size	Dias <i>et al.</i> (2004)
Canada	Population size, age, veterinary services	Perrin (2009)
Canada: Ontario	Breed, age, owner characteristics [†]	Leslie <i>et al.</i> (1994)
Chile: Coquimbo	Population size and density, ownership patterns, sex, age, mortality, fecundity, survival, life expectancy	Acosta-Jamett <i>et al.</i> (2010)
Chile: El Bosque	Population size, sex, age	Ibarra <i>et al.</i> (1997)
Chile: Valdivia	Population size, sex, age, owner characteristics	Martin <i>et al.</i> (1977)
Chile: Viña del Mar	Population size, sex, age, fertility	Morales <i>et al.</i> (2009)
Cyprus	Population size	Pappaioanou <i>et al.</i> (1984)
Ecuador: Galápagos	Population size, sex	Diaz <i>et al.</i> (2016)
Europe	Population size (by country) For UK: regional distribution, owner characteristics	Anderson (1983)
India: Madurai	Population size	Subbaraj <i>et al.</i> (1992)
Ireland	Sex, age, source, owner characteristics	Downes <i>et al.</i> (2009)
Italy: Teramo	Sex, age, source, reproductive history	Slater <i>et al.</i> (2008)
Japan	Breed, sex, age, morbidity	Inoue <i>et al.</i> (2015a,b,c)
Kenya: Kisumu, Siaya	Population size	Muriuki <i>et al.</i> (2016)
Kenya: Machakos	Population size and density, breed, sex, age, management, mortality	Kitala <i>et al.</i> (1993)
Malaysia	Breed, sex, litter size, seasonal distribution of whelping	Wong and Lee (1985)
Mexico: Mexico City	Population size, sex, age, veterinary care, management	Rangel <i>et al.</i> (1981)
Mexico: Yucatan	Population size, sex, age, management, household characteristics	Ortega-Pacheco <i>et al.</i> (2007)
The Netherlands	Population size*	Lumeij <i>et al.</i> (1998)
Nigeria: Jos	Population size	Okoh (1988)
Norway	For Bernese mountain dog, boxer, Bichon frisé: population size, sex, age, age-specific mortality	Moe <i>et al.</i> (2001)
Philippines	Density of rural dogs	Robinson <i>et al.</i> (1996); Childs <i>et al.</i> (1998)
Sri Lanka: Mirigama	Population size	Matter <i>et al.</i> (2000)
Sweden	Breed-specific mortality, disease-specific morbidity, breed-specific survival	Bonnett <i>et al.</i> (1997); Bonnet and Egenvall (2010)
	Breed, sex, age, owner characteristics	Egenvall <i>et al.</i> (1999); Hedhammar <i>et al.</i> (1999); Sallander <i>et al.</i> (2001)

(Continued)

Table 4.2 (Continued)

Country/region	Characteristics recorded	Source
UK	Sex, age, diet	Fennell (1975)
	Breed	Edney and Smith (1986)
	Breed, sex, age	Thrusfield (1989)
	Owner and household characteristics	Murray <i>et al.</i> (2010a, 2015); Westgarth <i>et al.</i> (2010a,b)
US	Types and characteristics of pet ownership [‡]	AVMA (2012 [§]); APPA (2013 [¶])
US	Age, health status	Lund <i>et al.</i> (1999)
	Free-roaming populations	Levy <i>et al.</i> (2003)
US: Boston	Age at death, breed, sex	Bronson (1981, 1982)
US: California	Breed, sex, age, owner characteristics	Dorn <i>et al.</i> (1967)
	Breed, sex, age [¶]	Franti and Kraus (1974)
	Owner characteristics	Schneider and Vaida (1975)
	Breed, sex, age, owner characteristics	Franti <i>et al.</i> (1974, 1980)
	Population size	Schneider (1975)
US: Illinois	Sex, age, reproductive history, owner characteristics	Griffiths and Brenner (1977)
US: Indiana	Population size	Lengerich <i>et al.</i> (1992); Patronek <i>et al.</i> (1997)
	Sex, age, owner characteristics	Teclaw <i>et al.</i> (1992); Patronek <i>et al.</i> (1997)
US: Kansas	Age, population dynamics	Nasser and Mosier (1980, 1982)
US: Nevada	Age, population dynamics	Nasser <i>et al.</i> (1984)
US: New Jersey	Breed, sex, age	Cohen <i>et al.</i> (1959)
US: Ohio	Breed, sex, age, owner characteristics	Schnurrenberger <i>et al.</i> (1961)
Zambia	Population size, sex, age, household characteristics	De Balogh <i>et al.</i> (1993)
Zimbabwe	Population size, sex, age, owner characteristics	Brooks (1990); Butler and Bingham (2000)

* Also reports ownership of birds and exotic species.

† Owner characteristics vary between studies, and include age, occupation, location (urban versus rural), number and age of children, and type of dwelling.

‡ Also reports ownership of horses.

§ Published quinquennially.

¶ Published biennially.

Hound, 1992), aerial counts (Barr *et al.*, 1986) and surveys of private households (McClintock, 1988; *Horse and Hound*, 1992). These methods have shortcomings. The first counted farriers, assuming that each shod 250 horses, and ignored unshod horses; the second assumed an average of four new sets of shoes per horse per year, again ignoring unshod horses; the aerial survey omitted horses indoors; and the household surveys excluded some categories of animals such as those in riding centres. However, each produced similar figures (for thoroughbred and non-thoroughbred horses, combined), ranging from 500 000 to 560 000 animals, suggesting valid, but conservative, estimates. More recently, a mailed questionnaire has been used to estimate the population and demographics of horses in Scotland and northern England (Mellor *et al.*, 1999).

In the US, sample surveys of equine operations have provided information on the age and type of horse (USDA, 1998, 2006).

The requirement for horse 'passports' within the European Union (Sluyter, 2001; EUROPA, 2008; Stationery Office, 2009) – although primarily designed to protect the health of those who eat horsemeat by preventing horses, dosed with any medicines that are not intended for use in food-producing animals, from entering the human food chain – should lead to better characterization of equine populations. Such data have been used, with other data, to estimate the size and spatial distribution of, and movements within, the horse population of Great Britain (Boden *et al.* 2012, 2013).

Populations of wild animals can be enumerated either **directly** or **indirectly**. Direct methods involve

observation of individual animals, and include aerial counts (manned and unmanned: Reilly and van der Vyver, 2000; Walter and Hone, 2003; Vermeulen *et al.*, 2013) and ground counts (Seber, 1973; Norton-Griffiths, 1978; Southwood, 1978; Buckland *et al.*, 1993)⁵. A common method is **capture–release–recapture** in which animals are caught, marked and released. A second sample is then captured. The numbers of marked animals recaptured in the second sample is then related to the number initially marked (e.g., Pilcher, 2010). Rather than physical capture, this approach also has been applied using camera-traps to identify animals through photographs (Jackson *et al.*, 2006).

The simplest index for estimating the number of individuals (Lincoln, 1930) is:

$$\hat{N} = \frac{an}{r},$$

where:

$\hat{N}$ = estimated population size;

a = number of individuals initially marked;

n = number of individuals in the second sample;

r = number of marked individuals recaptured in the second sample.

Several software programs are available to calculate abundance (and other parameters) from capture–release–recapture studies (e.g., Rexstad and Burnham, 1991; White and Burnham, 1999), and the correlation between capture–release–recapture estimates and estimates based on observation of individual animals can be good (Fagerstone and Biggins, 1986).

Capture–release–recapture techniques also have been applied to estimating the size of dog populations (Beck, 1973; Anvik *et al.*, 1974; Heussner *et al.*, 1978; Matter *et al.*, 2000; Shimozako and de Barros Couto, 2010); and these and other marking techniques also can provide information on the movement, home range and territories of wild animals, which can be relevant to disease transmission (see Chapter 7). The marking of bait, for example, has facilitated the identification of badgers' territories that overlap with dairy farms in the south-west of England on which tuberculosis is present, indicating that infected badgers could be the source of infection on these farms (Cheeseman *et al.*, 1981).

The validity of this method depends on several assumptions such as the complete mixing of marked animals in the population; that the population is not increased by immigration and birth; that marked and unmarked animals are equally likely to be captured in each sample; and that marks are not lost or overlooked (Seber, 1973; Southwood, 1978).

An alternative approach, with fewer assumptions, is **distance sampling**. This involves measurement of perpendicular distances between a series of transverse lines and animals observed from them, and then computation of animal density using an appropriate statistical model (Buckland *et al.*, 2001). The main assumptions are that animals are distributed randomly (see Chapter 8); the probability (see Chapter 12) of detecting animals from the lines is one; and distances are measured exactly. The technique is used to assess the size of wild-bird populations (Newson *et al.*, 2008, 2013). Childs and others (1998) provide an example relating to rural dog populations, as well as an assessment of marking methods.

Direct methods can be inaccurate when animals live in concealed habitats. The size of deer populations in woodland, for example, may be underestimated by a factor of four when direct methods are used (Ratcliffe, 1987; Langbein, 1996). Indirect methods are then more appropriate. These semi-quantitative techniques include assessing the effect of grazing and browsing on trees and plants ('impact levels') (Mayle *et al.*, 1999), dung and nest surveys (Marques *et al.*, 2001; Laing *et al.*, 2003), latrine use (Tuytens *et al.*, 2001) and counting tracks (Dzieciolowski, 1976; Keeping and Pelletier, 2014).

Separated populations

Separated populations occur as discrete units such as herds and flocks. They are particularly common in countries that practice intensive animal production, with many animals on one farm (e.g., many of the more-economically-developed countries). Nevertheless, contact may still occur between separated populations, both directly (e.g., at boundaries, through animal escapes, use of common land) and indirectly (e.g., via movement of people, wildlife or vehicles and equipment: Brennan *et al.*, 2008). Table 4.3 illustrates the various sizes of these units in the UK; most animals of all species are kept in larger units.

A separated population can be **closed**, with no movement of animals into or out of the unit (except to slaughter). An example is a dairy herd that raises its own replacements, or is under statutory control

⁵ Each method may be subject to biased estimates of population size, notably by underestimation (Caughley and Sinclair, 1994; Jachmann, 2002; Lubow and Ransom, 2009).

Table 4.3 Holdings by size of herd or flock: UK, June, 2011 (Adapted from DEFRA, 2012b.)

Dairy breeding herds	<i>1– < 10</i>	<i>10– < 50</i>	<i>50– < 100</i>	<i>100– < 150</i>	<i>150 and over</i>		<i>Total</i>
No. of holdings	7 200	3 300	4 900	3 400	3 800		22 600
No. of cows	19 000	96 000	362 000	415 000	921 000		1 813 000
Beef breeding herds	<i>1– < 10</i>	<i>10– < 20</i>	<i>20– < 30</i>	<i>30– < 50</i>	<i>50– < 100</i>	<i>100 and over</i>	<i>Total</i>
No. of holdings	23 700	11 700	7 500	8 000	6 700	2 800	60 500
No. of cows	98 000	167 000	183 000	307 000	459 000	457 000	1 671 000
Total cattle and calves	<i>1– < 10</i>	<i>10– < 30</i>	<i>30– < 50</i>	<i>50– < 100</i>	<i>100– < 150</i>	<i>150 and over</i>	<i>Total</i>
No. of holdings	11 700	16 300	10 700	16 400	9 500	21 500	86 200
No. of cattle	55 000	306 000	418 000	1 850 000	1 169 000	6 780 000	9 914 000
Female pig breeding herds	<i>1–4</i>	<i>5–24</i>	<i>25–99</i>	<i>100 and over</i>			<i>Total</i>
No. of holdings	3 300	1 300	600	900			6 000
No. of pigs	7 000	14 000	32 000	379 000			432 000
Fattening pigs	<i>1– < 10</i>	<i>10– < 50</i>	<i>50– < 300</i>	<i>300– < 1000</i>	<i>1000 and over</i>		<i>Total</i>
No. of holdings	3 900	1 800	1 100	1 000	1 200		9 000
No. of pigs	15 000	40 000	142 000	591 000	3 130 000		3 917 000
Total pigs	<i>1– < 10</i>	<i>10– < 50</i>	<i>50– < 300</i>	<i>300– < 1000</i>	<i>1000 and over</i>		<i>Total</i>
No. of holdings	5 100	2 200	1 200	1 000	1 300		10 900
No. of pigs	18 000	49 000	142 000	602 000	3 613 000		4 441 000
Female sheep breeding flocks	<i>1– < 20</i>	<i>20– < 50</i>	<i>50– < 125</i>	<i>125– < 500</i>	<i>500– < 1000</i>	<i>1000 and over</i>	<i>Total</i>
No. of holdings	12 300	12 400	15 100	19 900	5 900	2 500	68 100
No. of sheep	122 000	408 000	1 242 000	5 139 000	4 096 000	3 862 000	14 868 000
Total sheep and lambs	<i>1– < 20</i>	<i>20– < 50</i>	<i>50– < 125</i>	<i>125– < 500</i>	<i>500– < 1000</i>	<i>1000 and over</i>	<i>Total</i>
No. of holdings	9 800	9 800	13 100	21 700	9 300	9 300	73 000
No. of sheep	85 000	330 000	1 083 000	5 782 000	6 617 000	17 736 000	31 634 000
Broilers	<i>1–9 999</i>	<i>10 000–99 999</i>	<i>100 000 and over</i>				<i>Total</i>
No. of holdings	1 400	600	400				2 400
No. of birds	560 000	29 414 000	72 487 000				102 461 000

of movement. Two extreme examples of closed populations are SPF and gnotobiotic colonies of laboratory animals.

A separated population also can be **open**, with limited movement of individuals in and out. Examples include beef herds where animals are brought in from other farms and markets for fattening, and dairy herds that receive replacements from other farms.

Separated populations, especially of the closed type, are less likely to be infected with agents from other areas than contiguous populations. However, if infection enters separated populations, it may spread rapidly because the animal density frequently is high.

Assessing the size of separated populations

It is often easier to obtain information on the size of a separated than a contiguous population. The large

numbers of animals kept under conditions of intensive husbandry in a single separated unit usually have only one owner (i.e., the animal:owner ratio is high). Many demographic data about food animals are available as a result of regular censuses and estimations (annually, in the UK, for example⁶). The most extensive sources are the *Animal Health Yearbook* of the *Food and Agriculture Organization of the United Nations* and *Office International des Epizooties*; animal population sizes in *Tables 1.8* and *1.10* were obtained from the first source.

⁶ The first census of livestock in England was undertaken as early as 1086, as part of the 'Domesday Survey'. Unfortunately, though, when the data were edited, they also were abridged by omission of the livestock numbers from the final document, the *Liber de Wintonia* (*Book of Winchester*), for most parts of the country (Morris, 1976).

Thoroughbred horses are members of separated (usually open) populations. They are concentrated in stables in major training areas and can be counted easily. Published figures, however, may not be comprehensive. Thoroughbred horses in training in the UK, for example, are recorded annually, but they comprise only a small and variable age group. Stud books provide information on stallions, mares and foals, but may be incomplete.

Hall (2011) describes a method for deducing the number of registered cattle, sheep, pig, goat and horse breeds from annual registrations.

Measures of disease occurrence

Prevalence

Prevalence, P , is the number of instances of disease or related attributes (e.g., infection or presence of antibodies) in a known population, at a designated time, without distinction between old and new cases. When the time is not specified, prevalence usually refers to **point prevalence**; that is, the amount of disease in a population at a particular point in time.

Period prevalence refers to the number of cases that are known to have occurred during a specified period of time; for example, a year (**annual prevalence**). It is the sum of the point prevalence at the beginning of the period and the number of new cases that occur during the period, and therefore can be used when the development of a condition is insidious, the exact time of its onset thus not being known (e.g., some behavioural conditions). **Lifetime prevalence** is the number of individuals known to have had disease for at least part of their life.

Although prevalence can be defined simply as the number of affected animals, it is most meaningful when expressed in terms of the number of diseased animals in relation to the number of animals in the population at risk of developing the disease:

$$P = \frac{\text{number of individuals having a disease at a particular point in time}}{\text{number of individuals in the population at risk at that point in time}}.$$

For example, if 20 cows in a herd of 200 cows were lame on a particular day, then the prevalence of lameness in the herd on that day would be 20/200, that is, 0.1. This is a **proportion** that represents the **probability** of an animal having a specified disease at a given time. Prevalence can take values between 0 and 1, and is dimensionless. Sometimes, it is expressed

as a percentage. Thus, a prevalence of 0.1 = 10%. Additionally, if a disease is rare, its prevalence may be expressed as:

$$\frac{\text{number of cases of disease}}{\text{population at risk}} \times 10^n,$$

where n is an integer depending on the rarity of the disease. Thus, prevalence may be expressed per 10 000 population at risk ($n = 4$) or per 1 000 000 population at risk ($n = 6$).

Incidence

Incidence is the number of new cases that occur in a known population over a specified period of time. The two essential components of an incidence value are:

1. the number of new cases;
2. the period of time over which the new cases occur.

Incidence, like prevalence, can be defined simply in terms of the number of affected animals, but again is usually expressed in relation to the population at risk.

Cumulative incidence

The **cumulative incidence, CI** (also termed **risk**), is the proportion of non-diseased individuals at the beginning of a period of study that becomes diseased during the period:

$$CI = \frac{\text{number of individuals that become diseased during a particular period}}{\text{number of healthy individuals in the population at the beginning of that period}}.$$

It is therefore a proportion that can take values between 0 and 1 (or 0–100%), and is dimensionless.

Thus, if 20 animals in a cattery develop feline viral rhinotracheitis during a week, and there are 100 healthy cats in the cattery at the beginning of the week, then, for the week:

$$CI = \frac{20}{100} = 0.2.$$

The longer the period of observation, the greater the risk. Thus, if 10 more cats developed the disease during a second week of observation, the cumulative incidence would be 0.3 for the two-week period.

If the cumulative incidence has been estimated for one time period, x , it can be extrapolated to other periods, y :

$$CI_y = 1 - (1 - CI_x)^{y/x},$$

assuming that the risk is constant (Martin *et al.*, 1987). For example, if the cumulative incidence in one year is 0.30, and the value in three years is required, then $x = 1$, $y = 3$, and:

$$\begin{aligned} CI &= 1 - (1 - 0.30)^{3/1} \\ &= 1 - 0.7^3 \\ &= 1 - 0.34 \\ &= 0.66. \end{aligned}$$

Cumulative incidence is an indication of the average risk of developing disease during a particular period, in both the individual and the population. It is usually calculated only for the first occurrence of a disease (rather than for multiple occurrences). When calculating cumulative incidence, additional animals at risk cannot be added to the initial number at risk during the period of observation. However, if non-diseased animals are removed from the population during this period, the denominator is modified by subtraction of half the value of the number removed (Kleinbaum *et al.*, 1982). Cumulative incidence also is used in dynamic populations if the period of risk is short and related to a specific event (e.g., dystokia at calving in dairy herds), because all relevant animals can then be observed for the entire risk period. It also is appropriate for sessile populations such as corals (Thrusfield, 2016b).

Incidence rate

The **incidence rate**, I , measures the rapidity with which new cases of disease develop over time:

$$I = \frac{\text{number of new cases of disease that occur in a population during a particular period of time}}{\text{the sum, over all individuals, of the length of time at risk of developing disease}}.$$

The denominator is measured as 'animal-time at risk'. This is the sum of the periods of observation for each animal during which the latter is free from the disease (i.e., is at risk). As soon as an animal becomes diseased, it no longer contributes to this value. For example, six cows, free from disease, observed for one year would constitute 'six animal-years at risk'; equally, one cow observed for six years also would constitute 'six animal-years at risk'. An example of calculation of incidence rate is given in Table 4.4. Note that the incidence rate has a dimension, **time**⁻¹; the incidence rate is calculated **per animal-week**, **per animal-year**, and so on. This unit

Table 4.4 Example of calculation of incidence rate: enzootic bovine leucosis (EBL) (hypothetical data).

Cow number	Period of observation	Time of development of EBL after beginning of observation	Contribution to animal-years at risk
1	7 years	No disease	7 years
2	7 years	No disease	7 years
3	4 years	4 years	4 years
4	5 years	No disease	5 years
5	6 years	No disease	6 years
6	8 years	No disease	8 years
7	5 years	5 years	5 years
8	2 years	No disease	2 years
9	9 years	No disease	9 years
10	5 years	No disease	5 years
<i>Total</i>			58 years

Calculation:

Total number of cases = 2.

Incidence rate = 2 per 58 animal-years at risk

= 3.5 per 100 animal-years at risk, i.e., 0.035 per animal-year at risk.

of time sometimes is termed the **internal time component**.

If the incidence rate has been estimated for one time period, x , it can be extrapolated to other periods, y :

$$I_y = I_x(y/x),$$

assuming that the rate is constant (Martin *et al.*, 1987). For example, if the incidence rate has been estimated as two cases per animal-year at risk, and the rate per animal-month is required, then $y = 1$ and $x = 12$ (1 year = 12 months), and:

$$\begin{aligned} I_y &= 2(1/12) \\ &= 0.17 \text{ per animal-month at risk.} \end{aligned}$$

Similarly, an incidence rate of two cases per animal-month at risk is equivalent to 24 cases per animal-year at risk.

The technique is based on the idea that the movement to the diseased state depends on:

- the size of the population;
- the period of observation;
- the 'force of morbidity'.

The force of morbidity (also termed the 'hazard rate' and 'instantaneous incidence rate') is a theoretical measure of the risk of occurrence of disease at a point in time, and it is this that is estimated by the incidence

rate. The measure accommodates movement into and out of the population (e.g., heifers being brought into, and cows leaving, a dairy herd) during an observed time period, and is calculated when the population is relatively dynamic and disease is not restricted to a particular period of time.

Frequently the exact period of observation of individual animals cannot be recorded, and so an exact incidence rate cannot be calculated. An approximate calculation is then:

$$I = \frac{\text{number of new cases of disease that occur in a population during a particular period of time}}{(\text{number at risk at the start of the time period} + \text{number at risk at the end of the time period})/2},$$

using a denominator that represents the average number at risk (Martin *et al.*, 1987).

Thus, if a herd with an average size of 70 cows was observed for a year, and pneumonia was reported in seven cows, the incidence rate is approximated by:

$$\begin{aligned} &7/70 \text{ per animal-year at risk} \\ &= 0.10 \text{ per animal-year at risk.} \end{aligned}$$

The exact and approximate values are equal when the exact average period of risk for diseased animals is half of the period of observation. For example, if five animals are observed for one year, and three develop disease:

$$\begin{aligned} &\text{one at day 72 (0.20 year),} \\ &\text{one at day 180 (0.50 year),} \\ &\text{one at day 290 (0.80 year);} \end{aligned}$$

using the exact denominator:

$$\begin{aligned} I &= 3/(2 + 0.20 + 0.50 + 0.80) \\ &= 3/3.50 = 0.86; \end{aligned}$$

using the approximate denominator:

$$\begin{aligned} I &= 3/\{(5 + 2)/2\} \\ &= 3/(7/2) = 0.86. \end{aligned}$$

The exact and approximate incidence rates are equal, because the exact average period of risk for the diseased animals is six months: $(0.20 + 0.50 + 0.80)/3$ years = 0.50 years.

In contrast, if five animals are observed, and three develop disease:

$$\begin{aligned} &\text{two at day 120 (0.33 year),} \\ &\text{one at day 240 (0.67 year);} \end{aligned}$$

using the exact denominator:

$$\begin{aligned} I &= 3/(2 + 0.33 + 0.33 + 0.67) \\ &= 3/3.33 \\ &= 0.90; \end{aligned}$$

and, using the approximate denominator, again $I = 0.86$.

The approximate denominator underestimates the exact rate because the exact average period of risk of diseased animals is less than six months: $(0.33 + 0.33 + 0.67)/3$ years = 0.44 years.

Finally, if five animals are observed, and three develop disease:

$$\begin{aligned} &\text{one at day 120 (0.33 year),} \\ &\text{two at day 240 (0.67 year);} \end{aligned}$$

using the exact denominator:

$$\begin{aligned} I &= 3/(2 + 0.33 + 0.67 + 0.67) \\ &= 3/3.67 \\ &= 0.82; \end{aligned}$$

and, using the approximate denominator, again $I = 0.86$.

The approximate denominator overestimates the exact rate because the exact average period of risk of diseased animals is more than six months: $(0.33 + 0.67 + 0.67)/3$ years = 0.57 years.

When calculating an incidence rate, the internal time component should be short enough to allow only one bout of illness in each animal (after which time the animal ceases to be at risk). Thus, when measuring the incidence rate for bovine mastitis, an appropriate time unit could be one month, in which case the rate would be expressed in terms of animal-months at risk. However, in practice, some diseases may recur within the specified time unit (e.g., injuries). If the disease is of short duration, and subsequent recovery (and immunity) is brief, affected individuals can continue to be included in the population at risk, without interruption (Kleinbaum *et al.*, 1982). Alternatively, recovery periods can be defined, in which circumstance animals re-enter the population at risk after expiry of the periods (Bendixen, 1987). For example, a bovine mastitis recovery period of 17 days (typically three days of treatment plus 14 days recovery) may be defined; mastitis recurring within this period being considered to be the same case, and mastitis recurring outside this period being judged to be a separate case (Petrovski *et al.*, 2009).

The incidence rate is sometimes called, more fully, the **true incidence rate**, the **person-time incidence**

rate (from human epidemiology) or the **incidence density** (Miettinen, 1976). It relates to the population, and cannot be interpreted at the level of the individual.

Cumulative incidence can be estimated from the incidence rate thus:

$$CI = 1 - e^{-I},$$

where e is the base of natural logarithms, 2.718 (see Appendix II). For example, if the incidence rate is 0.03 per year, for one year:

$$\begin{aligned} CI &= 1 - 2.718^{-0.03} \\ &= 1 - (1/2.718^{0.03}) \\ &= 1 - 0.97 \\ &= 0.03. \end{aligned}$$

Note that, in this example, the cumulative incidence and incidence rate are equal; this equality is roughly maintained when $I < 0.10$ (Kleinbaum *et al.*, 1982). (Recall that cumulative incidence relates disease to the number at risk at the beginning of the period of observation, whereas an incidence rate relates disease to the average population at risk during the period – two different denominators. The rate is higher because the number under observation at the start of the period is reduced as cases occur and are therefore removed from the population at risk.)

Conversion of incidence rate to cumulative incidence is useful for comparing incidence between different studies of a disease that use each of the two parameters. For example, in a study of urinary incontinence in neutered bitches in the UK (Thrusfield *et al.*, 1998), an incidence rate of 0.0174 per animal-year at risk was estimated. This was the equivalent of a 10-year cumulative incidence of approximately 0.16, which was not dissimilar from the 0.20 value documented in a Swiss study (Arnold *et al.*, 1989).

Incidence is discussed in detail by Kleinbaum and others (1982) and Bendixen (1987).

Attack rate

Sometimes a population may be at risk for only a limited period of time, either because exposure to a causal agent is brief, or because the risk of developing the disease is limited to a narrow age range such as the neonatal period. Examples of the first reason would be the feeding of a batch of food contaminated with a mycotoxin to a herd of cattle (e.g., Griffiths and Done, 1991) and exposure to radiation during nuclear accidents. Even if observations were made on the animals for a long time, the incidence would not change. In these circumstances, when the period of risk is brief, the

term **attack rate** is used to describe the proportion of animals that develop the disease.

A **secondary attack rate** also can be defined (Lilienfeld and Lilienfeld, 1980). This is the proportion of cases of a transmissible disease that develop as a result of contact with the primary case:

$$\begin{aligned} \text{Secondary attack rate} &= \frac{\text{number of individuals exposed to the primary case that develop disease within the range of the incubation period}}{\text{total number of individuals exposed to the primary case}}. \end{aligned}$$

(Cases occurring outside the range of the incubation period are usually the result of contact with secondary cases, and are therefore called tertiary cases.) If the incubation period of the disease is not known, the numerator can be expressed in terms of a specified time period. The secondary attack rate is usually applied to closed aggregates of individuals such as pens and stables (and, in human populations, families and households) and is a useful measure of contagiousness (see Chapter 6).

The relationship between prevalence and incidence rate

A disease with a long duration is more likely to be detected during a cross-sectional survey than is a disease of short duration. For example, chronic arthritis, lasting for several months, could be detected by a cross-sectional abattoir survey that was undertaken any time during the several months that the arthritis was present. However, clinical louping-ill, lasting for a few days, could be detected by a cross-sectional survey only if the survey was conducted during the short period that the disease was apparent.

Prevalence, P , therefore depends on the duration, D , and the incidence rate, I , of a disease:

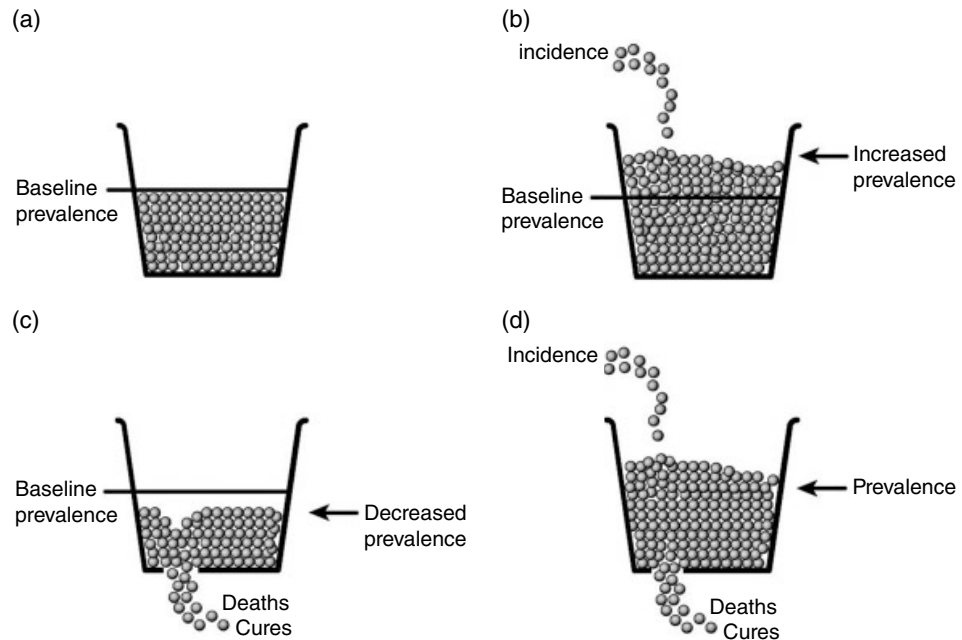
$$P \propto I \times D.$$

This means that a change in prevalence can be due to:

- a change in incidence rate;
- a change in the average duration of the disease;
- a change in both incidence rate and duration.

The relationship between prevalence and incidence rate can be likened to balls poured into a bucket (Figure 4.2). The bucket represents a population, and the current level of balls in the bucket is equivalent to prevalence (Figure 4.2a). The incidence rate is represented by balls poured into the bucket. If balls are poured into the bucket (representing the addition

Fig. 4.2 An analogy of the relationship between prevalence and incidence as beads poured into a bucket: (a) stable baseline prevalence; (b) increased prevalence by the addition of new cases; (c) decreased prevalence from either death or cure; (d) fluctuating prevalence resulting from the addition of new cases, and death or cure. (Adapted from Gordis, 2014.)



of new cases), the prevalence will increase (Figure 4.2b). If balls are lost through a hole in the bucket (representing removal of cases by either death or recovery), the prevalence will decrease (Figure 4.2c). Similarly, a decrease in duration of a disease (e.g., by improved therapy leading to accelerated recovery) also will decrease prevalence. However, an apparent paradox can occur if successful therapy decreases deaths and therefore prolongs life: prevalence can increase. For example, antibiotic treatment of acute bacterial pneumonia could decrease the fatality of the disease but also could increase the number of convalescent animals with chronic pneumonia. Most situations are dynamic, and the prevalence at a given time will depend on both incidence rate and the attrition rate of cases by either death or recovery (Figure 4.2d).

Calculation of incidence rate from prevalence

The exact relationship between prevalence and incidence rate is complex (Kleinbaum *et al.*, 1982), but a simple mathematical relationship can be derived under **steady-state** conditions (i.e., assuming a stable population and a constant incidence rate and prevalence):

$$P/(1-P) = I \times D.$$

When P is small (say, <10%), this simplifies to:

$$P = I \times D.$$

Therefore, if two components of the equation are known, the third can be calculated. For example, the annual incidence rate of occupationally-acquired

leptospirosis in New Zealand dairy farmers has been estimated (Blackmore and Schollum, 1983). Surveys had shown that 34% of farmers had serological reactions to leptospirosis (i.e., the prevalence of seropositive farmers was 0.34). Other work indicated that leptospiral titres of 1/24 or greater persist, on average, for 10 years (i.e., the duration of the infection, expressed as persistence of antibody at that titre, was 10 years). The number of notified cases of human leptospirosis had remained at approximately the same level for more than 10 years. Therefore, it could be assumed that a stable endemic level of disease existed in the human population. Thus:

$$P = 0.34,$$

$$D(\text{years}) = 10.$$

Rearranging the first equation above (recognizing that the prevalence, in this instance, is not small):

$$\begin{aligned} I &= \{P/(1-P)\}/D \\ &= \{0.34/(1-0.34)\}/10 \\ &= 0.052 \text{ (5.2\% per year).} \end{aligned}$$

This estimated annual incidence rate compares with a stable annual notification value of 1.1% in dairy farmers in a large dairy region of New Zealand. This suggests that, although individuals that seroconvert are likely to suffer clinical illness (Blackmore and Schollum, 1983), only approximately 20% are confirmed by medical diagnosis.

See Chapter 20 for a further discussion of seroconversion rates.

Application of prevalence and incidence values

Prevalence and incidence have different applications. Prevalence is useful if interest is focussed on existing cases; for example, in identifying disease problems for administrative purposes and for defining research priorities and long-term disease-control strategies, and in evaluating diagnostic tests (see Chapter 20). Cumulative incidence is used to predict an individual's change in health status because it indicates the probability of an individual becoming ill over a specified period of time. In contrast, an incidence rate cannot be directly interpreted at the individual level, but is appropriate when the speed of development of new cases in a population needs to be known.

Causal studies

Investigations of causal factors ideally require knowledge of incidence. This is because incidence values directly estimate the probability of developing disease during a specified period of time, and therefore permit the epidemiologist to determine whether the probability of developing disease differs in different populations or time periods in relation to suspected causal factors (Lilienfeld and Lilienfeld, 1980). The choice of the appropriate incidence measure (cumulative incidence versus incidence rate) in causal studies is discussed briefly in Chapter 15 and in detail by Kleinbaum and others (1982).

When attempting to identify causal associations, disease occurrence in the presence of a hypothesized causal factor is compared with disease occurrence in the absence of the factor. This can be conducted either by:

- comparing the *absolute* difference between values (e.g., the absolute difference between a cumulative incidence of 0.0010 over 10 years in a group 'exposed' to a factor, and of 0.0001 in an 'unexposed' group, is 0.0009); or
- comparing the *relative* difference between the two groups (0.0010/0.0001 = 10 in the above example).

A relative measure gives a clear indication of the **magnitude** of the difference, and is often applied in causal studies. This topic is discussed in detail in Chapter 15.

Mortality

Mortality measures are analogous to incidence measures, where the relevant outcome is death associated with, rather than new cases of, a specific disease.

Cumulative mortality

Cumulative mortality, *CM*, can be estimated in a similar way to cumulative incidence, but with the numerator comprising the number of deaths due to a particular disease over a specified period of time, and the denominator comprising the number of individuals at risk of dying during that period. Diseased animals present at the beginning of the period of observation are included in the denominator.

$$CM = \frac{\text{number of individuals that die during a particular period}}{\text{number of individuals in the population at the beginning of that period}}$$

Mortality rate

Mortality rate (mortality density), *M*, is calculated similarly to incidence rate. The numerator comprises the number of deaths. However, since an animal is at risk of dying *after* onset of disease, animals that develop disease continue to be included in the denominator until they die.

$$M = \frac{\text{number of deaths due to a disease that occur in a population during a particular period of time}}{\text{the sum, over all individuals, of the length of time at risk of dying}}$$

Death rate

The **death rate** is the total mortality rate for *all* diseases – rather than one specific disease – in a population. (Some authors do not distinguish between mortality rate and death rate. Thus, a disease-specific death rate may be encountered. Similarly, a crude mortality rate, referring to deaths from all causes, may be described.)

Case fatality

The tendency for a condition to cause the death of **affected** animals in a specified time is the **case fatality**, *CF*. This is the proportion of diseased animals that die:

$$CF = \frac{\text{number of deaths}}{\text{number of diseased animals}}$$

It measures the probability of death in diseased animals, is dimensionless, and can take values between 0 and 1 (or 0–100%).

The value of the case fatality depends on the time of observation, which can range from a brief period of hospitalization to several years. If the period of observation is long (e.g., in cases of chronic diseases such as cancer), it is more appropriate to quote **survival**.

Survival

Survival, S , is the probability of individuals with a specific disease remaining alive for a specified length of time.

$$S = \frac{N - D}{N},$$

where:

D = the number of deaths observed in a specified period of time;

N = the number of newly diagnosed cases under observation during the same period of time.

Survival is the complement of case fatality. Thus, for a given period of observation, the sum of the case fatality and survival should equal 1 (100%).

During observation, an animal may die, survive or be 'censored'. An animal is censored when follow-up ends before death or completion of the full period of observation (e.g., if an animal cannot be traced or the study is terminated)⁷.

One-year or two-year (and in humans, five-year or 10-year) survival times are often quoted for cancer cases. These times also provide a useful way of summarizing prognosis with, or without, treatment.

In domestic animals, survival depends not only on the characteristics of the disease but also on subjective factors, such as humane and economic reasons for euthanasia in pet animals and farm livestock, respectively. The effects of these factors can be removed when evaluating net effect of therapeutic procedures by including only animals that died from the disease or that were killed in its terminal stage.

Using the data in *Figure 4.3*, survival for at least one year can be estimated:

$$S = \frac{6}{7} = 0.86 \text{ (86\%)}.$$

However, two-year survival is more difficult to calculate because of the two censored observations (animals D and E). If these animals survived for at least two years, the two-year survival would again be 6/7 (0.86). However, if these two animals died within two years,

⁷ There are two types of censored observation resulting from an event not being observed on every subject. For example, if an aircraft-engine part is being tested, an experimenter may conduct the test on engine parts and decide to terminate observation at a pre-specified velocity. Thus, velocity disintegration is only known for those parts that break before the pre-specified velocity; this constitutes **Type I** censoring. Alternatively, the experimenter may decide observation is terminated when a given number of failures occurs, where this number is less than the number of parts observed; this is **Type II** censoring. In epidemiological studies, censoring is usually caused by time restrictions, and is therefore of Type I.

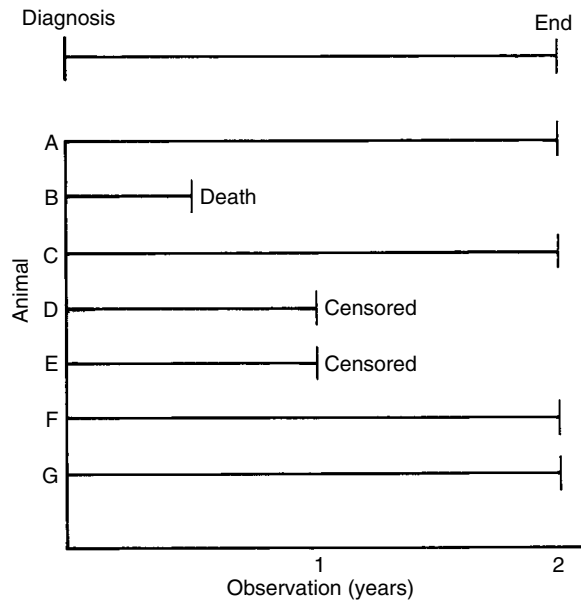


Fig. 4.3 Survival of seven diseased animals (hypothetical data).

the survival would be $4/7 = 0.57$. Statistical techniques, termed **survival analyses**, have been devised to deal with such incomplete data. A common method uses **life tables**.

Life tables were developed originally by demographers and actuaries to describe the lifetime of a theoretical population, based on the observed estimates from an actual population. They are, however, easily adapted to mortality and morbidity data.

The events being observed are divided into j discrete intervals of time, and, given a sample of N individuals, the cumulative probability of survival is calculated, using the following data for each interval (Lee, 1992):

- the number of survivors in the population at the beginning of the interval, n_j ;
- the number of censored observations during the interval (i.e., individuals lost to follow-up), c_j ;
- the population at risk during the interval $n_j - c_j/2^*$;
- the number of individuals in which death occurs during the interval, d_j ;
- the estimated probability of death occurring during the interval, $\hat{q}_j = d_j / (n_j - c_j/2)$;
- the estimated probability of death not occurring during the interval, $\hat{p}_j = 1 - \hat{q}_j$;
- the estimated cumulative probability of surviving all preceding intervals, $\hat{P}_j = \hat{p}_0 \times \hat{p}_1 \times \dots \times \hat{p}_{j-1} = \hat{P}_{j-1} \times \hat{p}_{j-1}$.

* Half the number of withdrawals, $c_j/2$, is applied on the assumption that the mean withdrawal time occurs at the midpoint of the time interval (Elandt-Johnson, 1977).

Table 4.5 Life table of 86 diabetic dogs. (Raw data supplied by Peter Graham and Andrew Nash, University of Glasgow Veterinary School.)

Time from diagnosis (months) (<i>j</i>)	No. survivors (<i>n_j</i>)	No. censored observations (<i>c_j</i>)	Population at risk (<i>n_j</i> – <i>c_j</i> /2)	No. dogs dying (<i>d_j</i>)	Estimated probability of death (<i>q_j</i>)	Estimated probability of survival (<i>p_j</i>)	Estimated cumulative probability of survival at beginning of interval (<i>P_j</i>)
0–1	86	1	85.5	5	0.058	0.942	1.000
>1–2	80	0	80.0	3	0.038	0.963	0.942
>2–3	77	0	77.0	2	0.026	0.974	0.907
>3–4	75	1	74.5	3	0.040	0.960	0.883
>4–5	71	1	70.5	3	0.043	0.957	0.847
>5–6	67	0	67.0	2	0.030	0.970	0.811
>6–12	65	3	63.5	5	0.079	0.921	0.787
>12–18	57	3	55.5	6	0.108	0.892	0.725
>18–24	48	7	44.5	6	0.135	0.965	0.647
>24–30	35	4	33.0	2	0.061	0.939	0.559
>30–36	29	5	26.5	2	0.075	0.925	0.525
>36–42	22	5	19.5	3	0.154	0.846	0.486
>42–48	14	5	11.5	3	0.261	0.739	0.411
>48–54	6	1	5.5	1	0.182	0.818	0.304
>54–60	4	2	3.0	0	0.000	1.000	0.249
>60	2	1	1.5	1	1.000*	0.000	0.083

* Logically, the probability of death beyond five years = 1.

Table 4.5 is a life table for 86 diabetic dogs presenting at a veterinary clinic.

Up to one month:

$$n_0 = 86$$

$$c_0 = 1$$

$$n_0 - c_0/2 = 86 - 0.5$$

$$= 85.5$$

$$d_0 = 5$$

$$\hat{q}_0 = d_0 / (n_0 - c_0/2)$$

$$= 5/85.5$$

$$= 0.058$$

$$\hat{p}_0 = 1 - \hat{q}_0$$

$$= 1 - 0.058$$

$$= 0.942.$$

$\hat{P}_0 = 1.000$ (all animals being alive at the beginning of the period of observation).

Thus, up to two months,

$$\hat{P}_1 = \hat{p}_0$$

$$= 0.942,$$

up to three months,

$$\hat{P}_2 = \hat{p}_0 \times \hat{p}_1$$

$$= 0.942 \times 0.963$$

$$= 0.907,$$

up to four months,

$$\hat{P}_3 = \hat{p}_0 \times \hat{p}_1 \times \hat{p}_2$$

$$= 0.942 \times 0.963 \times 0.974$$

$$= 0.883,$$

and so on.

The analysis reveals that diabetic dogs survive fairly well up to approximately 18 months after initial diagnosis (cumulative probability of survival = 0.725); survival then declines to below 25% five years after diagnosis.

Another common method of survival analysis is **Kaplan–Meier analysis** (Kaplan and Meier, 1958). Table 4.6 lists the one-year survival of cats with mammary carcinoma, after surgical removal of the tumour, estimated by the Kaplan–Meier technique, in relation to some prognostic factors (indicators). The results also can be presented graphically (Figure 4.4), and assist the clinician in determining prognosis. Thus,

Table 4.6 One-year survival of cats with mammary carcinoma according to some prognostic factors. (Based on Weijer and Hart, 1983.)

Prognostic factor	Number of cats	Survival
No tumour positive lymph nodes	107	0.58
Tumour positive lymph nodes	43	0.22
Diameter of primary tumour:		
1 cm	27	0.72
2 cm	31	0.41
3–5 cm	98	0.40
≥ 6 cm	44	0.23

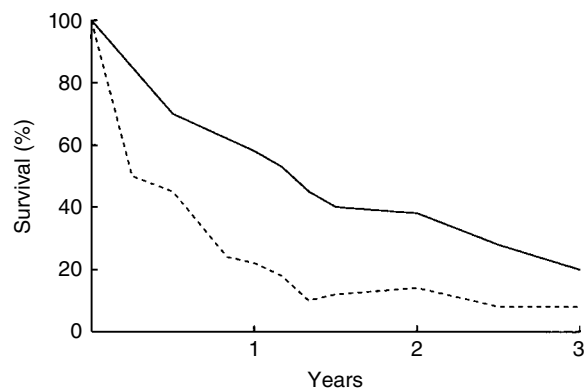


Fig. 4.4 Survival curve of cats with mammary carcinoma in relation to the presence of tumours in lymph nodes at initial diagnosis. The survival of cats was calculated at three-monthly intervals, and the points joined by straight lines. — no tumour-positive lymph nodes; - - - tumour-positive lymph nodes. (Adapted from Weijer and Hart, 1983.)

cats without secondary tumours in the lymph nodes when mammary cancer is diagnosed have a reasonable chance of surviving for one year (survival $\approx 60\%$), whereas cats with tumours in the lymph nodes have a much poorer prognosis (survival $\approx 20\%$).

Survival of animals is not comparable with survival of humans because animals are usually humanely destroyed, rather than being allowed to die naturally from conditions (Brodey and Abt, 1976; Fernandez-Mehler *et al.*, 2013). Moreover, the reasons for, and therefore the time of, euthanasia may vary widely for the same disease, and this may induce bias (see Chapter 10) in studies in which survival is the end-point (Belshaw and Dean, 2015). In such circumstances, it therefore may be appropriate to use other end-points, based on outcomes reported by owners; for example, quality of life (Wojciechowska and

Table 4.7 Some studies of the longevity of animals.

Species/breed, etc.	Source
Captive mammals	Comfort (1957); Weigl (2005)
Cat	Comfort (1955); Hayashidani <i>et al.</i> (1989)
Dog	Comfort (1960); Hayashidani <i>et al.</i> (1988); Michell (1999); Reid and Peterson (2000); Kraus <i>et al.</i> (2013); O'Neil <i>et al.</i> (2013); Inoue <i>et al.</i> (2015b)
Bernese mountain dog, boxer, Bichon frisé, Irish wolfhound	Moe <i>et al.</i> (2001)
Horse	King (1696); Comfort (1958)
Mammals	Flower (1931)
Poultry	Pease (1947)
Sheep	Carter (1939)

Hewson, 2005; Niessen *et al.*, 2010)⁸ or pain and mobility (Brown *et al.*, 2013; Gruen *et al.*, 2015).

Other measures of survival are also used in cancer studies (Misdorp, 1987). Thus, **tumour-free (disease-free) survival** can be estimated; however, this requires careful periodic examination for local recurrence or metastases. The success of local treatment can be expressed in terms of the **percentage recurrence** or **non-recurrence**. Systemic treatment (e.g., chemotherapy) can be assessed by the **percentage of complete or partial remission**.

Survival analysis also can be applied to periods of time during which animals are free from disease, and to studying longevity in healthy animals (Chiang, 1984); some examples are listed in Table 4.7. Methods for survival analysis are discussed in more detail in Dohoo and others (2009) and Kleinbaum and Klein (2012).

Example of calculation of prevalence, incidence, mortality, case fatality and survival

Suppose a veterinarian investigates a disease that runs a clinical course ending in either recovery or death, in a herd of cattle. On 1 July 2015 the herd is investigated

⁸ The definition of quality of life in animals is varied, and includes a balance between pleasant and unpleasant feelings (subjective and objective): a definition not dissimilar from that of animal welfare (e.g., Duncan, 1996; Clark *et al.*, 1997; Spruijt *et al.*, 2001). For reviews of the subject, see McMillan (2000) and Belshaw and others (2015).

when the disease is already present. The herd is then observed for the following year, during which period the herd size remains the same and all animals are followed up (i.e., there are no censored observations).

Total herd size on 1 July 2015: 600

Total number clinically ill on 1 July 2015: 20

Total number developing clinical disease between 1 July 2015 and 1 July 2016: 80

Total number dying from the disease from 1 July 2015 to 1 July 2016: 30

Prevalence on 1 July 2015 = $20/600 = 0.03$

Cumulative incidence 1 July 2015–1 July 2016 = $80/580 = 0.14$

Incidence rate = $80/\{(580 + 500)/2\}$
= 0.15 per animal-year at risk

Cumulative mortality 1 July 2015–1 July 2016 = $30/600 = 0.05$

Mortality rate = $30/\{(600 + 570)/2\}$
= 0.05 per animal-year at risk

Case fatality 1 July 2015–1 July 2016 = $30/100 = 0.30$

Survival 1 July 2015–1 July 2016 = $70/100 = 0.70$

(Note that in this calculation approximate incidence and mortality rates are calculated using the total population of susceptible animals as the denominator. Calculation of a true incidence rate and mortality rate would require observation of each animal so that the time of onset of disease, and occurrence of death, respectively, in each could be noted, and the number of animal-months at risk therefore recorded, as in *Table 4.4*.)

Ratios, proportions and rates

The preceding parts of this chapter have introduced some descriptive measures of disease occurrence as proportions and rates. These two terms and a third – ratios – have been widely and sometimes improperly used in veterinary and human medicine, and therefore require further discussion.

A ratio is a value obtained by dividing one quantity by another. For example, a male:female sex ratio might be 3:2, the upper figure being the **numerator** and the lower figure the **denominator**. A proportion is a special case of a ratio in which the numerator consists of some of (i.e., is a subset of) the individuals in the denominator. Thus, prevalence, cumulative incidence, case fatality and survival are proportions. In epidemiology, the term ratio is usually confined to measures where the numerator is *not* drawn from the denominator. For example:

$$\text{foetal death ratio} = \frac{\text{number of foetal deaths}}{\text{number of live births}}.$$

A rate is a ratio that expresses a change in one quantity (the numerator) with respect to another quantity (the denominator). ‘Time’ is usually included in the denominator. Velocity (e.g., 10 m per second) is a rate. True epidemiological rates have time as part of the denominator. Incidence rate is the commonest epidemiological rate.

However, ‘rate’ sometimes has been suffixed incorrectly to epidemiological ratios in which the numerator is a sub-set of the denominator (i.e., proportions). Thus, ‘prevalence rate’, ‘cumulative incidence rate’, ‘case fatality rate’ and ‘survival rate’ sometimes are used as synonyms for prevalence, cumulative incidence, case fatality and survival, respectively, although they are not true rates.

Table 4.8 lists some common epidemiological ratios and rates. In most instances, the rates are proportions, rather than true rates, but the terms are so well established that they are retained in this table.

Proportions and rates are expressed in three main forms: **crude**, **specific** and **adjusted**.

Crude measures

Crude prevalence, incidence and mortality values are an expression of the amount of disease and deaths in a population as a whole; they take no account of the structure of the population affected. For instance, the crude death rate of two laboratory colonies of mice could be 10/1000/day and 20/1000/day, respectively. Initially this might suggest that the second colony has twice the disease problem of the first, but this difference in crude rates might be due only to a difference in age structure. Mice have a lifespan of about two years and so if the second colony consisted of much older animals than the first, greater mortality would be expected in the second than the first colony, even without concurrent disease. Although crude rates may express the prevalence or incidence of a particular disease, they take no account of specific host characteristics, such as age, which can have a profound effect on the occurrence of disease in a population.

Specific measures

Specific measures of disease are those that describe disease occurrence in specific categories of the population related to host attributes such as age, sex, breed and method of husbandry, and, in man, also race, occupation and socio-economic group. They convey more information than crude measures on the pattern of disease. They can indicate categories of animal that are particularly at risk of disease, and therefore can provide evidence on its cause.

Specific measures are calculated in a similar manner to crude ones, except that the numerator and

Table 4.8 Some commonly used rates and ratios (indices usually refer to a defined population of animals observed for one year)*. (Modified and expanded from Schwabe *et al.*, 1977.)

Name	Definition
Rates	
No-return rate at n days:	$\frac{\text{No. animals bred that have not come back in heat in } n \text{ days after breeding}}{\text{No. animals bred}} \times 10^a$
Pregnancy rate at n days:	$\frac{\text{No. animals pregnant at } n \text{ days after breeding}}{\text{No. animals bred}} \times 10^a$
Crude live birth rate:	$\frac{\text{No. live births occurring}}{\text{Average population}} \times 10^a$
General fertility rate:	$\frac{\text{No. live births occurring}}{\text{Average no. female animals of reproductive age}} \times 10^a$
Crude death rate:	$\frac{\text{No. deaths occurring}}{\text{Average population}} \times 10^a$
Age-specific death rate:	$\frac{\text{No. deaths among animals in a specified age group}}{\text{Average no. in the specified age group}} \times 10^a$
Calf (lamb, piglet, puppy, etc.) mortality rate:	$\frac{\text{No. deaths under a specified age}^\dagger}{\text{No. live births}} \times 10^a$
Neonatal (calf, lamb, etc.) mortality rate:	$\frac{\text{No. deaths under a specified age}^\dagger}{\text{No. live births}} \times 10^a$
Foetal death rate (also called stillbirth rate):	$\frac{\text{No. foetal deaths}}{\text{No. live births plus foetal deaths}} \times 10^a$
Cause-specific death rate:	$\frac{\text{No. deaths from a specified cause}}{\text{Average population}} \times 10^a$
Proportional morbidity rate:	$\frac{\text{No. of animals with a specified disease}}{\text{Total no. of diseased animals}} \times 10^a$
Proportional mortality rate:	$\frac{\text{No. deaths from a specified cause}}{\text{Total no. deaths}} \times 10^a$
Ratios	
Foetal death ratio (also called stillbirth ratio):	$\frac{\text{No. foetal deaths}}{\text{No. live births}} \times 10^a$
Maternal mortality ratio:	$\frac{\text{No. deaths in dams from puerperal causes}}{\text{No. live births}} \times 10^a$
Zoonosis incidence ratio (ZIR):	$\frac{\text{No. new cases of a zoonotic disease in an animal species in a given geographical area in a stated time period}}{\text{Average human population in the same area during the same period} \times \text{time}} \times 10^a$
Area incidence ratio (AIR):	$\frac{\text{No. new cases of a disease in a given time period}}{\text{Unit geographical area in which the observations are made} \times \text{time}} \times 10^a$

* All rates could use other specified time periods.

[†] a = a whole number, usually between 2 and 6; for example, if $a = 3$, then $10^a = 10^3 = 1000$.

[‡] There is not a universal agreement on the age at which animals cease to be neonates in veterinary medicine.

denominator apply to one or more categories of a population with specific host attributes. For instance, a whole series of different age-specific incidence rates could be calculated that would cover the entire life-span of a particular population.

Age-specific incidence rates of many enteric diseases, such as salmonellosis and colibacillosis, can be higher in young than old animals. Sex-specific

incidence rates of diabetes mellitus are higher in females than in males. The breed-specific prevalence of squamous cell carcinoma of the eye in cattle reveals that Herefords have the highest prevalence: although there is a crude prevalence of 93/100 000 for all breeds of cattle, the specific prevalence for Herefords and Hereford crosses is 403/100 000. The prevalence of goitre in budgerigars is higher in pet birds than in

Table 4.9 Specific incidence rates of testicular neoplasia in cryptorchid dogs. (From Reif *et al.*, 1979.)

(a) Age-specific rates				
Age (years)	No. of dogs	Dog-years at risk	No. of neoplasms	Age-specific rate/1000 dog-years
≤2	262	411.3	0	0.00
2–3	153	288.8	0	0.00
4–5	93	199.4	0	0.00
6–7	49	103.0	7	67.96
8–9	31	59.2	4	67.57
≥10	21	43.3	3	69.28
Totals	609	1105.0	14	12.67

(b) Rates specific to location				
Location of testicles	No. of dogs	Dog-years at risk	No. of neoplasms	Incidence/1000 dog-years
Bilateral scrotal (controls)	329	680.0	0	0.00
Abdominal–scrotal	210	392.8	5	12.73
Inguinal–scrotal	188	372.2	9	24.18
Bilateral abdominal	54	96.8	0	0.00
Bilateral inguinal	27	52.5	0	0.00
Inguinal–abdominal	16	30.5	0	0.00
Cryptorchid unknown	114	160.2	0	0.00

breeders' birds (Blackmore, 1963). Similarly, the occupation-specific prevalence values of human Q fever in Australia indicate that meat workers are the group at greatest risk (Scott, 1981).

Table 4.9a illustrates that the crude incidence rate of testicular neoplasia in castrated dogs (12.67 per 1000 dog-years at risk) reveals much less about the period at risk in a dog's life than the age-specific values. Likewise, the rates specific to location (*Table 4.9b*) indicate at which sites the lesion is most likely to occur.

Calculation of age-specific incidence values has given further clues to the cause of bovine spongiform encephalopathy (see Chapters 2 and 3). The mode (most frequent value) of the disease's incubation period is four to five years, and very few cases occur

in two-year-old animals. A statutory ban on ruminant-derived protein (meat and bone meal), the suspected source of the infection, was introduced in July 1988. Its impact therefore would be detected initially in two-year-old animals two years later. None of the two-year-old animals affected then had been born after the ban (Wilesmith and Ryan, 1992). This finding added credence to the hypothesis that meat and bone meal was the source of the infection.

Adjusted (standardized) measures

Crude values can only be used to make *comparisons* between two different populations if the populations are similar with respect to all characteristics that might affect disease occurrence. If the populations are dissimilar with respect to such characteristics, erroneous conclusions might be drawn because these characteristics may act as confounders (see Chapters 3 and 15).

For example, *Table 4.10a* presents the results of a survey of the prevalence of leptospiral antibodies (seroprevalence) in samples of unvaccinated dogs taken in two cities, Glasgow and Edinburgh. The crude seroprevalence in Glasgow is 3% higher than in Edinburgh (0.27 compared with 0.24). Assuming that the rate of antibody decline (i.e., the duration of seropositivity) is constant, this might suggest that the incidence of leptospiral infection is greater in Glasgow than in Edinburgh (reasons might include a larger population of infected feral animals in the former than the latter city).

However, factors other than location might also affect seroprevalence. Male dogs, for instance, may run a higher risk of infection because of sexual behaviour, particularly the licking of the vulva of females (Dunbar, 1979), which is likely to bring them into contact with infected urine. Other studies have already shown that leptospiral seroprevalence is higher in male dogs than in bitches (Stuart, 1946; Cunningham *et al.*, 1957; Arimitsu *et al.*, 1989).

The confounding effect of sex can be removed by adjustment (standardization) by direct and indirect methods. This involves adjusting the crude values to reflect the values that would be expected if the potentially confounding characteristics were similarly distributed in the two study populations.

Direct adjustment In direct adjustment, the specific values in each population are required. The sex-specific seroprevalence values for the data in *Table 4.10a* are listed in *Table 4.10b*. Note that the seroprevalence in males is higher than that in females. Note, too, that the crude values are the **weighted** average of the specific values, with the number of males and females used as the weights:

Table 4.10 Numbers and proportions (seroprevalence) of urban dogs with microscopic agglutination test antibody titres $\geq 1:10$ to leptospires, Edinburgh and Glasgow, 1986–1988. (Based on van den Broek *et al.*, 1991.)

(a) Crude seroprevalence								
	<i>No. of dogs with positive titres</i>			<i>No. of dogs sampled</i>			<i>Seroprevalence</i>	
Edinburgh	61			260			0.24	
Glasgow	69			251			0.27	
Totals	130			511				

(b) Sex-specific seroprevalence								
	<i>No. of dogs with positive titres</i>			<i>No. of dogs sampled</i>			<i>Seroprevalence</i>	
	<i>Male</i>	<i>Female</i>	<i>Totals</i>	<i>Male</i>	<i>Female</i>	<i>Totals</i>	<i>Male</i>	<i>Female</i>
Edinburgh	15	46	61	48	212	260	0.31	0.22
Glasgow	53	16	69	180	71	251	0.29	0.23
Totals	68	60	130	228	283	511		

Edinburgh:

$$\text{crude seroprevalence} = \frac{(0.31 \times 48) + (0.22 \times 212)}{(48 + 212)} = 0.24;$$

Glasgow:

$$\text{crude seroprevalence} = \frac{(0.29 \times 180) + (0.23 \times 71)}{(180 + 71)} = 0.27.$$

To directly adjust these values, a **standard** population is selected, in which the frequency of the characteristic (sex, in this example) is known. Its choice is somewhat arbitrary, but it should be realistic and relevant. Thus, it may be a large population obtained from published demographic data, one of the two groups being compared, or the totals of the two groups. In this example, the totals of the two groups are used, that is, 228 males and 283 females.

The specific values in each group are now weighted by the frequency of the characteristic in the standard population:

$$\text{direct adjusted value} = sr_1 \times \frac{S_1}{N} + sr_2 \times \frac{S_2}{N},$$

where:

sr = specific value in study population;

S = frequency of characteristic in the standard population;

N = total number in the standard population ($S_1 + S_2 = N$).

For Edinburgh:

$$sr_1 \text{ (male)} = 0.31$$

$$S_1 = 228$$

$$sr_2 \text{ (female)} = 0.22$$

$$S_2 = 283$$

$$N = 511.$$

$$\begin{aligned} \text{Adjusted value} &= \{0.31 \times (228/511)\} + \\ &\quad \{0.22 \times (283/511)\} \\ &= 0.138 + 0.122 \\ &= 0.26. \end{aligned}$$

For Glasgow:

$$sr_1 \text{ (male)} = 0.29$$

$$S_1 = 228$$

$$sr_2 \text{ (female)} = 0.23$$

$$S_2 = 283$$

$$N = 511.$$

$$\begin{aligned} \text{Adjusted value} &= \{0.29 \times (228/511)\} + \\ &\quad \{0.23 \times (283/511)\} \\ &= 0.129 + 0.127 \\ &= 0.26. \end{aligned}$$

The adjusted values are similar. This suggests that sex is the factor responsible for the difference in seroprevalence between Edinburgh and Glasgow. If other characteristics (e.g., location or age) were responsible for the difference, the sex-adjusted values would be different.

In veterinary medicine, direct adjustment is indicated for age, sex, breed and other factors that may be confounders. However, the specific data are the 'facts'; the combination of specific values into a weighted average is merely a useful way of combining the values into a single index for the purpose of comparison of groups.

Indirect adjustment If specific values for one or both populations are not available, or the numbers with each characteristic are so small that large fluctuations in morbidity or mortality occur through the presence or absence of a few cases, the indirect method of adjustment can be used.

In indirect adjustment, only crude values are required for the two study populations, but the frequencies of the adjusting characteristic in these populations must be known. Additionally, specific values are required for the standard population. First, calculate the overall value *expected* in each population if the standard population's specific values applied:

$$\text{expected value} = Sr_1 \times \frac{s_1}{n} + Sr_2 \times \frac{s_2}{n},$$

where:

Sr = specific value in standard population;

s = frequency of characteristic in the study population;

n = total number in the study population ($s_1 + s_2 = n$).

In each population being compared, the ratio of the *observed* crude value to the *expected* value is termed the **standardized morbidity ratio** (or **standardized mortality ratio**, **SMR**, when mortality is being indirectly adjusted). These standardized ratios provide relative comparisons between two populations. They are multiplied by the crude value in the standard population to give the indirectly adjusted values for each population.

Age-adjusted SMRs, expressed as percentages, are commonly used in human medicine to relate mortality in particular occupations to the mortality experience of the general population (HMSO, 1958). The adjustment is made in five-year blocks for adults between 20 and 64 years of age.

The advantages, disadvantages and pitfalls associated with adjustment are discussed in detail by Fleiss and others (2003) and Kahn and Sempos (1989).

Mapping

A common method of displaying the geographical (spatial) distribution of disease and related factors is by drawing maps (cartography). This is of value not

only in the recording of areas where diseases exist but also in investigating the mode and direction of transmission of infectious diseases. For example, the spatial distribution of cases of foot-and-mouth disease during the British outbreak in 1967 suggested that the infection may have been disseminated by wind (see Chapter 6 and Smith and Hugh-Jones, 1969). Subsequent investigations supported this idea (Hugh-Jones and Wright, 1970; Sellers and Gloster, 1980).

Maps also can suggest possible causes of diseases of unknown aetiology. Mapping indicated that tumours (notably of the jaw) in sheep in Yorkshire clustered in areas where bracken was common (McCrea and Head, 1978). This led to the hypothesis that bracken causes tumours. Subsequently, the hypothesis was supported by experimental investigation (McCrea and Head, 1981). Similarly, comparison of the maps of hypocupraemia in cattle (Leech *et al.*, 1982) with a geochemical atlas (Webb *et al.*, 1978) has indicated areas in England and Wales where bovine copper deficiency may be caused by excess dietary molybdenum.

At their simplest, maps may be qualitative, indicating location without specifying the amount of disease. They also can be quantitative, displaying the number of cases of disease (the numerator in proportions, rates and ratios), the population at risk (the denominator), and prevalence and incidence (i.e., including both numerator and denominator).

Map bases

Maps can be constructed according to the shape of a country or region, in which case they are drawn to a **geographic base**. Alternatively, they can be drawn to represent the size of the population concerned, rather than land area; that is, to a **demographic (iso-demographic)** base (Forster, 1966), in which morbidity and mortality information is presented in relation to population size. This is particularly valuable if the characteristic that is being depicted is targeted at sub-groups of the population (e.g., particular age groups). Demographic maps require accurate information on both the numerators and denominators in morbidity and mortality values and are not common in veterinary medicine because this information often is missing. Moreover, they are generally used less often than they should be because they can be difficult to construct and to interpret by those who are unfamiliar with them.

Geographic base maps

Figure 4.5 is an example of a geographic base map. It is a 'conventional' map of Great Britain, showing the shape of the country. Most atlases consist of

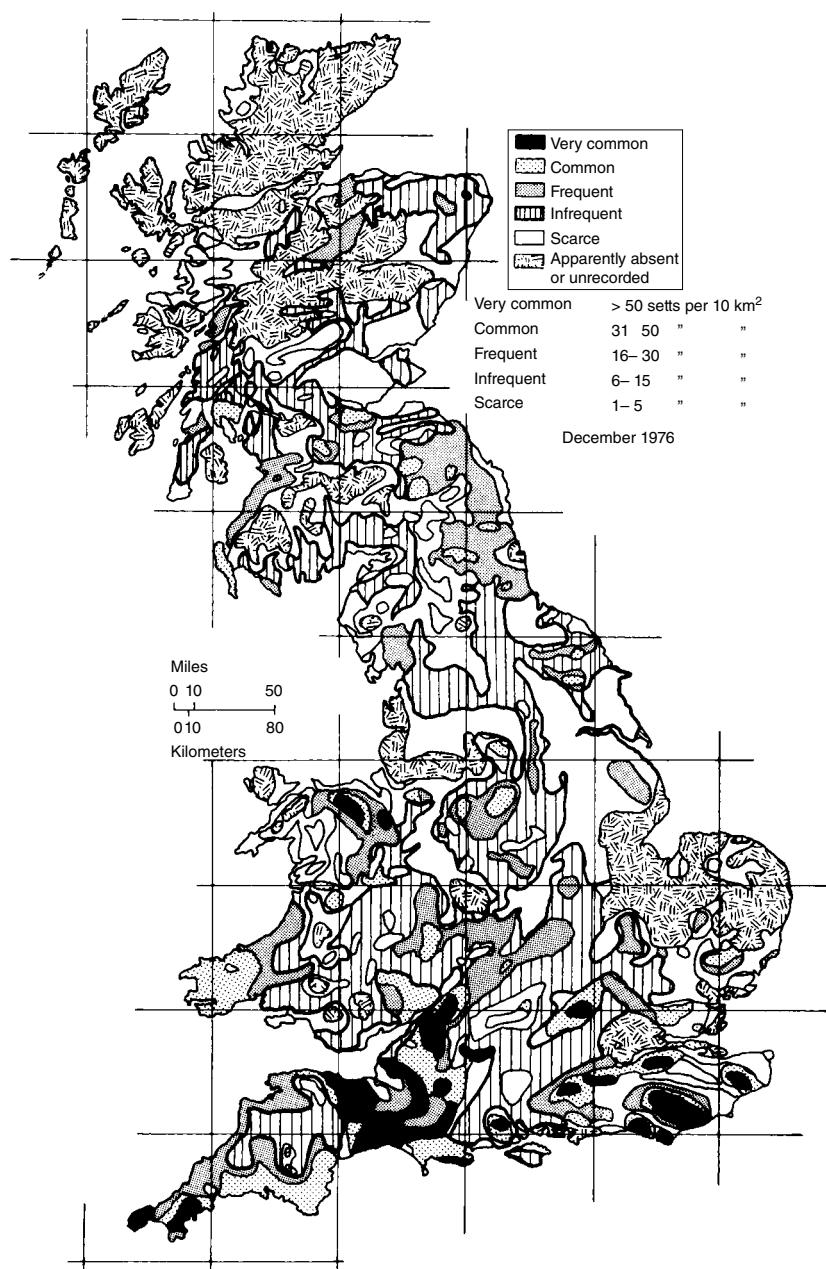


Fig. 4.5 Density of badger setts in Great Britain: an example of an isoplethic map (geographical base). (Source: Zuckerman, 1980.)

geographic base maps. There are several types of geographic base map, each with a different purpose, and displaying information in varying detail.

Point (dot or location) maps

These maps illustrate outbreaks of disease in discrete locations, by circles, squares, dots or other symbols. An example is *Figure 4.6*, where the solid circles with adjacent names indicate the sites of outbreaks of bluetongue in Portugal. Point maps are qualitative;

they do not indicate the extent of the outbreaks, which could each involve any number of animals. Point maps can be refined by using arrows to indicate direction of spread of disease. A series of point maps, displaying occurrence at different times, can indicate the direction of spread of an outbreak of disease. Additionally, point maps can be given a quantitative dimension (if data are available) by varying the density of the dots in proportion to the amount of disease (*Figure 4.7*).

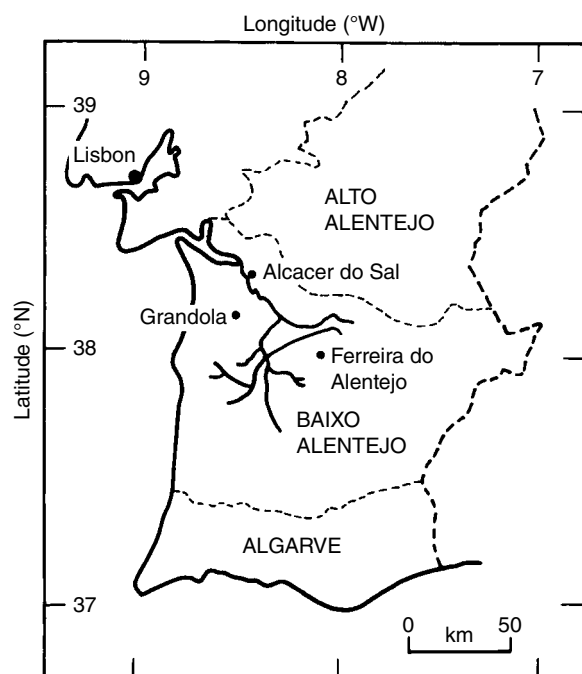


Fig. 4.6 Outbreaks of bluetongue in Portugal, July 1956: an example of a point map. (Source Sellers *et al.*, 1978. Reproduced with permission of Cambridge University Press.)

Distribution maps

A distribution map is constructed to show the area over which disease occurs. An example is given in *Figure 4.8*, illustrating areas in south-east Australia in which fascioliasis is continually present (endemic areas) and those that only experience the disease in wet years. Further examples, showing the world distribution of the major animal virus diseases, are presented by Odend'hal (1983).

Proportional circle maps

Morbidity and mortality can be depicted using circles whose area is proportional to the amount of disease or deaths (*Figure 4.9*). If the large values are substantially greater than the small values, the values can be represented by proportional spheres whose volume is proportional to the magnitude of the depicted characteristic. (Shading is used to give the impression of spheres on a two-dimensional map.)

Choroplethic maps

It is also possible to display quantitative information as discrete shaded units of area, graded in intensity to represent the variability of the mapped data. The units

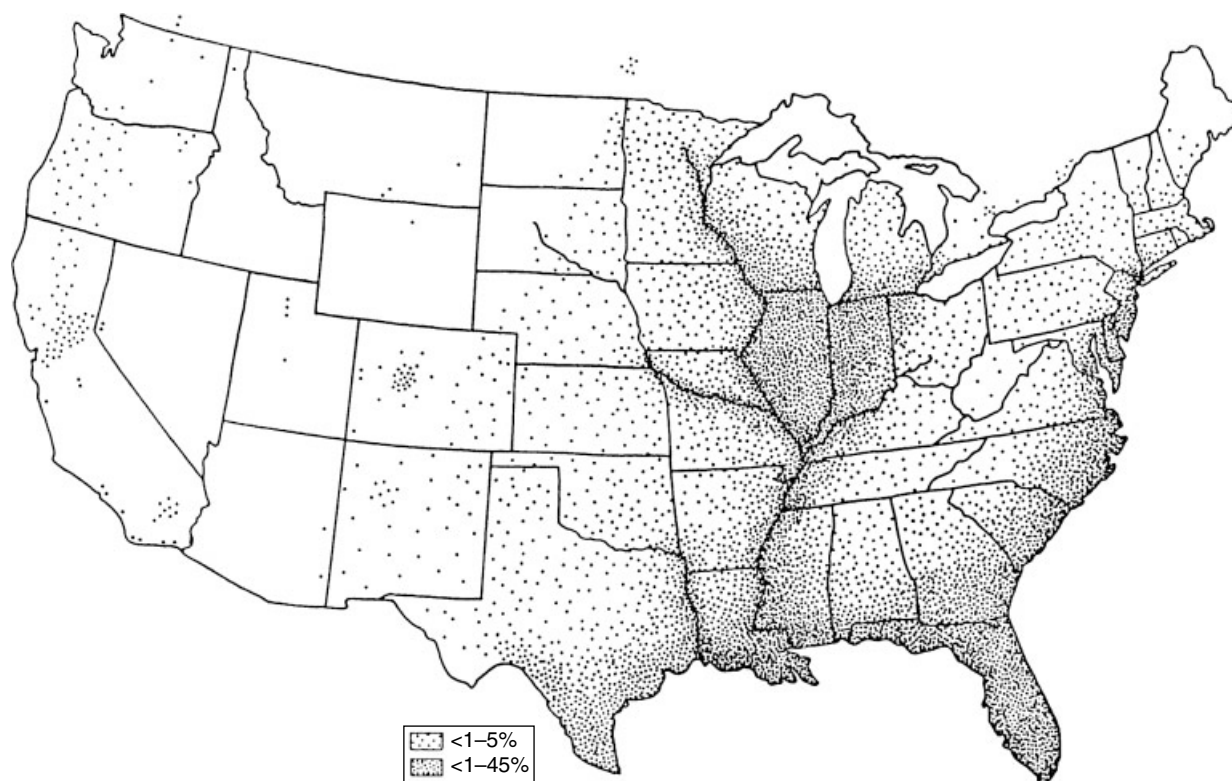


Fig. 4.7 Prevalence of heartworm infection in the US, 1995. (Source: Soll and Knight, 1995. Reproduced with permission of the American Heartworm Society.)

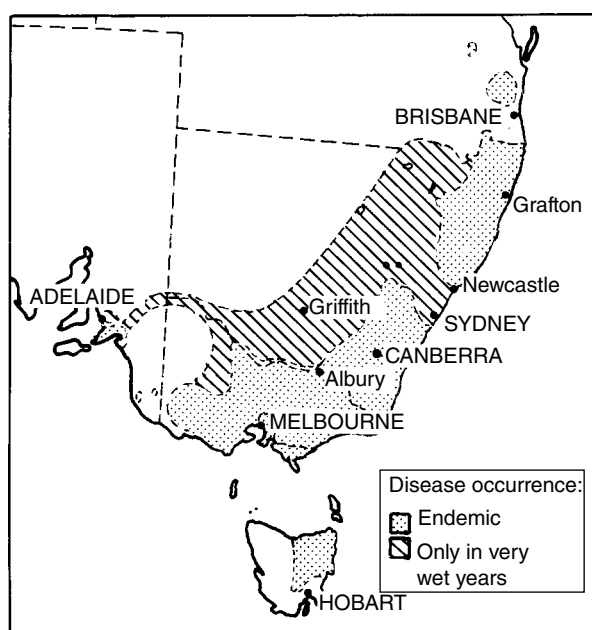


Fig. 4.8 Fascioliasis in Australia: an example of a distribution map. (Adapted from Barger *et al.*, 1978.)

can be formed from grid lines, but are commonly administrative areas such as parishes, shires, counties or states. Maps that portray information in this way are **choroplethic** (Greek: *choros* = 'an area', 'a region'; *plethos* = 'a throng', 'a crowd', 'the population'). Figure 4.10 is an example.

Choroplethic maps display quantitative data, but the boundaries between different recorded values are artificial. They are not the actual boundaries between, for example, high and low prevalence; they are merely the administrative boundaries of the areas over which the displayed values are averaged.

Isoplethic maps

True boundaries between different values can be depicted by joining all points of equal value by a line, such as joining points of equal height to produce the familiar contour map. Maps produced this way are **isoplethic** (Greek: *iso* = 'equal'). Lines joining points of equal morbidity are **isomorbs**, and those joining points of equal mortality are **isomorts**. If these lines are to be constructed, accurate estimates of both the

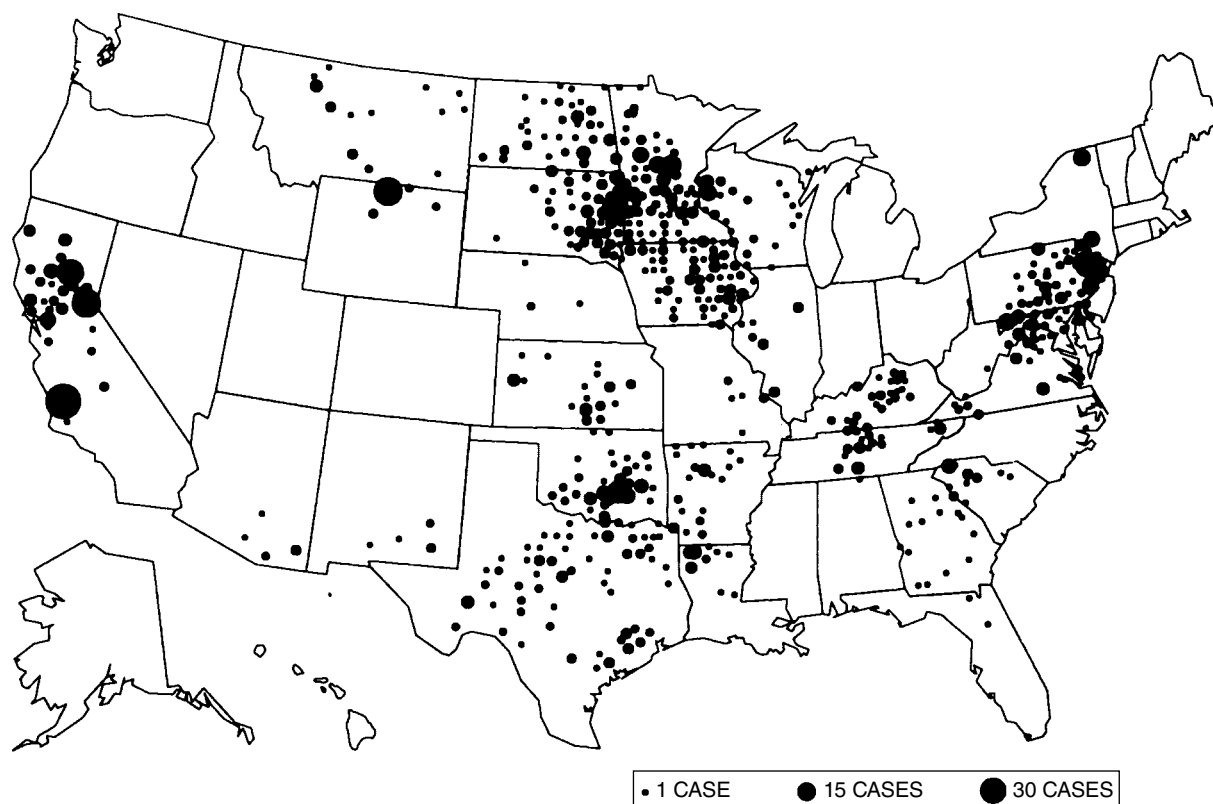


Fig. 4.9 Reported cases of rabies in skunks in the US, by county, 1990: an example of a proportional circle map. The area of each circle is proportional to the number of cases in each county. (Source: Uhaa *et al.*, 1992. Reproduced with permission of the American Veterinary Medical Association.)

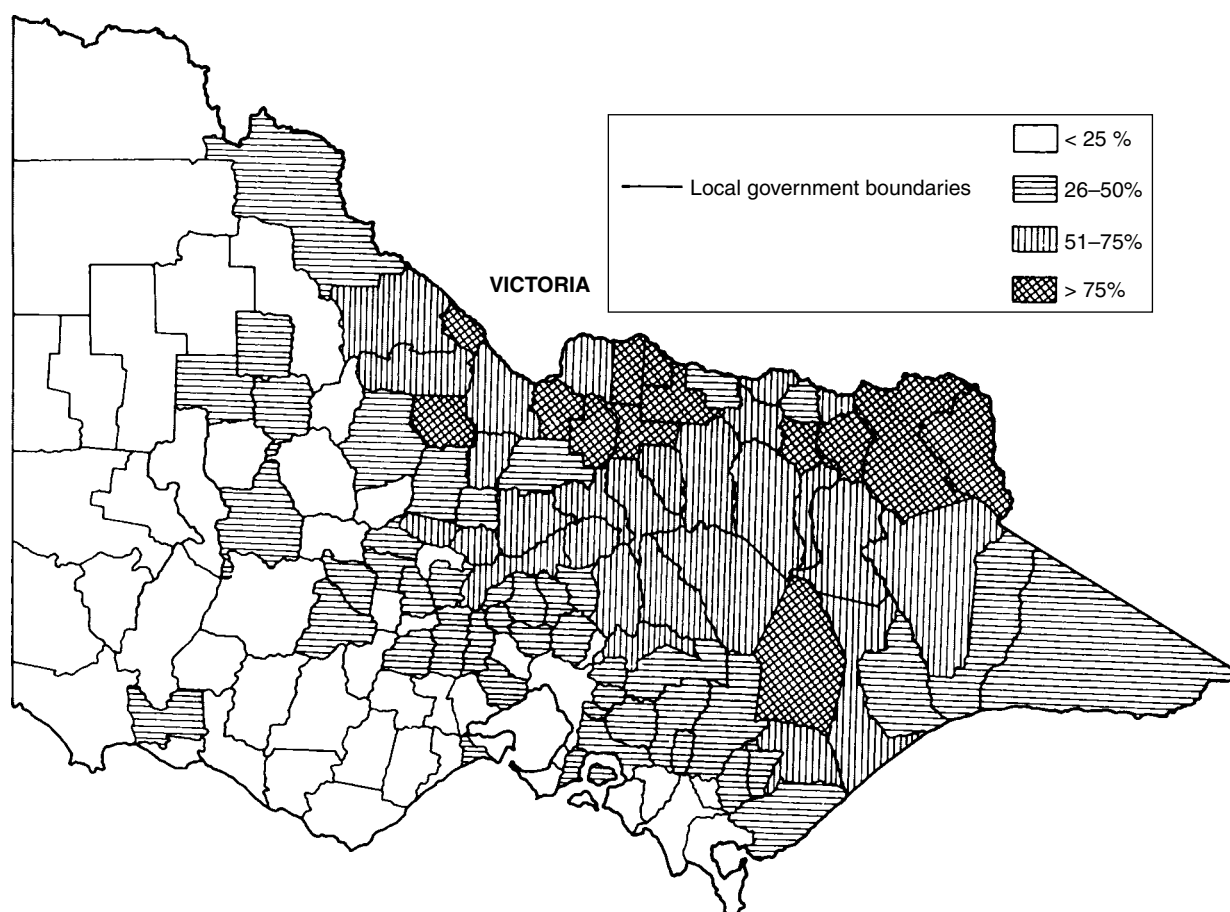


Fig. 4.10 Prevalence of fluke-affected livers by shire, Victoria, Australia, 1977–1978: an example of a choropleth map. (Source: Watt, 1980.)

number of cases of disease (numerator) and the size of the population at risk (denominator) over an area must be known.

Figure 4.5 is an isoplethic map showing badger density in Great Britain, drawn in relation to bovine tuberculosis. In this example, the ‘contours’ are the

boundaries between different ranges of badger density.

Medical mapping is discussed in detail by Cliff and Haggett (1988) and Koch (2005), and the use of maps in **geographical information systems** is outlined in Chapter 11.

Further reading

Bithell, J.F. (2000) A classification of disease mapping methods. *Statistics in Medicine*, **19**, 2203–2215
 Collett, D. (2014) *Modelling Survival Data in Medical Research*, 3rd edn. Chapman and Hall/CRC, Boca Raton. (A comprehensive text on survival analysis)
 Dawson, B. and Trapp, R.G. (2004) *Basic and Clinical Biostatistics*, 4th edn. McGraw-Hill, New York
 dos Santos Silva, I. (1999) *Cancer Epidemiology: Principles and Methods*. International Agency for

Research on Cancer, Lyon. (Includes a discussion of measures of disease and other health-related events)
 Downes, M.J., Dean, R.S., Stavisky, J.H., Adams, V.J., Grindlay, D.J.C. *et al.* (2013) Methods used to estimate the size of the owned dog and cat population: a systematic review. *BMC Veterinary Research*, **9**, 121; doi 10.1186/1746-6148-9-121
 Gordis, L. (2014) *Epidemiology*, 5th edn. Elsevier Life Sciences, London

- Koch, T. (2011) *Disease Maps: Epidemics on the Ground*. University of Chicago Press, Chicago. (*The history and contemporary applications of epidemic mapping*)
- O'Connell, A.F., Nichols, J.D. and Karanth, K.U. (Eds) (2011) *Camera Traps in Animal Ecology*. Springer, New York. (*Includes descriptions of the use of remote cameras to estimate animal abundance*)
- Plaza-Rodríguez, C., Appel, B., Kaesbohrer, A. and Filter, M. (2016) Discussing state-of-the art visualization techniques applicable for the epidemiological surveillance data on the example of *Campylobacter* spp. in raw chicken meat. *Zoonoses and Public Health*, **63**, 358–369. (*Includes a comparison of different mapping techniques*)
- Porta, M. (2014) *A Dictionary of Epidemiology*, 6th edn. Oxford University Press, Oxford

5

Determinants of disease

Chapter 3 introduced the concept that disease is caused by multiple factors. The factors are **determinants** of disease. A determinant is any characteristic that affects the health of a population. Diet, for example, is a determinant of bovine hypomagnesaemia: reduced food intake and low levels of plant magnesium, related to rapid grass growth, are associated with an increased incidence of the disease (see *Figure 3.7*). Knowledge of determinants facilitates identification of categories of animal that are at particular risk of developing disease. It therefore is a prerequisite for disease prevention, and is an aid to differential diagnosis. This chapter discusses the types of determinant and the interactions that can occur between them.

Classification of determinants

Determinants can be classified in three ways, as:

1. **primary** or **secondary**;
2. **intrinsic** or **extrinsic**;
3. associated with **host**, **agent** or **environment**.

Primary and secondary determinants

Primary determinants are factors whose variations exert a major effect in inducing disease. Frequently, primary determinants are necessary causes (see Chapter 3). Thus, exposure to distemper virus is a primary determinant of canine distemper.

Secondary determinants correspond to predisposing, enabling and reinforcing factors. For example, sex is a secondary determinant of canine heart valve incompetence: male dogs are more likely to develop incompetence than females (Buchanan, 1977; Thrusfield *et al.*, 1985; Toker *et al.*, 2011). The primary determinants may include other genetically determined factors such as the rate of ageing of the valves, which may be associated with breed. Primary and secondary determinants are listed in *Table 5.1*.

Intrinsic and extrinsic determinants

Table 5.1 also illustrates that some determinants (both primary and secondary) are internal to the host; for example, genetic constitution, including aberrant genes (which are the primary causes of genetic disorders), species, breed and sex. These determinants are **intrinsic**, also termed **endogenous** (Greek: *endon* = 'within'). In contrast, some determinants are external to the host; for instance, transportation, which may result in weight loss (D'Oliveira *et al.*, 2014) and physical trauma, producing bruising of carcasses (Jarvis and Cockram, 1994; Huertas *et al.*, 2010). Such determinants are **extrinsic**, also termed **exogenous** (Greek: *exo* = 'outside').

Table 5.2 exemplifies this scheme of classification in relation to canine pruritus (tendency to itch), a common problem in companion-animal practice.

Determinants associated with host, agent and environment

Many diseases include infectious agents in their sufficient causes. Most infectious agents enter the host as challenges from the environment (see *Figure 2.1*), and, when fetal infection occurs, the dam can be the 'environment' too. However, during the microbial revolution, the early emphasis on microbes as the primary causes of disease resulted in their being considered separately from other environmental factors such as husbandry, trauma and toxic agents. Thus, determinants commonly are classified into those associated with the **host**, the **agent** and the **environment**. These three groups of factors are sometimes called the **triad** (*Figure 5.1*). Some authors (e.g., Schwabe, 1984) consider that management and husbandry are important enough in intensive animal enterprises to be classified separately from the environment, as a fourth major group. Others have distinguished between the 'natural' and 'social' environments, with some authors defining the social environment as that arising among the animals themselves (e.g., the social organization of animal

Table 5.1 Primary and secondary determinants.

PRIMARY DETERMINANTS					
Intrinsic determinants	Extrinsic determinants				
	Animate		Inanimate		
	Endoparasitic	Ectoparasitic	Physical	Chemical	Allergic
Genetic constitution	Viruses	Arthropods	Trauma	Excess	Allergens
Metabolism	Bacteria		Climate	Deficiency	
Behaviour	Fungi		Radiation	Imbalance	
	Protozoa		Stressors	Poisons	
	Metazoa			Photosensitizers	
SECONDARY DETERMINANTS					
Intrinsic determinants			Extrinsic determinants		
Genetic constitution (including sex, species and breed)			Location		
Age			Climate		
Size and conformation			Husbandry (housing, diet, general management, animal use)		
Hormonal status			Trauma		
Nutritional status			Concurrent disease		
Immunological status			Vaccination status		
Functional status (e.g., pregnant, lactating)			Stressors		
Behaviour					

Table 5.2 Some determinants of canine pruritus. (Simplified from Mason, 1995; Logas, 2003; Tater, 2012; Sousa, 2013.)

Intrinsic determinants		Extrinsic determinants				
Host characteristics	Internal disease	Chemicals	Environment	Diet	Parasites	Bacteria, fungi and yeasts
Breed	Neoplasia:	Irritant	Solar	Adverse	Hookworm	Bacterial
Age	mast cell tumour	contact	dermatitis	food	dermatitis	folliculitis
	mycosis fungoides/ epitheliotropic lymphoma	dermatitis		reactions	<i>Pelodera</i> dermatitis	Deep pyoderma
	Immune-mediated	Calcinosis			Schistosomiasis	Dermatophytosis
	disorders: systemic lupus	cutis			Dirofilariasis	<i>Malassezia</i> dermatitis
	erythematosus	Adverse			Scabies	
	Hormonal hypersensitivity	drug			Pediculosis	
		reactions			Demodicosis	
					<i>Notoedres cati</i>	
					<i>Otodectes</i> <i>cyanotis</i>	
					Trombiculiasis	
					Cheyletiellosis	

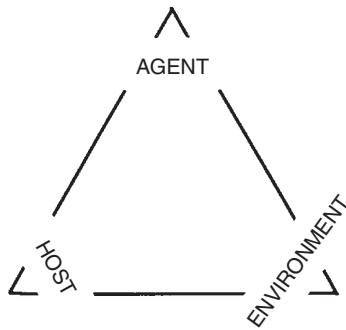


Fig. 5.1 The 'triad': three main headings under which determinants can be classified. (Adapted from Snieszko, 1974.)

groups may impact animals' immune status: Salak-Johnson and McGlone, 1991), whereas others refer to the 'human social environment' (e.g., farmers' social environments may impact their beliefs and actions regarding control measures for bovine tuberculosis: Enticott, 2008). The impacts of the human social environment are sometimes overlooked in veterinary epidemiology (at least explicitly), but a wide range of social, political, economic and other factors can be major influences on the occurrence of animal diseases and responses to them. Hence, within the triad (Figure 5.1) the 'environment' can include both the natural and the social (including human social) environments in which animals live.

In some diseases, an infectious agent is the main determinant, and host and environmental factors are often viewed as being of relatively minor importance. Such diseases are 'simple'; examples are major animal plagues such as foot-and-mouth disease and classical swine fever occurring in susceptible populations. In other diseases, termed 'complex', their multifactorial nature predominates and a clear **interaction** between host, agent and environment can be identified. Thus, 'environmental' mastitis involves an interaction between *Escherichia coli* or *Streptococcus uberis* (the agent), milking machine faults, and poor environmental hygiene (the environment) (Blowey and Edmondson, 2010). In addition, cows (the hosts) are most susceptible in early lactation. Similarly, it has been claimed that the binding of copper and manganese (trace elements found in the soil) to prion proteins may influence the pathogenesis of scrapie and other transmissible spongiform encephalopathies (Purdey, 2000).

However, drawing on the biosocial perspective¹ of Farmer and others (2006), even for apparently 'simple'

¹ The **biosocial (biopsychosocial)** model of disease propounds that a combination of biological, psychological and social factors interact to effect disease occurrence (Engel, 1977). It therefore follows a broad approach to causality, rather than seeking purely molecular, mechanistic/reductionist processes (see Chapter 3).

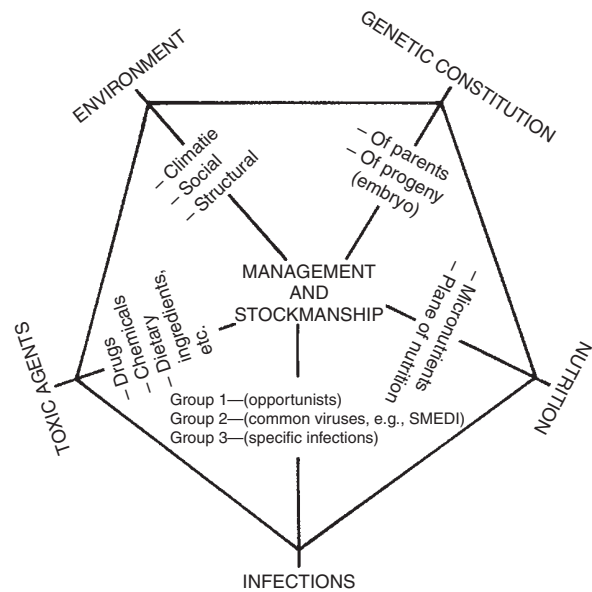


Fig. 5.2 Diagrammatic representation of causes of reproductive failure in a pig herd. (Adapted from Pritchard *et al.*, 1985.)

diseases an important role for the human social environment can be identified. For example, military campaigns, and their associated social disruption, frequently have facilitated the spread of important animal plagues (Karaszson, 1988), including rinderpest (see Table 1.2), with conflict and social unrest delaying its eradication in some parts of the world (Roeder and Rich, 2009). Similarly, the global distribution of foot-and-mouth disease can be attributed, at least partly, to the political and economic ability of some countries to eradicate the disease and to prevent subsequent re-entry (Forman *et al.*, 2009).

The complexity of a multifactorial disease depends upon how the disease is defined. Most 'diseases' with which the veterinarian is initially concerned are actually clinical signs presented by animals' owners. Thus, pruritus in a dog is a clinical sign, which can be caused by several different lesions, each with their own sufficient causes. Further examples of signs and lesions with different causes are given in Figures 10.3b and 10.3c. The causal web can become very complex when the 'disease' is defined in terms of a production shortfall in a herd or flock. Thus, 'reproductive failure' in a pig herd, reflected in unacceptably low numbers of piglets being born in a herd in a defined period of time, can be produced by decreased male or female fertility, or infection or metabolic derangement of the sow or fetus during pregnancy. Figure 5.2 divides the causes of reproductive failure in pigs into six main areas relating to genetic constitution, nutrition, infections, toxic

agents, environment and management; it therefore represents a subdivision of agent, host and environmental determinants. These six areas are discussed in detail by Wrathall (1975) and Rueff (2000).

Genetic factors include genetic defects affecting the parents (e.g., abnormal genitalia or gametes) and the offspring (e.g., malformations and inherited predisposition to disease).

Failure to supply nutritional requirements for specific micronutrients (vitamins, minor minerals and trace elements) can result in reduced litter sizes, embryonic death and delayed puberty. The plane of nutrition also can affect performance. Restriction of diet can delay puberty. In contrast, 'flushing' (increasing the amount fed) a few days before ovulation is due can increase the number of ova released in gilts.

Infections are classified into three groups:

1. opportunistic pathogens;
2. common pathogens;
3. specific infectious pathogens.

Group 1 pathogens are ubiquitous and frequently endogenous (see 'Exogenous and endogenous pathogens' later in this chapter). They cause disease sporadically when host resistance is lowered. Examples include *Staphylococcus* and *Listeria* spp. – infections causing prenatal deaths and abortions. Notable in group 2 are the porcine parvoviruses, also termed SMEDI viruses, an acronym for stillbirth, mummification, embryonic death and infertility, whose effects they produce. Group 3 infections include brucellosis, leptospirosis, toxoplasmosis and swine fever – infections caused by exogenous pathogens, which can produce low conception rates and induce abortion.

Toxic substances include those ingested in the food (e.g., mycotoxins, which can induce vulvovaginitis and abnormal oestrus), and environmental pollutants such as wood preservatives, which can cause abortion.

The environment affects reproduction through its climatic, social and structural components. High environmental temperatures, for example, can induce infertility in males.

Important management factors include herd age (young females may have a low ovulation rate and therefore small litter size), the boar:sow ratio, boar management, efficiency of heat detection and pregnancy diagnosis, breeding policy and record keeping.

The three schemes of determinant classification are not mutually exclusive. They are just three different ways of viewing the multifactorial nature of disease. Determinants are described below using the third system of classification: into those associated with host, agent or environment. This system also is followed

by Schwabe and others (1977), Martin and others (1987) and Smith (2006) in their discussion of determinants of animal diseases, and by Reif (1983) in his consideration of determinants of diseases of dogs and cats.

Host determinants

Genotype

The genetic constitution of a host is its **genotype**. Some diseases appear to have an almost totally genetic cause; that is, alterations in gene structure are considered to have a marked effect on their pathogenesis, and they may be inherited by succeeding generations; an example is haemophilia (von Willebrand's disease) in dogs, notably Doberman pinchers (Johnstone and Crane, 1981). Such diseases, in which aberrant genes are primary determinants, are traditionally termed **genetic diseases**. Other diseases, such as the simple infectious diseases, appear to have little or no genetic component. Many diseases, however, occupy positions intermediate to these two extremes; examples are bovine foot lameness (Peterse and Antonisse, 1981; Vermunt and Greenough, 1994) and mastitis (Rupp and Boichard, 2003).

Genetic diseases generally belong to one of three categories (Nicholas, 2010):

1. chromosomal aberrations;
2. Mendelian (simply inherited) disorders²;
3. multifactorial disorders.

The first two categories represent diseases that are almost totally genetic. The third category represents diseases that have a variable, complex, genetic component. Such diseases have been reported to occur more commonly than those belonging to the first two categories; their mode of inheritance will therefore be discussed in more detail.

Multifactorial inheritance

Many of the simply inherited disorders are qualitative, 'all-or-none' characteristics.

In contrast, other characteristics, such as muscle mass and the severity of some diseases (e.g., hip dysplasia: Morgan *et al.*, 2000), are quantitative, displaying continuous variation. The genetic component of this variation is explained by the cumulative (usually additive) effects of many genes at several sites (loci) on the chromosomes. This is **polygenic** inheritance. The

² Developments in molecular biology, however, have resulted in a reassessment of the classical Mendelian modes of inheritance (Zlotogora, 1998).

polygenic component additionally interacts with environmental factors; thus, there is a **multifactorial** cause to such traits. For example, canine hip dysplasia requires both a genetic weakness to the muscles supporting the hip joint, and conditions in early life that favour separation of the joint, resulting in the osseous changes that characterize the disease. These changes can be prevented by confining to cages pups that have genetically weak muscles, with their hindlimbs flexed and abducted. The development of the condition also is exacerbated by excess energy consumption (Fries and Remedios, 1995). Thus, there is clearly a non-genetic component relating to management and nutrition early in life. The relative (but not exclusive) occurrence of hip dysplasia in different giant breeds, between which there is no genetic movement, supports the hypothesis that large, poorly muscled breeds (whose conformation is genetically determined) are particularly at risk. Furthermore, litter mates can be affected to different degrees, suggesting a polygenic cause.

Some disorders, such as congenital heart defects, do not show continuous variation, but liability to the condition nevertheless can be interpreted as having a polygenic component. An individual can inherit the 'right' number and combination of genes, and be exposed to environmental determinants, to pass beyond a **threshold value**, beyond which the disease occurs (Falconer and Mackay, 1996). *Figure 5.3* illustrates this concept. The horizontal axis is the 'liability to disease', '+' indicating the presence of many, and '-' of few, of the right genes and environmental factors. The vertical axis is the relative frequency of values of liability to disease in the population. The congenital heart defect, patent ductus arteriosus, for example, is inherited multifactorially, and is discussed in more detail in Chapter 9 in the context of comparative epidemiology.

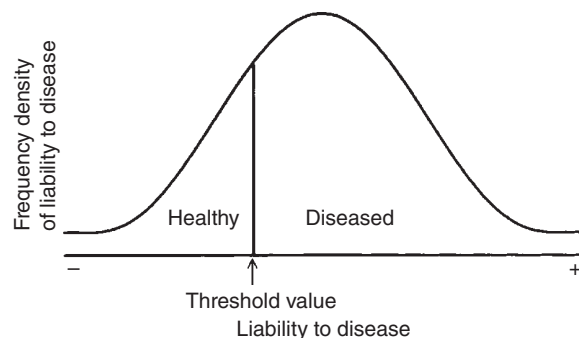


Fig. 5.3 Genetic model for the manifestation of a multifactorial disease. The disease occurs when the number and combination of genes, in association with environmental factors, exceeds the threshold value.

The likelihood of a relative inheriting the right combination of causal genes becomes less as a relationship becomes more distant. The likelihood of the right number and combination of genes being inherited also decreases as the number of genes required for manifestation of the disorder increases.

Initially, simple and polygenic modes of inheritance appeared to be mutually exclusive. However, it is now clear that the additive effects of many genes, inherited in a Mendelian manner, can be applied to the polygenic model for continuous traits. Conversely, a single-gene model can be applied to quantitative traits by converting the trait to a dichotomy, with an animal characterized as to whether its trait value is above or below a threshold value. Moreover, liability to disease can be applied to simple, single-gene traits where environmental factors have an effect (e.g., human phenylketonuria).

Although it is currently believed that many common diseases have a genetic component, it is unclear whether this amounts to a multifactorial (polygenic) predisposition or to the involvement of a single major chromosomal locus interacting with environmental factors. It also is difficult to infer a genetic component to the cause of a disease in the absence of data relating to genetically distinct groups (breeds, families, and sire and dam lines).

Foley and others (1979) describe heritable diseases of companion animals and livestock in detail, and Ostrander and Ruvinsky (2010) and Ackerman (2011) specifically address conditions in dogs. Identification of putative genes, or ones that map closely to them ('linked markers'), is an important component of the control of inherited disorders (see Chapter 27 and Patterson, 2000).

Age

The occurrence of many diseases shows a distinct association with age. Many bacterial and virus diseases, for instance, are more likely to occur, and to be fatal, in young than in old animals, either because of an absence of acquired immunity (particularly if deprived of colostrum) or because of a low non-immunological host resistance (Quinn *et al.*, 2011). Some protozoan and rickettsial infections, in contrast, induce milder responses in the young than in the old (Zintl *et al.*, 2005).

The absolute number of cases of a disease clearly is not an indication of the impact of disease in a particular age range because the ranges are present in different proportions in the total population. This is illustrated in *Figure 5.4*, which depicts **population pyramids** for dogs and cats. These show the age

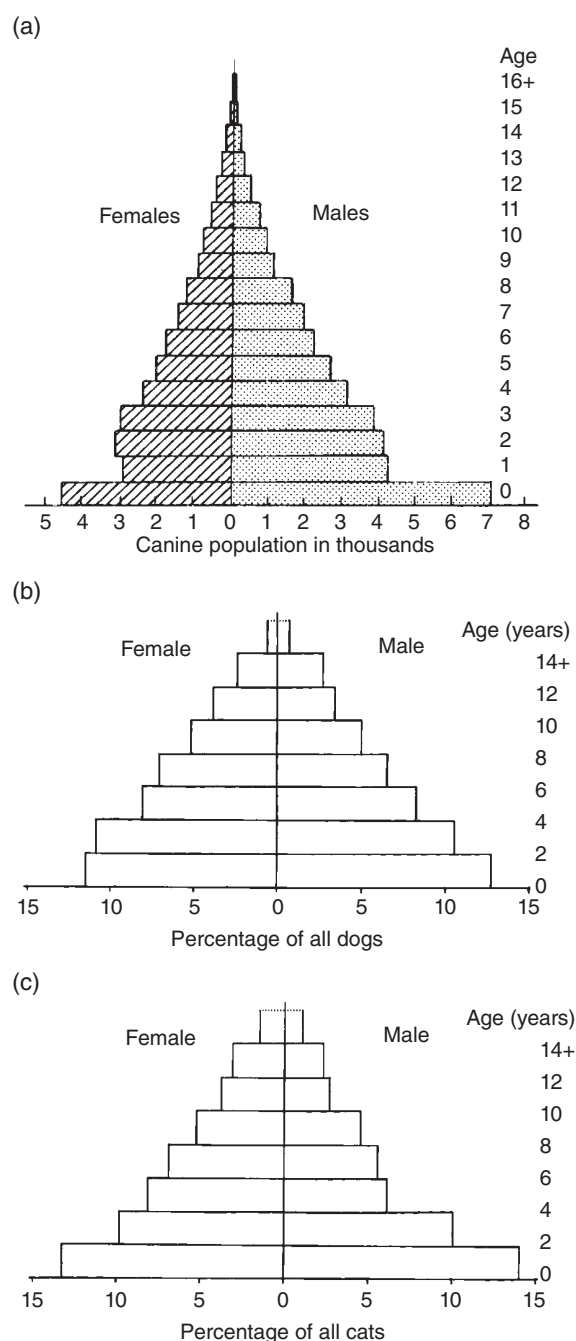


Fig. 5.4 Canine and feline population pyramids. (a) Canine: New Jersey, US, 1957; (b) canine: UK, 1986; (c) feline: UK, 1986. (Source: (a) From Cohen *et al.*, 1959. Reproduced with permission of the American Veterinary Medical Association. (b, c) Thrusfield, 1989. Reproduced with permission of John Wiley and Sons.)

distribution of animals (male and female indicated separately). Thus, age-specific rates (see Chapter 4) provide the most valuable information about disease in particular age groups, because they relate morbidity and mortality to a uniform size of population at

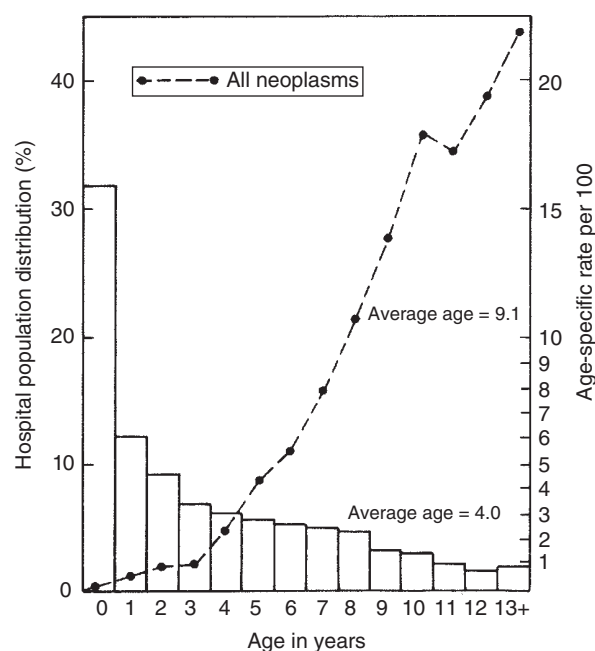


Fig. 5.5 Age distribution of a total canine hospital population (bars) and age-specific rates (graph) for canine neoplasia: University of Pennsylvania, 1952–1964. (Source: Reif, 1983. Reproduced with permission of Elsevier.)

risk. Figure 5.5 displays age-specific rates for canine neoplasia and shows that tumours are more common in old than in young animals. (There are some notable exceptions such as canine osteosarcoma and lymphoma, which can occur in young animals: Reif, 1983; Spodnick *et al.*, 1992.)

Sex

Sexual differences in disease occurrence may be attributed to hormonal, occupational, behavioural and genetic determinants.

Hormonal determinants

The effects of sex hormones may predispose animals to disease. Bitches are more likely to develop diabetes mellitus than dogs (Fall *et al.*, 2007) and signs often develop after oestrus, possibly related to the increased insulin requirements of diabetic bitches during oestrus. Similarly, it has been tendentiously suggested that the neutering of bitches decreases the likelihood of mammary carcinoma developing (Schneider *et al.*, 1969; Beauvais *et al.*, 2012), perhaps from the effect of oestrogens on this tumour (see Chapter 9).

Occupational determinants

Sex-associated occupational hazards, although more relevant to human than animal disease, can be

identified occasionally in animals, where animal use is equated with occupation. Thus, the increased risk of contracting heartworm infection by male dogs relative to bitches (Selby *et al.*, 1980) may result from increased 'occupational' exposure of male dogs during hunting to the mosquito that transmits the infection.

Genetic determinants

Genetic differences in disease incidence may be inherited either by being **sex-linked**, **sex-limited** or **sex-influenced**. Sex-linked inheritance is commonly associated with Mendelian inheritance, and occurs when the DNA responsible for a disease is carried on either the X or Y sex chromosomes. Canine haemophilia A and B, for example, are associated with the X chromosome and are inherited recessively, the defects being predominant in males (Patterson and Medway, 1966). Sex-limited inheritance occurs when the DNA responsible for the disease is not in the sex chromosomes, but the disease is expressed only in one sex, for example cryptorchidism in dogs. In sex-influenced inheritance, the threshold for the overt expression of a characteristic (based on the multifactorial genetic model: *Figure 5.3*) is lower in one sex than the other, therefore, there is an excess incidence in one sex over the other. Canine patent ductus arteriosus (see Chapter 9) may be an example.

In many diseases, there may be excess disease occurrence in one sex over the other, but either a genetic (or other) component has not been identified clearly or the method of inheritance has not been established. Examples that are reported to occur predominantly in male dogs include epilepsy (Kearsley-Fleet *et al.*, 2013) and tumours of the head and neck (Baba and Cătoi, 2007; Brønden *et al.*, 2009).

Some diseases may be apparently sex-associated, but are actually associated with other determinants that are related to gender. For instance (Schwabe *et al.*, 1977), the increased mortality rate in male dairy calves may appear to be sex-associated. However, the real association is with husbandry: male dairy calves may not be given as much attention as females because they are worth less (in this instance husbandry, therefore, is a confounder: see Chapters 3, 15 and 18).

Species and breed

Species and breeds vary in their susceptibility and responses to different infectious agents, and therefore in the role they play in disease transmission. Dogs, for example, do not develop heartwater. Pigs are harder to infect with foot-and-mouth disease virus via the respiratory tract than cattle and sheep (Sellers, 1971; Donaldson and Alexandersen, 2001). Thus, cattle are

the species most likely to be infected by between-farm spread of airborne virus, because of their extreme susceptibility to airborne infection and higher respiratory tidal volume (Donaldson *et al.*, 1982; Donaldson, 1987). Pigs are not only less susceptible to airborne virus but also, because of their lower tidal volume and the practice of housing them indoors, are less likely to encounter sufficient virus to initiate clinical disease. In contrast, if infection does occur, pigs excrete vast quantities of airborne virus (one pig being equivalent to 3000 cattle) and so are important sources of airborne virus during epidemics because they are often kept in large numbers. (See also Chapter 6.)

Rottweilers and Doberman pinschers react more severely to canine parvovirus enteritis than other breeds (Glickman *et al.*, 1985), and boxers, pointers, Australian shepherds, beagles and Scottish terriers appear to be more susceptible than other breeds to coccidioidomycosis (Davidson and Pappagianis, 1996) (for a distribution map of the causal fungus, see *Figure 7.2*). Different breeds of poultry vary in their susceptibility to a range of viruses (Bumstead, 1998; Hassan *et al.*, 2004).

The reasons for species' susceptibility are many and not fully understood. The efficacy of the immune mechanism against an infectious agent may be important. Thus, humans are not usually susceptible to infection with *Babesia* spp. but splenectomized individuals can develop the disease (Rosner *et al.*, 1984). Different species have been shown to have different receptors for infectious agents on the cell surface. This is particularly important with viruses, which must enter the host cell. Monkeys are not susceptible to poliovirus because they do not have the 'right' cell receptors. Removal of the virus capsid allows the virus to divide lytically in monkey cells if it is first made to enter them. Susceptibility can vary within a species, too. Thus, only certain pigs are susceptible to the strain of *E. coli* possessing the F4 (K88) fimbrial adhesin because susceptibility is determined by the presence of an intestinal receptor that is specified by one or more genes (Vögeli *et al.*, 1992).

Phylogenetically closely related animals are likely to be susceptible to infection by the same agent, albeit with different signs, because the 'fitness valley' that agents need to cross to colonize new hosts is shallow if hosts are genetically related (Kuiken, *et al.*, 2006). Herpesvirus B causes labial vesicular lesions in non-human primates, but fatal encephalitis in man. The rider to this – that phylogenetically closely related agents infect the same species of animal – is not, however, generally true. Measles and distemper are closely related paramyxoviruses, yet usually infect quite different species: man and dogs, respectively.

Apparently new diseases can develop when a species or breed is placed in a new ecosystem (see Chapter 7) that contains a pathogen that has a well-balanced relationship with local species or breeds. In such circumstances inapparent infection (discussed later in this chapter) is common in the local animals but clinical disease occurs in the exotic ones. This happened in South Africa, when European breeds of sheep were exposed to bluetongue virus. The agent did not produce clinical signs in the indigenous sheep, but caused severe disease in imported Merino sheep (Coetzee *et al.*, 2012). Resistance to the tick *Boophilus microplus* is greater in indigenous Zebu cattle (*Bos indicus*) than in European cattle (*Bos taurus*) (Brossard, 1998), and the importation of European cattle to West Africa also accentuated the problem of dermatophilosis.

There is also species and breed variation in the occurrence of non-infectious diseases. Thus, British breeds of sheep develop intestinal carcinoma more frequently than fine-wool breeds, Hereford cattle develop ocular squamous cell carcinoma more commonly than other breeds (see Chapter 2), and there is considerable variation in canine and feline breed predisposition to skin tumours (Goldschmidt and Schofer, 1994)³.

Many diseases having distinct associations with a particular familial line or breed are considered to be primarily genetic (Ubbink *et al.*, 1998b). Patterson (1980) has described over 100 such diseases in the dog⁴. A genetic causal component is more likely when disease incidence is higher in pedigree animals than in crossbreeds. Examples include congenital cardiovascular defects (Patterson, 1968) and valvular heart disease (Thrusfield *et al.*, 1985) in dogs.

Diseases may be present in a range of breeds because the breeds are genetically related. Thus, dogs of bulldog descent (boxers, Boston terriers, English bulldogs and pugs) show a high risk of developing mast cell tumours, which may be related to their common origin (London and Thamm, 2013). In contrast, the risk of a particular breed developing a disease may vary between countries, indicating different genetic 'pools'

³ For a general review of breed predispositions to cancer in pedigree dogs, see Dobson (2013).

⁴ The evolutionary history of dogs has involved at least two severe population 'bottlenecks' (Parker *et al.*, 2010). The first occurred when dogs were domesticated from wolves over 14 000 years ago (Germonpré *et al.*, 2009; see also Chapter 1). The second was most pronounced approximately 200 years ago, when most dog breeds were created by the selection of morphological and behavioural traits. Today, there are approximately 400 isolated populations or breeds. Breed creation has inadvertently selected many 'founder' mutations that are associated with specific traits and diseases. This translates into reduced disease and genetic heterogeneity, consistent with the fact that most breeds are predisposed to distinct sets of disease.

(or a different environment or method of management). Thus, Das and Tashjian (1965) found an increased risk of developing valvular heart disease in cocker spaniels in North America, whereas, in Scotland, Thrusfield and others (1985) did not detect an increased risk in that breed. In contrast, valvular heart disease is commoner in cavalier King Charles spaniels in Scotland (Thrusfield *et al.*, 1985) than in Australia (Malik *et al.*, 1992). Gough and Thomas (2010) document breed dispositions to diseases in dogs and cats.

Behaviour

Behaviour can affect the likelihood of transmission of infection from one species to another. Thus, in New Zealand, opossums stand their ground when confronted by cattle, thereby increasing the chance of aerosol transmission (see Chapter 6) of tuberculosis from infected opossums to cattle by inhalation. In contrast, in the UK, badgers' immediate response to threatening behaviour is to retreat, and so aerosol transmission of tuberculosis from infected badgers is less likely (Benham and Broom, 1989). In South Africa, mutual grooming is more likely than other forms of behaviour (e.g., aggression and foraging) to spread tuberculosis among meerkats because of its greater frequency (Drewe *et al.*, 2010). Behavioural patterns also may account for bite wound abscesses being more common in male than female cats.

In humans, cultural habits may predispose to disease transmission. For example, one reason for the spread of the highly-lethal Ebola virus during the outbreak in West Africa in 2014–2015 is the tradition at burial ceremonies for mourners to have direct contact with the deceased (Nielsen *et al.*, 2015).

Other host determinants

Size and conformation

Size, independent of particular breed associations, has been identified as a disease determinant. Hip dysplasia and osteosarcoma are more common in large than small breeds of dog (Ru *et al.*, 1998; LaFond *et al.*, 2002). Interestingly, the latter disease is also more common in large than small children (Longhi *et al.*, 2005). The conformation of animals may similarly increase the risk of some diseases. For instance, cows with a small pelvic outlet relative to their size (e.g., Chianina and Belgian blue) are predisposed to dystokia. Conformation also may have less direct effects. Thus, some calves cannot suckle their dams because the latter have large, bulbous teats (Logan *et al.*, 1974). This can result in the calves being hypogammaglobulinaemic, with the increased risk of fatal colibacillosis.

Coat colour

Predisposition to some diseases is associated with coat colour, which is heritable and a risk indicator. For example, white cats have a high risk of developing cutaneous squamous cell carcinoma (Dorn *et al.*, 1971) related to the lack of pigment, which protects the skin from the carcinogenic effects of the sun's ultraviolet radiation. In contrast, canine melanomas occur mainly in deeply pigmented animals (Brodey, 1970). White cats often have a genetic defect associated with deafness (Bergsma and Brown, 1971). White hair colour also is associated with congenital deafness in Dalmatians (Anderson *et al.*, 1968).

Agent determinants

Virulence and pathogenicity

Infectious agents vary in their ability to infect and to induce disease in animals⁵. The ability to infect is related to the inherent susceptibility of a host and whether or not the host is immune. The ability to induce disease is expressed in terms of **virulence** and **pathogenicity**. Virulence is the ability of an infectious agent to cause disease, in a **particular** host, in terms of **severity**⁶. For example, Newcastle disease virus is virulent to poultry, but the majority of infections in species of wild birds are not virulent (Miller and Koch, 2013). Virulence also is sometimes expressed quantitatively as the ratio of the number of clinical cases to the number of animals infected (Porta, 2014). Case fatality (see Chapter 4) is an indicator of virulence when death is the only outcome. Pathogenicity is sometimes incorrectly used as a synonym for virulence, with virulence reserved for variations in the disease-inducing potential of different strains of the same organism. However, 'pathogenicity' refers to the quality of disease induction (Stedman, 1989). Thus, the protozoan parasite *Naegleria fowleri* is pathogenic to humans in warm, but not in cold, water, because the infective trophozoites only predominate in warm water, pathogenicity therefore being governed in this instance by environment. Pathogenicity also may be quantified as the ratio of the

number of individuals developing clinical illness to the number exposed to infection (Porta, 2014).

Pathogenicity and virulence are commonly intrinsic characteristics of an infectious agent and are either **phenotypically** or **genotypically** conditioned. Phenotypic changes are transient, and are lost in succeeding generations. For example, Newcastle disease virus, cultivated in the chorioallantois of hens' eggs, is more virulent to chicks than virus that is cultivated in calf kidney cells. Genotypic changes result from a change in the DNA (and RNA, in RNA viruses) of the microbial genome. Most pathogenic bacteria express their virulence genes only when they are inside the host; the conditions in the host somehow facilitating expression of these genes.

Pathogenicity and virulence are determined by a variety of host and agent characteristics. Bacterial virulence and pathogenicity are determined by a relatively small group of factors ('common themes'), which may be either unique to specific pathogens or conserved across several different species (Finlay and Falkow, 1997; Wilson *et al.*, 2002). These include toxin and adhesin production, and common themes for invasion of the host and resistance to the clearance and defence mechanisms of the host (e.g., capsule production, intracellular lifestyles, and extracellular and intracellular invasion mechanisms). An agent may achieve pathogenicity, or increase virulence, by a change in antigenic composition to a type to which the host is neither genetically nor immunologically resistant. However, antigenic changes are not always the cause of changes in pathogenicity. They may simply be indicators of such changes, the determinants being associated with the production of inhibitory, toxic or other substances (e.g., exotoxins and endotoxins) and the immune-mediated damage that may ensue (McVey and Czurpynski, 2013). Genotypic changes also can alter the sensitivity of bacteria to antibiotics.

Traditionally, well-adapted pathogens were considered to be only mildly virulent, but this view now is rejected on theoretical and empirical grounds (Walther and Ewald, 2004). The current view is that evolved levels of virulence reflect trade-offs between microbial competition within individual hosts (favouring rapid reproduction and increased virulence) and requirements for successful transmission (promoting relatively benign organisms), and that the evolution of virulence is staged according to whether a pathogen either has newly emerged or is established (Ebert and Bull, 2008).

Types of genotypic change

Various genotypic changes can occur in infectious agents (Table 5.3). Major ones include **mutation**,

⁵ This also is true for plant pathogens (Mills *et al.*, 1995).

⁶ The conventional wisdom was that parasites evolve into less virulent forms because, in killing their hosts, lethal parasites appear to 'commit suicide'. However, recent evidence (Ebert and Mangin, 1995; Kochin *et al.*, 2010) suggests that parasite evolution involves changes in response to the host's immunity, and a subtle balance between virulence and transmission rate. Galvani (2003) discusses the complex interactions between evolutionary, ecological and epidemiological processes that determine virulence.

Table 5.3 Potential sources of genetic variability in bacterial and virus populations. (Adapted from Schrag and Weiner, 1995. Reproduced with permission from Elsevier.)

Bacteria	Viruses
Mutation: Mutation rate is approximately 10^{-9} to 10^{-6} per base pair. Point mutations, deletions, insertions and inversions can lead to significant changes in virulence factors (e.g., adhesion ability, toxin production). Antigenic drift can allow bacterial pathogens to 'hide' from mammalian immune systems Transposition: Transposons are segments of DNA that can be integrated into new sites on the same or different DNA molecules from their origin; conjugative transposons are elements that can promote their own transfer from one bacterial cell to another Transformation: Uptake and integration into bacterial chromosome of exogenous DNA Plasmid exchange: Transfer of plasmids between bacterial cells Conjugation: Plasmid-mediated chromosome transfer between bacterial cells Lysogeny: Incorporation of phage genes into bacterial genome via phage integration (e.g., <i>Escherichia coli</i> O157) Transduction: Phage-mediated transfer of small portions of bacterial DNA	Mutation: RNA viruses have significantly higher mutation rates than DNA viruses or bacteria, because of the greater instability of RNA molecules and the higher error rates of RNA replication enzymes. Point mutations can generate rapid antigenic variation Recombination between viruses: Intraspecific and interspecific recombination has been observed in some RNA and DNA viruses Recombination with host genes: Recombination can occur in DNA viruses that integrate into host chromosomes or in the DNA provirus step of retrovirus replication. If the host chromosome has previously incorporated pieces of virus DNA, recombination can result in the integration of both host and virus genes into virus genetic material Reassortment of virus segments: In RNA viruses with segmented genomes (e.g., influenza viruses) reassortment of segments among progeny rapidly leads to high levels of genetic variability within populations

recombination, conjugation, transduction and transformation (Griffiths *et al.*, 2005).

Mutation Alteration in the sequence of nucleic acids in the genome of a cell or virus particle is called mutation. There may be either **point mutation** of one base, resulting in misreading of succeeding codon triplets, or **deletion mutation**, where whole segments of genome are removed. Deletion mutants are more likely to occur because they result in changes without redundant genetic material. Frequent mutation may produce antigenic diversity, which may induce recurrent outbreaks of disease in a population that is not immune to the new antigen. Within the same organism, mutation rates can vary for different genetic markers by a factor of 1000. Sites within the genome that frequently mutate are termed 'hot spots'. If these spots code for virulence determinants and antigens in the infectious agent, then the agent can change virulence and antigens frequently. If mutation occurs at sites that are not associated with either virulence or antigenic type, then changes in these two characteristics are rare. Sometimes only one or two mutations suffice to convert a relatively harmless bacterium or virus into a highly virulent form (Rosqvist *et al.*, 1988; Conenello *et al.*, 2007).

Mutation may not only result in changes in pathogenicity and virulence but also change the range of hosts

and vectors. For example, a point mutation in the genome of chikungunya virus has allowed it to extend infection from its recognized mosquito vector, *Aedes aegypti*, to *Ae. albopictus* (Tsetsarkin *et al.*, 2007). This has implications for the geographical range of the infection, which is traditionally limited to the tropics. *Aedes albopictus* is now established in Europe and the Americas (Medlock *et al.*, 2006) and cases of chikungunya virus infection have been identified recently in southern Europe (Enserink, 2007).

The switch from virulence to non-virulence – which is reversible – is sometimes termed **phase variation**. This can occur with a high frequency; approximately one in 10^6 in *Bordetella pertussis* (the cause of whooping cough in children), for example. The phase variation is manifested in changes in colonial characteristics, and similar changes occur in *B. bronchiseptica*, the cause of canine 'kennel cough', (Thrusfield, 1992), in which infections may range from the clinically inapparent to the overt (Bemis *et al.*, 1977; McKiernan *et al.*, 1984; and see 'Gradient of infection' later in this chapter).

Recombination The reassortment of segments of a genome that occurs when two microbes exchange genetic material is termed recombination. Thus, influenza A viruses have a genome that is packaged in each virion (virus particle) as eight strands of RNA. Influenza viruses are divided into groups based on the

structure of two major antigens: the haemagglutinin and neuraminidase (see also Table 20.6). Reassortment between current human and avian strains of the virus (possibly in pigs) is likely to produce recombinants with novel haemagglutinin and neuraminidase combinations (Webster *et al.*, 1992; Wahlgren, 2011). Major changes are referred to as 'shift' and minor changes as 'drift' (Webster *et al.*, 1982). The major changes are responsible for the periodic – approximately decadal – pandemics of influenza in man (Kaplan, 1982; Webster, 1993). (Note that antigenic drift also occurs in trypanosomes, but by a totally different mechanism. The superficial cell membrane is shed to reveal new antigens⁷. The epidemiological result, though, is similar: new antigens, therefore a partially or totally susceptible population.) Recombination also may occur in the orbiviruses (e.g., African horse sickness and bluetongue) where the precise mechanism is not known, and the term 'genetic reassortment' has been applied (Belaganahalli *et al.*, 2015).

Recombination also may be associated with viruses expanding their host range (da Cruz *et al.*, 2013).

Conjugation Conjugation involves transmission of genetic material – usually in the form of a **plasmid**⁸ – from one bacterium to another, by a conjugal mechanism (i.e., they touch) through a sex pilus⁹. The greatest practical effect of conjugation is in conferring resistance to antibiotics in both 'established' and emerging and re-emerging pathogens (McCormick, 1998); for example, gentamycin resistance in *Staphylococcus* spp. (Schaberg *et al.*, 1985), which may pose a particular problem in nosocomial infections¹⁰. Conjugally-mediated drug resistance therefore may be an important determinant of the effectiveness of therapy when infections occur, and there is increasing evidence that some antibiotic-resistant strains of zoonotic bacteria

have evolved in farm livestock (Fey *et al.*, 2000; Will-ems *et al.*, 2000)¹¹. Conjugation occurs in many bacteria, including *Bordetella bronchiseptica*, *E. coli* and *Clostridium*, *Mannheimia* (*Pasteurella*), *Proteus*, *Salmonella*, *Shigella* and *Streptococcus* spp.

Transduction The transfer of a small portion of genome from one bacterium to another, 'accidentally', by a bacterial virus (phage)¹², is labelled transduction. Again, resistance factors, as well as surface antigens, may be transferred in this way. It occurs, for example, in *Shigella*, *Pseudomonas*, *Vibrio* and *Proteus* spp. (Sack *et al.*, 2001). Sometimes, relatively large blocks of genes are required to alter virulence and pathogenicity via both phage and plasmid transfer; these are termed 'pathogenicity islands' (Elwell and Shipley, 1980; Cheetham and Katz, 1995).

Transformation Release of DNA from one bacterial cell and then its entry into another cell of the same bacterial species is called transformation. It occurs spontaneously in *Neisseria gonorrhoeae* (Hamilton and Dillard, 2006) but, to occur in other bacterial species, DNA has to be extracted in the laboratory¹³. (This type of transformation should not be confused with the *in vitro* production of malignant cells, which is also called transformation.)

In addition to these five methods of genetic alteration, infection by more than one type of virus particle may be necessary to produce disease. Such infections do not strictly involve a change in a virus genome, rather a complementation of it, which may render a non-pathogenic virus particle pathogenic. This occurs in some plant virus infections because several plant viruses have split genomes that are packaged in separate particles. Each particle carries a portion of the total genome which is, itself, non-infectious, but which contributes to the whole infectious unit. For example, tobacco rattle virus has two virions: one containing a promoting gene, and the other containing replication and maturation genes. All three genes, and therefore both types of virion, are necessary to instigate the successful infection of a tobacco plant (Sanger, 1968). In

⁷ African trypanosomes can spend a long time in the blood of their mammalian host, where they are exposed to the immune system and are thought to take advantage of it to modulate their own numbers. Their major immunogenic protein is the variant surface glycoprotein (VSG). Trypanosomes escape antibody-mediated destruction through periodic changes of the expressed VSG gene from a repertoire of 1000 genes (Barry and McCulloch, 2001; Gibson, 2001; Vanhamme *et al.*, 2001; Barry and Carrington, 2004; Hall *et al.*, 2013).

⁸ Plasmids are small, autonomously replicating molecules in bacterial cells.

⁹ Plasmids also can be transferred non-conjugally (Storrs *et al.*, 1988).

¹⁰ Nosocomial infections are those that are acquired in hospitals or clinics (Greek: *nosokomeian* = 'hospital'). In human and (probably) veterinary hospitals, the most common are those of the urinary tract, followed by pneumonias, surgical site infections and bacteraemias (Emori and Gaynes, 1993; Greene *et al.*, 2012).

¹¹ There is also evidence that the process is reversible, when in-feed antibiotics are subsequently banned (Ferber, 2002).

¹² There are two types of transduction: **generalized** and **specialized**. In the former, *any* region of the bacterial DNA can be transferred; whereas, in the latter, only certain *specific* parts of the bacterial chromosome can be transferred.

¹³ There also is evidence that damaged environmental DNA can survive for millions of years, and may drive bacterial evolution by natural transformation (Overballe-Petersen *et al.*, 2013).

Increasing severity of disease →

Signs in animal	No signs	No signs (Subclinical disease)	Clinical signs		Death
			(Mild disease)	(Severe disease)	
Type of infection	No infection	Inapparent infection	Overt infection		
Status of animal	Insusceptible or immune	Susceptible			

Fig. 5.6 Gradient of infection: the various responses of an animal to challenge by an infectious agent.

animals, Rous sarcoma virus has capsid proteins that are genetically determined by a separate helper virus (Rubin, 2011). Similarly, some adeno-associated viruses require an adenovirus for infectivity (Berns and Bohezky, 1987).

Infection with immunosuppressive viruses can exacerbate other infections (e.g., rinderpest-infection-aggravated haemoprotozoan infections). Conversely, infection by one virus may prevent infection by, or lessen the virulence of, a second virus. This occurs when the first virus induces the host's cells to release an inhibitory substance known as interferon (Mossman, 2011).

The ways in which virulence and pathogenicity affect the transmission and maintenance of infection are discussed in Chapter 6.

Genotypic variability and microbial taxonomy

Initially, classification of microbes was based on physical and chemical properties; for example, buoyant density and susceptibility to heat inactivation to define genera of the virus family, Picornaviridae, which includes the foot-and-mouth disease virus species. Subsequently, nucleotide sequencing and other microbial characteristics have been used to classify microbes in a manner that may reflect, more accurately, variation in pathogenicity and virulence. Thus, foot-and-mouth disease virus occurs as seven **serotypes** – A, O, C, Southern African Territories (SAT) 1, SAT 2, SAT 3 and Asia 1 – defined by the inability of infection or vaccination to confer immunity against other serotypes. Substantial genetically determined antigenic variation

exists within each serotype (particularly types A and O), and is associated with varying virulence¹⁴.

The genetic variation in microbes also may provide valuable clues to the origin of epidemics. The type O foot-and-mouth disease viruses, for instance, exhibit genetically and geographically distinct evolutionary lineages (**topotypes**) (Samuel and Knowles, 2001; Rweyemamu *et al.*, 2008). Using a 15% nucleotide-sequence difference as the upper limit defining a single topotype, eight topotypes of type O have been identified: European/South American (Euro-SA), Middle East/South Asia (ME-SA), South East Asia (SEA), Cathay, East Africa (EA), West Africa (WA), Indonesia 1 (ISA-1) and Indonesia 2 (ISA-2). The 2001 epidemic in the UK was caused by the PanAsia strain, a genetic sub-lineage of the ME-SA topotype, which emerged in India in 1990 before spreading throughout Asia and into the Middle East, South Africa and southern Europe (Cottam *et al.*, 2006). Identification of this strain therefore supported the hypothesis that meat, illegally imported from the Far East, was the source of the epidemic (DEFRA, 2002b).

Gradient of infection

'Gradient of infection' refers to the variety of responses of an animal to challenge by an infectious agent (Figure 5.6) and therefore represents the combined effect of an agent's pathogenicity and virulence,

¹⁴ The variation is so substantial that each isolate may be unique (Clavijo and Kitching, 2003).

and host characteristics such as susceptibility and pathological and clinical reactions. These responses affect the further availability of the agent to other susceptible animals, and the ability of the veterinarian to detect, and therefore to treat and control, the infection. If an animal is either insusceptible or immune, then infection and significant replication and shedding of an agent do not usually occur and the animal is not important in the transmission of infection to others.

Inapparent (silent) infection

This is infection of a susceptible host without clinical signs¹⁵. The infection may run a similar course to that which produces a clinical case, with replication and shedding of agent. The inapparently infected animal poses a considerable problem to the disease controller because it is impossible to detect the infection without auxiliary diagnostic aids such as antigen detection or serology. For example, sheep may show either no, or transient, clinical signs of infection with foot-and-mouth disease virus (Kitching and Hughes, 2002), although they may excrete virus (Sharma, 1978). Demonstration of infection by serology therefore is recommended in this species (Donaldson, 2000).

Subclinical infection occurs without overt clinical signs. Some authors use this term and inapparent infection synonymously. Others ascribe a loss of productivity to subclinical infection, which is absent from inapparent infection. 'Subclinical' also can be applied to non-infectious conditions, such as hypomagnesaemia, where there may be no clinical signs.

Clinical infection

Clinical infection produces clinical signs. Disease may be **mild**. If the disease is very mild with an illness too indefinite to permit a clinical diagnosis, then it is termed an **abortive reaction**. Thus, the spectrum of response to foot-and-mouth disease virus infection in sheep may result in only mild signs of foot-and-mouth disease (Bolton, 1968; Kitching and Hughes, 2002), leading to difficulty in unequivocal diagnosis (Ayres *et al.*, 2001; de la Rua *et al.*, 2001). Laboratory diagnosis is therefore prudent (and probably necessary) to confirm a diagnosis in this species, and to avoid misclassifying unaffected sheep as affected¹⁶.

¹⁵ Some authorities use the term 'unapparent' synonymously (e.g., Suttmoller and Olascoaga, 2002).

¹⁶ This is particularly important if animals incorrectly classified as diseased on clinical examination alone ('false-positives': see Chapter 20) are subject to mandatory slaughter.

There is a gradation to severe disease, which is called a **frank clinical reaction**, when the intensity is sufficient to allow a clinical diagnosis. The spectrum of response to foot-and-mouth disease virus infection in sheep therefore may also include clear clinical signs, often associated with times of stress such as parturition (Brown, 2002; Reid, 2002; Tyson, 2002).

The severest reaction results in death. Paradoxically, death is the logical climax of some infections because it is the only means by which the agent can be released to infect other animals. An example is infection with *Trichinella spiralis*, which is transmitted exclusively by flesh eating (Campbell, 1983).

Inapparent and mild clinical infections may indicate an adaptation of some antiquity between host and parasite; the relationship between bluetongue virus and indigenous South African sheep already has been cited.

Outcome of infection

Clinical disease may result in the development of a long-standing chronic clinical infection, recovery, or death. Chronically infected cases are potential sources of an infectious agent. Death usually removes an animal as a source of infection, although there are important exceptions such as infection with *T. spiralis*, and anthrax infection where carcasses contaminate the soil. Recovery may result in **sterile immunity** following an effective host response, which removes all of the infectious agent from the body. Animals that have sterile immunity no longer constitute a threat to the susceptible population.

Two states, however, are important determinants:

1. the carrier state;
2. latent infection.

The carrier state

'Carrier' is used loosely to describe several situations. In a broad sense, a carrier is any animal that sheds an infectious agent without demonstrating clinical signs. Thus, an inapparently or subclinically infected animal may be a carrier, and may shed agent, either continuously or intermittently. The periods for which animals are carriers vary, but carriers may be important sources of infection to susceptible animals during these periods. Cattle, for example, are important carriers of haemorrhagic septicaemia, and may introduce the disease into disease-free areas, causing explosive

outbreaks of the disease in the resident cattle population (De Alwis, 1999)¹⁷.

Incubatory carriers are animals that excrete agent during the disease's incubation period. For instance, dogs usually shed rabies virus in their saliva for up to five days before clinical signs of rabies develop (Fox, 1958), and periods as long as 14 days have been reported (Fekadu and Baer, 1980). Thus, in countries in which rabies is endemic, the *World Health Organization* recommends that dogs and cats that have bitten a person should be confined for 10 days; this protocol is designed to determine if the bitten person was exposed to rabies virus.

Convalescent carriers are animals that shed agent when they are recovering from a disease, and the agent may then persist for prolonged periods.

The carrier state in foot-and-mouth disease A carrier of foot-and-mouth disease virus is precisely defined as an animal from which the virus can be isolated from oropharyngeal fluid samples, collected by probang, for periods greater than 28 days after virus challenge (Salt, 1994). It occurs in cattle and sheep following clinical and subclinical disease, the latter commonly following challenge with low titres of virus, and in cattle with partial immunity (Sutmoller *et al.*, 1968), such circumstances occurring in areas in which the disease is endemic. It also can be induced experimentally (Chang *et al.*, 2013).

Persistence also may occur in vaccinated cattle that are subsequently challenged. The oropharynx is the primary site of replication of the virus in cattle, whereas, in sheep, virus is most frequently isolated from the tonsillar region. However, virus titres are low, and decline, falling below the level considered necessary for transmission (Donaldson and Kitching, 1989).

The duration of the carrier state varies between species, with sheep and goats carrying foot-and-mouth disease virus for up to nine months, cattle for up to three years, and the African buffalo for at least five years; whereas pigs do not act as carriers. The frequency of development of the carrier state in nature also appears to be species-related. Thus, up to 50% of cattle have been recorded as carriers for six months

following epidemics (Sutmoller and Gaggero, 1965), whereas the carrier state in sheep and goats has been rarely reported naturally (Anderson *et al.*, 1976; Hancock and Prado, 1993; Donaldson, 2000).

Although virus-laden oropharyngeal fluid from carrier animals can infect cattle and pigs experimentally (van Bekkum, 1973), many experiments have failed to demonstrate transmission from carrier animals to in-contact susceptible animals (Davies, 2002; Sutmoller and Olascoaga, 2002). Similarly, there is a paucity of evidence that carriers can transmit infection in the field, relating largely to African buffalo infected with SAT serotypes, and circumstantial evidence of transmission from carrier domesticated livestock in outbreaks in Zimbabwe due to the SAT 2 serotype (Thomson, 1996).

Collectively, this information suggests that, although the carrier state may be a clearly identified phenomenon, which may be responsible for the generation of new virus variants in the field (Malirat *et al.*, 1994), its role as a determinant of transmission and maintenance of infection during epidemics is uncertain.

Latent infection

A latent infection is one that persists in an animal, and in which there are no overt clinical signs. Thus, the distinction between latency, chronic infection and the carrier state is blurred. Latency may or may not be accompanied by transmission to other susceptible animals. In persistent bacterial infections (e.g., tuberculosis), a balance occurs between host and agent such that the agent replicates, but the disease may not progress for a long time. In virus and rickettsial infections, persistence is not usually associated with demonstrable replication of the agent unless the latter is reactivated. Many examples of virus and rickettsial latency are known (notably in herpesviruses: Burrows, 1977; Arzul *et al.*, 2002), but the role of latency in perpetuating infection in a population, except in a minority of infections such as bovine virus diarrhoea virus infection (Lindberg, 2003), is still unclear.

The likelihood of persistence can depend not only on the particular infectious agent and host species but also on the host's age at the time of infection. For instance, all kittens transplacentally infected with feline leukaemia virus become permanently infected (Jarrett, 1985). However, from eight weeks of age, an increasing proportion of cats resist infection: by four to six months of age only about 15% of naturally infected cats become permanently infected. Latently infected cats do not appear to transmit infection (except possibly female cats to their kittens, in milk).

Unidentified latent infections can be obstacles to the control of disease. For example, the bacterium

¹⁷ A notorious human example is Mary Mallone, an Irish immigrant to New York at the turn of the 19th century, who carried typhoid, and whose poor personal hygiene resulted in her infecting many people while she was working in the catering industry. Despite periods in enforced quarantine, she persisted in working in catering (sometimes changing her name to do so) and became known as 'Typhoid Mary' (Leavitt, 1996). As a lifelong carrier, she was eventually forcibly quarantined for life. (She refused surgical removal of her gall bladder – a major repository of the causal bacterium.)

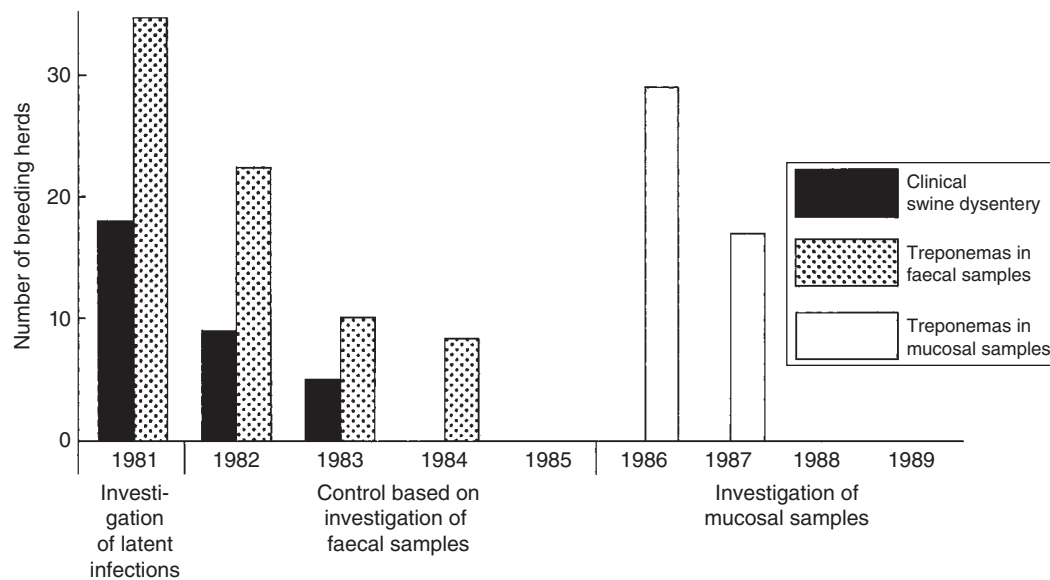


Fig. 5.7 Control and eradication of swine dysentery: Thuringia, Germany, 1981–89. (Adapted from Blaha, 1992.)

Brachyspira (previously *Serpulina*, *Treponema*) *hyodysenteriae*, the cause of swine dysentery, can latently infect pigs. Attempts to control swine dysentery in the Thuringia region of Germany in the 1960s and 1970s were unsuccessful because only pigs in clinically affected herds were treated, and the infection was maintained in latently infected animals. However, when latently infected herds were identified by examining faecal samples for presence of the bacterium, and control measures were instituted in these herds, the disease declined (Figure 5.7). Pathogen-free status was subsequently monitored by examination of mucosal scrapings from the colon.

Microbial colonization of hosts

Infectious agents enter a host at varying times during its life. Some – the vertically-transmitted ones (see Chapter 6) – infect the host before birth. Initial infection may or may not be followed by the immediate development of disease.

Exogenous and endogenous pathogens

The previous description of microbes involved with reproductive failure in pigs illustrates that pathogenic infectious agents can be classified into two groups: **exogenous** and **endogenous** (Dubos, 1965).

The exogenous pathogens are not usually present in the host. They cannot normally survive for a long time in the external environment (soil, water, etc.). They do not usually form persistent relationships

with the host. They are generally acquired by exposure to an infected animal, and usually produce disease with clearly identifiable clinical signs and pathological lesions. Examples include canine distemper, rinderpest and the other traditional animal plagues.

The endogenous pathogens often are found in healthy animals, commonly in the gastrointestinal and respiratory tracts, and usually do not cause disease unless the host is stressed. An example is *E. coli*, which is commonly found in the intestinal tract of calves, and which may cause disease only when a calf is immunodeficient, for example due to deprivation of colostrum (Isaacson *et al.*, 1978).

This twofold classification is somewhat simplistic. Some pathogens possess characteristics of both groups. For example, *Salmonella* spp. usually produce distinct clinical signs when they infect animals. However, some animals can permanently carry and intermittently excrete the agent without showing signs, as carriers. The bacterium also can be transmitted by methods other than direct contact with infected animals; for example, in contaminated food.

Opportunistic pathogens

Some organisms only become pathogenic following perturbations in their host (e.g., immunodeficiency, ageing, prior infection, medication or concurrent disease). Such organisms are **opportunistic**. They may emerge from among endogenous agents, or may be acquired exogenously from the environment. Examples include bacterial infections of surgical

wounds in dogs and cats (Boerlin *et al.*, 2001) and the group 1 pathogens described earlier that can cause prenatal deaths and abortions in pigs.

Zoonoses are also sometimes recognized as opportunists in the sense that they cause disease in one host but regularly exploit other hosts for their maintenance (e.g., rabies acquired in humans from bats and domestic animals).

All opportunistic pathogens therefore can be compartmentalized into environments where they cause disease (e.g., human burn wounds for *Pseudomonas aeruginosa*; humans for rabies) and those where they do not (e.g., the soil for *P. aeruginosa*; bats for rabies) (Brown *et al.*, 2012b).

Environmental determinants

The environment includes location, climate and husbandry. Particular attention has been paid to environmental determinants of disease in livestock enterprises, where intensive production systems expose animals to unnatural environments (e.g., chicken battery houses), and in human medicine, where social and occupational exposure to possible causal factors (e.g., to smoke in relation to lung cancer: see *Table 3.2*) can occur. Moreover, the health and welfare of captive wild animals also may be affected by their environment (Kirkwood, 2003).

Location

Local geological formations, vegetation and climate affect the spatial distribution of both animals and disease. Thus, the incidence of jaw tumours in sheep is associated with the distribution of bracken (McCrea and Head, 1981), and illustrates the value of maps in identifying causes of disease. Non-specific chronic canine pulmonary disease in middle-aged and old dogs has been shown to be associated with urban residence in the US (Reif and Cohen, 1970); urban residence being defined in relation to atmospheric pollution (see Chapter 9). This investigation has been refined by demonstrating an urban/rural gradient in the occurrence of canine pulmonary disease (Reif and Cohen, 1979): an example of the application of the method of concomitant variations (see Chapter 3) in inferring cause. The effects of proximity of livestock to oil and natural-gas sites are equivocal (Waldner *et al.*, 2001b,c; Scott *et al.*, 2003a,b).

Noise is associated with location and may also be considered as being related to 'occupation' and husbandry. The impact of noise on human health has been investigated extensively (Kryter, 1985; Butler

et al., 1999; Goines and Hagler, 2007), but its effect on animal health has not been studied in detail. Most investigations have been conducted on humans and laboratory animals where, apart from the obvious induction of temporary or permanent deafness, noise has been shown to cause a general stress reaction (discussed later in this chapter) with altered secretion of adrenocortical hormones (Castelhano-Carlos and Baumans, 2009). In a review of the effects of noise on animal health, Algers and others (1978) describe leucopenia (which could be associated with immunosuppression), decreased milk production in dairy cows, and oligospermia and an increased incidence of abortion in rats. Studies in horses suggest that impulse noises (sonic booms) have no effect on animal health or behaviour (Ewbank and Cullinan, 1984).

The temporal distribution of disease is also affected by location because of the seasonal effects of climate. These effects, and methods of identifying them, are discussed in Chapter 8.

Climate

Two types of climate can be identified: **macroclimate** and **microclimate**.

Macroclimate

The macroclimate comprises the normal components of weather to which animals are exposed: rainfall, temperature, solar radiation, humidity and wind, all of which can affect health (Webster, 1981). Temperature may be a primary determinant; for example, low temperatures in the induction of hypothermia, to which newborn animals are particularly prone. Low temperatures also have been associated with an increase in arthritic pain in humans (Aikman, 1997). Wind and rain increase heat loss from animals. Cold stress predisposes animals to disease, for example by reducing efficiency of digestion, which may predispose to infectious enteritis. Wind also can carry infectious agents (e.g., foot-and-mouth disease virus) and arthropod vectors (e.g., *Culicoides* spp. infected with bluetongue virus) over long distances (see Chapter 6).

Solar radiation can act as a primary determinant: the carcinogenic effect of solar ultraviolet radiation has already been mentioned in relation to cutaneous squamous cell carcinoma. It is also an important component of the sufficient cause of infectious bovine keratoconjunctivitis, in which the primary determinant is infection with *Moraxella bovis* (Hughes *et al.*, 1965), the ultraviolet radiation 'loosening' the corneal epithelium, thereby facilitating colonization

of the cornea by the bacterium (George, 1993). The bacterium alone only produces mild clinical signs; the classical disease only occurs when high levels of ultraviolet radiation are present. The disease therefore tends to be seasonal, occurring most frequently during summer, and occasionally in winter, when ultraviolet radiation is reflected from fresh snow (Hubbert and Hermann, 1970). Stratospheric ozone depletion, with the associated increase in ultraviolet radiation, may therefore cause an increase in the incidence of this and other diseases, including Uberreiter's syndrome (chronic superficial keratoconjunctivitis) in dogs, and cataracts and skin lesions in farmed fish (Mayer, 1992).

The macroclimate also can affect the stability of infectious agents, in which circumstance it is a secondary determinant¹⁸. Bovine rhinotracheitis virus survives well when humidity is low, whereas rhinoviruses survive when it is high. Porcine transmissible gastroenteritis virus is sensitive to solar ultraviolet radiation, and therefore is more likely to be inactivated in summer than winter. The geographical distribution of *Angiostrongylus vasorum* (the 'French heartworm') appears to be limited by cold (Jeffery *et al.*, 2004). The statistical association between respiratory disease and cool damp weather conditions is probably due to a build-up of pathogens, rather than a reduction in host resistance by climatic stress (Webster, 1981).

Drought can have mixed effects. The one in England and Wales in the summer of 1976, for example, was associated not only with decreased milk yield and decreased weight gain in cattle, and an increase in the prevalence of warble fly infestation in sheep, but also with a beneficial reduction in levels of fascioliasis and parasitic bronchitis (SVS, 1982). Ticks are generally adversely affected by drought. Thus, the resumption of rains following severe drought in East Africa is associated with increased tick burdens in buffalo, and therefore high numbers of *Babesia* spp. A complex interaction between the consequent infection of lions with *Babesia* spp. and immunosuppression induced by coincidental infection with canine distemper virus therefore results in high mortality levels in affected lions following drought (Munson *et al.*, 2008). Mosquitoes are highly adaptable, laying drought-resistant eggs and colonizing alternative water sources, with

consequent increases in mosquito-borne infections following re-wetting of habitats (Brown *et al.*, 2014).

Climatic variation also can induce changes in disease occurrence over vast distances. For example, the epidemic of Rift Valley fever that occurred in East Africa in 1997–1998 was the result of an increase in the size of the mosquito vector population following increased rainfall stemming from the El Niño phenomenon¹⁹ (Brown and Bolin, 2000). Large-scale episodic epidemics and mass mortalities in marine environments since the 1970s have been attributed to climatic changes, resulting in transportation of iron-rich dust from Africa to the Atlantic and Caribbean basin, which favours growth of opportunistic organisms and increased virulence of pathogens (Hayes *et al.*, 2001). Historically, fluctuations in climate in Asia may have been responsible for reintroductions of plague into Western Europe (see Chapters 1 and 4: the Black Death) because of increases in prevalence of the disease in a major Asiatic host, the great gerbil (*Rhombomys opimus*), associated with warm springs and wet summers, and subsequent maritime import into Europe, providing an alternative explanation for the reappearance of the disease than persistence in European rodent reservoirs (see Chapter 7 and Schmid *et al.*, 2015).

A common method of measuring climatic impact is calculation of the **wind-chill index**. This combines the effects of temperature and wind speed, and is especially important at temperatures below freezing, where convective heat losses are magnified by wind. The index can influence the occurrence of conditions such as bovine respiratory disease (Cernicchiaro *et al.*, 2012).

The macroclimate, in conjunction with geological features, determines vegetation and affects the spatial distribution of disease because of the resultant distribution of hosts and vectors; this is discussed in Chapter 7.

Climate change The effects of climate change are currently of general widespread concern²⁰, and one of these is its impact on the frequency and distribution of disease in both humans and animals, which may be either direct (e.g., increasing temperatures or more extreme events) or indirect (e.g., through water shortage, famine and coastal flooding) (McMichael *et al.*,

¹⁸ A tragic historical example of the effect of weather on disease occurrence is the 19th century Irish Potato Famine. A sustained pattern of cool rainy weather in 1845 and 1846 led to the emergence and spread of *Phytophthora infestans*, the fungus-like cause of potato late blight, leading to yield reductions of 40% and 90%, respectively. Over one million people died of starvation, and a further million emigrated, out of a population of eight million (Money, 2007).

¹⁹ El Niño is a disruption of the oceanic–atmospheric system in the tropical Pacific (Philander, 1990). Other related oceanic–climatic phenomena that appear to affect disease occurrence include the Southern Oscillation (Nicholls, 1986), the Indian Ocean Dipole (Bennett, 2009) and the North Atlantic Oscillation (Morand *et al.*, 2013).

²⁰ Tyokumbur (2014) reviews their ecological consequences.

2003; Patz *et al.*, 2005). This impact is not 'unidirectional'; that is, climate change may be responsible for decreases, as well as increases, in disease occurrence.

It has been speculated for some time that global warming, resulting from an increase in the 'greenhouse effect' caused by the high industrial output of carbon dioxide emissions, may be an important determinant (Aitken, 1993). For example, a mean rise in temperature of 2–4°C in Europe would extend the growing season in the UK and the rainfall pattern would alter. Extension of the grazing season could increase the incidence of bovine hypomagnesaemia (see Figure 3.7) and selenium and cobalt deficiency in lambs. A concomitant change to 'softer' grazing with more lush pasture also could lead to an increase in dental problems in sheep.

The distribution and dynamics of biological vectors also may alter, resulting in changes in vector-transmitted diseases; for instance, climate change is expected to increase the risk of bluetongue across much of Europe, not only because of extension of the northern range limit of the traditional vector, *Culicoides imicola*, but also because of extension of transmission beyond this midge to other species of *Culicoides* (Purse *et al.*, 2005; Wilson and Mellor, 2009; Guis *et al.* 2012)²¹. Similarly, louping-ill, Lyme disease and tick-borne fever could become more common as the pattern of activity of the agents' vector, the tick *Ixodes ricinus*, shifts; the elevated temperature increasing the rates of infection and transmission between the different stages of the tick and its hosts (Brownstein *et al.*, 2005; Randolph, 2009). Likewise, the incidence of human malaria is predicted to increase at higher altitudes in the densely-populated highlands of Africa and South America (Siraj *et al.*, 2014).

Recent increases in levels of helminthiasis in the UK (e.g., Kenyon *et al.*, 2009) have been attributed to climate change because survival of free-living stages of helminths is mainly affected by temperature and moisture, and larval development is temperature-dependent (Armour, 1980).

Wildlife also is not immune to climatic adjustments. Warmer temperatures are likely to increase survival of nematodes such as *Parelaphostrongylus tenuis*, for example, which therefore may pose an increased risk

to the North American moose, a species that is already heat stressed and therefore is more likely to succumb to parasites and microbial diseases (see 'Stress' later in this chapter; and Murray *et al.*, 2006; Lenartz *et al.*, 2009).

The influence of climate change on disease also extends beyond the land to the sea and other aquatic environments. Thus, global warming has been targeted as a major factor in the increase in diseases of coral and other marine species (Harvell *et al.*, 1999; Rosenberg and Ben-Haim, 2002). The two- to three-year cycles in coral bleaching reported between 1979 and 1988, for example, may be explained by increased global temperatures modulated by cyclical El Niño phenomena (Williams and Bunkley-Williams 1990). Similarly, anticipated rises in water temperature are likely to increase the occurrence of some diseases of fish (e.g., white spot in salmonids), decrease the frequency of others (e.g., infectious haematopoietic necrosis virus in carp) and increase the geographical range of others (Marcos-López *et al.*, 2010).

Climate change, however, should not be viewed as the sole determinant of changes in the patterns of disease (Lafferty, 2009; Medlock and Leach, 2015), which may include other factors, including social changes (Chaves *et al.*, 2008), urbanization (Alirol *et al.*, 2011) and alterations in land use (Lindblade *et al.*, 2000; Jones *et al.*, 2013; Medlock and Vaux, 2015). Such issues are discussed more fully in Chapter 7.

The impact of climate change on animal disease is discussed further by de la Rocque and others (2008), Baylis and Morse (2012) and Heffernan and her colleagues (2013). Kuhn and others (2005) address the use of climate to predict epidemics of infectious disease, and Rohr and others (2011) elucidate the research that is required to incriminate unequivocally climate change as a determinant of changing patterns of disease.

Microclimate

A microclimate is a climate that occurs in a small, defined space. This may be as small as within a few millimetres of a plant's or an animal's surface or as large as a piggery or calf house. In the former, microclimate may be **terrestrial** (e.g., over the surface of leaves) or **biological** (e.g., over the surface of a host's body). The terrestrial microclimate affects the development of arthropods and helminths. The biological microclimate can change during the course of a disease, assisting in its spread. For instance, sweating during the parasitaemic phase of human malaria increases the humidity of the body's surface and attracts more

²¹ Although the understanding of the dynamics of vector-borne diseases is generally greater than the understanding of other infectious diseases, evidence for the role of climate in changing disease patterns of the former is not always unequivocal (Rogers and Randolph, 2006), being clearer for some diseases (e.g., bluetongue) than for others (e.g., malaria).

Table 5.4 Occupational exposure limits of gases and dust in livestock buildings. (Adapted from Wathes, 1994.)

	Human occupational exposure limits		Animal exposure limits (maximum continuous)
	Long-term (eight-hour time-weighted average)	Short-term (10 min)	
Gases (ppm)			
Ammonia, NH ₃	25	35	20
Carbon dioxide, CO ₂	5 000	15 000	3 000
Carbon monoxide, CO	50	300	10
Formaldehyde, HCHO	20	30	–
Hydrogen sulphide, H ₂ S	10	15	0.5
Methane, CH ₄	Asphyxiant		–
Nitrogen dioxide, NO ₂	3	5	–
Dusts (mg m ⁻³)			
Grain dust*			
Total inhalable fraction	10	–	–
Non-specific dust			
Total inhalable fraction	10	–	3.4 [†]
Respirable fraction	5	–	1.7 [†]

* Maximum exposure time.

† 24-hour time-weighted average.

mosquitoes to the humid skin surface at a time when the protozoon is readily available (Cork, 1996).

The microclimate in intensive animal production units is an important determinant of disease. Stable dust is associated with respiratory hypersensitivity and non-allergic pulmonary disease, and can act as a vehicle for microorganisms (Collins and Algers, 1986; Couëtil, 2009). High levels of ammonia are associated with keratoconjunctivitis in hens (Charles and Payne, 1966) and turbinate atrophy in pigs (Drummond *et al.*, 1981). Poor ventilation is associated with chronic equine respiratory disease (Clarke, 1987). Therefore, adequate ventilation is recommended to remove stale air, microbial aerosols and dust, and to reduce humidity (Wathes *et al.*, 1983; Wathes, 1989). Exposure limits for dust and gases, for both humans and animals in livestock buildings, have been suggested (Table 5.4), noting that levels can vary according to the species of animal being reared and the husbandry system (Barrasa *et al.*, 2012). Although the effects of high concentrations of non-pathogenic airborne bacteria on livestock are unclear, there is evidence (Pritchard *et al.*, 1981) that reduced levels of airborne bacteria are associated with a reduced incidence of clinical and subclinical respiratory disease.

Domestic microclimates also may be determinants. High levels of household humidity, for example, are

related to increased levels of canine mite allergens (Randall *et al.*, 2003).

Husbandry

Housing

The importance of well-designed ventilation in an animal house has already been mentioned. The structure of bedding materials and surfaces is also a determinant. Thus, claw lesions are more common and severe in pigs reared on aluminium slats than in pigs reared on steel or concrete slabs, or on soil (Fritschen, 1979). Limb lesions are more common in pigs reared on concrete than on asphalt-based floors (Kovacs and Beer, 1979); and hoof lesions and vulva biting (associated with aggression) are common problems in group-housed sows (Kroneman *et al.*, 1993). Smith and Straw (2006) suggest that excessive floor slope may predispose to rectal prolapse in pigs because of the increased effect of gravity.

Diet

Diet has obvious effects in diseases caused by energy, protein, vitamin and mineral deficiencies. Sometimes the effects are less clearly defined. There is evidence, for example, that increased dietary levels of biotin reduce the incidence of foot lesions in sows (Penny *et al.*, 1980). However, there is no evidence of an

association between a deficiency of the vitamin and an increased incidence of the lesions.

Feeding regimens may be a determinant. Thus, gastric torsion in sows kept in sow stalls has been associated with once-a-day rather than twice-a-day feeding, which may indicate that the ingestion of a relatively large amount of food is a causal factor (Crossman, 1978).

Management (including animal use)

Management determines stocking density and production policy. Increased densities increase the challenge of microbial pathogens. An internal replacement policy (i.e., maintaining a 'closed' population: see Chapter 4) is less likely to introduce pathogens into an enterprise than a policy involving buying in animals from outside the herd.

The use to which an animal is put (its 'occupation') can affect disease occurrence. Equine limb injuries occur in racehorses, being partly related to race distance and weight of jockey (Pinchbeck *et al.*, 2004b). 'Hump-sore' ('yoke gall': infestation of the skin of cattle by the filarial worm, *Stephanofilaria assamensis*) occurs more frequently in draught zebus than in non-working cattle (Agrawal and Shah, 1986). Apparently sex-related differences in disease occurrence, which are actually related to animal use, have been described earlier.

Stress

No general theory of stress has been universally accepted (Moberg, 1985; Moberg and Mench, 2000), and there has been conflict and confusion over interpretation of the related physiological responses (Becker, 1987). In human medicine, it is used to describe emotional conflicts and displeasure. In veterinary medicine, it is often considered as arising from factors such as weaning, overcrowding, transportation, changes in diet and other environmental factors such as excessive heat (Kick *et al.*, 2012; Lara and Rostagno, 2013). However, in such circumstances, stress may just be a convenient term to describe an area of interest or particular conditions of management, and may mask understanding of underlying principles and mechanisms (Rushen, 1986). A similar vagueness also may be demonstrated in human medicine (Stansfield, 2002).

Stress was initially considered in relation to the physiological 'flight or fight' reaction (Cannon, 1914). Factors such as physical exertion and fear stimulate secretion of catecholamines by the adrenal medulla. These in turn assist muscular exercise by

redistributing blood to the muscles, enabling animals to respond by either 'flight' or 'fight'.

A more comprehensive theory of stress was formulated by Selye (1946), who described the effects of noxious stimuli (e.g., cold, heat, immobilization and severe infections) in laboratory animals. These stimuli were called **stressors**, and their effects were later collectively termed the **general adaptation syndrome**. This was divided into three parts:

1. general alarm reaction;
2. phase of resistance (phase of adaptation);
3. phase of reaction.

Initial responses included a decrease in the size of the liver, lymph nodes, spleen and thymus, and the development of erosions in the digestive tract. The phase of resistance began after approximately 48 hours, and included adrenal enlargement. If this was unsuccessful in dealing with the stimuli, death could occur in the final stage. Selye's focus was on physical stressors, rather than on emotional triggers of stress, the latter being of particular relevance to humans (Jackson, 2013)²².

Selye argued that there was a similar, that is, **non-specific**, response to all stressors, which always involved adrenal activity (e.g., increased secretion of cortisol into the circulation). However, later studies (Mason *et al.*, 1968) demonstrated that the adrenal response is closely related to psychological stimuli, and that it does not occur when some stressors (e.g., fasting) are applied in the absence of psychological stressors. This resulted in the rejection of a non-specific stress response (Mason, 1971). Consequently, the particular stimulus responsible for an observed reaction should be identified; this is particularly important in practical situations (e.g., transportation) where several stimuli are involved. Changes in variables such as plasma cortisol are found in response to a variety of psychological stimuli; some of these may be considered aversive, whereas others may be considered pleasurable. The changes in biological function in response to a stressor can be sufficient to remove the threat. However, when the response is prolonged and extensive, some aspects of biological function may be affected, leaving the animal vulnerable to detrimental effects; for example, endocrine events vital for reproductive success may be disrupted,

²² Participation in epidemic disease control has been identified as a cause of short-term and long-term stress in veterinarians (Nusbaum *et al.*, 2007; Noordman and Endenburg, 2008; Hibi *et al.*, 2015; Makita *et al.*, 2015). Stress is particularly noticeable when killing healthy animals (Rohlf and Bennett, 2005; Whiting and Marion, 2011).

immune function may be altered, resulting in susceptibility to infectious disease, and abnormal behaviour may develop (Moberg, 1985).

There is large variation in how animals respond to stressors. One individual may respond to some stimuli, whereas others may not, and some animals, compared with other animals, may show a completely different type of response to the same stressor. Factors such as experience, genetics and 'coping style' will affect whether or not an animal perceives a stimulus as a stressor, and the type of biological response initiated by the animal in response to the stimulus (Moberg, 1985).

Evidence for the role of stress as a determinant of disease has usually been in the context of particular conditions of management, rather than in relation to a well-understood, unequivocal physiological response. It is in the former context that stress is now discussed.

Stress can be a primary determinant. A notable example is when stress results from the capture of animals, where it can produce a **postcapture myopathy syndrome**. This syndrome is reported in wild animals in South Africa (Basson and Hofmeyer, 1973). It is characterized by ataxia, paresis or paralysis, the production of brown urine, and asymmetrical muscular and myocardial lesions. A similar disease occurs in red deer following capture (McCalum, 1985).

Stress is also a primary determinant of the **porcine stress syndrome** (O'Brien and Ball, 2006). This is the inability of susceptible pigs to tolerate the usual environmental stressors (e.g., castration, vaccination, movement and high ambient temperatures) that are associated with normal management. The syndrome occurs rapidly – often within minutes – after exposure to a stressor. The disease, which is considered to be identical to malignant (fulminant) hyperthermia in humans, dogs, cats, horses and pigs, is characterized initially by muscle and tail tremors. Further stress can produce dyspnoea, cyanosis, increased body temperature and acidosis. The final stage is marked by total collapse, muscle rigidity, hyperthermia and death. The condition is a genetically determined error of metabolism, which induces a switch of energy utilization in muscles of affected pigs from aerobic to anaerobic metabolism. It is inherited through an autosomal recessive gene with high or complete penetrance. Susceptible pigs can be detected by the rapid development of malignant hyperthermia under halothane anaesthesia, and by certain blood-group-linked genes (Archibald and Imlah, 1985).

Stress may be a secondary determinant of several diseases. The immune system can be suppressed by stressors (Dohms and Metz, 1991). Although the significance of such immunosuppression in predisposing animals to infectious disease is unclear (Kick *et al.*, 2012), epidemiological studies have provided evidence that it is functionally relevant²³. Thus, shipping fever (see Chapter 1) is associated with transportation, dehorning, castration and winter weather, and the increased incidence of malignant catarrhal fever and yersiniosis in deer in winter may be due to prolonged exposure to cold and a low plane of nutrition. Stress and related psychosocial factors also may be determinants of cancer (Trainor *et al.*, 2009).

Interaction

Determinants associated with host, agent or environment do not exert their effects in isolation, but **interact** to induce disease. 'Interaction' refers to the interdependent operation of factors to produce, prevent, control, mediate or otherwise influence the occurrence of an event (Porta, 2014). Thus, factors that result in net decreased magnesium intake interact with those that induce net increased loss to produce hypomagnesaemia (see Figure 3.7). Nutritional intake of protein and gastrointestinal parasitism interact because the parasitism induces protein deficiency by increasing the demands for amino acids in the alimentary tract; this, in turn, affects the development and maintenance of immunity, which is sensitive to protein supply (Sykes and Coop, 2001). Bovine alimentary papillomas, caused by a papillomavirus (the agent), can transform to carcinomas in areas where bracken fern (the environment) is common, indicating an interaction between agent and environment (Jarrett, 1980). There is an interaction between a gene (the host) and stressors (the environment), which induces the porcine stress syndrome.

Diseases caused by mixed agents

An important example of interaction between agents is the **mixed infection**, that is, an infection with more than one type of agent. The main infectious

²³ In humans, it has been hypothesized that stress-induced immunosuppression actually may be beneficial by reducing the risk of autoimmune diseases (Råberg *et al.*, 1998).

Table 5.5 Examples of diseases caused by mixed infections. (Adapted and expanded from Rutter, 1982.)

<i>Disease</i>	<i>Classification*</i>	<i>Agents</i>
Enteric disease (most species)	I (?II)	Enterotoxigenic <i>Escherichia coli</i> Rotavirus Coronavirus Calicivirus <i>Cryptosporidium</i> spp.
Respiratory disease (calves)	I	<i>Mannheimia (Pasteurella)</i> spp. Bovine respiratory syncytial virus Parainfluenza-3 virus Bovine virus diarrhoea virus
Atrophic rhinitis (pigs)	I	<i>Bordetella bronchiseptica</i> <i>Pasteurella multocida</i>
Footrot (sheep)	II	<i>Trueperella (Arcanobacterium, Corynebacterium) pyogenes</i> <i>Fusobacterium necrophorum</i> <i>Dichelobacter nodosus</i> Motile fusobacteria
Pneumonia (sheep)	II	Parainfluenza-3 virus <i>Mannheimia (Pasteurella) haemolytica</i>
Swine dysentery (pigs)	II	<i>Treponema hyodysenteriae</i> Gut anaerobes
'Coli septicaemia' (chickens)	II	<i>Escherichia coli</i> Infectious bronchitis virus
Respiratory disease (bovine)	?II	<i>Mycoplasma bovis</i> <i>Mycoplasma dispar</i> Parainfluenza-3 virus Respiratory syncytial virus Infectious bovine rhinotracheitis virus <i>Mannheimia (Pasteurella)</i> spp. Other bacteria
Summer mastitis (bovine)	?II	<i>Trueperella (Arcanobacterium, Corynebacterium) pyogenes</i> <i>Peptococcus indolicus</i> <i>Streptococcus dysgalactiae</i> Micro-aerophilic cocci

* I = Single agents can reproduce clinical signs but mixed infections frequently occur.

II = Mixed infections are essential with cooperative or synergistic interactions.

? = Insufficient evidence for definitive classification.

disease problems in intensive production systems relate to the body surfaces. Common problems are enteric and respiratory diseases and mastitis. These diseases are frequently caused by mixed infections. Two categories of diseases can be identified (Rutter, 1982):

1. diseases in which clinical signs can be reproduced by single agents independently, although mixed infections usually occur in animals;
2. diseases in which two or more microbial components are necessary to induce disease.

Some examples are listed in *Table 5.5*. Category I agents include *E. coli*, rotaviruses, caliciviruses and

Cryptosporidium spp., all of which can induce diarrhoea. Category II agents are exemplified by those that cause calf pneumonia, which include five viruses, four mycoplasmas and 19 bacterial species. The precise mechanism of the interaction is unclear, but investigations in mice (Jakab, 1977) suggest that pulmonary phagocytosis of bacteria may be reduced by virus inhibition of intracellular killing mechanisms in macrophages, and that increased bacterial adherence to virus-infected cells may increase.

Similarly, respiratory disease in calves is often associated with mixed infections with *Mannheimia (Pasteurella)* spp., bovine respiratory syncytial virus, parainfluenza-3 virus and bovine virus diarrhoea

virus (Fulton *et al.*, 2000; Ridpath, 2010). Although the first two viruses can induce respiratory disease when acting individually, bovine virus diarrhoea virus interacts with them and *Mannheimia* spp. through its immunosuppressive effect (Yates, 1982; also see Figure 3.6).

Footrot in sheep also demonstrates interaction. If the interdigital skin of the sheep's foot is either wet or damaged for prolonged periods of time, the common soil bacterium, *Fusobacterium necrophorum*, may invade the tissue. Alone, this bacterium may cause interdigital dermatitis and release necrotic toxins, but this may then allow other bacteria, including *Trueperella* (*Arcanobacterium*, *Corynebacterium*) *pyogenes* and *Dichelobacter nodosus*, to become established, resulting in digestion of the dermis and the characteristic severe lesions of footrot (Green and George, 2008).

Other examples of interactions demonstrated experimentally include intestinal coccidiosis in chickens, which may predispose to necrotic enteritis with the associated proliferation of *Clostridium perfringens* (Shane *et al.*, 1985), and haemorrhagic enteritis virus infection of turkey poults, which increases their susceptibility to colibacillosis (Larsen *et al.*, 1985). A full discussion of microbial interactions is presented by Woolcock (1991), and Fenton (2013) discusses interactions between helminths, bacteria, viruses and protozoa.

Occasionally, mixed infections may lead to interactions that benefit the host. For example, experimental mixed infections with different trypanosome strains result in competition (see Chapter 7) and mutual suppression that prolongs host survival (Balmer *et al.*, 2009), although the significance of this finding to natural infections in cattle and humans is unclear (Gillingwater *et al.*, 2010).

In addition to this general concept of interaction, two specific meanings are attached to interaction, defining **biological** and **statistical** interaction, both of which have been tendentiously redacted (Rothman *et al.*, 2008).

Biological interaction

Biological interaction involves dependence between two factors based on an underlying physical or chemical association and reaction – also therefore termed ‘mechanical interaction’ (Rothman *et al.*, 2008). For instance, there is a chemical interaction between the K88 antigen of *E. coli* (the agent) and receptors in the intestines of some pigs (the host),

described earlier in this chapter, which results in the bacteria that possess the antigen being pathogenic to pigs with the receptors. There appears to be a physical interaction, also mentioned earlier, between the presence of bacteria and poor ventilation in the induction of calf respiratory disease, associated with the density of airborne bacteria. Biological interaction similarly is demonstrated by category II mixed agents. Biological interactions therefore relate to identifiable stages in a causal pathway, and represent many of the known general qualitative interactions²⁴.

Two or more factors also can interact biologically to produce an effect that is greater than that expected of either factor alone; this is **synergism**. An example would be the potentiating effect of combinations of antibiotics. Similarly, there is synergism between the component organisms of ovine footrot, described earlier.

‘Synergism’ should be reserved to describe **biological** mechanisms. In the epidemiological literature, however, synergism also has been used to describe certain types of statistical, rather than biological, interactions, and this has led to some confusion (Kleinbaum *et al.*, 1982). The use of synergism in a statistical context is discussed later in this chapter.

Syndemics

The recent term, **syndemic**, describes the synergistic interaction between two or more coexisting diseases and the resultant excess burden of disease in the population (Singer and Clair, 2003; Singer, 2009). The synergism also involves an interaction between disease and the human social environment (defined earlier in this chapter). For example, co-infection of humans with HIV and *Mycobacterium tuberculosis* reduces survival time, compared with infection with either microbe alone (Dye *et al.*, 1999), and co-infections are concentrated in low-income and ethnic-minority populations (Rose *et al.*, 2002).

‘Syndemic’ thus encompasses the general concept of the multifactorial nature of disease in the specific context of multiple conditions (infectious and non-infectious) and social determinants, and their consequent impact in populations. The term also has been expanded to include connections between

²⁴ Although efforts have been made to explain biological interactions (notably in relation to cancer: e.g., Moolgavkar, 1986; Knox, 2010), proposed mechanisms are unlikely to explain all cases of disease because of unknown factors and biological interactions, noting also that this approach is reductionist and therefore may be unable to offer complete causal explanations (see Chapter 3).

human health and animal health (Rock *et al.*, 2009). Additionally, it can be legitimately extended to include other contexts, such as interaction between factors that influence disease occurrence in human and animal populations within the background of climate change (Heffernan, 2013).

Statistical interaction

Statistical interaction is a quantitative effect involving two or more factors. Often disease occurrence does not depend simply on the presence or absence of a factor; there may be continuous variation in the frequency of occurrence of disease associated with both the strength of a factor (e.g., the frequency with which dairy farm personnel milk cows and infection with *Leptospira* spp.: see Table 3.1) and the number of factors involved. There is often a 'background' frequency of occurrence associated with none of the factors under consideration. When two or more factors are associated with disease, the frequency of disease may be proportional to the occurrence of disease resulting from the *separate* frequencies attributable to each factor (i.e., the frequency when each factor is present singly, minus the background frequency). Alternatively, the frequency may be either in excess of or less than that expected from the *combined* effects of each factor, in which case **statistical interaction** occurs. For example, Willeberg (1976), in his study of the feline urological syndrome (FUS), showed that castration and high levels of dry cat food intake, when present simultaneously, resulted in a frequency of the FUS in excess of that expected from the combined effects of each factor, indicating positive statistical interaction between the two factors.

When several component causes are present simultaneously, their joint effect can be explained quantitatively in terms of two causal models: **additive** and **non-additive** (Kupper and Hogan, 1978). The additive model interprets disease occurrence, when two or more factors are present, as the *sum* of the amount of disease attributable to each factor. If no interaction exists, then, for example:

when X and Y are both absent, suppose 'background' disease occurrence = γ ,
 when cause X is present alone, disease occurrence = $2 + \gamma$,
 when cause Y is present alone, disease occurrence = $5 + \gamma$,
 when X and Y are both present, disease occurrence = $7 + \gamma$.

If positive interaction occurs, then the level of disease occurrence, when X and Y are present, will be greater than $7 + \gamma$.

The commonest non-additive model is the **multiplicative**. This interprets disease occurrence, when two or more factors are present, as the *product* of the amount of disease attributable to each factor. If no interaction exists, then, for example:

when X and Y are both absent, suppose 'background' disease occurrence = δ ,
 when cause X is present alone, disease occurrence = 2δ ,
 when cause Y is present alone, disease occurrence = 5δ ,
 when X and Y are both present, disease occurrence = 10δ .

If positive interaction occurs, then the level of disease occurrence, when X and Y are present, will be greater than 10δ .

Disease occurrence can be measured in terms of incidence (see Chapter 4) or other measures of the risk of disease, such as the relative risk or odds ratio (see Chapter 15). The type of model depends on the means of expressing disease occurrence; for example, a multiplicative model may become additive if log transformation of the measure of occurrence is conducted. The additive model commonly is applied when differences in incidence are used; whereas the multiplicative model is employed when the relative risk or odds ratio are used.

In epidemiology, the additive model is of particular relevance to assessing the impact of a factor on disease occurrence in a population, whereas the multiplicative model has a role in the elucidation of causes (Kleinbaum *et al.*, 1982). When there is evidence of positive interaction based on the additive model, the model has sometimes been described as **synergistic**. However, there are arguable differences between interaction and synergism (Blot and Day, 1979). Evidence of a positive statistical interaction does not necessarily imply a causal relationship. However, if it can be inferred that the factors are part of an aggregate of causes with a common causal pathway, then synergism is said to have occurred (MacMahon, 1972). Synergism, in a statistical context, therefore may be thought of as a positive statistical interaction where a causal pathway may be inferred. Thus, castration and high levels of dry cat food intake (usually associated with overfeeding and sometimes related to insufficient water intake) are synergistically associated in the FUS: both may result in inactivity, thereby reducing blood flow to the kidneys, impairing renal function, and therefore promoting changes in the urine that are conducive to the formation of uroliths.

The value of assessing statistical interaction lies in its ability to identify the *degree* to which various determinants interact. It then may be possible to predict the extent to which disease incidence may be reduced by modification of the determinants. Thus, the value of interaction often lies in its ability to *predict* outcome, rather than to explain biological interaction (Kupper and Hogan, 1978). Statistical interaction is described in more detail in Chapters 15 and 22.

The cause of cancer

The cause of cancer exemplifies interaction between host, agent and environment. The abnormal, unrestricted multiplication of cells produces a tumour. Tumours may be benign, in which growth is restricted, and spread to other parts of the body does not occur, or malignant, in which growth is unrestricted, and spread (metastasis) may occur. Malignant tumours are commonly termed cancers, the word taking its meaning from the zodiacal constellation of the crab, because malignant tumours 'put out' extensions like the limbs of a crab.

The induction of cancer

The mass of cancerous cells that constitutes a malignant tumour originates from a single 'founder' cell, which once was normal, but which has undergone a fundamental change. This change is manifested in several abnormal characteristics such as excessive dependence on anaerobic metabolism and the presence of unusual tumour antigens, in addition to the disregard for normal territorial boundaries, which is a cancer's most obvious characteristic.

For many years, cancer researchers have considered a random model of cancer development. In this, cancer formation is the phenotypic end result of a whole series of changes that may have taken a long period of time to develop. Following an initiation step produced by a cancer-forming agent on a cell, there follows a period of tumour promotion. The initiating step is a rapid step, which affects the genetic material of the cell. If the cell does not repair this damage, then promoting factors may progress the cell toward a malignant phenotype. In contrast to initiation, progression may be a very slow process, and may not even be manifested in the lifetime of the animal.

Over the past four decades, cancer research has generated a rich and complex body of information revealing that cancer is a disease involving dynamic changes in the genome. Each stage of multistage carcinogenesis reflects genetic changes in the cell with a selection advantage that drives the progression towards a highly malignant cell. The age-dependent incidence of cancer

suggests a requirement for between four and seven rate-limiting, random events to produce the malignant phenotype.

More recently, the random model of cancer development has been challenged by a contemporary 'stem cell' model (Kreso and Dick, 2014). In this model, a cancer is driven by a small population of cancer stem cells that have the ability to self-renew. The implication of this is that any new treatments developed would have to be able to target the bulk population of cancer cells as well as this highly resistant population of cells, and that the stem-cell population contributes significantly to the cause of drug resistance.

Epidemiological investigations, conducted over many years, have revealed that microbes (predominantly viruses), as well as chemical and physical agents, can induce cancer²⁵. For example, infection with polyomavirus is linked to cancer in laboratory animals (Weinberg, 1983), raccoons (Dela Cruz *et al.*, 2013) and humans (Chang and Moore, 2012), and human papillomavirus is implicated in cervical cancer (Bosch *et al.*, 2002). There are also well-established relationships between viruses and avian leucosis and feline leukaemia (Teich, 1982). Bacteria also have been linked to cancer; notably, *Helicobacter pylori* in relation to human gastric cancer (Parsonnet, 1995)²⁶; and the helminth, *Schistosoma haematobium*, has been implicated in causing bladder cancer (Gelfand *et al.*, 1967). Moreover, infections may occur jointly to induce cancer (Ewald and Swain Ewald, 2014); for example, Epstein–Barr virus and *Plasmodium falciparum* in relation to Burkitt's lymphoma.

Hydrocarbons in soot were the first chemicals to be incriminated. In 1775, Percivall Pott, a London surgeon, recorded an increased incidence of scrotal epithelioma in chimney sweeps (Brown and Thornton, 1957)²⁷ and, in 1939, Nieberle reported the appearance of ethmoid tumours in sheep living close to industrial plants emitting arsenic, arguably concluding that arsenic was carcinogenic (Cotchin, 1956). Since then, a range of chemical carcinogens has been identified (USDHHS, 2011), including hydrocarbons, aromatic amines (associated with bladder cancer in dyestuff workers), *N*-nitroso compounds (associated with liver

²⁵ Currently, an infectious cause is accepted for about 20% of human cancers (zur Hausen, 2008; de Martel *et al.*, 2012).

²⁶ Before, and after, the discovery of *Mycobacterium tuberculosis* in 1882, cancer and tuberculosis both were considered to be infectious (Onuigbo, 1975), with some cancers being traced from tuberculosis (MacBride, 1772).

²⁷ The clustering of a rare disease in a very specific host population enabled the 'method of difference' to be used in unequivocally inferring causality, before the method was formalised by Mill (see Chapter 3).

cancer in fish, birds and mammals), steroids (e.g., oestrone, inducing mammary cancer in mice), inorganic products such as asbestos (associated with mesothelioma in man; see Chapter 9) and some natural products (e.g., the fungal aflatoxins, which are contaminants of peanut oil, implicated in liver cancer in humans and in fish exposed to contaminated foodstuffs).

'**Co-carcinogen**' is a general term for a factor that furthers the action of a carcinogen, such as chronic inflammation or a chemical promoter. Squamous cell carcinoma of sheep in northern Australia occurs predominantly on the ears, the prevalence increasing with decreasing latitude, and solar ultraviolet radiation has been incriminated as a physical carcinogen. A substantial proportion of the lesions are associated with ear marks or punch holes (Ladds and Entwistle, 1977), supporting a long-established opinion that inflammation associated with ear marks may be a co-carcinogen (Dodd, 1923).

The advent of modern genomics has revealed an extremely wide range of mechanisms underlying the mutational processes of cancer (Garraway and Lander, 2013). Although it was initially believed that the cancer **mutation rate** was relatively constant (approximately 1/Mb), in fact there is marked variation, from <0.1/Mb (in some paediatric cancers) to approximately 100/Mb (in lung cancer and melanoma). Sequencing also has revealed a wide array of mutational patterns within and across specific tumour types. These **mutational spectra** may arise due to extrinsic factors, both physical (e.g., ultraviolet light) and chemical (e.g., tobacco smoke), or intrinsic factors (e.g., DNA repair deficiencies). Cancer cells typically have large-scale chromosomal gains or losses, affecting as much as 10% of their genome. **Chromosomal shattering (chromothripsis)** is believed to occur due to inaccurate chromosome segregation during mitosis. This may result in tens or hundreds of rearrangements localized to one or a few chromosomes. Rearrangements also may take the form of **chromosomal chains**. These are rearrangements consisting of around four to 12 break-point junctions²⁸, with the break-points forming closed chains, which are distributed across multiple chromosomes. Other mutational mechanisms also have been identified (Garraway and Lander, 2013).

Until quite recently, it was believed that there were two main types of cancer-causing genes: **proto-oncogenes** and **tumour suppressing genes (tumour suppressor genes)** (Lee and Yeilding,

2003). Proto-oncogenes code for the products that directly support cell proliferation. These products form a group of cell-regulatory molecules, including growth factors, membrane-associated growth factor receptors, GTP-binding proteins, tyrosine and serine kinases, and transcription factors. These molecules are vital for normal modulation of cell behaviour. When modified (e.g., by mutation) through interaction with chemical carcinogens or ionizing radiation, they acquire the potential to transform cells directly towards the cancerous state. Such modified forms of the proto-oncogenes are called **oncogenes**. Many transforming animal retroviruses contain versions of such oncogenes, presumably acquired during the sojourn of the virus within host cells.

Tumour suppressor genes have the property of restraining growth. In association with proto-oncogenes, such genes exert extremely important roles in physiological cell regulation. One such gene (the retinoblastoma susceptibility gene, *RBI*) regulates cell movement into and around the replication cycle. Another (p53) initiates cell-cycle arrest in response to genotoxic injury, so permitting DNA repair. Others may modulate the passage of signals from cell to cell or from substratum to cell, via adhesion molecules, and so adjust cell behaviour and proliferation in response to prevailing cell density within a tissue. In carcinogenesis, these genes may be silenced, usually by mutation or by acquired loss of part of the chromosome containing the gene. Normal diploid cells contain two copies of each tumour suppressor gene, and in general both must be inactivated to achieve the full change of phenotype that is recognized as cancer. However, these paired events do not necessarily occur at the same time. They may be acquired one after the other through continuous exposure to a carcinogen; alternatively, only one may be acquired, the other being inherited in the germ line. Animals that inherit such defective tumour suppressor genes are clearly at higher risk of acquiring cancer through subsequent exposure to environmental carcinogens, and several well-known inherited cancer-susceptibility syndromes in humans (e.g., familial retinoblastoma and familial polyposis coli) are the result of this type of inherited gene defect. Interestingly, the transforming genes of several animal DNA viruses (e.g., in the papovavirus, papillomavirus and adenovirus groups) appear to act through binding to the products of endogenous tumour suppressor genes.

Recent advances in cancer genomic research have revealed a vast array of new cancer genes. Although some of these encode classical signalling proteins, many cancer genes are now known to be involved in processes not previously associated with cancer,

²⁸ Break-point junctions are regions where 'break-points' (locations on a chromosome where DNA might get deleted, inverted, or swapped around) appear to be near one another in the genome being sequenced, although they are separated on a reference genome.

including, but not limited to, cell signalling; chromatin and epigenomic regulation; RNA slicing; protein homeostasis; metabolism; and lineage maturation. For many of these genes, the ways in which they are involved in cancer remains unknown. Further study of these genes and the ways they link to known, or currently unknown, cancer processes will increase understanding of cancer and may reveal new treatments. Although the myriad of pathways identified as being involved in cancer formation and progression would appear to be confusing, it has been suggested that cancer pathogenesis can be simplified in terms of the acquisition of fundamental characteristics. Hanahan and Weinberg (2000, 2011) suggest that cancer can be defined as the acquisition of eight fundamental characteristics, namely: limitless replicative capacity, independence of anti-growth signals, ability to evade apoptosis, self-sustainable growth signals, ability to promote neoangiogenesis, ability to metastasize, evasion of host immunity and deregulation of cellular energetics (anaerobic metabolism). This is a rapidly evolving area, full discussion of which is beyond the scope of this book; Garraway and Lander (2013) provide an excellent review.

Investigating the cause of cancer

Investigation of the causes of cancer requires collaboration across a wide range of disciplines. Epidemiologists have identified risk factors using observational studies. Two groups of factors are defined (Gopal, 1977):

1. specific causal agents;
2. modifying factors.

Specific physical causal agents that have been incriminated include ultraviolet and ionizing radiation (the latter experimentally inducing thyroid tumours and leukaemia in dogs), chronic irritation (associated with some horn cancers in Indian cattle: Somvanshi, 1991) and parasites (e.g., *Spirocerca lupi* associated with canine oesophageal osteosarcoma and fibrosarcoma: Seibold *et al.*, 1955). Specific chemical and biological initiators, such as viruses, already have been described. Modifying factors are not incriminated as initiators, but in some way affect the incidence of cancer, and include co-carcinogens. The genetic composition of the host is the most important modifying factor, and may be related to the presence of suitable proto-

oncogenes. Some cancers can be hereditary; for example, porcine lymphosarcoma (McTaggart *et al.*, 1979).

Interactions have been demonstrated in which chemical carcinogens enhance the production of tumours *in vitro* by oncogenic viruses. One outstanding example is the interaction between bracken fern and infection with bovine papillomavirus, described earlier in this chapter. Some non-oncogenic viruses also are reported to interact with chemical carcinogens (Martin, 1964). Thus, chickens infected with pox viruses, and mice infected with influenza virus, are more susceptible to chemical carcinogenesis than non-infected animals.

Doll (1977) contended that most cancers have environmental causes as either initiators or promoters. Recently, it has been reasoned that most are due largely to random genetic mutations (Tomasetti and Vogelstein, 2015), but this view has been contested in favour of extrinsic factors (Wu *et al.*, 2016).

Investigation of the cause of cancer therefore involves cooperation between several disciplines: biochemistry, pathology, molecular biology, genetics and epidemiology; and attempts have been made to produce conceptual models that link molecular to epidemiological studies (Moolgavkar, 1986). However, Rothman and others (2008) comment that:

‘Description of a mechanism whereby such [biological] interactions take place does not lead immediately to precise predictions about epidemiologic observations. One reason is that rarely, if ever, is a mechanism proposed that can account for all observed cases of disease, or all effects of all risk factors, measured and unmeasured. Background noise, in the form of unaccounted-for effects and biologic interactions with other factors, can easily obliterate any pattern sought by the investigator.’

The various disciplines should therefore be considered as complementary suppliers of different pieces of the ‘cancer jig-saw puzzle’.

Tumours of domestic animals and some of their causes are reviewed by Meuten (2002) and Baba and Cătoi (2007). Domestic animals also can be useful biological models of human cancer; this topic is discussed in Chapter 9.

Further reading

Altizer, S., Ostfield, R.S., Johnson, P.T.J., Kutz, S. and Harvell, D. (2013) Climate change and infectious diseases: from evidence to a predictive framework. *Science*, **341**, 514–519

Baba, A.I. and Cătoi, C. (2007) *Comparative Oncology*. The Publishing House of the Romanian Academy, Bucharest. (Includes a discussion of carcinogens and carcinogenesis in animals and humans)

- Baylis, M. and Morse, A.P. (2012) Disease, human and animal health and environmental change. In: *The Sage Handbook of Environmental Change*, Vol 2. Eds Matthews, J.A., Bartlein, P.J., Briffa, K.R., Dawson, A.G., de Vernal, A., *et al.*, pp. 387–405. Sage Publications, London
- Benson, J.R. and Liao, S.-S. (2010) The nature and development of cancer. In: *Oxford Textbook of Medicine*, 5th edn. Eds Warrell, D.A., Cox, T.M. and Firth, J.D., pp. 333–357. Oxford University Press, Oxford
- Blancou, J. and Lefèvre, P.-C. (2010) Influence of climate and general considerations on the epidemiology of animal diseases in livestock raised in extensive systems. In: *Infectious and Parasitic Diseases of Livestock*, Vol. 1. Eds Lefèvre, P.-C., Blancou, J., Chermette, R. and Uilenberg, G., pp. 53–61. Lavoisier, Paris
- Clarke, A. (1987) Air hygiene and equine respiratory disease. *In Practice*, **9**, 196–204
- Clayton, D. and McKeigue, P.M. (2001) Epidemiological methods for studying genes and environmental factors in complex diseases. *The Lancet*, **358**, 1356–1360
- Cochran, G.M., Ewald, P.W. and Cochran, K.D. (2000) Infectious causation of disease: an evolutionary perspective. *Perspectives in Biology and Medicine*, **43**, 406–448
- Cox, D.R. (1984) Interaction. *International Statistical Review*, **52**, 1–31
- Darcel, C. (1994) Reflections on viruses and cancer. *Veterinary Research Communications*, **18**, 43–61
- Dennis, M.J. (1986) The effects of temperature and humidity on some animal diseases – a review. *British Veterinary Journal*, **142**, 472–485
- Dobson, A.P. (2009) Climate variability, global change, immunity, and the dynamics of infectious diseases. *Ecology*, **90**, 920–927
- Ekesbo, I. (1988) *Environment and Animal Health*. Proceedings of the 6th International Congress on Animal Hygiene, Skara, Sweden. 2 vols. Swedish University of Agricultural Sciences, Report No. 21
- Engleberg, N.C., DiRita, V.J. and Dermody, T.S. (2013) *Schaechter's Mechanisms of Microbial Disease*, 5th edn. Wolters Kluwer/Lippincott Williams and Wilkins, Baltimore
- Ewald, P.W. (1983) Host-parasite relations, vectors, and the evolution of disease severity. *Annual Review of Ecology and Systematics*, **14**, 465–485
- Furuuchi, S. (1993) Weather and animal diseases. In: *Agricultural Meteorology CAgM Report No. 52, Report of the RA II Working Group on Agricultural Meteorology*, Geneva, January 1993. WMO/TD-No. 524, pp. 1–37. World Meteorological Organization, Geneva
- Gage, K.L., Burkot, T.R., Eisen, R.J. and Hayes, E.B. (2008) Climate and vectorborne diseases. *American Journal of Preventive Medicine*, **35**, 436–450
- Gale, P., Drew, T., Phipps, L.P., David, G. and Wooldridge, M. (2009) The effect of climate change on the occurrence and prevalence of livestock diseases in Britain: a review. *Journal of Applied Microbiology*, **106**, 1409–1423
- Garraway, L.A. and Lander, E.S. (2013) Lessons from the cancer genome. *Cell*, **153**, 17–37
- Gough, A. and Thomas, A. (2010) *Breed Predispositions to Disease in Dogs and Cats*, 2nd edn. Wiley Blackwell, Oxford
- Greene, C.E., Weese, J.S. and Calpin, J.P. (2012) Environmental factors in infectious disease. In: *Infectious Diseases of the Dog and Cat*, 4th edn. Ed. Greene, C.F., pp. 1078–1100. Elsevier Saunders, St Louis
- Hartigan, P.J. (2004) Stress and disease. In: *Bovine Medicine: Diseases and Husbandry of Cattle*, 2nd edn. Eds Andrews, A.H., Blowey, R.W., Boyd, H. and Eddy, R.G., pp. 1133–1148. Blackwell Science, Oxford
- Heffernan, C., Salman, M. and York, L. (2013) Livestock infectious disease and climate change: a review of selected literature. In: *Animal Science Reviews 2012*. Ed. Hemming, D., pp. 19–44. CAB International, Wallingford
- Hoberg, E.P. and Brooks, D.R. (2007) How will global climate change affect parasite-host assemblages? *Trends in Parasitology*, **23**, 571–574
- Jovanović, S., Savić, M. and Živković, D. (2009) Genetic variation in disease resistance among farm animals. *Biotechnology in Animal Husbandry*, **25**, 339–347
- Lactera, N., Bernabucci, U., Khalifa, H.H., Ronchi, B. and Nardoni, A. (Eds) (2003) *Interactions between Climate and Animal Production*. Wageningen Academic Publishers, Wageningen
- Lafferty, K.D. (2009) The ecology of climate change and infectious diseases. *Ecology*, **90**, 888–900
- Lindahl, J.F. and Grace, D. (2015) The consequences of human actions on risks for infectious diseases: a review. *Infection Ecology and Epidemiology*, **5**, 30048; doi 10.3402/iee.v5.30048
- MacMahon, B. (1972) Concepts of multiple factors. In: *Multiple Factors in the Causation of Environmentally Induced Disease*. Fogarty International Center Proceedings No. 12. Eds Lee, D.H.K. and Kotkin, P., pp. 1–12. Academic Press, New York and London
- Madwell, B.R. and Theilen, G.H. (1987) Etiology of cancer in animals. In: *Veterinary Cancer Medicine*, 2nd edn. Eds Theilen, G.H. and Madwell, B.R., pp. 13–26. Lea and Febiger, Philadelphia
- McMichael, A.J., Woodruff, R.E. and Hales, S. (2006) Climate change and human health: present and future risks. *The Lancet*, **367**, 859–869
- Meier, H. (Ed.) (1963) Epizootiology of cancer in animals. *Annals of the New York Academy of Sciences*, **108**, 617–1326

- Mims, C.A., Nash, A. and Stephen, J. (2000) *Mims' Pathogenesis of Infectious Disease*, 5th edn. Academic Press, London
- Modiano, J.F., Henry, C.J., and Macy, D.W. (2013) The etiology of cancer. In: *Withrow & MacEwan's Small Animal Clinical Oncology*, 5th edn. Eds Withrow, S.J., Vail, D.M. and Page, R.L., pp. 1–29. Elsevier Saunders, St Louis
- Morrison, W.B. (1998) Environmental causes of naturally occurring cancer in dogs and cats. In: *Cancer in Dogs and Cats: Medical and Surgical Management*, Ed. Morrison, W.B., pp. 31–41. Williams and Wilkins, Baltimore
- Mukherjee, S. (2011) *The Emperor of All Maladies*. Fourth Estate, London. (A history of cancer investigation, including identification of its causes)
- Muller, M. and Brem, G. (Coordinators) (1998) Genetic resistance to animal diseases. *Revue Scientifique et Technique, Office International des Epizooties*, **17**, 1–378
- Pascual, M. and Bouma, M.J. (2009) Do rising temperatures matter? *Ecology*, **90**, 906–912
- Patterson, D.F. (1993) Understanding and controlling inherited diseases in dogs and cats. *Tidjschrift voor Diegeneeskunde*, **118**, Suppl. 1, 23S–27S
- Philips, C. and Piggins, D. (Eds) (1992) *Farm Animals and the Environment*. CAB International, Wallingford. (Includes discussions of environment-dependent diseases, stress and the effects of ventilation on health and performance)
- Pinto, J., Bonacic, C., Hamilton-West, C., Romero, J. and Lubroth, J. (2008) Climate change and animal diseases in South America. *Revue Scientifique et Technique, Office International des Epizooties*, **27**, 599–613
- Reif, J.S. (1983) Ecologic factors and disease. In: *Textbook of Veterinary Internal Medicine. Volume 1: Diseases of the Dog and Cat*, 2nd edn. Ed. Ettinger, S.Y., pp. 147–173. W.B. Saunders Company, Philadelphia
- Relman, D.A. and Falkow, S. (2015) A molecular perspective of microbial pathogenicity. In: *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 8th edn, Vol. 1. Eds Bennett, J.E., Dolin, R. and Blaser, M.J., pp. 1–10. Elsevier Saunders, Philadelphia
- Rosenthal, J. (2009) Climate change and the geographic distribution of infectious diseases. *Ecohealth*, **6**, 489–495
- Sainsbury, D.W.B. (1991) Environmental factors affecting susceptibility to disease. In: *Microbiology of Animals and Animal Products*. World Animal Science, A6. Ed. Woolcock, J.B., pp. 77–94. Elsevier, Amsterdam
- Sainsbury, D.W.B. (1998) *Animal Health*, 2nd edn. Blackwell Science, Oxford. (A concise account of factors affecting the health of livestock in intensive production systems)
- Schottenfeld, D. and Fraumeni, J.F. (Eds) (2006) *Cancer Epidemiology and Prevention*, 3rd edn. Oxford University Press, New York
- Semenza, J.C. and Menne, B. (2009) Climate change and infectious diseases in Europe. *The Lancet Infectious Diseases*, **9**, 365–375
- Singh, S.K. (Ed.) (2014) *Viral Infections and Global Change*. John Wiley and Sons, Hoboken. (Includes a discussion of the effects of climate change on the occurrence of virus infections)
- Swerdlow, A.J., Peto, R. and Doll, R. (2010) Epidemiology of cancer. In: *Oxford Textbook of Medicine*, 5th edn. Eds Warrell, D.A., Cox, T.M. and Firth, J.D., pp. 299–332. Oxford University Press, Oxford. (A mainly medical discussion, which also includes general principles)
- Wathes, C.M. (1992) Ventilation. In: *Farm Animals and the Environment*. Eds Phillips, C. and Piggins, D., pp. 83–91. CAB International, Wallingford. (A discussion of the biological consequences of ventilation, including its effects on health and performance)
- Wathes, C.M. and Charles, D.R. (Eds) (1994) *Livestock Housing*. CAB International, Wallingford. (A broad review of the housing requirements of production species in relation to health)

6

The transmission and maintenance of infection

Infectious disease is the result of the invasion of a host by a pathogenic organism. The continued survival of infectious agents, with or without the induction of disease, depends on their successful transmission to a susceptible host, the instigation of an infection therein and replication of the agents to maintain the cycle of infection. The complete cycle of infection of an infectious agent is its **life-cycle**. A knowledge of the life-cycle of an infectious agent is essential when selecting the most applicable control technique (see Chapter 27). This involves knowledge of:

- the modes of transmission and maintenance of infection;
- the ecological conditions that favour the survival and transmission of infectious agents.

This chapter is concerned with the first topic; the second is considered in Chapter 7 with reference to basic ecology.

Transmission may be either **horizontal (lateral)** or **vertical**. Horizontally-transmitted infections are those transmitted from any segment of a population to another; for example, influenza virus from one horse to a stable-mate. Vertically-transmitted infections are transmitted from one generation to the next by infection of the embryo or fetus while *in utero* (in mammals) or *in ovo* (in birds, reptiles, amphibians, fish and arthropods). Transmission by milk to offspring also is considered, by some, to be vertical¹.

Horizontal transmission

Infections can be transmitted horizontally either **directly** or **indirectly** (Figure 6.1).

Direct transmission occurs when a susceptible host contracts an infection, either by physical contact with

an infected host or by contact with the latter's infected discharges (e.g., the transmission of canine distemper in infected urine and faeces).

Indirect transmission involves an intermediate vehicle, living or inanimate, that transmits infection between infected and susceptible hosts. This vehicle generally may be termed a **vector**, although the term is usually restricted, by common usage, to living carriers (see 'Vector' following). Indirect transmission can involve a vector of a different species from that of the initially infected host. The life-cycle of infectious agents therefore may be complex, with several different hosts. Details of specific life-cycles are not presented in this chapter, but a basic knowledge of veterinary microbiology and parasitology is assumed.

Airborne transmission of infectious agents, frequently over long distances, is often defined as indirect, although it is more correctly classified as direct because no intermediate vehicle is involved.

Types of host and vector

A variety of terms describe the range of host/parasite relationships, and are used by the epidemiologist, protozoologist, entomologist, helminthologist and microbiologist. Each of these may use terms specific to his or her discipline that have the same general meaning, from the point of view of the life-cycle of the disease, as different words from other disciplines; for example, **intermediate host** in helminthology and **biological vector** in entomology (see later in this chapter).

Hosts

Host A plant, animal or arthropod that is capable of being infected with, and therefore giving sustenance to, an infectious agent. Replication or development of the agent usually occurs in the host.

Definitive host A parasitological term describing a host in which an organism undergoes its sexual phase

¹ The term, 'pseudovertical', also is used to refer to transmission through suckling, as well as to transmission via contaminated egg surfaces or fluids (Wilkinson, 1999).

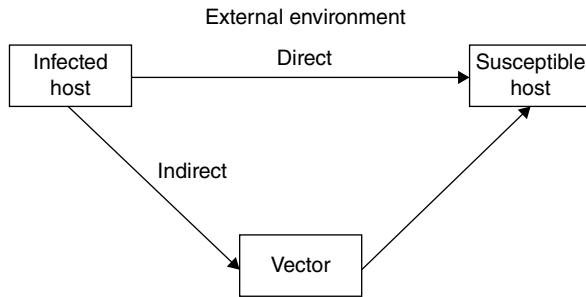


Fig. 6.1 Basic mechanisms of transmission of infectious agents.

of reproduction (e.g., *Taenia pisiformis* in dogs; *Plasmodium* spp. in mosquitoes).

Final host A term used in a more general sense (i.e., in connection with all types of infectious agent) as a synonym for **definitive host**. Both 'final' and 'definitive' imply the 'end of the line'; in other words, the termination of a dynamic process. They are, in most cases, therefore improperly used.

Primary (natural) host An animal that maintains an infection in the latter's endemic area (e.g., dogs infected with distemper virus). Since an infectious agent frequently depends upon a primary host for its long-term existence, the host also is called a **maintenance host**. Wildlife acting as such hosts may be sources of infection to domestic livestock (e.g., wildebeest as sources of infection with malignant catarrh for cattle).

Secondary (aberrant) host A species that additionally is involved in the life-cycle of an agent, especially outside typical endemic areas (e.g., cattle infected with strains of foot-and-mouth virus that usually cycle in buffaloes²). A secondary host sometimes can act as a maintenance host³.

² Transmission of infections between wildlife hosts and domesticated livestock can be variable. Thus, African buffalo infected with foot-and-mouth disease are responsible for outbreaks in domestic livestock in South Africa (Vosloo *et al.*, 2002), whereas the virus appears to cycle separately in buffalo and cattle populations in East Africa (Wekesa *et al.*, 2015).

³ The distinction between primary and secondary hosts is important in relation to microbial evolution. For instance, although influenza viruses can infect a wide variety of birds and mammals, the primary hosts are wild waterfowl, shorebirds and gulls. The evolutionary rate of influenza virus in the primary hosts is believed to be slow, while in mammals, chickens and turkeys the rate is much higher (Suarez, 2000; Chen and Holmes, 2006). The higher rate of evolution in mammals is thought to be a result of selective pressure on the virus to adapt to an aberrant host species.

Paratenic host A host in which an agent is transferred **mechanically**, without further development (e.g., fish, containing *Diphyllbothrium* spp. larvae, which are preyed upon by larger fish). This term is exclusive to helminthology, and could be considered to have its entomological analogue in the term **mechanical vector**.

Intermediate host An animal in which an infectious agent undergoes some development, frequently with **asexual reproduction** (e.g., *Cysticercus pisiformis* in rabbits and hares). This term is parasitological in origin.

Amplifier host An animal which, because of temporally-associated changes in population dynamics that produce a sudden increase in the host population size, may suddenly increase the amount of infectious agent. Multiplication of the agent occurs in this type of host. This term is most commonly used in relation to virus diseases. Examples are pigs and wild birds infected with Japanese encephalitis virus (Figure 6.2) and transmission cycles of West Nile virus between birds and ornithophilic mosquitoes (Jourdain *et al.*, 2008).

Hibernating host An animal in which an agent is held, probably without replication, in a state of 'suspended animation' (e.g., hibernating snakes infected with either Western, Eastern or Japanese encephalitis virus).

Incidental (dead-end or accidental) host One that does not usually transmit an infectious agent to other animals (e.g., bulls infected with *Brucella abortus*). 'Final' and 'definitive' can be applied validly to this type of host.

Link host A host that forms a link between other host species (e.g., pigs linking infected herons to man in Japanese encephalitis: Figure 6.2).

Reservoir A term commonly used as a synonym for, or prefix to, 'host' (**reservoir host**). A reservoir host is one in which an infectious agent normally lives and multiplies, and therefore is a common source of infection to other animals; thus, it is frequently a primary host. Reservoirs should be defined in relation to specific 'target' populations (Haydon *et al.*, 2002). In Kenya, for example, buffalo and waterbuck are reservoirs of bovine ephemeral fever virus, acting as a source of infection for cattle (Davies *et al.*, 1975). Similarly, cattle are reservoirs of bluetongue virus, and therefore can be sources of infection for sheep (Schwartz-Cornil *et al.*, 2008).

Animals may be important reservoirs of infection for humans. For example, in West Africa, the multimammate mouse, *Mastomys natalensis*, is the primary

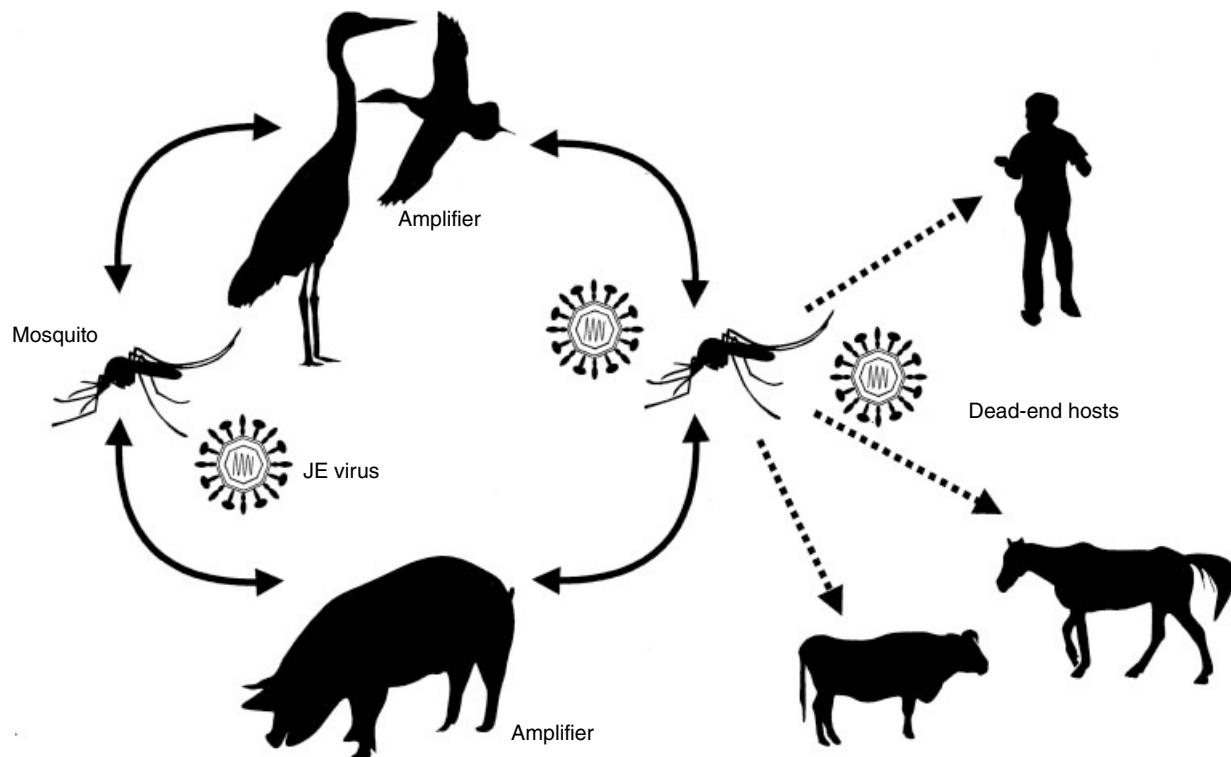


Fig. 6.2 Transmission cycle of Japanese encephalitis virus between amplifiers (pigs and wild birds) and mosquito vectors (especially *Culex tritaeniorhynchus*), including infection of dead-end hosts (humans, horses and cattle). (Source: Morita, *et al.*, 2015. Reproduced with permission of the World Organisation for Animal Health (OIE).)

host and reservoir of Lassa fever, a virus disease with a high case fatality in humans (Lecompte *et al.*, 2006). The mouse is adapted to life both within houses and in fields, and comes into contact with people in rural areas, particularly during the wet season, when it may seek shelter in houses and thus may transmit the infection to humans. Likewise, birds are reservoirs of Eastern equine encephalitis, and so farmed birds can be used as 'sentinels' for surveillance of the infection (see Chapter 21). Bats, too, harbour a range of viruses, including Hendra virus, Nipah virus, severe acute respiratory syndrome (SARS) and lyssaviruses, their long life-span relative to their size possibly facilitating circulation of viruses in bat colonies (Calisher *et al.*, 2006), their higher body temperature during flight possibly being associated with a better-adapted immune response (O'Shea *et al.*, 2014), and the relatively large number of sympatric species (see Chapter 7) promoting inter-species transmission (Luis *et al.*, 2013). Reservoirs may be primary or secondary hosts.

'Reservoir' also is used to refer to any substance that is a common source of infection (e.g., soil as a source of anthrax spores).

Vector An animate transmitter of infectious agents. By common usage, vectors are defined as invertebrate animals – usually arthropods – that transmit infectious agents to vertebrates. The dictionary definition of the term implies independent movement; that is, a living vehicle. Inanimate carriers of agents (e.g., feed concentrates contaminated with *Salmonella* spp.) usually are called 'fomites' (singular: fomes, from the Greek meaning 'tinder'; because fomites were thought metaphorically to be the 'tinder' by which the 'fire' of an epidemic was ignited).

Mechanical vector An animal (usually an arthropod) that physically carries an infectious agent to its primary or secondary host (e.g., mosquitoes and fleas transmitting myxomatosis virus between rabbits). The infectious agent neither multiplies nor develops in the mechanical vector.

Biological vector A vector (usually an arthropod) in which an infectious agent undergoes either a necessary part of its life-cycle, or multiplication, before transmission to the natural or secondary host.

Table 6.1 Susceptibility of vertebrate hosts to Rift Valley fever virus. (From Animal Health Australia, 2016. *Disease Strategy: Rift Valley fever (Version 4.0)*. Australian Veterinary Emergency Plan (AUSVETPLAN), Edition 4. National Biosecurity Committee, Canberra.)

<i>Extremely susceptible</i> (70–100% mortality)	<i>Highly susceptible</i> (20–70% mortality)	<i>Moderately susceptible</i> (<10% mortality)	<i>Resistant (inapparent infection)</i>	<i>Refractory (not susceptible)</i>
Newborn lambs	Calves	Adult cattle	Equines	Birds
Newborn kids	Adult sheep	Adult goats	Pigs	Reptiles
Puppies	Certain rodents	Camels	Adult dogs	Amphibians
Kittens		Buffalo	Adult cats	
Mice		Asian monkeys	African monkeys	
Hamsters		South American monkeys	Guinea pigs	
Certain other rodents		Humans	Rabbits	
			Certain other rodents	

Three types of biological transmission occur:

1. **developmental transmission:** with an essential phase of development occurring in the vector (e.g., *Dirofilaria immitis* in mosquitoes);
2. **propagative transmission:** when the agent multiplies in the vector (e.g., louping ill virus in ixodid ticks);
3. **cyclopropagative transmission:** a combination of 1 and 2 (e.g., *Babesia* spp. in ticks).

Development in the vector involves migration of the infectious agent. Thus, two types of transmission are identified in the life-cycles of members of the protozoan genus *Trypanosoma* (Maudlin *et al.*, 2004). The African trypanosomes that parasitize the blood and tissues of infected animals are ingested by insects of the genus *Glossina*, in which they undergo a developmental cycle that involves migration from their initial focus of infection in the midgut, then back to the salivary glands, from which infective forms are released; this is **salivarian** transmission. In contrast, members of the species *T. cruzi* (the cause of Chagas' disease in humans, dogs and cats in South America, with wild animals implicated as reservoirs) are ingested by Triatomid bugs, from which infective forms are shed in the faeces, human infection occurring by contamination of wounds and the eyes, and infection in dogs and cats also by ingestion of the bugs; this is **stercorarian** transmission.

Biological vectors are frequently either definitive or intermediate hosts; for example, mosquitoes are biological vectors and the definitive hosts of *Plasmodium* spp. (the cause of malaria).

Factors associated with the spread of infection

Three factors are important in the transmission of infection (Gordon Smith, 1982):

1. characteristics of hosts;
2. characteristics of pathogens;
3. effective contact.

Characteristics of hosts

A host's **susceptibility** (**vulnerability**) and **infectiousness** determine its ability to transmit infection. Susceptibility to infection may be limited to a single species or group of species. For example, only equids are naturally susceptible to equine rhinopneumonitis virus infection. Alternatively, several widely different species may be susceptible to an infection (*Table 6.1*).

Susceptibility within a species may vary markedly and may be associated with age (*Table 6.1*) or selection of genetically-resistant animals following exposure to an infectious agent; for example, the mortality in rabbits, exposed experimentally to a standard dose of myxomatosis, fell from 90% to 25% over a seven-year period (Fenner and Ratcliffe, 1965).

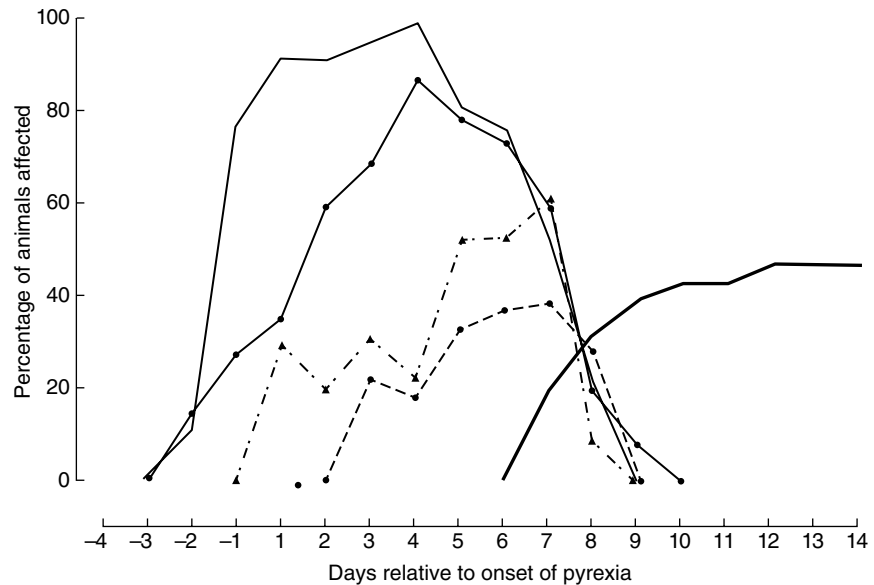
'Infectiousness' refers to:

- the duration of the period when an animal is infective;
- the relative amount of an infectious agent that an animal can transmit.

An animal is not infectious as soon as it is infected – a period of time lapses between infection and the shedding of the agent; this is a parasite's **prepatent** period, a virus's **eclipse phase** and a bacterium's **latent period**⁴. In contrast, the **incubation period** is the period of time between infection and the development

⁴ 'Latent period' also is used for this time interval for infectious diseases in general. For non-infectious diseases, it is the interval between exposure to a cause and appearance of manifestations of disease. The latent period of canine bladder cancer, for example, is approximately four years (see Chapter 9).

Fig. 6.3 Correlation of viraemia and virus excretion in cattle infected experimentally with virulent rinderpest virus (strain RGK/1: — viraemia; ●—● nasal excretion of virus; ▲—▲ urinary excretion of virus; ○—○ faecal excretion of virus; — mortality. (Source: Liess and Plowright, 1964. Reproduced with permission of Cambridge University Press.)



of clinical signs. Thus, inapparent infections have a prepatent period, but do not have an incubation period. The **generation time** is the period between infection and maximum infectiousness. This is considered by some authorities (Porta, 2014) to be synonymous with the **serial interval**: the period of time between analogous phases of an infectious illness (e.g., infection, onset of clinical signs) in successive cases of a chain of infection spread from animal to animal. These periods, for a given agent and host species, are not the same for all animals, but show natural variation. The frequency distribution of incubation periods, for example, often follows a lognormal statistical distribution (see Figure 12.4 and Sartwell, 1950, 1966; Armenian and Lilienfeld, 1983)⁵.

Figure 6.3 plots the excretion of rinderpest virus in a group of experimentally infected cattle. It illustrates that nasal excretion is the most common form of shedding of the virus, that virus is shed *before* the appearance of clinical signs, and that, for *the group*, the period of maximum infectiousness is four days after the onset of clinical signs (i.e., pyrexia).

Diseases with short incubation periods run a clinical course, terminating in either recovery or death, relatively quickly. Thus, a high host density is required to ensure that the agent's life-cycle can be perpetuated (Figure 6.4). An example is distemper virus infection of dogs with an incubation period of four to five days. This disease therefore is endemic only in urban areas where there is a high density of dogs. In contrast, infectious diseases with long incubation periods can

		Characteristics of host population		
		Low density	Mixed or changing density	High density
Characteristics of infectious agents	Short incubation period	□	■	■ ■ ■ ■ ■ ■ ■ ■ ■ ■
	Mixed incubation period	■ ■ ■ ■	■ ■ ■ ■ ■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■ ■ ■ ■ ■
	Long incubation period	■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■

Fig. 6.4 The relationship between duration of incubation period of an infectious agent, density of the host, and the potential of the infectious agent to exist in a population. □: conditions unfavourable for the existence of infectious agent; ■: conditions favourable for the existence of infectious agent; the number of solid squares indicates the relative degree to which the conditions are favourable. (Adapted from Macdonald and Bacon, 1980.)

maintain their cycles of infection in varying animal densities (Figure 6.4); rabies is an example.

The time between infection and availability of an infectious agent in an arthropod vector is the agent's **extrinsic incubation period**.

For transmission to occur between a vertebrate host and an arthropod vector, an infectious agent must be present to a minimum concentration in the vertebrate host's circulation. This is the **threshold level**. Some vertebrate hosts may become infected, but are unable

⁵ Other distributions also have been explored (Nishiura, 2007).

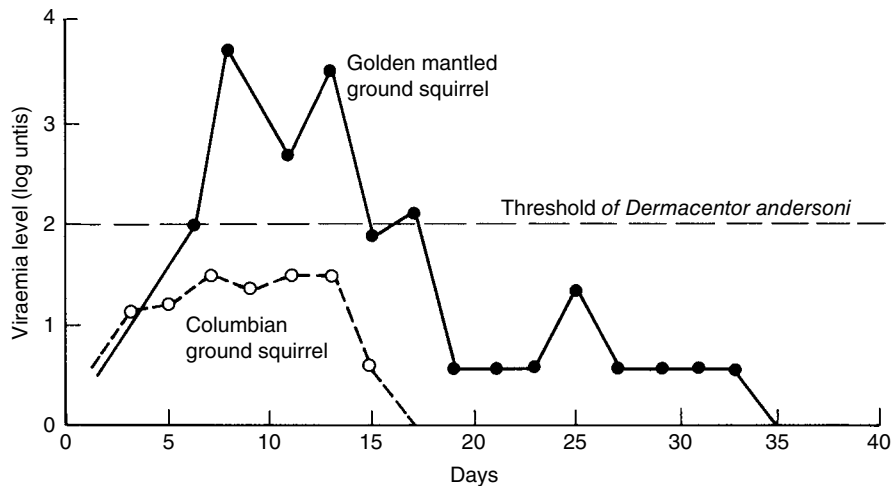


Fig. 6.5 Colorado tick fever: the relationships between viraemia levels in a maintenance host (the golden mantled ground squirrel) and an accidental host (the Columbian ground squirrel) and the threshold of infection of the arthropod maintenance host (*Dermacentor andersoni*). (Source: Gordon Smith, C.E. (1976) *Epidemiology and Infections*. Meadowfield, Shildon. After Burgdorfer, 1960.)

to transmit infection to arthropods because the threshold level is not achieved. These are therefore incidental hosts. For example (Figure 6.5), the Columbian ground squirrel is an incidental host for Colorado tick fever. However, when the virus infects and multiplies in the golden mantled ground squirrel, the threshold level is attained; therefore the vector, the tick *Dermacentor andersoni*, can ingest virus particles, and this squirrel can act as a maintenance host.

Characteristics of pathogens

Three important characteristics of pathogens that affect transmission of infectious agents are **infectivity**, **virulence** and **stability**.

Infectivity relates to the amount of an organism that is required to initiate infection. The infectivity of different organisms varies considerably. For example, the **particle:infectivity ratio** (the number of virus particles required to instigate infection) of bacterial viruses (bacteriophages) in tissue culture is approximately 1:1, indicating a high degree of infectivity. Lower degrees of infectivity are demonstrated by animal viruses, with ratios of between 10:1 and 100:1. Plant viruses have an even lower infectivity, with a ratio of approximately 1000:1. Infectivity can vary between different strains of the same organism, and can depend upon the route of the infection, and the age and innate resistance of the host.

When an agent is capable of infecting more than one species, its infectivity for different hosts is often quite different. For instance, the infective dose of strains of *Campylobacter jejuni* isolated from chickens is only 500 bacteria for chickens, whereas the infective dose of strains of the same bacterium isolated from seagulls is above 10^7 for chickens. This example illustrates that

the infectivity of an agent cannot be specified without reference to the host that it infects.

Virulence (see Chapter 5) also affects transmission and can change. Repeated passage through the same species of animal tends to increase virulence for that species but to simultaneously lower virulence for the original natural host. Thus, serial passage of Ross River virus in suckling mice increases its virulence for mice (Taylor and Marshall, 1975), but alternate passage in mice and the mosquito *Aedes aegypti* does not alter virulence. In contrast, when Edwards (1928) passaged rinderpest virus of bovine origin serially through several hundred goats, its virulence for cattle dramatically decreased, enabling production of the first veterinary-modified live-virus vaccine.

The length of time for which an organism can remain infective outside its host is the organism's **stability**. Some organisms survive only for short periods of time; that is, they are very **labile** (e.g., *Leptospira* spp. in dry environments), whereas others are more resistant (Table 6.2). Stability is frequently facilitated by protective capsules, such as those forming the outer layer of bacterial spores (e.g., *Bacillus anthracis*). The hazards presented to infectious agents by the external environment, and techniques of achieving stability, are discussed later in this chapter.

Effective contact

Effective contact describes the conditions under which infection is likely to occur. For a particular infection it depends on the stability of the organism and the routes by which the organism leaves an infected host and enters a susceptible one.

Effective contact may be very short (e.g., seasonally-transmitted, vector-borne diseases) or potentially of

Table 6.2 The effect of temperature and pH on time for 90% foot-and-mouth disease virus inactivation. (From Pharo, 2002a.)

Effect of temperature (at pH 7.5)		Effect of pH (at 4°C)	
Temperature	Inactivation time (90%)	pH	Inactivation time (90%)
61°C	30 seconds	10.0	14 hours
55°C	2 minutes	9.0	1 week
49°C	1 hour	8.0	3 weeks
43°C	7 hours	7.0–7.5	>5 weeks
37°C	21 hours	6.5	14 hours
20°C	11 days	6.0	1 minute
4°C	18 weeks	5.0	1 second

many years' duration (e.g., anthrax spores in soil: Dragon and Rennie, 1995). The duration of infectiousness determines the number of susceptibles that can be infected by an infected animal. Thus, upper respiratory tract infections (e.g., kennel cough in dogs) result in short periods of infectiousness of several days' duration, whereas cows infected with bovine tuberculosis may excrete the bacterium in their milk for several years.

Behaviour, which may be changed during infection⁶, also can affect the likelihood of effective contact. Thus, wild animals that are naturally shy of man may enter houses when they contract rabies, therefore increasing the likelihood of human infection. Humans infected with Guinea worm are driven to soothe affected limbs in rivers, thus facilitating release of the parasite from lesions, and transmission to other individuals. Sticklebacks infected with cestodes become sluggish, and so are likely to be caught by predatory birds, in which the life-cycle is completed.

⁶ Host behavioural changes following infection are widespread throughout the Animal Kingdom (Poulin, 1995), also involving intermediate hosts. For example, *Leucochloridium*, a helminth parasite with birds as its definitive hosts, has a snail as its intermediate host. The tentacles of infected snails change in size, shape and colour, and pulsate in response to light (Kagan, 1951), thereby attracting the definitive hosts. Similarly, infection of rats with *Toxoplasma gondii* has been tendentiously argued to change their behaviour such that they are more likely than uninfected rats to be caught by cats, thereby perpetuating transmission of the parasite (Worth *et al.*, 2014). The magnitude of the effect of parasites on host behaviour appears to be similar in vertebrate and invertebrate hosts; among helminth parasites, nematodes appear to induce the greatest behavioural changes (Poulin and Forbes, 2012).

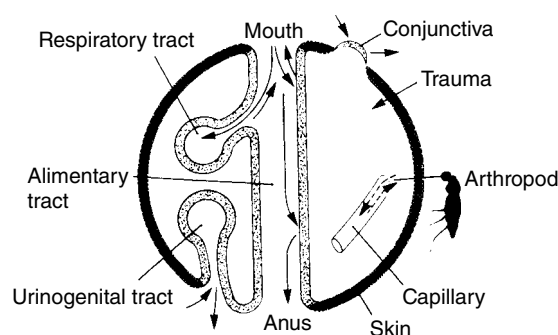


Fig. 6.6 Body surfaces as sites of horizontal infection and shedding of infectious agents. (Adapted from Mims *et al.*, 2000.)

The pathogenesis of disease may increase the likelihood of transmission; for example, respiratory diseases may induce coughing and sneezing, thereby spreading respiratory pathogens to near neighbours.

Routes of infection

The site or sites by which an infectious agent gains entry to a host (Figure 6.6), and by which it leaves the host (Table 6.3), are the agent's routes of infection.

The oral route

Infection via the mouth is one of the more common routes of entry, especially in relation to the enteric organisms, which often 'escape' from an infected animal in the faeces. Organisms such as rotaviruses, *Salmonella* spp. and gastrointestinal parasites may contaminate water and foodstuffs, which then act as fomites. Ingested agents may be excreted in the faeces, producing the **faecal–oral** transmission cycle.

Agents that gain entry to the body orally may be disseminated from the infected animal by a variety of routes, apart from in the faeces. *Brucella abortus* often infects cows orally but is excreted later in the milk and uterine discharges. Similar circumstances occur in relation to infection of ruminants with the rickettsia, *Coxiella burnetii*, the cause of Q fever. Such agents may then be retransmitted by both the oral and other routes. Although some organisms can be transmitted by the oral route, the low pH of gastric secretions is an effective barrier against this method of transmission for a wide variety of organisms.

Table 6.3 Examples of routes by which animal pathogens are shed from infected hosts. (Adapted from Wathes, 1994.)

Site of exit	Contaminated tissue or fluid	Pathogen	Disease	Host
Body surface	Hair	<i>Microsporum canis</i>	Ringworm	Dog, human, etc.
	Lesion crusts	Poxviruses	Cowpox	Ox, sheep, etc.
	Exudate (e.g., pus)	<i>Staphylococcus aureus</i>	Abscesses	
Nose	Secretions	Paramyxovirus spp.	Distemper	Dog
	Exudate	Orthomyxovirus spp.	Influenza	Pig, horse, birds
	(bloodstained)	<i>Bacillus anthracis</i>	Anthrax	Ox, sheep
Mouth	Saliva	Foot-and-mouth virus	Foot-and-mouth disease	Ox, sheep
		Lyssavirus spp.	Rabies	Dog
	Sputum	<i>Mycobacterium tuberculosis</i>	Tuberculosis (pulmonary)	Ox, human
	Tonsil	<i>Erysipelothrix rhusiopathiae</i>	Erysipelas	Pig
Mammary	Milk	<i>Streptococcus agalactiae</i>	Mastitis	Ox
Anus	Faeces	<i>Mycobacterium johnei</i>	Johne's disease	Ox, sheep
		Rotavirus spp.	Enteritis	Pig
		<i>Salmonella dublin</i>	Enteritis, septicaemia	Ox
Urogenital tract	Urine, semen	<i>Leptospira canicola</i>	Leptospirosis	Dog
		<i>Campylobacter fetus</i>	Infertility	Ox
	Eggs	<i>Salmonella pullorum</i>	Pullorum disease	Poultry
Eyes	Tears	<i>Haemophilus influenzae</i>	Pink eye (New Forest disease)	Ox
Wound (tick vector)	Blood	<i>Rickettsia burnetti</i>	Q fever	Ox

The respiratory route

The respiratory route is also a common method of transmission for many infectious agents, including those that are not restricted to the respiratory tract (e.g., *Salmonella typhimurium*: Wathes *et al.*, 1988). Infectious agents seldom occur as individual airborne particles, but usually are associated with other organic matter in the form of droplets or dust. The nature and size of such composite particles affect their dispersal, stability, site of deposition in the body, and pathogenesis of disease (Thomas, 2013).

Infections spread by the respiratory route are more likely to occur where population densities are high and ventilation is poor. Examples of such conditions are enzootic pneumonia in intensively reared pigs, and occupationally-acquired brucellosis in meat workers. In environmental extremes, diseases that are spread rarely by the respiratory route become transmissible by this method. These circumstances arise in the airborne transmission of rabies from insectivorous bats to animals and humans within the confines of a cave. Similarly, African swine fever virus, which is usually transmitted by *Ornithodoros* spp. ticks, spreads rapidly by the oral route in piggeries. In crowded and poor living conditions, pneumonic plague is transmitted directly between people, rather than by the bites of infected fleas, the

latter method of transmission inducing the less severe bubonic plague.

Infection via skin, cornea and mucous membranes

Transmission via the skin is **percutaneous** (Latin: *per* = 'through', *across*; *cut* = 'skin'). Certain agents infect only the skin, and transmission is always by direct contact with either another infected animal or a fomes; examples are ringworm and ectoparasitic infestations. The incidence of such infections and infestations is particularly influenced by the population density of the susceptible hosts. Intact skin acts as an effective barrier to the majority of infectious agents, but some, particularly the immature stages of some nematodes and trematodes, can penetrate this barrier and cause infection. Examples include blood fluke (*Schistosoma* spp.) and hookworm (*Ancylostoma* spp.) infections, the latter infection also being zoonotic and the cause of cutaneous larva migrans in man.

If the skin is cut or abraded, then infection by a variety of organisms can occur, resulting in localized infections of the skin (e.g., by *Staphylococcus* spp. and the cutaneous form of human anthrax). Other agents, such as leptospires and swine vesicular disease virus, may gain entry to the body percutaneously and then develop a more generalized infection.

Another important form of percutaneous infection is from bites by both vertebrates and arthropods. Agents that are present in the saliva, such as the viruses of rabies and lymphocytic choriomeningitis, and bacteria such as *Streptobacillus moniliformis* (a common inhabitant of the oropharynx of rats), are transmitted by animal bites. Diseases transmitted by the bites of infected arthropod vectors constitute a particular class of infections that was introduced earlier in this chapter during the description of hosts and vectors.

Infection of the cornea may remain localized; for example, bovine keratoconjunctivitis caused by *Moraxella bovis*. Alternatively, the infection may spread to other parts of the body; for instance, corneal infection of birds with Newcastle disease virus.

Although few diseases can be transmitted through intact skin, several can infect undamaged mucous membranes. An important class of such agents is those that are very labile in the external environment, and require intimate sexual contact during coitus to be transmitted to the urogenital tract, for example *Trypanosoma equiperdum* in horses.

Methods of transmission

Six main methods of transmission, which bring infectious agents into contact with the sites of infection, can be identified:

1. ingestion;
2. aerial transmission;
3. contact;
4. inoculation;
5. iatrogenic transmission;
6. coitus.

Ingestion

This may occur via a mechanical vehicle (fomes), for example contaminated water, or by ingestion of intermediate hosts, such as cestode cysts in meat. Ingested agents usually are excreted in the faeces, producing the faecal–oral transmission cycle. In such instances, transmission sometimes can occur by direct ingestion of faeces (coprophagia); for example, herpesvirus in parrots (Widén *et al.*, 2012). Some agents are excreted only faecally because they are localized to the intestine (e.g., the Johne's disease bacillus in cattle). Other agents, if they invade the bloodstream, can be excreted by additional means, such as the urine (e.g., *Salmonella* spp.). Sometimes, agents are excreted on the breath (e.g., reoviruses).

Aerial transmission

This involves airborne transmission of infectious agents via contaminated air⁷. It is the usual method of transmission with the hardy spores of fungi and some bacteria, and also occurs with pathogens of the respiratory tract that are expired on the breath of infected animals and enter susceptible ones during inspiration.

Quasi-stable suspensions of liquids or solids in gases, that are capable of floating for some time, are formed only when droplet diameters do not exceed 5 nm. Expiratory droplets range in size from 15 to 100 nm and thus even the smallest sediment rapidly (within three seconds). Therefore, they cannot travel far. Direct infection from expiratory droplets is thus limited to the area directly in front of the infected individual (the 'expiratory cone'). Very localized droplet infection can occur on food bowls and by sniffing.

Aerosol transmission is a type of airborne transmission involving transmission via an aerosol, which is defined variously as (1) any solution in the form of a fine spray in which the droplets approximate colloidal size (1–100 nm), and (2) finely divided virus particles hanging or floating in air. Thus, both quasi-stable suspensions and expiratory droplets can be involved in aerosol transmission. The three essential elements for aerial transmission by aerosol are: (1) 'take-off', (2) 'dispersal', and (3) 'landing' (Cox, 1987).

Some agents that are not primarily pathogens of the respiratory tract can contaminate the air and therefore also may be airborne. An example is foot-and-mouth disease virus shed from ruptured vesicles (take-off), which, under conducive meteorological conditions, then may be wind-borne (dispersal) and inhaled by susceptible cattle (landing) (Henderson, 1969; see also 'Long-distance transmission of infection' later in this chapter). Similarly, some *Salmonella* spp. infections are airborne and infect animals via the conjunctiva (Moore, 1957). Airborne transmission also is incriminated in the spread of hospital-acquired infections such as *Clostridium difficile* (Roberts *et al.*, 2008; Eames *et al.*, 2009).

⁷ Interest in aerial transfer of disease dates back to its fallacious role as the carrier of miasmata (see Chapter 1). More soundly, when the 19th-century London Metropolitan Asylums Board began to isolate cases of smallpox in hospitals, local residents' fear of aerial transmission of the disease was so great that cases were moved to hospital ships in the Thames Estuary (Mortimer, 2008). This is an early example of the application of the 'precautionary principle' to disease control (see Chapter 24).

Table 6.4 Common pathogens of pigs and poultry known to be transmitted aerially. (Adapted from Wathes, 1987.)

Bacteria	
<i>Bordetella bronchiseptica</i>	<i>Mycoplasma gallisepticum</i>
<i>Brucella suis</i>	<i>Mycoplasma hyorhinus</i>
<i>Erysipelothrix rhusiopathiae</i>	<i>Mycoplasma suis</i>
<i>Escherichia coli</i>	<i>Pasteurella multocida</i>
<i>Haemophilus gallinarum</i>	<i>Pasteurella pseudotuberculosis</i>
<i>Haemophilus parasuis</i>	<i>Rhodococcus</i>
<i>Haemophilus pleuropneumoniae</i>	<i>(Corynebacterium) equi</i>
<i>Leptospira pomona</i>	<i>Salmonella pullorum</i>
<i>Listeria monocytogenes</i>	<i>Salmonella typhimurium</i>
<i>Mycobacterium avium</i>	<i>Staphylococcus aureus</i>
<i>Mycobacterium tuberculosis</i>	<i>Streptococcus suis</i> type II
Fungi	
<i>Aspergillus flavus</i>	<i>Coccidioides immitis</i>
<i>Aspergillus fumigatus</i>	<i>Cryptococcus neoformans</i>
<i>Aspergillus nidulans</i>	<i>Histoplasma farcinorum</i>
<i>Aspergillus niger</i>	<i>Rhinosporidium seeberi</i>
Rickettsia	
<i>Coxiella burnetii</i>	
Protozoa	
<i>Toxoplasma gondii</i>	
Viruses	
African swine fever	Infectious nephrosis of fowls
Avian encephalomyelitis	Infectious porcine encephalomyelitis
Avian leukosis	Marek's disease
Foot-and-mouth disease	Newcastle disease
Fowl plague	Ornithosis
Hog cholera	Porcine enterovirus
Inclusion body rhinitis	Swine influenza
Infectious bronchitis of fowls	Transmissible gastroenteritis of swine
Infectious laryngotracheitis of fowls	

Table 6.4 lists some common pathogens of pigs and poultry that can be transmitted aerially.

Contact

Contact transmission is transmission without transmission factors (e.g., mechanical vectors) and without participation of an external medium. This is particularly important in relation to infectious agents that are shed from the body surfaces, such as vesicular viruses, and with agents that gain entry through the body surface. Very few agents are transmitted merely by touch; some degree of trauma is necessary, albeit microscopic. Transmission may be by bites (e.g., rabies and rat bite fever), or by scratches (e.g., cat scratch fever).

Diseases transmitted by contact may be described as 'contagious' (Latin: *contagio* = 'to touch closely') but

this term now is used less precisely than previously, and has been used to describe any highly infectious disease (Porta, 2014).

Inoculation

Inoculation (Latin: *inoculatus* = 'engrafted' or 'implanted') is the introduction into the body, by puncture of the skin or through a wound, of infectious agents.

Although classified separately here, inoculation is frequently associated with contact transmission (e.g., bites from rabid dogs). Arthropods that act as vectors may inoculate infectious agents into the blood by biting (e.g., tsetse flies infected with *Trypanosoma* spp., in which development of the parasite occurs in the salivary gland, gut and mouthparts).

Iatrogenic transmission

Iatrogenic literally means 'created by a doctor'. Thus, an iatrogenically-transmitted infection is one that is transferred during surgical and medical practice.

There are two main types, involving:

1. introduction of pathogens by dirty instruments, (e.g., during non-aseptic surgery and tattooing: Jafari *et al.*, 2010) or by contaminated body surfaces;
2. introduction of pathogens contaminating prophylactic or therapeutic preparations (e.g., bovine leucosis virus in *Babesia* spp. vaccines: Hugoson *et al.*, 1968⁸; *Pseudomonas aeruginosa* in intramammary dry-cow antibiotic preparations: Nicholls *et al.*, 1981; porcine reproductive and respiratory syndrome virus in needles: Otake *et al.*, 2002; lumpy skin disease in anaplasmosis vaccine; scrapie in louping-ill vaccine; human hepatitis B virus and HIV/AIDS in serum preparations and blood⁹) and, more rarely, by organ transplantation (e.g., rabies virus by corneal transplants).

Coitus

Some infectious agents may be transmitted during coitus. Certain diseases are transmitted only in this way.

⁸ Inferred from a statistical association between the occurrence of leucosis and vaccine use.

⁹ Vaccines – while not actually being contaminated with pathogens – also may induce disease themselves (e.g., attenuated bluetongue vaccine causing teratogenic defects: Murray and Eaton, 1996). They also may be responsible for acute hypersensitive reactions (Tizard, 1990), as well as being determinants of chronic immune-mediated diseases (Hogenesch *et al.*, 1999; Dodds, 2001).

These were called venereal diseases (Latin: *venereus* = 'pertaining to sexual love'). In human medicine they now are referred to as sexually-transmitted diseases (STDs). Sexual transmission can occur not only in vertebrates but also in arthropods. For example, African swine fever virus can be sexually transmitted from male to female ticks of the genus *Ornithodoros* (Plowright *et al.*, 1974).

The mode of transmission of agents frequently governs the epidemic picture. Thus, agents that are transmitted by the faecal–oral and airborne modes often produce sudden explosive epidemics, whereas coitally-transmitted diseases spread more slowly, over a long period of time.

Long-distance transmission of infection

Infectious diseases can be transmitted by the methods just described over long distances as a result of the mobility of infected animals, microorganisms and parasites, vectors and fomites.

Host mobility

Previously, transportation by sea provided a period of quarantine, but epidemics, nevertheless, did occur. For example, the Black Death probably entered mediaeval Europe via trade routes from the Orient (Bartsocas, 1966; McNeill, 1977; Harrison, 2012) and cholera spread globally along trade routes in the 19th century (Rushton, 2005). Rinderpest was brought into Italy in the early 18th century by traders importing cattle from Hungary (Mantovani and Zanetti, 1993) and spread throughout Europe (see *Table 1.1*), and Italian colonists accidentally introduced a few cattle infected with the virus into the Horn of Africa in 1880, resulting in a pandemic that swept through Sub-Saharan Africa, from Somalia to Cape Town (Scott, 1964). Rabies spread throughout the Dutch East Indies' archipelago during the late 19th and early 20th century by the movement of dogs via the military forces, for trade and as pets (Ward, 2014). The contemporary expansion of air, sea and land networks increases the likelihood of infectious disease pandemics, invasion of new regions by vectors, and importation of vector-borne pathogens (Tatem *et al.*, 2006)¹⁰.

¹⁰ In earlier times, geographical isolation was considered to be a useful defence against disease: for example, the British looked upon their island as 'a fortress built by nature for herself against infection and the hand of war' (Blair, 1963). Remoteness also protected Pacific islanders, Aboriginal Australians and the Khoisan of southern Africa from Eurasian microbes such as measles, influenza, syphilis, gonorrhoea and tuberculosis, until contact with Europeans was established (Diamond, 1997).

Table 6.5 Movement of species among free-ranging and captive wildlife populations that has exacerbated the spread of disease. (Adapted from Cleaveland *et al.*, 2002. Reproduced with permission of Oxford University Press.)

Disease	Species of concern	Movement
Canine distemper	Black-footed ferret	Wild to captive
Haematozoa (<i>Plasmodium kempti</i>)	Wild turkeys	Wild to wild
Lungworm (<i>Bronchonema magna</i>)	Bontebok, springbok	Wild to wild
<i>Mycoplasma</i> spp.	Gopher and desert tortoises	Captive to wild; wild to wild
Parvovirus	Racoons	Wild to wild
Rabies	Racoons, skunks	Wild to wild
Rinderpest (globally eradicated)	Cloven-hoofed artiodactyls (e.g., wildebeest, eland, buffalo, bushbuck)	Domestic to wild; wild to wild
Tuberculosis	Arabian oryx	Captive to wild
Warble flies, nostril flies	Caribou	Captive to wild
'Whirling disease'	Rainbow trout	Captive to wild

The increasing use of air transport now means that animals incubating infections can arrive at their destination before clinical signs have appeared. The movement of horses and equine semen, in connection with sale, breeding and competition, has spread a variety of equine infections, including African horse sickness, contagious equine metritis, equine infectious anaemia, equine viral arteritis, piroplasmiasis, Venezuelan equine encephalomyelitis and influenza, between continents (Timoney, 2000; Dominguez *et al.*, 2016). International livestock trade also poses a clear risk of disease transmission: enzootic bovine leucosis, *Mycoplasma bovis*, *M. canadensis*, *Haemophilus somnus* and infectious bovine rhinotracheitis may have been introduced into the UK in this way (Scudamore, 1984). The translocation of farmed fish for angling and food production poses similar risks (Cable and Harris, 2003), and disease can spread between free-ranging and captive wildlife as a result of movement between the two types of population (*Table 6.5*).

The importation of animals infested with the Old World screw-worm fly, *Chrysomya bezziana*, has spread the disease from Uruguay to Libya; the giant fluke, *Fascioloides magna*, was introduced into

European elk from North America; and duck plague was introduced into North American wild ducks and geese through the importation of infected domestic ornamental waterfowl (Leighton, 2002). There is a risk of the introduction of heartwater into the US associated with the importation of tick-infected reptiles and wild game from Africa, and from infected livestock imported from the Caribbean (Burrige *et al.*, 2002).

Transmission also can involve more complex relationships. Thus, Crimean–Congo haemorrhagic fever virus infects its vector, *Hyalomma* spp. ticks, which are then carried by migrating birds from the Crimea to Africa where they are responsible for outbreaks of the disease (Hoogstraal, 1979).

There is unease that *C. bezziana*, which is endemic in Papua New Guinea, may be imported into Australia as an inadvertent passenger on international flights or by animal movement between the Torres Strait Islands (see the case study in Chapter 25 for further details), and there is concern that *Culicoides* spp., the vectors of bluetongue, also may disseminate infection in this way (Saegerman *et al.*, 2008).

The movement of people by aeroplane also can distribute human diseases over all parts of the world (Prothro, 1977), a notable example being human SARS, where transmission on board flights also probably occurs (Mangili and Gendreau, 2005). Another, recent, example is Zika virus, which may have been imported into Brazil either by fans attending the 2014 soccer World Cup (Zanluca *et al.*, 2015) or by French Polynesians competing in the Va'a World Sprint Championship canoe race (Musso, 2015).

Additionally, infective vectors can introduce diseases to non-endemic countries via intercontinental flights; for example, mosquitoes carrying malaria (Zucker, 1999)¹¹. Although smallpox has been globally eradicated (see Chapter 27), it is believed that unofficial virus stocks¹² exist (Henderson, 1998) and, if intentionally released, could be widely distributed by air travel (Graiss *et al.*, 2003).

The movement of animals may also contribute to epidemics of human disease. For example, epidemics of human trypanosomiasis ('sleeping sickness') occur in distinct foci in Africa, in contrast to trypanosomiasis in animals, which tends to occur throughout the tsetse fly region. Cattle are also reservoirs of infection of human trypanosomes, and the movement of infected

cattle (e.g., to market) appears to be responsible for translocating human infective trypanosome strains into regions previously free from the disease (Hide *et al.*, 1996; Fèvre *et al.*, 2001; Hide, 2003).

Airborne (atmospheric) transmission

Airborne transmission of infection (atmospheric transmission) is a specific category of long-distance transmission. It cannot occur with expiratory droplets because they sediment rapidly (see 'Aerial transmission' earlier in this chapter). Transmission of respiratory and vesicular infections indirectly through the air over long distances (atmospheric transmission) must, therefore, be effected by other means. The evaporation of water from droplets (which can occur when droplets are airborne or on the ground) produces desiccated **droplet nuclei**, ranging in diameter from 2 to 10 nm. The smallest of these are quasi-stable and can travel over long distances, assisted by wind. The rate of formation of these nuclei depends on the temperature and relative humidity. Rain sediments the nuclei.

Foot-and-mouth disease Atmospheric transmission of foot-and-mouth disease has received considerable attention. Thus, during the 1967–1968 foot-and-mouth disease epidemic in the UK, a series of secondary outbreaks followed the primary one (*Figure 6.7a*). Initially, it was suggested that this was due to infected imported lamb, because no human or mechanical links could be established between the secondary outbreaks and the primary outbreak at Bryn Farm. However, a complex meteorological hypothesis has been presented (Tinline, 1970, 1972) suggesting that the secondary outbreaks were caused by virus particles being pulled downwards in a current of air, which was forced into vertical oscillation as it flowed over a hill. This phenomenon is called a **lee wave** (*Figure 6.7b*)¹³.

Another, more general model of airborne transmission of foot-and-mouth disease virus uses the meteorological **Gaussian plume** model for atmospheric diffusion (Hanna *et al.*, 1982), which is a basic model for dispersion calculations. This predicts horizontal elliptical spread, the shape of the virus-laden plume depending on wind speed, downwind distance from the source of infection, and air stability (Gloster *et al.*, 1981, 1982). More recent model formulations include the **ALOHA** (**A**erial **L**ocation of **H**azardous

¹¹ There are also other health-related risks associated with flying, such as deep-vein thrombosis and additional risks to sufferers of pulmonary disease (Buik, 2004).

¹² Stocks are officially confined at the Centers for Disease Control and Prevention, Atlanta, Georgia, US, and Vector, Koltsovo, Novosibirsk, Russia.

¹³ For fuller discussions of lee waves, see Casswell (1966) and Bradbury (1989b). Recent studies (Gloster *et al.*, 2005) suggest that the role of lee waves may have been exaggerated.

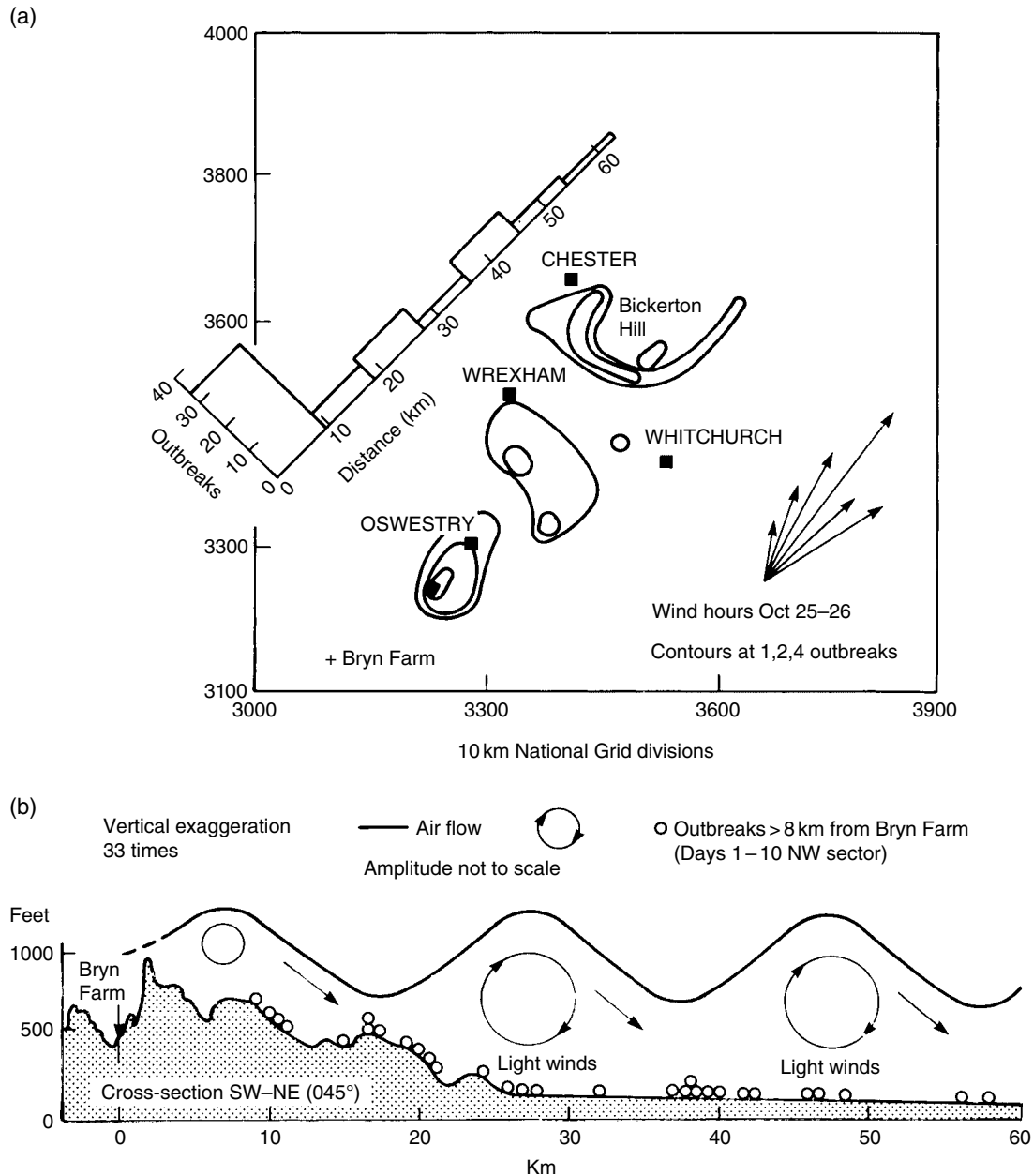


Fig. 6.7 The 1967–1968 English foot-and-mouth disease epidemic: (a) outbreaks from days 1–10; (b) profile through Bryn Farm illustrating the lee wave hypothesis of the aerial spread of virus. (Source: Tinline, 1970. Reprinted with permission).

Atmospheres) system (Casal *et al.*, 1997), initially developed to predict the dispersal of toxic gases from chemical engineering plants; and the **Rimpuf** model (Sørensen *et al.*, 2000). The latter model – which considers topography and factors that affect virus survival (e.g., relative humidity) – simulates plumes by breaking them into independent, three-dimensional concentration fields ('puffs'), transportation of which depends on atmospheric stability. This model predicts that the distance at which susceptible animals are at

risk of infection from infected animals depends on the species of each, and the number of infected animals (Table 6.6)¹⁴. The distance over which animals are at

¹⁴ The modelled infection is *between* populations, where it is generally argued that the size of an infected population affects transmission to other populations (Willeberg *et al.*, 1994; Van Nes *et al.*, 1998). This contrasts with transmission of infection *within* a population, where transmission depends on the density of susceptible individuals, rather than population size (Bouma *et al.*, 1995; de Jong *et al.*, 1995) (see Chapter 8).

Table 6.6 Effect of species and number of animals excreting foot-and-mouth virus on the risk for different species downwind. ((a) From Sørensen *et al.*, 2000. (b) From Donaldson *et al.*, 2001.)

(a) C Noville strain

Species excreting virus	Distance (km) downwind at which species are at risk		
	Cattle	Sheep	Pigs
1000 infected animals			
Pigs	300	90	20
Cattle	3	0.5	<0.1
Sheep	3	0.5	<0.1
100 infected animals			
Pigs	120	15	5
Cattle	0.7	<0.1	<0.1
Sheep	0.7	<0.1	<0.1
10 infected animals			
Pigs	30	4	1
Cattle	<0.1	<0.1	<0.1
Sheep	<0.1	<0.1	<0.1
1 infected animal			
Pigs	5	1	0.3
Cattle	<0.1	<0.1	<0.1
Sheep	<0.1	<0.1	<0.1

(b) PanAsia strain

Species excreting virus	Distance (km) downwind at which species are at risk		
	Cattle	Sheep	Pigs
1000 infected animals			
Pigs	6	2	<0.2
Cattle	0.7	0.2	<0.1
Sheep	0.7	0.2	<0.1
100 infected animals			
Pigs	2	0.4	<0.1
Cattle	0.2	<0.1	<0.1
Sheep	0.2	<0.1	<0.1
10 infected animals			
Pigs	0.5	0.1	<0.1
Cattle	<0.1	<0.1	<0.1
Sheep	<0.1	<0.1	<0.1
1 infected animal			
Pigs	<0.1	<0.1	<0.1
Cattle	<0.1	<0.1	<0.1
Sheep	<0.1	<0.1	<0.1

risk also depends on the infecting strain: virus production by animals infected by the C Noville strain (Table 6.6a) being approximately 300 times greater than from animals infected by the PanAsia strain (Table 6.6b).

Models of airborne spread generally predict similar levels of risk to livestock, with differences between them being related to the meteorological data that are used, determination of the sequence of events on infected premises, and assumptions about virus release and susceptibility to infection (Gloster *et al.*, 2010).

Long-distance spread of foot-and-mouth disease virus is most likely under the following conditions:

- high output of virus from the infected animals: pigs are an important source of airborne virus because they excrete vast quantities of airborne virus (see Chapter 5) and are often kept in large numbers (Donaldson *et al.*, 1982; Donaldson, 1987);
- steady wind direction so that animals may be exposed for several hours;
- relative humidity greater than 60%, allowing high survival of virus (Donaldson *et al.*, 1982); however, heavy rains wash the virus out of the air;
- low levels of atmospheric turbulence associated with a flat landscape, stable surface air (particularly over water) and light winds, which result in low dispersion of virus (Gloster *et al.*, 1982).

Extensive windborne spread, however, is not common, probably because the likelihood of all the predisposing factors occurring together is small¹⁵. Analysis of field data has shown the most usual pattern of airborne spread of the virus is from pigs at source to cattle downwind (Henderson, 1969; Hugh-Jones and Wright, 1970; Sellers, 1971; Sellers and Forman, 1973; Donaldson *et al.*, 1982; Gloster *et al.*, 1982). The 1967–1968 epidemic in the UK occurred in one of the most densely stocked dairy areas in the country. During the first three weeks, around 350 separate outbreaks occurred and more than 2000 farms were affected over a period of nine months (see Figure 4.1a). The size of the outbreak was attributed to its explosive start, which occurred following extensive airborne transmission from an infected pig unit (HMSO, 1969). The 2001 epidemic (see Figure 4.1b) began in similar fashion by airborne spread from a pig unit to nearby cattle and sheep farms (DEFRA, 2002b; see Chapter 4).

Some other diseases The identification of isolates of Aujeszky's disease virus using molecular epidemiological techniques has also suggested that airborne

¹⁵ Concerns that virus might be spread by smoke rising from incinerated infected carcasses, for example, are probably unfounded (Gloster *et al.*, 2001; Jones *et al.*, 2004b).

transmission of this virus over long distances can occur (Christensen *et al.*, 1990, 1993), and this suggestion has been supported by dispersion models (Gloster *et al.*, 1984; Grant *et al.*, 1994). Similarly, studies of outbreaks of vesicular stomatitis, based on nucleotide fingerprinting and trajectory analysis of winds, suggest that wind could have been responsible for carrying infection from Mexico to the US (Sellers and Maarouf, 1990). There is also evidence for airborne transmission of porcine enzootic pneumonia (Goodwin, 1985; Dee *et al.*, 2009), Newcastle disease (Smith, 1964; Dawson, 1973) and the virus causing porcine reproductive and respiratory syndrome (Beilage *et al.*, 1991; Mortensen and Madsen, 1992; Dee *et al.*, 2009).

Wind also can carry arthropod vectors over long distances (Pedgley, 1983). The outbreak of bluetongue in Portugal in 1956 (Sellers *et al.*, 1978) may have been caused by windborne transfer of the African vector, *Culicoides imicola*, from North Africa¹⁶, although the latter now is permanently established in Portugal (Mellor *et al.*, 1985). Windborne transfer also has been incriminated in the introduction of the disease into Cyprus (Polydorou, 1980), Israel (Braverman and Chchik, 1993), the Balearic Islands (Alba *et al.*, 2004), northern Spain (García-Lastra *et al.*, 2012) and northern Europe (Burgin *et al.*, 2013). African horse sickness (Sellers *et al.*, 1977) and epizootic haemorrhagic disease virus (Kedmi *et al.*, 2010) also may be transferred in a similar way.

Vertical transmission

Types and methods of vertical transmission

There are two types of vertical transmission: **hereditary** and **congenital**.

Hereditarily-transmitted diseases are carried within the genome of either parent. Thus, retroviruses, which have integrated DNA copies of the virus in the host's genome, are transferred hereditarily.

Congenitally-transmitted diseases are, literally, those present at birth. According to strict etymology, hereditarily-transmitted diseases are a part of this group. However, by common usage, 'congenital' refers to diseases *acquired* either *in utero* or *in ovo*, rather than inherited.

Transmission can occur at various stages of embryonic development. It may produce either abortion, if incompatible with life, or teratoma (literally 'monsters'). Alternatively, infection that is inapparent and continuous after birth (**innate infection**) can occur.

Germinative transmission

This involves either infection of the superficial layers of the ovary, or infection of the ovum itself. Examples include the chicken leukosis viruses, spontaneous lymphoid leukaemias of mice (Gross, 1955), murine lymphocytic choriomeningitis and avian salmonellosis.

Transmission to the embryo

This is via the placenta (transplacentally) or via the fetal circulation, through the placenta, to the fetus. For example, kittens can be transplacentally infected with feline panleucopenia virus (Csiza *et al.*, 1971). Viruses, being small, cross the placenta with greater ease and earlier in pregnancy than larger microbes. The fetal circulation, however, can carry most microbes. Infection of the placenta does not always produce infection of the fetus. Q fever particles, for instance, may be found in large quantities in bovine placentae without infection of the developing calves.

Ascending infection

This is infection that is transmitted from the lower genital canal to the amnion and placenta (e.g., some *Staphylococcus* and *Streptococcus* spp. infections).

Infection at parturition

This is infection acquired from the lower genital canal at birth (e.g., human herpes simplex infection).

Immunological status and vertical transmission

The immunological status of the fetus is important when agents are transmitted vertically. Immune tolerance of microbial antigens by the fetus can be detrimental in postnatal life, because 'non-self' antigens are then recognized as 'self'. The result is a lack of a protective immune response, sometimes with the development of a carrier state with the subsequent dangers to other susceptible animals, as in the case of feline panleucopenia. However, immune tolerance by the fetus can occasionally be advantageous when infections have clinical and pathological effects mediated by the immune response. The paradigm

¹⁶ The wind is capable of transporting the midges as 'aerial plankton' up to 700 km (Wittmann and Baylis, 2000).

of this is lymphocytic choriomeningitis (LCM) infection of mice (Oldstone, 1998). In adults the disease is mediated by a lethal infiltration of the brain by responsive T lymphocytes. Prenatal infection induces a tolerance to LCM virion antigens; therefore no lymphocytic infiltration occurs in adult infections; thus, there is no clinical disease.

Transovarial and trans-stadial transmission in arthropods

Some arthropods, notably ticks and mites, transmit bacteria, viruses and protozoa from one generation to another via their eggs; this is **transovarial** transmission. Examples of transovarially-transmitted infections include bovine anaplasmosis (a protozoan disease causing anaemia in cattle in the tropics and sub-tropics, transmitted by several genera of ticks) and canine babesiosis (another protozoan disease causing anaemia in dogs, transmitted by ticks of the genera *Dermacentor* and *Haemaphysalis*).

In contrast, some arthropods only transmit infections from one developmental stage to another (e.g., in ticks: larva to nymph, nymph to adult); this is **trans-stadial** transmission. An example of a trans-stadially-transmitted disease is theileriosis, caused by protozoa of the genus *Theileria*, occurring in cattle, sheep and goats, and transmitted by ticks of the genus *Rhipicephalus*.

Some infections are spread both transovarially and trans-stadially, for example Nairobi sheep disease, a virus infection transmitted by the brown tick, *R. appendiculatus*. Investigations of tick-transmitted diseases are revealing that many infections, once thought to be transmitted by only one of these methods, are transmitted by both.

Some infections involve transovarial, trans-stadial and sexual transmission in arthropods. Thus, in Africa, African swine fever virus is primarily maintained in the tick *Ornithodoros moubata* by these three methods of transmission (Figure 6.8). Warthogs are infected by the bite of the tick, but there is neither horizontal

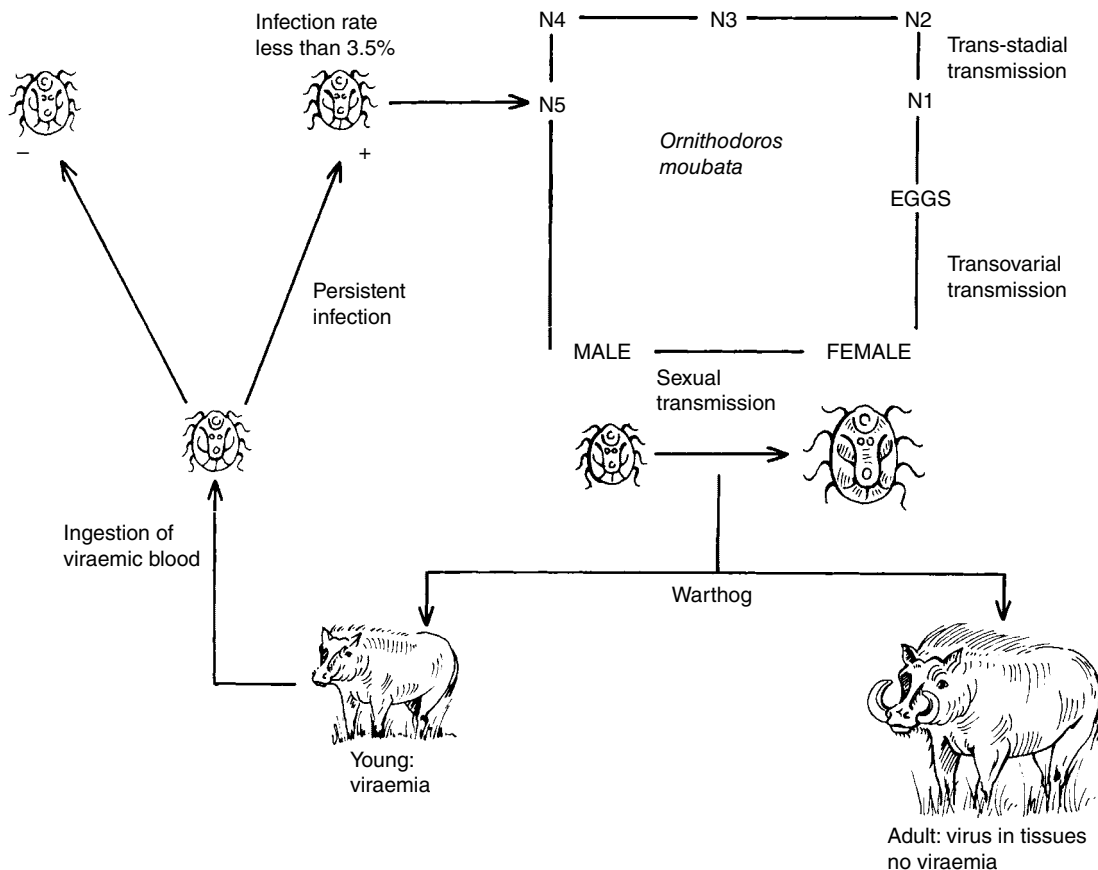


Fig. 6.8 Transmission cycle of African swine fever virus in warthogs and *Ornithodoros moubata*. N = nymphal stage. (Adapted from Wilkinson, 1984.)

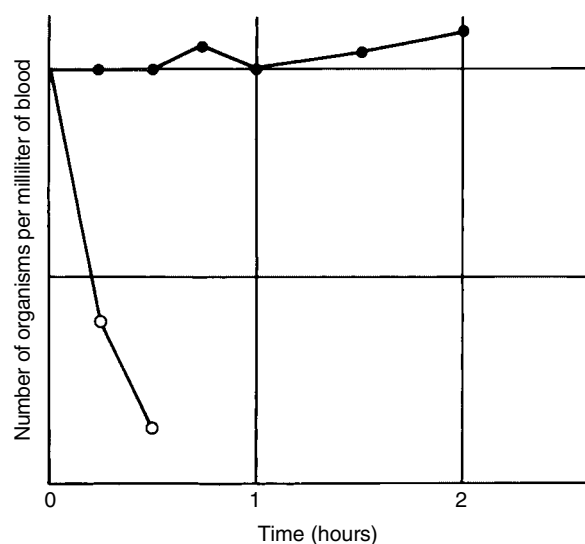


Fig. 6.9 The protective action of bacterial capsules: pneumococci, capsulated (●) and not capsulated (○), injected intravenously into mice; blood of mice then sampled every 15 minutes. (Source: Boycott, 1971.)

nor vertical transmission between them, and only a small proportion of nymphal ticks are persistently infected when they bite young warthogs during a brief viraemic phase.

Maintenance of infection

Hazards to infectious agents

The transmission of infection involves some stages when the infectious agent is in the host, and others when it is in the external environment or in a vector, or in both (Figure 6.1). Both internal and external environments present hazards to infectious agents.

The environment within the host

The host has its natural defence mechanisms: surface-active chemicals, specific reactive cells, phagocytes and humoral antibodies. The successful parasite must be able to avoid, in part, these mechanisms, and must also avoid competition with other agents that may simultaneously infect the host in a similar niche (see Chapter 7). Parasites have evolved strategies to resist the host's protective mechanisms, such as acid-resistant helminth cuticles (to resist gastric acid), an intracellular mode of life, encystment,

Table 6.7 The comparative fecundity of *Platyhelminthes* and *Nemathelminthes*. (From Dobson *et al.*, 1992.)

Species	Fecundity	Host
Free-living species (young/adult/breeding season)		
Turbellaria		
<i>Polycelis nigra</i>	2.5	
<i>P. tenuis</i>	1.0	
<i>Dugesia polychroa</i>	0.8–1.7	
<i>Planaria torva</i>	4.9–15.2	
<i>Dendrocoelum lacteum</i>	4.9–10.3	
<i>Bdellocephala punctata</i>	16.0	
Parasitic species (eggs/worm/day)		
Digenea		
<i>Schistosoma mansoni</i>	100	Hamster
Cestoda		
<i>Echinococcus granulosus</i>	600	Dog
<i>Hymenolepis diminuta</i>	200 000	Rat
<i>Taenia saginata</i>	720 000	Man
Acanthocephala		
<i>Moniliformis moniliformis</i>	5 000	Rat
Nematoda		
<i>Ascaris lumbricoides</i>	200 000	Pig
<i>Enterobius vermicularis</i>	11 000	Man
<i>Wucheria bancrofti</i>	12 500	Man

immunosuppression and reduced immunogenicity (Capron and Dessaint, 1992). Some bacteria possess capsules that protect them against phagocytosis, for example, *Pneumococcus* spp. (Figure 6.9). Many parasitic nematodes have a greater fecundity than their free-living counterparts (Table 6.7), thus ensuring that some offspring will survive the host's immune response and potentially lethal conditions in the external environment.

The external environment

The two main hazards presented by the external environment are desiccation and ultraviolet light. Desiccation is not always lethal, but frequently inhibits multiplication. Low temperatures are not usually lethal, but can inhibit multiplication. The high temperatures attained in temperate climates are probably not lethal but those reached in tropical countries may be more effective. Many agents may be partially protected from desiccation by being discharged in moist carriers such as faeces and urine. They also may persist by being shed into favourable surroundings; leptospires, for example, persist longer in paddy fields than in semi-arid regions. Some viruses that survive loss of water and become desiccated remain viable for

Table 6.8 Foot-and-mouth disease virus survival times in animal products, and on some common fomites and surfaces. (Compiled from Mebus *et al.*, 1993; Sanson, 1994; Bartley *et al.*, 2002; and Pharo, 2002a.)

Product/material	Survival time
Lymph and haemal nodes at 4°C	120 days
Bone marrow at 4°C	210 days
Skeletal muscle (pH<6) at 4°C	2 days
Frozen carcass (no <i>rigor mortis</i>)	6 months
Milk pH 2.0 at 4°C	1 minute
Milk pH 5.8 at 4°C	18 hours
Milk pH 7.0 at 4°C	15 days
Dried casein	2 months
Cheese (manufactured from raw milk) pH 6	30 days
Cheese (manufactured from raw milk) cured at >2°C	120 days
Cured ham	6–26 weeks
Frozen or liquid manure	>6 months
Soil surface up to 30°C	<5 days
Soil surface under snow	>184 days
Wool	14 days
Ox's hair	4–6 weeks
Houseflies	10 weeks
Contaminated footwear	11–14 weeks
Wood, hay, straw, feed sack, etc.	15 weeks

months or years. Other viruses (e.g., the poxviruses) are resistant to desiccation and can survive for long periods in dry infected scab material. Agents may also survive in inanimate material in the environment, which may therefore act as a fomes; for example, animal foodstuffs contaminated with *Salmonella* spp. Foot-and-mouth disease virus can survive for several weeks on a variety of fomites (Table 6.8) and contaminate the environment (Sellers *et al.*, 1971; Bravo de Rueda *et al.*, 2015), necessitating thorough cleansing and disinfection of premises after outbreaks (Quinn, 1991)¹⁷.

¹⁷ The following description of the cleansing and disinfection procedures used in Scotland during the 2001 foot-and-mouth disease epidemic exemplifies the intensity of effort that is required to effectively remove the hazard of further infection from environmental contamination. First, and usually within a day of slaughter of affected animals, preliminary cleansing and disinfection of affected farms was undertaken by teams of six: two with pressure washers at the gate to disinfect all leaving vehicles, and four to help with the carcass disposal pyre, carcass moving and preliminary

Maintenance strategies

The ways in which infectious agents are maintained can be considered as **strategies** for maintenance. Five main strategies can be identified:

1. avoidance of a stage in the external environment;
2. the development of resistant forms;
3. a 'rapidly in, rapidly out' strategy;
4. persistence within the host;
5. extension of host range.

Avoidance of a stage in the external environment

Some agents avoid transfer via the environment. There are four main methods:

1. by vertical transmission;
2. by venereal transmission;
3. by vector transmission;
4. by transmission by sarcophagia (flesh eating); for example, the helminth *Trichinella spiralis* occurs in cysts in the muscle of pigs, rats and other animals, and is only transmitted when these animals are eaten by predators and scavengers, including man; 'salmon poisoning' is another example (see Figure 7.9).

disinfectant spraying of the premises. It was often necessary to provide water bowlers to assist with this. All areas that had been in contact with susceptible stock (especially the slaughter area) were thoroughly soaked with a disinfectant. If immediate removal of carcasses was not possible, then they, also, were soaked with disinfectant and covered until removed. On removal, the area on which the carcasses were located, and any other areas used for transportation and removal, were again sprayed with disinfectant. Next, premises were visited by specially trained cleansing-and-disinfection officers to discuss implementation of cleansing and disinfection, either by the farmer alone, or in combination with a contractor, or by the latter alone. A proforma of work to be carried out on approximately 800 premises was completed. Faeces were removed, stacked to heat up, and sprayed with disinfectant. Slurry, by preference, was treated with caustic soda on farms to raise the pH to 11, and then spread on fields; 193 premises were treated in this way. Slurry in towers was left for at least 90 days, before spreading. Some slurry in pits was partially treated and removed to give space for wash water. Some was left for at least 150 days, and then spread. A further 150 premises were dealt with either by these timed methods, or by caustic-soda treatment by the farmers themselves. All areas in contact with susceptible livestock were first hosed down with water. A detergent degreaser then was applied to remove grease and encrusted faeces. These areas were washed down again, and further degreased and washed, if required. Particularly dirty roofs were also washed or vacuumed. The clean areas were then sprayed with disinfectant, and, finally, seven days later, the areas were disinfected once more. Additionally, approximately 800 3 km pre-emptively-culled premises (see Figures 11.11, 12.12 and Chapter 27) had to have all areas in contact with livestock sprayed with disinfectant.

Resistant forms

The harshness of the external environment can be buffered by surrounding the infectious agent with a shell that is resistant to heat and desiccation. Some bacteria form such shells (spores). Examples include members of the genera *Clostridium* and *Bacillus*, which can survive boiling water, even flames, for short periods of time, and may survive in the external environment for decades. Fungi also may produce spores. Generally these are less resistant than bacterial spores. Some helminths and protozoa form resistant shells (cysts). These can protect the agent from the host's defence mechanisms; the protozoan parasite, *Toxoplasma gondii*, for example, can survive for many years in its cystic form in the host, until the latter is eaten. Thick-shelled helminth eggs can resist the external environment and may overwinter on pasture.

'Rapidly in, rapidly out' strategy

Some agents enter the host, replicate and leave very quickly, before the host has time to mount an immune response or die. Many viruses of the upper respiratory tract can do this within 24 hours. The strategy requires a continuous supply of susceptible hosts. This may be one reason why respiratory and enteric infections, such as the common cold virus in man, are not present in primitive societies of low population density, and may not have occurred in small prehistoric societies (Brothwell and Sandison, 1967; Black, 1975)¹⁸.

Persistence within the host

Infectious agents may persist within the host, sometimes for life. Persistence occurs because the host's defence mechanisms fail to eliminate agents. This failure may arise because microorganisms adapt to the host's phagocytic cells, or develop strategies for avoiding the host's immune response (Mims *et al.*, 2000). The latter include **immunosuppression** and **tolerance**.

Immunosuppression results in the agents being maintained in the host for varying periods of time. It may be general or antigen-specific. General immunosuppression is demonstrated by some viruses (e.g., rinderpest) and protozoa (e.g., *T. gondii*), and facili-

tates survival of these and other agents in the host. Antigen-specific immunosuppression only involves the response to the infecting microorganism, the responses to other agents being unaffected. Human leprosy and tuberculosis induce this type of immunosuppression.

Tolerance is due to a primary lack of responsiveness by the host, rather than active suppression. This can occur prenatally; the example of LCM has been given earlier in this chapter. It also can occur when there are large amounts of circulating antigen or antigen-antibody complexes, for instance, in human leishmaniasis and cryptococcosis. Tolerance is sometimes found in infections with microorganisms that have antigens similar to normal host antigens (e.g., *Bacterioides* spp. infections in mice). It has been suggested that such 'molecular mimicry' is generally associated with tolerance, but the evidence is equivocal (Karlsen and Dyrberg, 1998).

Other means of avoiding the host's immune response are **antigenic variation** (see Chapter 5), **intracellular parasitism** (see Chapter 7), multiplication in **sites inaccessible** to the immune response (e.g., mammary tumour virus of mice, infecting the luminal surface of the mammary gland), and the induction of **ineffective antibodies** (e.g., Aleutian disease virus of mink). Some nematodes induce an **anti-inflammatory** response (Perry, 2012).

Other examples of persistent infections are *Mycobacterium johnei*, tapeworm infections of the gut and *T. spiralis* in tissues. Specific types of persistent infection (latent and chronic infection, and the carrier state) have already been discussed in Chapter 5.

Persistence can be associated with a long incubation or prepatent period. A group of virus diseases, termed 'slow virus diseases' because of their long incubation period, fall into this category. Maedi-visna, for example, is a slow virus disease of sheep, producing neurological and respiratory signs, with an incubation period of two to eight years. Similarly, scrapie is a virus-like disease, also of sheep, which produces neurological signs and has an incubation period of one to five years. Its persistence within its host facilitates vertical and possibly horizontal transmission in a flock.

Alternatively, an agent's prepatent period may be relatively short, but excretion of the agent may continue for a long time (i.e., the period of infectiousness is long). Excretion may be intermittent; for example, *Salmonella* spp. infection can be associated with intermittent clinical episodes or subclinical infection, both

¹⁸ Grenfell and others (2004) and Boldin and Kisdi (2012) present a detailed discussion of the evolution of the dynamics of infection.

Table 6.9 Some characteristics of host/parasite relationships between fleas, acting as vectors, and infectious agents. (Simplified from Bibikova, 1977.)

<i>Disease and pathogen</i>	<i>Site of pathogen in flea</i>	<i>Reproduction of pathogen in flea</i>	<i>Duration of pathogen in flea</i>	<i>Pathogenic effect on flea</i>
Myxomatosis <i>Fibromavirus myxomatosis</i>	Digestive tract	No	Up to 100 days	Yes
Tularaemia <i>Francisella tularensis</i>	Digestive tract	No	Several days	No
Murine typhus <i>Rickettsia mooseri</i>	Digestive tract	Yes	Lifetime	No
Murine trypanosomiasis <i>Trypanosoma lewisi</i>	Digestive tract	Yes	Lifetime	No
Salmonellosis <i>Salmonella enteritidis</i>	Digestive tract	Yes	Up to 40 days	Yes
Plague <i>Yersinia pestis</i>	Digestive tract	Yes	Several months to over one year	Yes

associated with occasional excretion of the bacterium. Infection also may result in continuous excretion. For instance, infection of cattle by *Leptospira* serovar Hardjo, results in urinary excretion of the bacterium that can last for 12–24 months. The long period of infectiousness of hosts infected with such agents ensures that a susceptible population, resulting from regular births, is always available. Some endogenous agents (see Chapter 5) may persist as the bacterial flora of hosts.

Agents may persist not only within vertebrate hosts but also in arthropod vectors. African swine fever virus, for example, persists in ticks for up to eight months (Haresnape and Wilkinson, 1989). *Table 6.9* lists the duration of infection of fleas by various microbial agents. Note that some agents (e.g., murine typhus) can persist in fleas for the latter's lifetime which can be very long – over 500 days in the case of unfed *Pulex irritans* (Soulsby, 1982). Additionally, agents can persist in flea excreta for long periods; murine typhus, for example, can persist for over nine years (Smith, 1973).

Extension of host range

Many infectious agents can infect more than one host. Indeed, their number exceeds that of one-host agents. For example, over 50% of pathogenic infectious agents to which humans are susceptible are shared by other species of animal, and are likely to be over-represented among emerging

and re-emerging pathogens (Woolhouse and Gowtage-Sequeria, 2005). An important role of the veterinarian is to control these zoonoses (e.g., tuberculosis and canine ascarid infections). Some infections of various hosts are inapparent, increasing the difficulty of control. For example, the bacterium *Borrelia burgdorferi*, the tick-transmitted cause of Lyme disease in man and other animals, also inapparently infects several wild and domestic mammals and birds, which maintain the infection in Europe and the US (Anderson, 1988). *Table 6.10* lists some infectious diseases with wildlife as maintenance hosts that transmit infection to domestic livestock.

Extension of host range is an obvious way of maintaining infection, and is facilitated by the presence of the various hosts in the same area. However, if an agent is present in different species in the same region, it should not be assumed that transfer between them always occurs. For example, 18 southern African species of wildlife have been found to have antibodies to foot-and-mouth disease virus (Condy *et al.*, 1969; Keet *et al.*, 1996), but only the African wild buffalo has been shown to be an efficient maintenance host (Condy *et al.*, 1985) and occasional transmitter of infection to cattle (Thomson, 1996) and some cloven-hoofed species of wildlife (Bastos *et al.*, 2000). In contrast, several species of wild animals, such as the elephant, act as dead-end hosts and are incapable of transmitting the virus to other

Table 6.10 Some infectious diseases commonly transmitted from wildlife to domestic livestock. (Adapted from Bengis *et al.*, 2002.)

<i>Disease</i>	<i>Wildlife reservoir</i>	<i>Domestic animals affected</i>	<i>Epidemic potential</i>
African horse sickness	Zebra	Horses and donkeys	Moderate
African swine fever	Argasid ticks; warthogs	Domestic pigs	Major
African trypanosomiasis	Elephant; wild ruminants and wild suids	Cattle, horses, pigs, sheep, goats and dogs	Moderate
American trypanosomiasis (Chagas' disease)	Triatomid bugs; opossums; lagomorphs; rodents; primates	Dogs and cats	Moderate
Bluetongue	Various artiodactyls (uncertain)	Sheep and cattle	Moderate
Classical swine fever	Wild boar	Pigs	Major
Foot-and-mouth disease	African buffalo	Cattle, pigs, sheep and goats	Major
Heartwater	Suspect buffalo; other artiodactyls; chelonians and gallinaceous birds	Cattle, sheep and goats	Moderate
Lumpy skin disease	Uncertain	Cattle	Moderate
Malignant catarrhal fever	Blue and black wildebeest	Cattle	Limited (seasonal)
Newcastle disease	Wild birds; exotic pet birds	Poultry	Major
Theileriosis or Corridor disease (<i>Theileria parva</i> group)	African buffalo	Cattle	Moderate

animals (Brooksby, 1972). Thus, apart from Sub-Saharan Africa, foot-and-mouth disease in wildlife is considered to be an extension of the infection in livestock (Thomson *et al.*, 2003).

Transboundary diseases

Transboundary animal diseases are '*those [diseases] that are of significant trade and/or food security importance for a considerable number of countries; which can easily spread to other countries and reach epidemic proportions; and where control/management, including exclusion, requires cooperation between several countries*' (Otte *et al.*, 2004). Increasing use of the term has led to its application more widely, to include novel or infrequent incursions of diseases into new territory, without necessarily being associated with major epidemics (e.g., the outbreak of equine infectious anaemia in the Republic of Ireland in 2006: More *et al.*, 2008).

Transboundary diseases include both well-established and emerging diseases. They were traditionally described as 'exotic', and have a long history (e.g.,

rinderpest: see Chapter 1). However, the term exotic is now considered anachronistic in the context of a 'globalized' world (Albrow and King, 1990), with flourishing transportation and the consequent exchange of products, and interdependence of economic and cultural activities. These activities are likely to escalate as unit costs of transportation continue to fall (WTO, 1998).

Table 6.11 lists some transboundary diseases and their known or hypothesized disseminating factors, notably among which is host mobility in one form or another (Siembieda *et al.*, 2011; Basagoudanavar and Hosamani, 2013). The ability to control them effectively is directly related to their modes of transmission and maintenance. Thus, restricting the spread of equine infectious anaemia is facilitated by strict adherence to well-established control procedures; namely, testing horses to detect carriers before importing them from countries in which the disease is endemic. However, other transboundary diseases are virtually impossible to limit because of the refractory nature of their disseminating factors (e.g., *Aspergillus sydowii* infection of corals).

Table 6.11 Some transboundary diseases.

<i>Disease</i>	<i>Disseminating factors</i>	<i>Source</i>
African horse sickness	Nomadic movement of animals; importation of carrier zebras; El Niño-induced rainfall fluctuations; extension of vector range and distribution	Mellor and Hamblin (2004)
African swine fever	Non-regulated movements of pigs, pork and pig products; wild boar	Costard <i>et al.</i> (2013b)
<i>Anguillicola crassus</i> infestation of eels	Importation of live eels	Koops and Hartmann (1989)
<i>Aspergillus sydowii</i> infection of corals	Atmospheric transmission of African dust	Shinn <i>et al.</i> (2000)
Avian influenza	Wild-bird migration; smuggling of birds; export of captive birds	Alexander (2007)
Bluetongue	Climate-change-induced virus persistence; extension of vector range and distribution	Purse <i>et al.</i> (2005)
Classical swine fever	Direct or indirect contact with local or imported wild boar; illegal feeding of swill; importation of pigs	Edwards <i>et al.</i> (2000); Moennig <i>et al.</i> (2003)
Contagious bovine pleuropneumonia	Transboundary trafficking of cattle	Geering and Amanfu (2002)
Equine infectious anaemia	Importation of carrier horses	DEFRA (2011b)
Foot-and-mouth disease	Non-regulated movements of susceptible species and products; transboundary trafficking of susceptible species	EMPRES (2003); Hartnett <i>et al.</i> (2007)
Haemorrhagic septicaemia	Importation of carrier animals; susceptible wild animals	De Alwis (1999); Abusalab <i>et al.</i> (2003)
Newcastle disease	Trade in poultry and poultry hatching eggs; smuggling of pet birds; feral birds; racing pigeons; contaminated meat; poultry food	Alexander (1988); Cobb (2011)
Peste des petits ruminants	Transboundary trafficking of small ruminants	EMPRES (2009)
Rift Valley fever	Importation of animals; animal migration; wind-borne vectors; natural and dam-induced flooding; El Niño-induced rainfall fluctuations	Balkhy and Memish (2003); Anyamba <i>et al.</i> (2008); Rolin <i>et al.</i> (2013)
White spot disease of shrimp	Translocation of live brood shrimps and post-larvae; feeding of unprocessed shrimp meal	Hill (2002)

Further reading

- Anderson, R.C. (2000) *Nematode Parasites of Vertebrates: Their Development and Transmission*, 2nd edn. CABI Publishing, Wallingford
- Appel, M.J. (Ed.) (1987) *Virus Infections of Vertebrates, Volume 1. Virus Infections of Carnivores*. Elsevier, Amsterdam
- Blaha, T. (Ed.) (1989) *Applied Veterinary Epidemiology*. Elsevier, Amsterdam. (Includes details of the transmission and maintenance of the major infectious diseases of domestic animals)
- Brooksby, J.B. (Ed.) (1983) The aerial transmission of disease. *Philosophical Transactions of the Royal Society of London, Series B*, **302**, 439–604
- Desowitz, R.S. (1997) *Who Gave Pinta to the Santa Maria?: Torrid Diseases in a Temperate World*. W.W. Norton and Company, New York and London. (A popular historical account of the global spread of infectious diseases)
- Dieckmann, U., Metz, J.A.J., Sabelis, M.W. and Sigmund, K. (2002) *Adaptive Dynamics of Infectious Diseases: In Pursuit of Virulence Management*. Cambridge University Press, Cambridge
- Dinter, Z. and Morein, B. (Eds) (1990) *Virus Infections of Vertebrates, Volume 3. Virus Infections of Ruminants*. Elsevier, Amsterdam
- Domenech, J., Lubroth, J., Eddi, C., Martin, V. and Roger, F. (2006) Regional and international approaches on prevention and control of animal transboundary and emerging diseases. *Annals of the New York Academy of Sciences*, **1081**, 90–107
- Fèvre, E.M., Bronsvoort, B.M. de C., Hamilton, K.A. and Cleaveland, S. (2006) Animal movements and the

- spread of infectious diseases. *Trends in Microbiology*, **14**, 125–131
- Fernández, P.J. and White, W.R. (2010) *Atlas of Transboundary Animal Diseases*. World Organisation for Animal Health, Paris
- Gavier-Widén, D., Duff, J.P. and Meredith, A. (Eds) (2012) *Infectious Diseases of Wild Mammals and Birds in Europe*. Wiley Blackwell, Chichester
- Geering, W.A., Forman, A.J. and Nunn, M.J. (1995) *Exotic Diseases of Animals: A Field Guide for Australian Veterinarians*. Bureau of Resource Sciences, Australian Government Publishing Service, Canberra. (A general description of exotic diseases, with a discussion of eradication in the context of Australia)
- Gibbs, E.P.J. and Bokma, B.H. (Eds) (2002) *The Domestic Animal/Wildlife Interface: Issues for Disease Control, Conservation, Sustainable Food Production, and Emerging Diseases*. *Annals of the New York Academy of Sciences*, Vol. **969**. The New York Academy of Sciences, New York. (Includes discussions of wild animal reservoirs of infection)
- Goering, R.V., Dockrell, H.M., Wakelin, D., Zuckerman, M., Chiodini, P.L. *et al.* (2008) *Medical Microbiology*, 4th edn. Mosby Europe, London. (A description of host/parasite relationships, host defence mechanisms and microbial strategies for transmission)
- Gordon Smith, C.E. (1982) Major factors in the spread of infections. In: *Animal Disease in Relation to Animal Conservation*. Symposium of the Zoological Society of London No. 50. Eds Edwards, M.A. and McDonnell, U., pp. 207–235. Academic Press, London and New York
- Hurd, H. (1999) Manipulative parasites. *Biologist*, **46**, 162–166. (A concise outline of transmission strategies adopted by parasites)
- Ishibashi, T. and Berlingieri, F. (2010) Animal diseases and international trade. In: *Infectious and Parasitic Diseases of Livestock*, Vol. 1. Eds Lefèvre, P.-C., Blancou, J., Chermette, R. and Uilenberg, G., pp. 53–61. Lavoisier, Paris
- Karesh, W.B., Cook, R.A., Bennett, E.L. and Newcomb, J. (2005) Wildlife trade and global disease emergence. *Emerging Infectious Diseases*, **11**, 1000–1002
- Matumoto, M. (1969) Mechanisms of perpetuation of animal viruses in nature. *Bacteriological Reviews*, **33**, 404–418
- McFerran, J.B. and McNulty, M.S. (Eds) (1993) *Virus Infections of Vertebrates, Volume 4. Virus Infections of Birds*. Elsevier, Amsterdam
- Mims, C.A., Nash, A. and Stephen, J. (2000) *Mims' Pathogenesis of Infectious Disease*, 5th edn. Academic Press, London
- Nathanson, N. (2005) Virus perpetuation in populations: biological variables that determine persistence or eradication. In: *Infectious Diseases from Nature: Mechanisms of Viral Emergence and Persistence*. Eds Peters, C.J. and Calisher, C.H., pp. 3–15. Springer-Verlag, Wien/New York
- Pastoret, P.-P., Thiry, E., Brochier, B., Schwerts, A., Thomas, I. *et al.* (1988) Diseases of wild animals transmissible to domestic animals. *Revue Scientifique et Technique, Office International des Epizooties*, **7**, 705–736
- Pensaert, M.B. (Ed.) (1989) *Virus Infections of Vertebrates, Volume 2. Virus Infections of Porcines*. Elsevier, Amsterdam
- Osterhaus, A. (Ed.) (1994) *Virus Infections of Vertebrates, Volume 5. Virus Infections of Rodents and Lagomorphs*. Elsevier, Amsterdam
- Quinn, P.J., Markey, B.K., Donnelly, W.J.C., Leonard, F.C., Hartigan, P. *et al.* (2011) *Veterinary Microbiology and Microbial Disease*, 2nd edn. Wiley Blackwell, Oxford
- Roche, B., Rohani, P., Dobson, A.P. and Guégan, J.-F. (2013) The impact of community organization on vector-borne pathogens. *The American Naturalist*, **181**, 1–11
- Sewell, M.M.H. and Brocklesby, D.W. (Eds) (1990) *Handbook on Animal Diseases in the Tropics*. 4th edn. Baillière Tindall, London. (A description of tropical infectious diseases of animals, including concise summaries of transmission and maintenance)
- Simpson, V.R. (2002) Wild animals as reservoirs of infectious diseases in the UK. *The Veterinary Journal*, **163**, 128–146
- Simpson, V. (2008) Wildlife as reservoirs of zoonotic diseases in the UK. *In Practice*, **30**, 286–294
- Studdert, M.J. (Ed.) (1996) *Virus Infections of Vertebrates, Vol. 6. Virus Infections of Equines*. Elsevier, New York
- Taylor, M.A., Coop, R.L. and Wall, R.L. (2007) *Veterinary Parasitology*, 3rd edn. Blackwell Publishing, Oxford
- Williams, E.S. and Barker, I.K. (Eds) (2001) *Infectious Diseases of Wild Mammals*, 3rd edn. Manson Publishing, London
- Woldehiwet, Z. and Ristic, M. (Eds) (1993) *Rickettsial and Chlamydial Diseases of Domestic Animals*. Pergamon Press, Oxford
- Woodford, M.H. and Rossiter, P.B. (1993) Disease risks associated with wildlife translocation projects. *Revue Scientifique et Technique, Office International des Epizooties*, **12**, 115–135

7

The ecology of disease

The study of disease in populations requires an understanding of the relationships between organisms (hosts and agents) and their environment. These relationships govern the spatial and temporal occurrence of disease. Climate, for example, affects the survival of hosts and infectious agents and the distribution of the latter's vectors and reservoirs either directly or, more subtly, by regulating the occurrence of plants that support the organisms. Similarly, the type of plant can affect the availability of minerals and trace elements, and therefore the occurrence of disease associated with a deficiency, an excess or an imbalance of these chemicals. For instance, white clover (*Trifolium repens*) absorbs relatively small amounts of selenium, whereas brown top (*Agrostis tenuis*) absorbs large amounts (Davies and Watkinson, 1966). Therefore, when pasture is top-dressed with selenium salts to prevent selenium deficiency in animals, the risk of selenium toxicity is greater if the latter plant predominates. Thus, natural factors (type of plant) and agricultural practices (pasture management) both exert an effect.

The study of animals and plants in relation to their habits and habitation (**habitat**) is **ecology** (Greek: *oikos* = 'house'; *logo-* = 'discoursing')¹. Ecology developed as a discipline relating to animals and plants, but has been extended to include microorganisms (e.g., Atlas and Bartha, 1998) and has a qualitative and quantitative framework similar to epidemiology (Dodson *et al.*, 1998).

Ecology also has been extended to encompass not only the natural (i.e., physical and biological) but also the social, cultural and historical contexts in which populations exist, including local and global factors,

such as urbanization and globalization (McLaren and Hawe, 2005). Infectious agents often 'capitalize' on ecological and social disturbance (Weiss and McMichael, 2004)². Thus, the severe consequences of the bubonic plague (the Black Death) epidemic in the 14th century in Europe (see Chapters 1 and 4) have been linked to a chain of natural and social events over the preceding decades, including unusual weather leading to crop failure, with subsequent impoverishment and malnutrition of the population, all compounding the emerging deterioration of the feudal social system (Gottfreid, 1983). More recently, changes in human ecology, including rural-to-urban migration, changes in personal behaviour, the social disruption of war and conflict, and increasing long-distance mobility (see Chapter 6) have been responsible for the emergence and spread of new diseases (e.g., HIV) and resurgence of old ones (e.g., tuberculosis and cholera). Agricultural changes (e.g., intensification of pig farming and expansion of farming into new areas) combined with, and linked to, ecological disruption (e.g., deforestation) are critical to the emergence of some infectious diseases (e.g., Nipah virus).

In the context of animal disease, the scale of ecological studies therefore ranges from an investigation of leptospires in the 'environment' of the renal tubules to the distribution and dynamics of sylvatic hosts of foot-and-mouth disease in the African savannahs, and to the impact of human activities on the emergence and spread of disease.

¹ The term 'ecology' was first used in 1866 by Ernst Haeckel in his book *General Morphology*, in support of the ideas propounded by Charles Darwin, seven years earlier, in his seminal *Origin of Species*.

² The radical precept that human social factors are the major drivers of negative ecological changes (including changing patterns in the occurrence of infectious and non-infectious diseases) is encapsulated in **social ecology** (Bookchin, 1995). Ecohealth (see Chapter 2) focusses on the effect of such changes on human health, and awareness of the extent of such changes and their global impact on human health has driven the emergence of planetary health (see Chapter 2), which aims at a multidisciplinary solution to the problem (Almada *et al.*, 2017).

Table 7.1 Koppen's system of classification of climate based on de Candolle's plant groups. (From Colinvaux, 1973.)

<i>De Candolle's plant group</i>	<i>Postulated plant requirements</i>	<i>Formation</i>	<i>Koppen's climatic division</i>
Megatherms (most heat)	Continuous high temperature and abundant moisture	Tropical rain forest	A (rainy with no winter)
Xerophiles (dry-loving)	Tolerate drought, need minimum hot season	Hot desert such as Sonoran	B (dry)
Mesotherms (middle heat)	Moderate temperature and moderate moisture	Temperate deciduous forest	C (rainy with mild winters)
Microtherms (little heat)	Less heat, less moisture, tolerate long cold winters	Boreal forest	D (rainy climates with severe winters)
Hekistotherms (least heat)	Tolerate polar regions 'beyond tree-line'	Tundra	E (polar climates with no warm season)

The study of a disease's ecology (also termed its natural history) is frequently a part of epidemiological investigations. This has two objectives:

1. an increase in the understanding of the pathogenesis, maintenance and, for infectious agents, transmission of disease;
2. the use of knowledge of a disease's ecology to predict when and where a disease may occur, to enable the development of suitable control techniques.

This chapter introduces basic ecological concepts and relates them to epidemiology.

Basic ecological concepts

Two major factors that determine the occurrence of disease are the distribution and size of animal populations. The former depends on the distribution of suitable food; the latter depends on availability of food, mates and the species' breeding potential. Both may be under the influence of natural and human forces.

The distribution of populations

Vegetational zones

Botanists were among the first to note the division of the Earth into different vegetational zones. In some parts of the world this division is clear; for example, the border between forest and tundra in northern regions, and the zoning of forests as one ascends mountains. In other areas the change is more gradual, for instance, the transition from deserts to prairies. Early 18th-century naturalists suggested that the world was divided into discrete **formations** of vegetation, such as tundra, savannah and desert, and they drew maps that neatly but erroneously separated formations by lines.

The first serious attempt to explain these apparently neat formations was made by de Candolle (1874), who argued that climate, particularly temperature, dictated vegetation³. He drew the first vegetational map based on isotherms. Rain forests were described as formations of **megatherms**, deciduous forests of **mesotherms**, and deserts of **xerophiles**. At the beginning of the 20th century Koppen used de Candolle's classification as the basis of the modern system (*Table 7.1*), which provides a good correlation between climatic and vegetational regions. Climate may dictate boundaries, but in a much more complex way than merely by ground-level temperature changes and rainfall. Meteorological work using satellites, and long-term studies of the thermal composition of air masses, however, have suggested that the mean positions of air fronts over the Earth roughly coincide with vegetational types.

Biomes

In the 19th century, zoologists noted that the broad divisions of the Earth were populated by similar animals. Even if the divisions were discontinuous (e.g., Africa and South America), some animals, especially birds, showed similar features. This assisted the evolutionists in adding credence to their theory of **convergent evolution**, which states that animals of different ancestral stock evolve similar features to suit similar environments.

Zoologists attempted to classify different areas of the world according to the types of animal and plant that were present, because the distribution of animals appeared to be related to vegetation. Wallace (1860, 2017) and Huxley (1868) defined zoological boundaries (their 'Lines') separating Asia from the Asian–

³ Alexander von Humboldt had earlier noted the relationship between altitude-related temperature differences and the range of plants (Egerton, 2009).

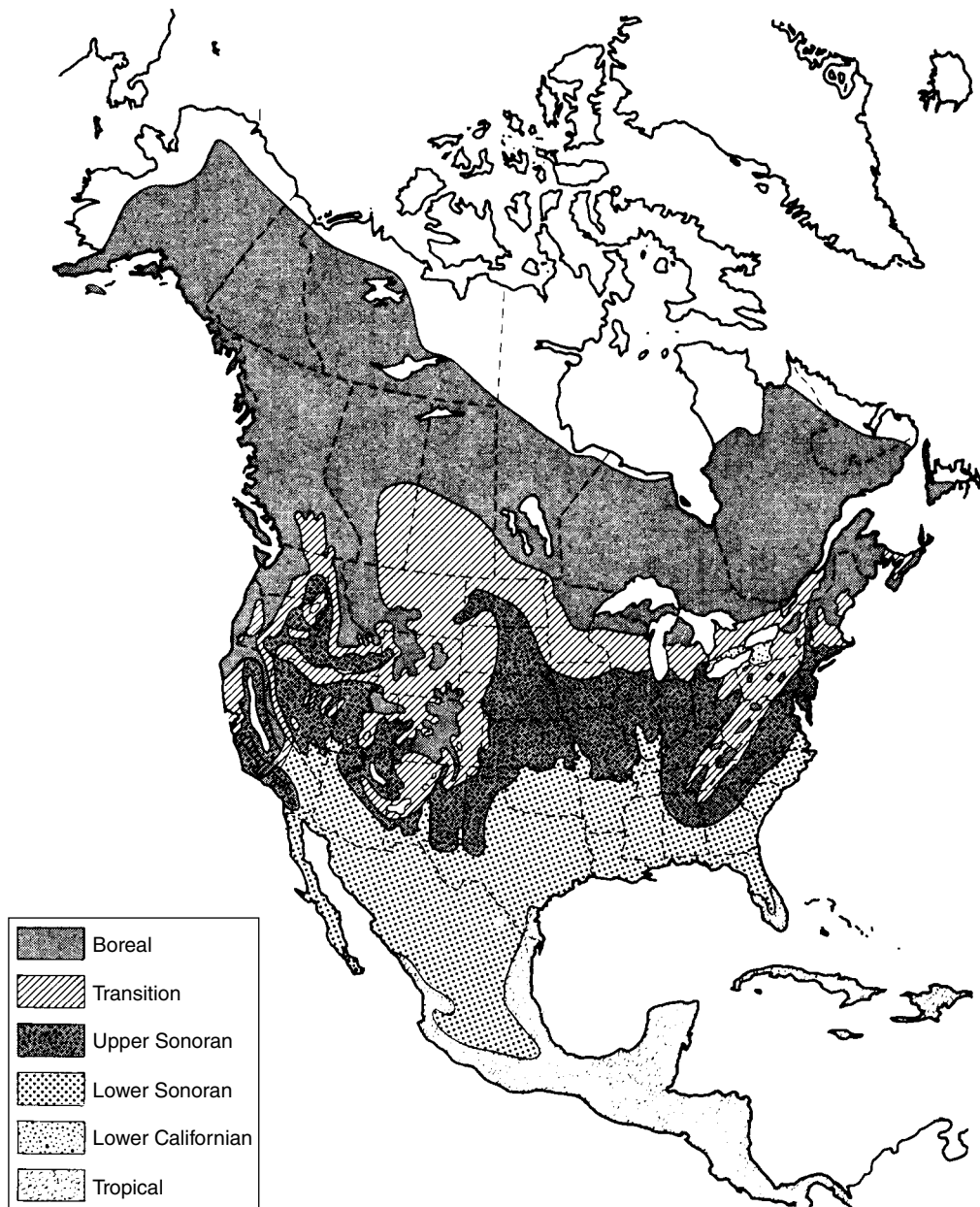


Fig. 7.1 Map of the life zones of North America proposed by Merriam. (Source: Merriam, 1893. Public domain.)

Australian transitional region⁴. Later, Merriam (1893) defined **life zones** in North America after studying the distribution of animals and plants at various altitudes on mountains.

⁴ Although zoological boundaries may suggest barriers to disease transmission, some infected species may cross them (McCallum *et al.*, 2008). Moreover, boundaries based on the distribution of animals, alone, may be of little use to botanists (Van Welzen *et al.*, 2011).

Merriam proposed four main life zones (*Figure 7.1*):

1. Boreal (northern), involving the Canadian, Hudsonian and Alpine Arctic;
2. Transition, containing animals and plants from the Boreal and Sonoran;
3. Sonoran (named after Sonora, a state in NW Mexico), comprising the Upper and Lower;
4. Tropical.

A fifth, minor one (the Lower Californian), also is indicated in *Figure 7.1*.

The first impression of the vegetation in a life zone is termed its **vegetational physiognomy**; for example, the boreal life zone is dominated by conifers. However, it is important to note that there is a gradual transition from one zone to another; the apparent boundary on the life-zone map is set sharply by the cartographer. African ecological zones, based on climate, vegetation and potential for agricultural use, are described by Pratt and others (1966), White (1983) and FAO (1999), and global zones by FAO (2014).

Merriam, like de Candolle, thought that temperature governed the distribution of animals. He argued that, in the northern hemisphere, an animal's northern boundary was drawn by the threshold temperature below which reproduction was not possible. The animal's southern boundary was drawn by the threshold temperature above which the heat was intolerable. Although Merriam spent much time measuring mean temperatures, he was never able to match isotherms with life zones. Although reasons for the transition from one life zone to another are not available, the existence of these zones is clear. They are now commonly called **biomes**⁵. Thus, tropical rain forest, savannah and tundra are biomes, each with its own particular range of plants and animals (the boreal biome is colloquially called the 'Spruce-Moose' biome).

The notion of zones also is associated with the concept of an **ecotope**. This is the smallest mappable landscape unit that is associated with a uniform or homogeneous landscape (Naveh and Lieberman, 1994; also see 'Landscape' later in this chapter). Some ecologists consider this to be functionally defined; for example, an olive grove is a Mediterranean ecotope for wintering birds (Farina, 2000a).

The distribution of infectious agents and their vectors, and therefore of the diseases produced by the former, may be limited by the environmental conditions of biomes and ecotopes. The distribution of Rift Valley fever, for example, is associated with the wetter African ecological zones. This may be related to the abundance of mosquito vectors in these zones (Davies, 1975). Likewise, commercial coconut palms

in villages in Venezuela are ecotopes for the vectors of Chagas' disease in humans, dogs and cats (Morocoima *et al.*, 2010), thus indicating specific areas of increased risk of the disease. The fungus *Coccidioides immitis*, which systemically infects humans, dogs, cattle and pigs, producing primary respiratory signs in humans and dogs, appears to be endemically limited to the Lower Sonoran life zone (Figure 7.2). This zone is characterized by hot summers, mild winters, sparse vegetation, an annual rainfall of 15–20 cm (6–8 inches), an alkaline soil pH and wind conditions that are conducive to maintenance and dissemination of the fungus (Egeberg, 1954). Although the disease was first reported over 100 years ago (Deresinski, 1980), the number of cases increased dramatically, and can be particularly severe in immunosuppressed individuals, such as those infected with human immunodeficiency virus (HIV), the cause of AIDS (Kirkland and Fierer, 1996).

The prevalence of heartworm (*Dirofilaria immitis*) infection of dogs in California is related to plant climatic zones (Figure 7.3). The seven zones are based on average temperatures during the day and night. The time required for the parasite's microfilariae to develop to infective L₃ larvae in the mosquito vector varies considerably between each zone, thus limiting the 'window' of transmission. The high prevalence in the Interior Valley areas also may be explained by the large amount of agricultural land devoted to rice production, which is known to increase the abundance of mosquitoes (Hill and Cambournac, 1941)⁶.

The nature of an ecological zone also has a major influence on animal husbandry, which, in turn, can affect morbidity and mortality (Carles, 1992). For example, animals reared on rangelands throughout the world experience marked climatic and nutritional changes (e.g., in forage type and availability), and inadequate nutrition therefore occurs. Fertility is particularly sensitive to the status of the body's reserves, and so infertility is a common problem on rangelands (Carles, 1986). The practice of trekking may pose additional stress, and so diseases in which stress is a component cause (e.g., pasteurellosis; see Figure 3.6b) are also rangeland problems. Localized environments, such as night enclosures and watering points, become heavily contaminated with microorganisms that are responsible for septicaemias in neonatal kids and lambs in some African pastoral systems. The high prevalence of brucellosis among livestock kept on rangelands may be associated with difficulties in the adequate disposal of aborted fetuses. Pre-weaning and post-weaning

⁵ The term **microbiome** has been used to refer to a characteristic microbial community occupying a well-defined habitat with distinct physical and chemical properties (Whipps *et al.*, 1988), such a collection of microbes being a **microbiota**, when the community comprises commensal, symbiotic and pathogenic microorganisms in an individual. More recently, microbiome has referred to the collection of genomes of microbes in a particular environment, the idea specifically being that a comprehensive genetic view of humans also should include the genes of the microbiota that are present (Hooper and Gordon, 2001). The virus component of a microbiome is sometimes termed its **virome** (e.g., Wylie *et al.*, 2012; Smith and Wang, 2013).

⁶ The relationship between malaria and rice cultivation is well documented (Service, 1989).

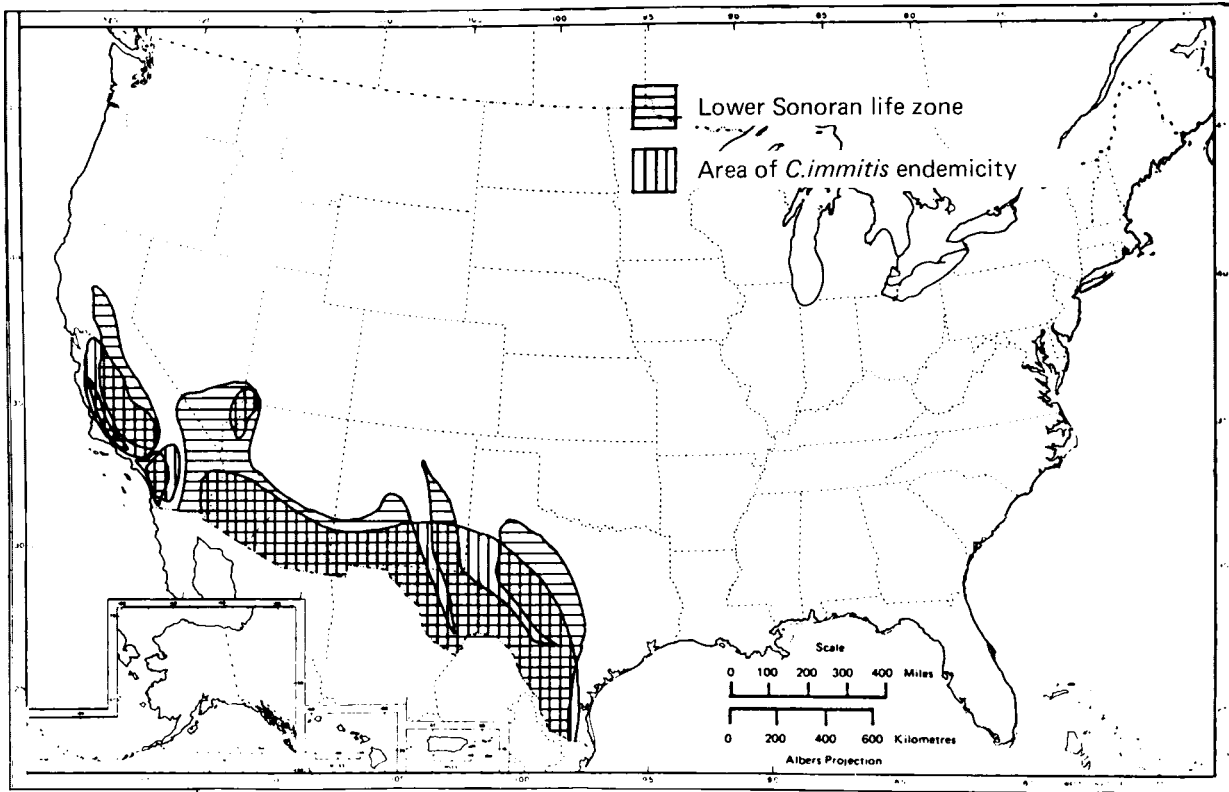


Fig. 7.2 Lower Sonoran life zone and area of endemic *Coccidioides immitis* (nosoarea) in the US. (Source: Schmelzer and Tabershaw, 1968. Reproduced with permission of Sheridan.)

mortality rates are therefore often high: in sheep and goats reared extensively on tropical rangelands in Africa, India and Australia they can exceed 30%.

Regulation of population size

The 'balance of nature'

The early biologists were impressed by the stability of animal and plant populations. Populations grow, reach a certain size, and then stop growing. The population becomes stable and **balanced**, with the rate of reproduction equalling the death rate. The theory of the 'balance of nature' has been a classical assumption in ecology for centuries, dating back to Ancient Greece (Egerton, 1973). It conveys the notion that populations, communities, ecosystems, and even the entire earth (the *Gaia hypothesis*: see Chapter 2), are potentially self-regulating systems that are kept in a stable equilibrium, if left alone⁷. However, it is disputed by some

⁷ This justified the conservationists' view that nature should be 'unmolested', although there are examples of new ecosystems resulting from human intervention without major adverse effects (e.g., the conversion of dry rain forest into savannah by indigenous Australians: Jones, 1969; Bird *et al.*, 2008).

contemporary ecologists, who argue in favour of more complex models of equilibrium (Kricher, 2009).

Control of population size by competition

Two hypotheses have been formulated to explain the 'balance of nature'. Chapman (1928) argued that there was **environmental resistance**. Animal populations had an intrinsic rate of increase but there was some quality of the environment that resisted the increase. This theory may be good but there is no evidence to support it. The currently accepted theory is that populations are brought into balance by **competition** for the resources of the habitat, the most common of which is food⁸. Competition therefore is **density-dependent**.

In order to test this hypothesis it is necessary to conduct an experiment with food supply controlled. Such an experiment was conducted by Gause (1934), using one species of *Paramecium* with a constant supply of food. The growth curve for this protozoon is a sigmoid

⁸ Competitive strategies, however, can be more complex than competition for food; for example, those between bacterial populations (Hibbing *et al.*, 2010).

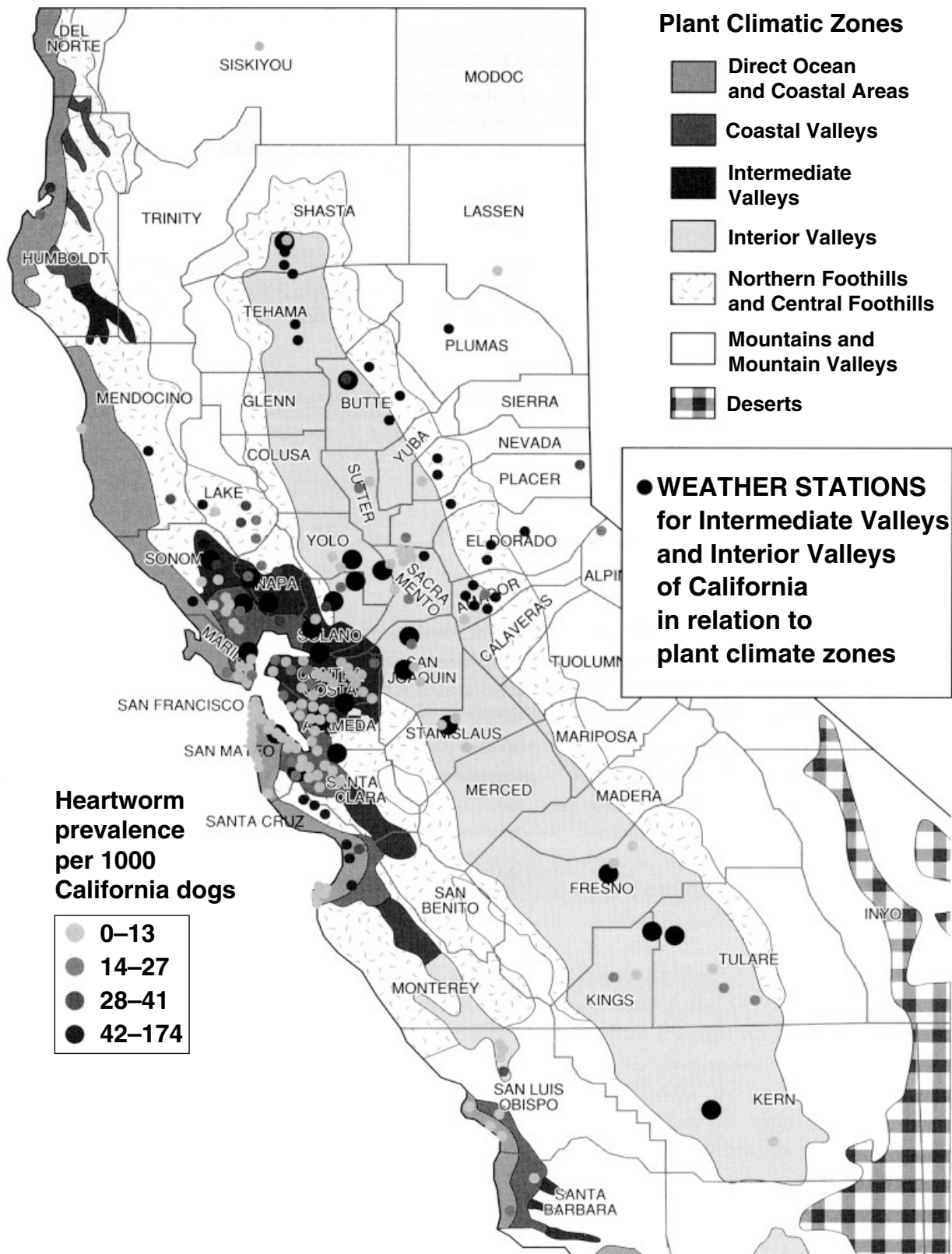


Fig. 7.3 Prevalence of canine heartworm in California. (Source: Theis *et al.*, 1998.)

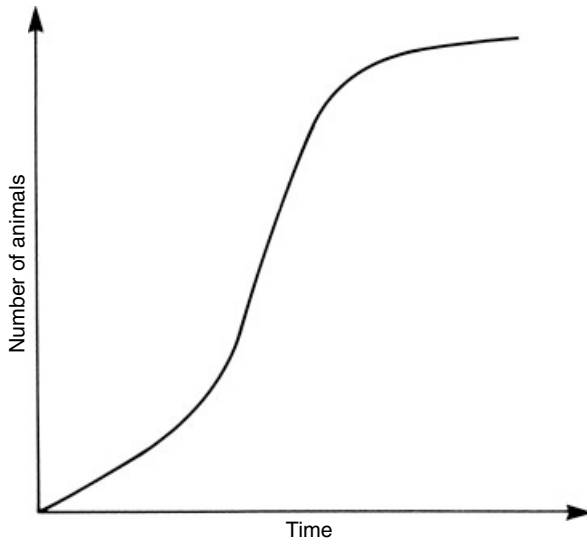


Fig. 7.4 The sigmoid growth curve for a simple population in a confined space with limited input of energy.

(Figure 7.4) that approximates to a mathematical equation, called the **logistic equation** (see also Chapter 22).

The logistic equation is derived thus: if R = observed rate of increase, r = intrinsic rate of increase, N = number of animals, then, for the slope of the curve:

$$R = rN.$$

If K = saturation number, then, with increasing competition, when $N = K$, $R = 0$.

The complete history of the sigmoid curve is:

$$R = rN \left(1 - \frac{N}{K} \right).$$

Early in growth, when growth is rapid because much food is available, N is small; therefore the quotient N/K is small:

$$1 - \frac{N}{K} \text{ approaches } 1,$$

and R approaches rN .

Later, as growth decreases (because less food is available):

$$\frac{N}{K} \text{ approaches } \frac{K}{K} = 1.$$

Thus $\frac{N}{K}$ approaches 0.

Thus $R = rN \times 0$
 $= 0$.

Further laboratory studies, for example with the fruit fly *Drosophila*, supported the hypothesis of density-dependent competition for food. Parasite populations

also may be regulated by this mechanism. Reproduction of *Ascaris* spp., for example, is density-dependent (Croll *et al.*, 1982). However, experiments with other insects, notably the flour beetle, showed that density-dependent factors other than food availability may control the size of populations; for instance, the build-up of metabolic excretory products, and decreased reproduction due to crowding. Thus, the competition model is a useful theoretical concept that has aided understanding of regulatory⁹ mechanisms, but in the real world density dependence is rarely the result of just a single factor: various factors may operate at different times to control population size.

Dispersal

In some parts of the world, there may be dramatic seasonal variations in climate. An Australian species of grasshopper overwinters in its egg. The warmth of spring causes the eggs to hatch. The adults that develop then lay eggs as long as the weather is wet. A drought kills all of the adults. This is not density-dependent; it occurs long before competition occurs. Such insects survive only by **dispersal** over large areas to different climates so that at least some are in an area that is wet. This phenomenon led Andrewartha and Birch (1954) to suggest that large animals, too, were controlled by climate, not by competition for food. They emphasized the dangers of oversimplification using the logistic model.

Forced dispersal of animals has been advocated as a means of controlling disease; for example, relocating wild bison away from an anthrax epidemic (Novakowski *et al.*, 1963) and moving waterfowl away from botulism outbreaks (Parrish and Hunter, 1969). However, this may be problematic if it conflicts with animals' natural movement tendencies (Meagher, 1989).

The Moran effect

Populations may be regulated by climatic and other environmental factors over large areas, and if two similar populations have the same density-dependent structure then their sizes over these areas may be synchronized; this is termed the **Moran effect** (Moran, 1953; Royama, 1992; Hudson and Cattadori, 1999). This phenomenon can be a determinant of disease transmission. Thus, in Kazakhstan, the prevalence of plague among great gerbils (*Rhombomys opimus*) is increased in warmer springs and wetter summers

⁹ 'Population regulation' and the 'balance of nature' are synecdoches, which imply that there is active control with a particular purpose. However, both terms are used by ecologists simply to describe the processes that affect population dynamics – no purpose is required.

(Stenseth *et al.*, 2006) and correlates with the population density of the gerbils and the flea vectors (Reijnen *et al.*, 2012). When climatic conditions uniformly affect large geographical areas, they can synchronize gerbil densities regionally (Kausrud *et al.*, 2007). This synchronization of high gerbil densities facilitates gradual transmission of plague across the region (Davis *et al.*, 2008). The collapse of gerbil populations in response to climatic changes results in an increase in the density of fleas per gerbil, facilitating further the spread of plague within the rodent population (Reijnen *et al.*, 2014) and causing fleas to seek out alternative hosts, including humans and domestic animals (Samia *et al.*, 2011). Such periods of heightened plague activity in Asia may explain the historical pattern of plague in Western Europe during and after the Middle Ages, resulting from trade with Asia (see Chapters 1, 4 and 5: the Black Death).

Population synchrony is strongly correlated with the probability of global extinction and so is particularly relevant to conservation (Hanski and Woiwod, 1993; Heino *et al.*, 1997)¹⁰. Synchrony also can be induced by dispersal (Ranta *et al.*, 1995) and the relative contributions of the Moran effect and dispersal to synchrony influence the probability of extinction. If dispersal is the main cause of synchrony and a local population is wiped out (e.g., by a disease epidemic) then the empty habitat will be recolonized. In contrast, if the Moran effect dominates and dispersal is rare then the empty habitat might not be repopulated. At a local scale, dispersal can dominate over the Moran effect, whereas the latter tends to dominate at global scales (Ranta *et al.*, 1995; Heino *et al.*, 1997). However, the two mechanisms are not mutually exclusive, and the precise causes of synchrony may require an experiment or complex analyses (e.g., Grenfell *et al.*, 1998). If the Moran effect is known to dominate, it may be possible to eradicate local pest and pathogen populations, knowing that recolonization is unlikely.

Predation

Predation has an obvious plausible role in controlling the size of populations but, in large animals, there is contradictory evidence. For example, the reintroduction of wolves into Yellowstone National Park in the US in the 1990s was subsequently associated with a dramatic decline in the number of elk, suggesting that the predatory wolves had an impact on the elk

population (Ripple *et al.*, 2010)¹¹. Similarly, predation has a role in controlling hare populations, although other influences, such as the availability and quality of food, also exert an effect (Krebs and Boutin, 1995; Krebs *et al.*, 2001). In contrast, in the Serengeti of Africa there were about 200 000 wildebeests in the 1960s, but lions killed only 12 000–18 000 each year, an insignificant number (mostly of newborn animals, of which only a small proportion were taken because reproductive synchrony resulted in a glut of animals: Estes, 1976), whilst the migratory pattern of some ungulates may allow them to escape predators (Fryxell *et al.*, 1988).

However, small predators, notably of insects, are efficient controllers of populations. Predators have been used to control insect pests; for example, the use of ladybirds to control cotton cushiony scale insects (DeBach and Rosen, 1991).

Infectious disease

Pandemics with high case fatality rates, such as the Black Death and rinderpest (see Chapter 1) clearly had a severe impact on populations. However, considerable interest is developing in the role of infectious disease in determining and regulating the size of populations more generally (Anderson and May, 1986, 1991). There is circumstantial evidence, for instance, that the approximate decadal fluctuations in populations of forest-dwelling Lepidoptera are caused by epidemics of disease (Myers, 1988), and there is evidence of similar effects in vertebrate populations. The chytrid fungus, *Batrachochytrium dendrobatidis*, for example, probably caused population decline in rain-forest-dwelling frogs in Australia and Panama (Berger *et al.*, 1999). Intentional release of rabbit haemorrhagic disease virus appears to be an effective method of controlling rabbit populations (Sanson *et al.*, 2000), and myxomatosis appears to have a regulatory effect, too (Ross *et al.*, 1989).

There are theoretical reasons why infectious disease actually may lead to extinction of a species (de Castro and Bolker, 2005), and infections are a major risk to endangered species (McCallum and Dobson, 1995; Pedersen *et al.*, 2007) when they 'spill over' from other species. Canine distemper virus, for example, has killed over 70% of the last remaining free-living colony of black-footed ferrets (see also Table 6.5). Historical infectious-disease-induced extinctions most likely include species of Hawaiian land birds, caused by avian malaria in the 19th century, and native species of rats

¹⁰ The phenomenon of synchrony is widespread in natural systems and includes not only populations but also disease epidemics (Satake and Koizume, 2008).

¹¹ Predation may not have been the only factor related to the decline, other influences may have included drought and culling by humans (Dobson, 2014).

on Christmas Island, caused by trypanosomiasis around the turn of the 20th century (McCallum, 2012). Currently, the Tasmanian devil is threatened with extinction due to the infection causing facial tumour disease (McCallum *et al.*, 2009).

Infectious agents can be divided into two groups according to their generation dynamics: **microparasites** and **macroparasites** (May and Anderson, 1979); this is a useful classification when considering the effects of parasites on host populations. The microparasites multiply directly when inside the hosts, increasing the level of parasitism. They include viruses, bacteria and protozoa. Macroparasites, in contrast, do not increase the level of parasitism: they *grow* in the host, but multiply by producing infective stages, which are then released from the host to infect new hosts. They include helminths and arthropods.

Microparasitic infections can obviously depress host population size when they occur as epidemics or pandemics with high case fatality rates. Such effects are usually transient, but they do indicate a potential for more general regulation of the host population akin to that of predators.

The intrinsic growth rate of a population must be influenced by parasites if they are to regulate population size. This rate is governed by host survival and reproductive capacity. Microparasites are frequently pathogenic organisms of high virulence. Their obvious regulatory potential therefore lies in their capacity to kill hosts, but they also may have less direct effects such as inducing susceptibility to opportunistic infections and rendering infected hosts 'less competitive' than their peers.

There are certain experimentally- and theoretically-identified conditions under which microparasites can regulate host population size (Anderson, 1981). Disease-induced mortality must be high compared with the disease-free rate of growth. The influence of pathogenicity and virulence, however, is often moderated by a short period of infectiousness because fewer individuals are likely to be infected. Moreover, enduring immunity in recovered individuals prevents recurrence of disease. These last two factors therefore mitigate the regulatory effects of virus and bacterial infections in vertebrates. (Invertebrates cannot develop acquired immunity, and the capacity of pathogenic bacteria and viruses to regulate invertebrate populations is generally acknowledged.) Early epidemiological studies indicated that the HIV/AIDS pandemic could be so severe as to reverse positive population growth (Anderson and May, 1988), and subsequent evidence supported this (Whiteside, 1998; Barnett and Whiteside, 2002). Characteristics of the infection that contribute to this effect are the virus' high virulence, the lack of an immune response

Table 7.2 The average parasite burdens of North American mammals.* (From Dobson *et al.*, 1992.)

	Mean burden per individual host	Mean number of species per host population
Trematodes	108	1.8
Cestodes	140	2.8
Nematodes	117	5.3
Acanthocephalans	1	0.3
Number of parasite species	3	10

* The data are from 76 populations of mammals comprising 10 species of lagomorphs, 22 species of rodents, 35 species of carnivores and 11 species of artiodactyls. Carnivores generally had the most diverse range of parasites, and lagomorphs the least. Gregarious species had the highest mean parasite burdens.

in the host (and, consequently, in many countries, the fatal outcome of the disease without treatment), the long period of infectiousness, and vertical transmission. These factors are compounded by the social distribution of HIV/AIDS, with the people most at risk (such as those living in poverty) also being those least able to access treatment (Farmer, 1999)¹².

Infections with macroparasites, particularly helminths, are ubiquitous in the Animal Kingdom (Table 7.2). Therefore, they could have a widespread regulatory effect on animal populations, although the effect may not be as overt as those induced by microparasites. Helminths not only significantly decrease an animal's growth rate but also can reduce host survival and reproductive capacity. For example, sheep mortality is related to intensity of infection with *Fasciola hepatica* (Smith, 1984); and red grouse infected with *Trichostrongylus tenuis* produce smaller clutch sizes than uninfected birds (Hudson, 1986).

Infections also may act more subtly; for example, by increasing a host's susceptibility to predation (Anderson, 1979). Thus, cormorants capture a disproportionately large number of roach infected with the tapeworm, *Ligula intestinalis*, compared with the prevalence of infected fish in the population as a whole (Dobben, 1952).

Microparasites and macroparasites have been identified as suitable candidates for the control of rats, dogs, cats and goats that are introduced to oceanic islands; parasitic nematodes and viruses of low to intermediate

¹² There also may be less direct effects of disease on population size. The 18th-century English demographer, the Rev. Thomas Malthus, argued that disease served as a 'positive check' on population growth by affecting growth in food supply (Malthus, 1798), and the effect of the Black Death on food production has already been noted (see Chapter 4).

pathogenicity being the most appropriate (Dobson, 1988). It also has been postulated that microparasites might be used less directly to control vertebrate pest populations by being genetically engineered to express genes encoding gamete proteins, thus 'immunosterilizing' the pests (Tyndale-Biscoe, 1994).

The population dynamics of microparasites are discussed by Nokes (1992), Bulmer (1994), Ganusov and others (2002) and Nath and others (2008). Dobson and others (1992) discuss macroparasites.

Home range

Certain animals have a natural restriction to the area over which they roam to undertake normal activities (e.g., foraging, hunting and breeding); this is their **home range** (Burt, 1943). For example, Arctic birds have a larger home range when food is scarce than when it is plentiful. This may control the population, and has implications for the transmission of infectious disease (Dürr and Ward, 2014); infected animals may transmit infection over their home range, but no further. For example, rats are the maintenance hosts of the rickettsial disease scrub typhus. Trombiculid mites (chiggers) are the vectors; they parasitize mammals and birds. The small home range of the rats results in the mite's life-cycle being restricted to small areas, called 'mite islands' (Audy, 1961). When the mites are infected with the rickettsia, localized endemic areas of scrub typhus, associated with the mite islands, occur. Extension of the infection occurs only by dispersal of infected mites by wider-ranging incidental hosts such as birds and man, in whom the infection causes serious disease.

Home range is a useful guide to areas over which disease surveillance in wildlife should be conducted (see Chapter 21). For example, in the US, coyotes are efficient sentinels (see Chapter 21) of bovine tuberculosis, and so it is recommended that sampling commences in this species at least one home range from the perimeter of the infected premises to distinguish coyotes that ingested infected livestock on the known infected premises from those that ingested bovine tuberculosis from either wildlife or infected livestock from neighbouring premises (USDA, 2011)¹³.

¹³ Home ranges are often expressed areally (i.e., in square miles or square kilometres), but surveillance is usually undertaken within a fixed radius (e.g., Figure 11.11). The surveillance radius, r , can be calculated from the home range, thus: $r = 2 \times \sqrt{(a/\pi)}$, where a is the home range (USDA, 2011). For example, if a species' home range is five square miles, then $r = 2 \times \sqrt{(5/3.142)} = 2.5$ miles. This converts home range in square miles to circular equivalent areas: the diameter of the circular home range then becomes the radius of the surveillance zone. However, this should not be the only means of determining the appropriate area to sample. Consideration also

Domesticated species also may demonstrate home ranges if they are allowed to roam free. For example, free-ranging domestic pigs in Kenya have home ranges of over 10 000 m², increasing their risk of exposure to African swine fever, toxoplasmosis, trypanosomiasis and trichinellosis (Thomas *et al.*, 2013).

Territoriality

The part of an animal's home range that it defends aggressively from invaders is the animal's **territory**. This behavioural response is **territoriality** (Maher and Lott, 1995), which is particularly conspicuous in birds. This has an advantage, economizing movement when searching for food. Territoriality also may control the population because there is a minimum size to a territory and a finite amount of space, and therefore a finite number of animals that can exist in the territory. The conditions that favour territoriality over non-territorial organization may depend on the 'economics' of the strategy (Brown, 1964). If a resource (food) is scarce, widely dispersed or subject to seasonal fluctuation, then it may be too costly to defend by territorial behaviour. These factors interact to determine optimum territorial size (Carpenter *et al.*, 1983).

Although territoriality might be expected to be an effective buffer against incursion and spread of infectious diseases, territorial populations can include mobile, non-resident individuals, which may be highly effective spreaders of infection (Craft *et al.*, 2011). Additionally, infection may spread between different territorial groups of the same, or different, species, at, or *en route* to, habitat 'hot spots' (e.g., watering holes: Benavides *et al.*, 2012).

Social dominance

In the 1920s, a social hierarchy called the 'peck order' was discovered among birds. Some gregarious species, especially rodents, inhabit favourable places. When crowding occurs, the socially weaker animals are forced out. This may be a population-control mechanism.

The 'Wynne-Edwards' hypothesis

The population-control consequence of territoriality, social hierarchy and behaviour may be just a side-effect. The Aberdeen zoologist, Wynne-Edwards, suggested that population control was the main purpose of **group behaviour** (Wynne-Edwards, 1962), which sometimes causes physiological stress (see Chapter 5). The crowding of rats results in associated fighting, cannibalism and reduced fecundity. Such experimentally-stressed animals, and those that are naturally stressed (e.g.,

should be given to the ecology of the species that is being targeted (e.g., whether or not it is migratory).

		Grouping of foxes				
		Solitary	Pair	Family	Social groups	Mixed society
Behaviour of rabid foxes	Dumb	□ □ □ □	■	■ ■	■ ■ ■	■ ■
	Furious	■	■ ■	■ ■ ■	■ ■ ■ ■	■ ■ ■
	Mixed	■	■ ■	■ ■ ■ ■ ■	■ ■ ■ ■ ■ ■ ■	■ ■ ■ ■ ■

Fig. 7.5 Social group behaviour and behaviour of rabid foxes as determinants of rabies in foxes.

□: conditions unfavourable for existence of rabies virus; number of open squares indicates the relative degree to which conditions are unfavourable. ■: conditions favourable for the existence of rabies virus; number of solid squares indicates the relative degree to which conditions are favourable. (Source: Macdonald and Bacon, 1980.)

sewer-dwelling rats), have hypertrophied adrenals, indicative of the general adaptation syndrome (see Chapter 5).

At certain times of the year animals congregate, although there are no obvious spatial restrictions (e.g., deer during the rutting season). Wynne-Edwards suggested that this 'head count' of the population evoked, by feedback, the general adaptation syndrome and controlled reproduction. There are two problems with this theory: evolution tends to select individuals, not groups, and the hypothesis suggests that birth-controllers are favoured, whereas evolution usually selects the efficient producers. This theory is not fashionable today.

For whatever reason animals congregate, the increased contact can aid transmission of infectious agents, and can produce seasonal trends in disease occurrence (see Chapter 8). Thus, when North American leopard frogs congregate to spawn during the winter, there is seasonal transmission of Lucke's frog virus (McKinnell and Ellis, 1972).

It is difficult to produce a general theory of population control. Food availability is important, and energy availability limits the supportable biomass (see 'The distribution of energy between trophic levels' later in this chapter). Infectious disease also may have a role. Different mechanisms probably operate in different circumstances.

Implications for disease occurrence of the distribution and control of populations

The distribution, the home range of animals, and other behavioural activities of hosts of infectious agents affect the latter's transmission. An example is vulpine rabies. The infection is maintained in Europe in foxes

(Wachendörfer and Frost, 1992). The behaviour of foxes during the year alters the association between foxes. The animals may be solitary, paired or part of a family unit. Similarly, rabid foxes' behaviour depends on the type of rabies that they display; foxes with dumb rabies may seek a solitary existence, whereas furious rabies may cause foxes to approach other animals readily. *Figure 7.5* illustrates how these different behaviour patterns affect the survival and spread of rabies virus between animals.

Increases in home range also may increase spread of infection. Thus, during the summer months, rabies may be confined to Arctic foxes in the northern tundra and forests, but in winter, as food supplies become scarce, infected foxes may invade more southerly regions and introduce rabies to such areas.

The niche

Gause's work with *Paramecium*, mentioned earlier in this chapter, is an example of **intraspecific competition**; that is, competition between members of the same species. **Interspecific** competition also can occur when two species live together, in which circumstance they either might both thrive, or one may be exterminated by the other.

The solving, simultaneously (in the mathematical sense), of the logistic equation for each species to find the relative size of each population produces pairs of equations that were derived independently in the US by Lotka (1925) and in Italy by Volterra (Chapman, 1931). These equations therefore are called **Lotka–Volterra** equations. They can be derived for varying degrees of competition. The conclusion drawn from these equations is of fundamental importance in

ecology. It is that *the coexistence of two strongly competing species is impossible*. Coexistence is possible only if competition is weak. This was tested, again by Gause, using two different species of *Paramecium* in a test-tube culture. He found that either one or the other species triumphed, depending on the composition of the environment. This led to the principle of **competitive exclusion**: that competition will exclude all but one species from a particular position defined by an animal's feeding habits, physiology, mechanical abilities and behaviour. This position is an animal's **niche**¹⁴ (Elton, 1927). The principle of competitive exclusion therefore can be summarized as 'one species, one niche'. (This implies that Charles Darwin's original concept of the survival of animals most suited to their environment, as a result of competition, should be modified to one of survival by the avoidance of competition.)

There are examples of competition leading to exclusion in the world as a result of strong competition, although they are few. Probably the best-documented one is of the Abington turtles. Abington is an island in the South Atlantic that had an indigenous species of turtle. During the 19th century, sailors introduced goats to the island. The goats had exactly the same requirements as the turtles for food. Therefore there was strong competition, which led to the extinction of one of the species, in this case the turtles, according to the Lotka–Volterra prediction. Competitive exclusion is known to operate among virus infections at the cellular level (Domingo *et al.*, 1999), whereas some soil (Shtark *et al.*, 2010) and human (Zipperer *et al.*, 2016) bacteria compete with others by releasing antibiotics.

Competitive exclusion has been used as a means of disease control. The snail, *Biomphalaria glabrata*, which is an intermediate host for schistosomiasis, has been replaced by the more competitive snail *Marisa cornuarietis*, which is not an intermediate host for the helminth (Lord, 1983). *Marisa cornuarietis* is reared and then released into streams and ponds, which are the habitat of *B. glabrata*. *Marisa cornuarietis* dominates within a few months of its release, and *B. glabrata* virtually disappears.

Competitive exclusion also offers prospects for control of *Salmonella* and *Campylobacter* spp. and *Escherichia coli* infections in poultry (Mead, 2000; La Ragione and Mead, 2013) and *E. coli* infections in pigs (Genovese *et al.*, 2000) (see also Chapter 27). There is also evidence that coyotes exclude red foxes from their domestic family territories (Voigt and Earle, 1983; Sargeant *et al.*, 1987; Harrison *et al.*, 1989), and it has therefore been

suggested that such interspecific competition could be used to control the vulpine vector of rabies, and therefore the disease (Kappeler, 1992).

However, the 'real world' is very diverse and there are many opportunities for animals to avoid competition by finding their own niche. Sometimes the mechanism of avoidance is not obvious; for example, marine zooplankton are all filter feeders but actually filter particles of different sizes and therefore do not compete.

Avoidance of competition is usual in **sympatric species**; that is, species found in the same country or area. Giraffe, Thompson's gazelles and wildebeests are sympatric species in East Africa. They avoid competition for food: the giraffe, with its long neck, feeds high up; the gazelle and wildebeest, although of similar stature, eat differently: the gazelle eats ground-hugging leaves, while the wildebeest eats side shoots.

There are two sympatric species of cormorant in England: the common cormorant and the shag. Both species look alike, occupy the same stretches of shore, are submarine feeders, nest on cliffs and are fairly abundant. They appear to occupy the same niche, but do not. The common cormorant has a mixed diet, but excluding sand eels and sprats. It fishes out to sea and nests high on cliffs on broad ledges. The shag, in contrast, eats mostly sand eels and sprats, fishes in the shallows, and nests low on cliffs or on shallow ledges.

There are many other examples of sympatric species occupying different niches, ranging from cone shells that occupy different sublittoral zones, to warblers that occupy different parts of the same tree. Short-lived animals, for example insects, can occupy the same niche and avoid competition by pursuing their activities during different seasons.

Disease can affect relationships between sympatric species when avoidance of competition does not occur (De Leo *et al.*, 2002). Parasites can indirectly tip the balance of competition and allow one host species to exclude another from a potentially sympatric range. Thus, the meningeal helminth parasite, *Parelaphostrongylus tenuis*, has prevented moose and caribou from becoming established in areas in the eastern US, easing competition for white-tailed deer (Schmitz and Nudds, 1993).

Although competition may be avoided by sympatric species, their inevitably close proximity can provide the opportunity for transmission of infection between them; these may include endangered species, whose populations may consequently decline (Table 7.3).

Gause noted a laboratory example of the development of a mechanism to avoid competition. During an experiment with two species of *Paramecium*, he noticed that both species survived in the same test tube because one species had changed its mode of living to

¹⁴ For a detailed discussion of the distinction between 'niche' and 'habitat', see Whittaker and others (1973).

Table 7.3 Infections transmitted from more common sympatric populations that have caused population declines in threatened mammals. (Source: Adapted from Cleaveland *et al.*, 2002. Reproduced with permission of Oxford University Press.)

Threatened host	Infection	Reservoir host
Ethiopian wolf	Rabies, canine distemper virus	Domestic dogs
African wild dog	Rabies, canine distemper virus	Domestic dogs
Baikal seal	Canine distemper virus	Domestic dogs
Caspian seal	Canine distemper virus	Possibly terrestrial carnivores
Black-footed ferret	Canine distemper virus	Various wild carnivores
Arctic foxes	Otodectic mange	Domestic dogs
Chimpanzee	Polio	People
Bighorn sheep	<i>Pasteurella</i> spp.	Domestic sheep
Chamois	Infectious keratoconjunctivitis	Domestic sheep
Monk seal	Morbillivirus	Possibly dolphins
Mountain gorilla	Measles	People
Rain forest toads	Chytridiomycosis	Cane toads

inhabit only the top half of the test tube, while the other species had moved to the bottom of the tube, thus avoiding competition. This process was explained first by Darwin when he developed the concept of **divergence of character**: characters must diverge when closely-related species live in the same region, be it test tube or prairie. The synonymous term **character displacement** was first used in the 1950s.

One would expect displacement to be more common than exclusion because the world offers many ways of subtly changing niches. Displacement is also a mechanism of increasing species diversity. One example illustrates this phenomenon. Two species of nuthatch occur in Greece, Turkey and other parts of Asia: *Sitta neumayer* and *S. tephronota*. The external appearance of *S. neumayer* in Greece, and *S. tephronota* in Central Asia, where the species do not overlap, is similar. However, in Iran, where the two species overlap and coexist, the external appearance of each species is different. This external divergence of characters probably reflects other changes that avoid competition.

Some examples of niches relating to disease

Louse infestations

Lice tend to be host-species-specific; pig lice do not live on humans or dogs, and vice versa. By being host-specific, species of lice avoid competition: they have their own niche. The human louse also demonstrates character displacement. Two types of louse live on humans: the head louse and the body louse. These each parasitize the two different parts of the body,

rather like Gause's two species of *Paramecium* living in the top and the bottom of the test tube.

Internal parasitism

An animal comprises a connected set of organ systems, which are potential niches for many internal parasites with complex life-cycles. The **fundamental niche** is the range of sites that can be occupied by a parasite; whereas **realized niches** are the sites that are actually occupied (Poulin, 1998). Realized niches may be the optimal sites of a parasite within the fundamental niche, if competition with other species does not occur. Alternatively, if there is potential competition, realized niches may be those that are occupied to avoid competition, and such 'interactive site segregation' (Holmes, 1973) is known to occur. For example, several species of helminth parasitize the intestine of grebes, but, on average, occupy different sites along the length of the intestine (Figure 7.6).

Intracellular parasitism

Intracellular parasites comprise all viruses, some bacteria (e.g., *Brucella* spp., *Mycobacterium tuberculosis*, and *Rickettsia*, *Ehrlichia*, *Coxiella* and *Chlamydia* spp.) and some protozoa (e.g., *Babesia* spp.). They occupy a variety of niches in cells, including the cytoplasm, endosomes, lysosomes and vesicles. There are several advantages to this type of existence, such as safety from humoral antibodies and the avoidance of competition with extracellular agents. The intracellular environment, however, is harsh; this is reflected in the relatively low generation times of intracellular parasites compared with extracellular ones. The

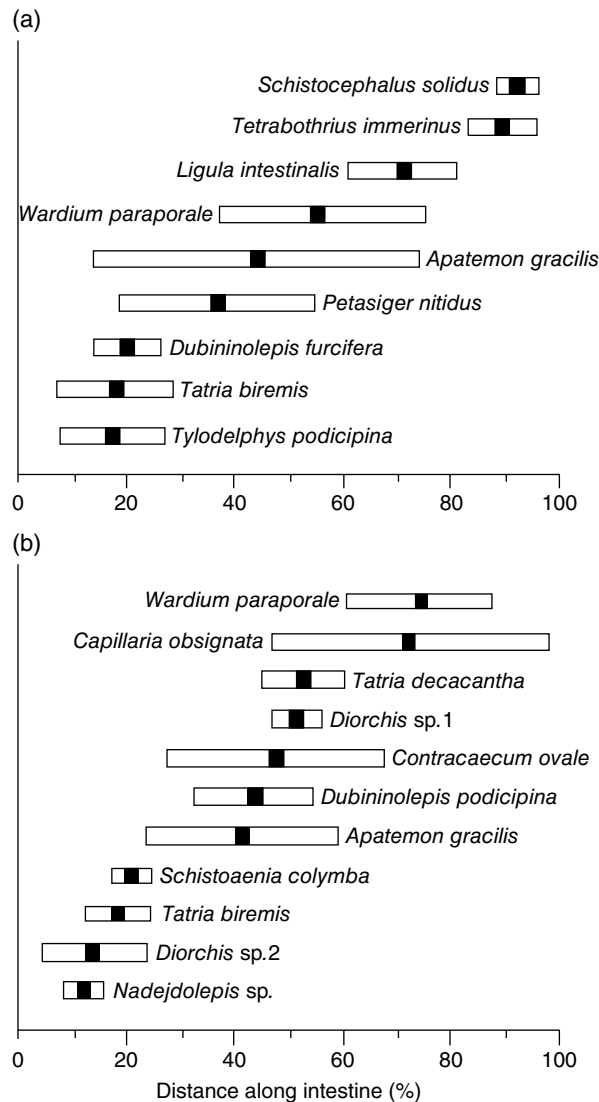


Fig. 7.6 Distribution of intestinal helminth parasites in two species of grebes (a) *Aechmophorus occidentalis*, and (b) *Podiceps nigricollis*. ■: mean; □: standard deviation. (Reproduced with permission of Poulin, 1998, Chapman and Hall; data from Stock and Holmes, 1988.)

intracellular environment shares this characteristic with larger extreme environments such as snowfields and salt lakes (Table 7.4).

Intracellular parasites therefore have evolved diverse mechanisms for evading the host's cellular defences (Hackstadt, 1998; Sibley, 2004). These include rapid escape from phagocytic vacuoles (*Rickettsia* spp.), blocking of maturation of endosomes (*Mycobacterium* and *Ehrlichia* spp.) and adaptation to lysosomal conditions (*Coxiella* spp.).

Epidemiological interference

Studies in India have shown that the presence, in a human community, of one type of respiratory adenovirus prevents infection with other types, even though the latter are common in surrounding communities (Bang, 1975). This is because the first type occupies a niche (the lower respiratory tract), which therefore cannot be filled by other agents. Similarly, there is evidence that infection of laboratory animals and domestic livestock with one serodeme (a population demonstrating the same range of variable antigens: WHO, 1978) of *Trypanosoma congolense* delays the establishment of infection with a different serodeme in the same animals (Dwinger *et al.*, 1986). This phenomenon is **epidemiological interference**, which initially had been identified experimentally in virus infections of embryonated chicken eggs (Duffy, 1944), and which exemplifies competitive exclusion.

Interference can affect the time of occurrence of disease. An epidemic caused by one agent may suppress epidemics caused by other similar agents. This is true of human respiratory infections in North America and India (Bang, 1975); measles and whooping cough (Rohani *et al.*, 2003); influenza in Norway, where epidemics appear to have been delayed by earlier infections with norovirus, and vice versa (Ånestad *et al.*, 2009); influenza in Sweden, where interruption of the spread of the infection may have been caused by spread of rhinoviruses (Linde *et al.*, 2009); and measles in Hungary, which is influenced by influenza epidemics (Berencsi *et al.*, 2010). Interference also is

Table 7.4 Comparison of the intracellular environment with terrestrial extreme climates. (After Moulder, 1974.)

	Terrestrial extreme environments (e.g., deserts, salt lakes, hot springs, snowfields)	The cell
Diversity of inhabiting species is low	+	+
Dominant forms have evolved unique fitness traits	+	+
Dominant forms dependent on species diversity limiting factors	Factor is abiotic , e.g., heat, salinity, dryness	Factor is biotic : the cell

reported to occur between distemper-like viruses and canine distemper virus (Green and Stulberg, 1946), and between Rift Valley fever virus and yellow fever virus in monkeys (Findlay and MacCallum, 1937). The clearest evidence of interference is when epidemics of two diseases oscillate out of phase (Huang and Rohani, 2006).

Some diseases are common in the young. Interference by other agents during early life causes the diseases of the young to occur in older age cohorts, altering the age-specific incidence rates. There is evidence that this occurs with certain virus infections in humans (Bang, 1975). In addition to occupying a niche, the antecedent infections also may induce interferon production, which has a protective effect against succeeding infections (Khaitov *et al.*, 2009).

Interference also can affect the rate of natural immunization. If an infectious agent is present at continued high levels, and infection is followed by immunity, then there is usually a decreased incidence in older age groups. However, if other agents interfere with the agent in the young, then immunity induced by the agent is delayed, producing continued infection in older subjects. There is evidence, for example, that interference by other enteroviruses delays natural poliovirus immunization in humans (Bang, 1975). Interference also may explain why vaccination against influenza can be associated with a higher risk of non-influenza respiratory virus infections, because of the lack of interference by influenza infections in vaccinated individuals (Cowling and Nishura, 2012).

Epidemiological interference may be a general phenomenon. It has an obvious place in the evolution of disease, preventing massive multiple and possibly fatal infections of the young. An example of the application of epidemiological interference to the control of enteric diseases is given in Chapter 27.

The relationships between different types of animals and plants

A particular biome contains different types of animals and plants. Some are common, others are scarce. Some are large, others are small. Reasons for these variations have been suggested by ecological studies.

Animals tend to move about *en masse*, and so it is difficult to study them all simultaneously. Ecologists have therefore chosen to look in detail at one species of animal, in conditions that favoured easy observation. Charles Elton (1927) visited Bear Island near Spitzbergen and observed Arctic foxes, with particular reference to what they ate. Bear Island was essentially a tundra biome, thus foxes were easy to observe.

Food chains

Elton noted what the foxes ate in the summer and the winter. In the summer, the foxes ate birds (e.g., ptarmigan and sandpiper). The birds ate berries, tundra, leaves and insects. The insects also ate leaves. Thus, Elton noted that there was a **food chain**: tundra–insects–birds–fox. In addition, the foxes ate marine birds, which in turn ate smaller marine animals, which in turn ate marine plants. Thus, there was a further food chain: marine plants–marine animals–seabirds–fox.

In the winter, the birds migrated to the south, leaving only polar bear dung and the remains of carcasses of seals that had been killed by polar bears. Thus, in the winter, there was a different food chain: marine animal–seal–polar bear dung–fox. In animal communities, therefore, a complex system has evolved, with food chains linking animals.

The size of animals and food webs

Elton observed that animals fed at different levels in the food chain. These levels he termed **trophic levels**. He also noted that animals occupying different trophic levels generally were of different sizes. The foxes were the largest, and the birds (one level down) were smaller. Similarly, those further down the pyramid (e.g., the insects) were even smaller. Also, moving down the food chain (e.g., from foxes to insects), the animals were *more abundant*. There were more birds than foxes, and more insects than birds. A histogram depicting animal size against number of individuals is shown in *Figure 7.7a*. If the vertical axis of the histogram is moved to the centre, and the bars are arranged symmetrically, a pyramid is produced (*Figure 7.7b*); this is the Eltonian **pyramid of numbers**. As animals become larger and rarer, they have larger home ranges and therefore, if shedding an infectious agent, transmit infections over larger areas than small animals. Thus, although hedgehogs (relatively small animals) can be infected with foot-and-mouth disease virus (McLauchlan and Henderson, 1947), they have relatively small home ranges, and so probably play only a minor role in the dissemination of the virus during epidemics.

The food chain is a simplistic view of the relationship between an animal and its food. In reality, an animal usually eats a variety of food, and so there are generally many linked food chains radiating outwards from the lower plant trophic levels to the herbivores, and then inwards towards the top carnivores¹⁵, producing **food**

¹⁵ The introduction of predatory top carnivores into an ecosystem can affect the abundance of animals at various lower trophic levels through a **trophic cascade** (Terborgh and Estes, 2010). Thus, the recovery of wolves in parts of the US is considered to have reduced the number of elk, which fostered the growth of willows, which, in turn, resulted in increased numbers of beavers and birds (Mech, 2012).

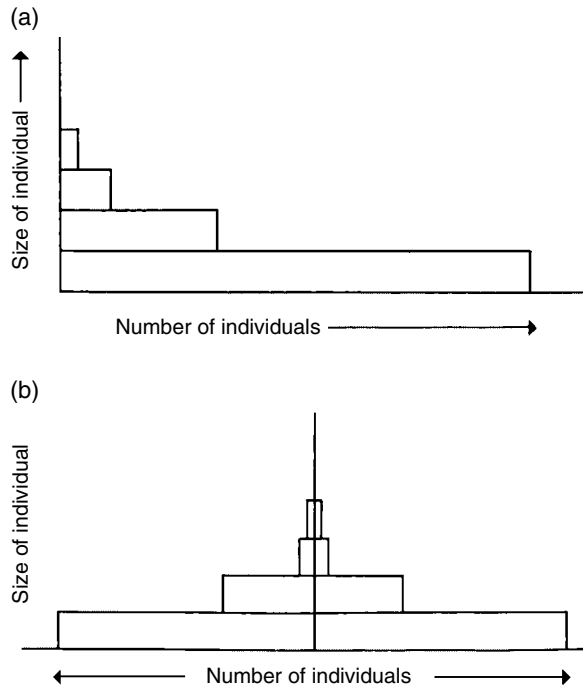


Fig. 7.7 (a) The relationship between size of animals and size of populations. (b) The Eltonian 'pyramid of numbers'.

webs (Figure 7.8). In addition, parasitic food webs can be identified in which the small parasites occupy a level in the food web higher than the organisms that they parasitize, and in which parasites with complex life-cycles may feed on several different trophic levels (Lafferty *et al.*, 2008)¹⁶.

Although early ecological studies highlighted the complexity of food webs, recent studies suggest that all webs follow relatively simple rules, involving such characteristics as the length of the food chain, similarities between trophic levels, and 'connectance'¹⁷ (Williams and Martinez, 2000).

An animal's feeding habits and its place in the trophic hierarchy place restrictions on its mode of life; this led Elton to define the niche as: '*an animal's place in the biotic environment; its relation to food and enemies*'.

¹⁶ There are also complex marine food webs involving parasites (Poulin and Chappell, 2002).

¹⁷ 'Connectance' or 'connectivity' refers to the extent to which the different components of the web interact. It is part of the wider issue of the structure of **networks**, which pervades modern science (e.g., power grids, the Internet, and cellular and metabolic pathways: Strogatz, 2001). Connectivity is also of relevance to disease spread, where the degree of contact between populations of susceptible animals is an important determinant of disease transmission (May and Lloyd, 2001; Webb and Sauter-Louis, 2002; Christley and French, 2003; Weber *et al.*, 2004; Gurarie and Seto, 2009; see also Chapter 23: 'Contact-network modelling').

The significance of food webs to disease transmission

The food web of an animal can determine to which orally-transmitted infectious agents an animal acts as host, and from which food poisoning toxins it is at risk. Predators, for instance, risk being infected with microbes ingested with infected food. For example, captive raptors (some of which are endangered species) can be fatally infected with viruses through consumption of food of avian origin (Forbes *et al.*, 1997; Rideout *et al.*, 1997). It therefore has been suggested that captive raptors that are likely to be released into the wild should be fed with rodents, rabbits and other small mammals – rather than chickens – to reduce the risk of released birds transmitting infection to wild populations (Höffle *et al.*, 2002).

Helminth diseases, for which there are definitive and intermediate hosts, frequently are transmitted via food webs. For example, the tapeworm *Echinococcus granulosus* includes the sheep as an intermediate host and the dog as the definitive host. The cysts in the liver and lungs of the intermediate host are transmitted to dogs when the latter eat sheep offal; hence the recommendations that raw sheep offal should not be fed to dogs.

Figure 7.9 illustrates the complex life-cycle of *Neorickettsia helminthoeca*¹⁸. This rickettsia produces a febrile disease in dogs and foxes. The disease is called 'salmon poisoning' because it is associated with the feeding of salmon to dogs. This agent's life-cycle also illustrates a parasitic food chain, in which the smallest member, the rickettsia, parasitizes a fluke which in turn parasitizes a snail for part of its life-cycle. The snail in turn releases infected miracidia which parasitize the salmon. Feeding salmon to dogs transmits the rickettsia to the latter, where the microbe produces clinical disease.

¹⁸ Complex life-cycles have evolved in wide and distantly related groups of parasitic nematodes (Adamson, 1986). The simplest explanation is that, at first, only single-host cycles in vertebrates occurred, with intermediate hosts (initially, paratenic hosts: see Chapter 6) being added later. Intermediate hosts offer an advantage by protecting the parasite from the environment, and offering it to the host in the food chain (Anderson, 1984). Alternatively, there may be an advantage to the evolution of multiple-host cycles by conferring greater 'fitness' on the parasite when one host is used for food and the other for dispersion (Morand, 1996). This is supported by the greater virulence of helminth parasites in the intermediate host (the food base) than in the definitive host (the mode of dispersion) (Ewald, 1995). Moreover, studies in sympatric species of cestodes, with only the life-cycles – one-host versus two-host – varying, indicate that the two-host species are more abundant than the one-host species (Robert *et al.*, 1988; Morand *et al.*, 1995). In addition, marine organisms appear to have more complex life histories than terrestrial species, and their pathogens have a greater number of intermediate host species than is typical for terrestrial diseases (McCallum *et al.*, 2004).

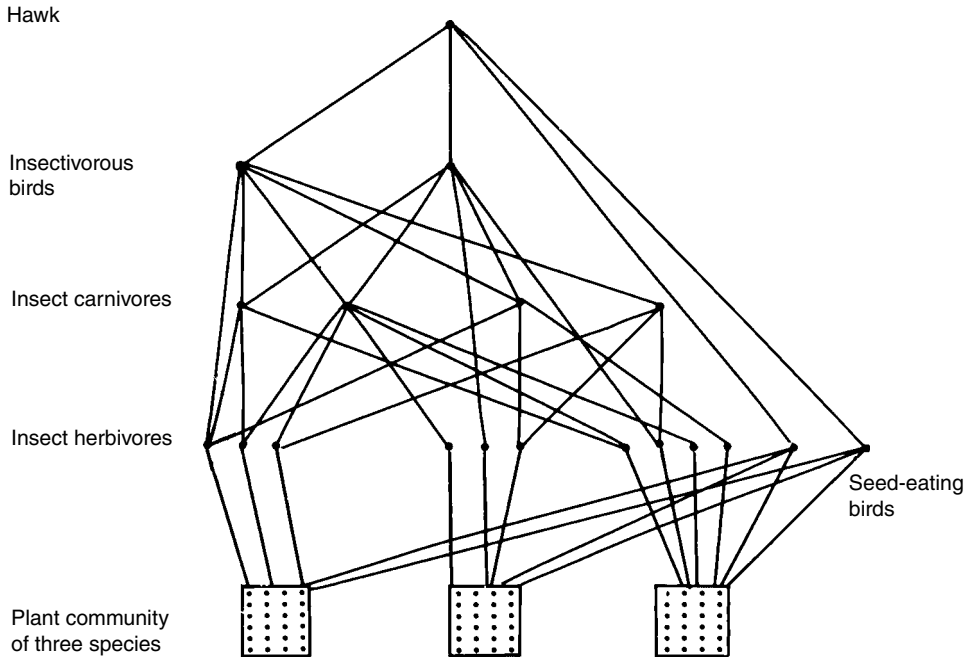


Fig. 7.8 Hypothetical food web involving carnivorous, insectivorous and herbivorous birds, carnivorous and herbivorous insects, and plants.

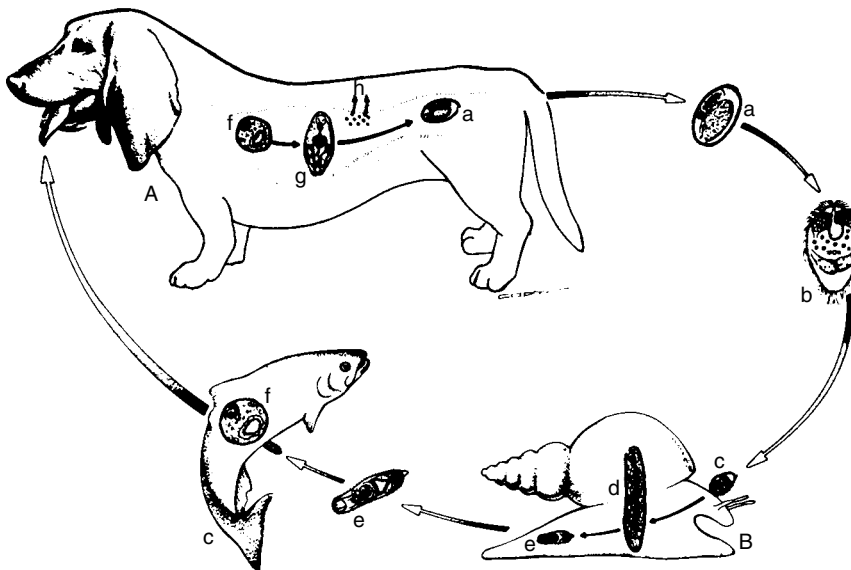


Fig. 7.9 Life cycle of the fluke *Nanophyetus salmincola salmincola* and the microorganism *Neorickettsia helminthoeca*. A, dog (definitive host); B, *Oxytrema silicula* (first intermediate host); C, Salmonidae fish (second intermediate host); a, *N. s. salmincola* egg in dog faeces; b, miracidium; c, miracidium enters snail by skin penetration; d, redia; e, cercaria; f, encysted metacercaria in salmon, ingested by the dog in raw salmon meat; g, adult fluke develops in duodenal mucosa; h, *Neorickettsia helminthoeca* leave the fluke and infect the dog systemically. (Source: Booth *et al.*, 1984. Reproduced with permission of the Canadian Veterinary Medical Association.)

Ingestion of intermediate stages of parasites sometimes may control, rather than transmit, infection. For example, members of the genus *Utricularia* (carnivorous bladderworts) have been shown to ingest cercariae and miracidia of *Schistosoma mansoni* (Gibson and Warren, 1970). It has been suggested (Lord, 1983) that the absence of schistosomiasis from Cuba, which has 17 species of *Utricularia*, may be related to this activity.

Parasitic food chains also might be exploited in the control of other helminth parasites (Waller, 1992). Some bacteria are effective controllers of plant nematode parasites (Tribe, 1980). Predaceous fungi might be used to control nematode parasites of livestock; *Duddingtonia flagrans*, for example, is able to trap and destroy free-living stages of trichostrongylid larvae (Larsen, 1999). Similarly, *Arthrotrix oligospora*, a predator of infective larvae of the

bovine nematode *Ostertagia ostertagi*, has reduced the level of parasitism in cattle when applied to dung pats (Grønvold *et al.*, 1989, 1993), although oral administration has had little benefit yet (Hashmi and Connan, 1989).

The distribution of energy between trophic levels

Elton's theory explained why animals occupying different trophic levels were of different sizes, but it did not explain why there were so few animals higher up the pyramid. There could be geometric restrictions: more small animals can be packed into a fixed space than larger ones. However, the sea contains few predators (e.g., sharks) at the top of the pyramid even though the sea is very large.

Lindemann (1942) explained population density at different levels of the pyramid by considering the food chain, not in terms of particulate food, but in terms of **calorific energy flow**. According to the second law of thermodynamics, the process of converting energy from one state to another is wasteful; that is, there is not 100% conversion of energy. Thus, moving up from one level to another in the Eltonian pyramid, conversion at each level wastes energy and so less protoplasm can be supported at progressively higher levels. Therefore, even if animals at different levels were of the same size, there would be fewer higher up. Since those higher up are larger, the packaging of a supportable amount of protoplasm produces even fewer animals.

It is because of the greater availability of energy at lower levels that ungulates are often bigger than their carnivorous predators. The biggest animals tend to be those that feed very low in the pyramid (e.g., filter feeders like blue whales) because much energy is available to them. When civilization dawned and man ceased to be a hunter/gatherer and began cultivating crops, he 'climbed down' the pyramid, tapping more energy. This is one reason for the increase in the world's population in the cradle of civilization in early Egypt and the associated development of horticulture and livestock farming (see Chapter 1).

The analysis of predation

The association between predator and prey is a special case of interaction in a food chain. Many mathematical models have been devised to analyse predator/prey interactions. The one to be discussed here is that devised independently by Lotka and Volterra (Lotka, 1925; Chapman, 1931). They reasoned that predator/prey interactions were similar to interactions between competing species, described earlier in this chapter, and so adapted their formulae accordingly.

The three predictions of this model are:

1. the fluctuation of two species, one of which feeds on the other, are periodic, and the periods depend only on the coefficient of growth;
2. the ultimate mean values of the numbers of individuals of the two species are, with fixed coefficients, independent of the initial numbers of individuals;
3. if individuals of the two species are eliminated in proportion to their total number, then the recovery potential of the prey is greater than that of the predator; conversely, increased protection of the prey from all risks, including the predator, allows both species to increase.

Thus, applying the first prediction, the prevalence of rabies in foxes is related to the population density of foxes (a predator) and, therefore, to the population density of mice (a prey). *Figure 7.10* illustrates this relationship, using demographic and disease prevalence data collected in Germany.

There are also similarities between predator/prey interactions and parasite/host interactions (Hall *et al.*, 2008). For example, cyclic patterns of measles and other childhood diseases (Yorke and London, 1973) are equivalent to Lotka–Volterra cycles because the development of immunity by infected individuals is equivalent, in its effect on parasite populations, to the effects of prey removal on predator populations.

Ecosystems

The relationship between animals linked by food chains defines the variety of animals in a particular area. Similarly, climate and vegetation govern the distribution of plants and therefore of the animals that live off them. These areas are characterized by the animals and plants that occupy them, and by their



Fig. 7.10 The relationship between the population density of mice and the prevalence of fox rabies. The relationship arises from a predator/prey relationship between foxes and mice (see text). (Source: Sinnecker, 1976. Reproduced with permission of John Wiley and Sons, Ltd.)

physical and climatic features. This unique interacting complex is called an **ecosystem** (Tansley, 1935). The components of an ecosystem can be considered separately, and ecosystems themselves can vary in size. Various terms have been devised to describe these components and their relationships (Schwabe, 1984) including **biotope**, **biocenosis** and **landscape**.

Biotope

A biotope is the smallest spatial unit providing uniform conditions for life. An organism's biotope therefore describes its location. This contrasts with a niche, which describes the functional position of an organism in a community. A biotope can vary in size. For example, it may be the caeca of a chicken for coccidia, or an area of poorly drained land for *Fasciola hepatica* infection of cattle.

Biocenosis

A biocenosis is the collection of living organisms in a biotope. The organisms include plants, animals and microorganisms in the biotope. Sometimes **biotic community** is used synonymously with biocenosis. On other occasions, biotic community refers to a large biocenosis. Major biotic communities are biomes.

Landscape

'Landscape' is a term in wide general circulation and therefore can encompass different concepts¹⁹. In ecology, a landscape is a recognizable and repeated cluster of ecosystems; and, just as ecosystems vary in size, so do landscapes (Forman and Godron, 1981). The boundary between landscapes is relatively distinct, particularly in relation to the structure of vegetation. Ecologically, landscape structure is measured by the distribution of energy, nutrients, and numbers and kinds of component ecosystems. The configurations of the component ecosystems are rarely random and so species tend to be aggregated or clustered. This explains why assemblages of communities or species occur in **patches**, surrounded by a **matrix** with a dissimilar community structure or composition.

The origins of patches are determined by five distinct causal mechanisms, producing five types of patch:

1. **spot disturbance patches**, resulting from disturbance of a small area of matrix (e.g., patches produced by a small fire in a grassland);

2. **remnant patches**, caused by widespread disturbance surrounding a small area (e.g., a shrub-covered island produced by flooding a valley);
3. **environmental-resource patches**, resulting from the normal heterogeneous distribution of resources (e.g., concentration of reptiles and amphibians in a desert oasis);
4. **introduced patches**, stemming from introduction by human activity (e.g., golf courses, wheat fields);
5. **ephemeral patches**, determined by normal short-lived fluctuations in resources (e.g., localized blooming of annual plants in the desert).

Patches should be viewed as dynamic, not static, structures (Wu and Loucks, 1995).

Types of ecosystem

Three types of ecosystem can be identified, according to their origin: **autochthonous**, **anthropurgic** and **synanthropic**.

Autochthonous ecosystems

'Autochthonous' derives from the Greek adjective *autos*, meaning 'oneself' or 'itself'; the Greek noun *chthon*, meaning 'the earth' or 'the land'; and the adjectival suffix *-ous*, meaning 'deriving from'. Hence an autochthonous ecosystem is one 'coming from the land itself'. Examples are to be found in biomes such as tropical rain forests and deserts.

Anthropurgic ecosystems

'Anthropurgic' is derived from the Greek noun *anthropos*, meaning 'man'; and the Greek verb root *erg*, meaning 'to work at, to create, to produce'. Thus, an anthropurgic ecosystem is one created by man²⁰. Examples are those found in cultivated pastures and towns. Some authors use 'anthropogenic' (Greek: *gen-* = 'be produced') in a similar context.

Synanthropic ecosystems

'Synanthropic' originates from the Greek preposition *syn*, meaning 'along with, together with'; and the Greek noun *anthropos*, meaning 'man'. Thus, a synanthropic ecosystem is one that is in contact with man. An example is a rubbish tip, harbouring a variety of vermin. It follows that some synanthropic ecosystems, such as rubbish tips, are anthropurgic.

Synanthropic ecosystems facilitate the transmission of zoonotic infections from their lower animal hosts to humans. For example, the brown rat, *Rattus norvegicus*, inhabits rubbish dumps and can be inapparently

¹⁹ Artists, for example, commonly portray the landscape as what the eye can see, and therefore may limit the idea to natural landforms or communities.

²⁰ Strictly, anthropurgic also can mean 'creating man'.

infected with *Leptospira* serovar Ballum. Humans in proximity to rubbish dumps that harbour infected rats therefore may be infected with the bacterium.

An ecological climax

An ecological climax traditionally is said to have occurred when plants, animals, microbes, soil and macroclimate (see Chapter 5) have evolved to a stable, balanced relationship, and originally was used in relation only to plants (Clements, 1916)²¹.

Characteristically, when infections are present, they, too, are stable and therefore are usually endemic. Also, the balance between host and parasite usually results in inapparent infections. Such stable situations can be disrupted, frequently by humans, resulting in epidemics. For example, bluetongue was recognized only after the importation of European breeds of sheep to South Africa towards the end of the 19th century (Neitz, 1948). The virus, however, was present in indigenous sheep before that time, but was part of an ecological climax in which it only produced inapparent infections. The importation of exotic sheep represented a disturbance of the stable climax.

A climax involving endemic infectious agents indicates that all factors for the maintenance and transmission of the agent are present. Sometimes changes in local ecology may tip the balance in favour of parasites, thus increasing disease incidence. For example, the seasonal periodicity of foot-and-mouth disease in South America may result from seasonal increases in the size of the susceptible cattle population when animals are brought into an endemic area for fattening (Rosenberg *et al.*, 1980).

Some ecologists have considered 'ecological climax' to be an outdated term, arguing that the various species that inhabit an ecosystem tend to be transitory (Whittaker, 1953), while other ecologists still consider it to be a valid construct (Roughgarden *et al.*, 1989).

Ecotones

Ecotones are the transitional boundaries between two adjacent biomes or smaller landscapes (Gosz, 1993), ranging from a few centimetres to a few kilometres. In landscape ecology, an ecotone is the marginal area where two patches meet. Ecotones originally were defined visually as stable delineations between vegetational zones, and were viewed as relatively static areas (Clements, 1905). However, they are now considered to include all elements of an ecosystem, including animals (Burgess *et al.*, 2005), and to be dynamic and

complex components that have a disproportionately large effect on biological diversity ('biodiversity') and ecosystem function (Risser, 1995). They also have been termed **ecological interfaces**, and are logical locations for sympatric species (Kark and Rensburg, 2006)²². They can be either naturally created (e.g., flooded areas caused by beaver activity) or anthropurgic (e.g., flooded areas around artificial reservoirs) (Farina, 2000b), and may be associated both with increased species diversity²³ (Kernaghan and Harper, 2001) and decreased diversity (Backeus, 1993). The factors affecting species diversity are complex: they include a greater array of soil types and moisture (leading to increased diversity: Gosz, 1992; Risser, 1995) or increased environmental fluctuations rendering regions unfavourable for plant growth (resulting in decreased diversity: Maarel, 1990).

Ecotones are regions where rates of infectious disease may be high; for example, parasitic diseases in wildlife, which also may increase due to climate-driven shifts in the ecotones (Svobodova *et al.*, 2004). Tick vectors of zoonotic infections, such as Lyme disease, also cluster in ecotones (Lindström and Jaenson, 2003), and competent rodent reservoirs (the white-footed mouse, in the case of Lyme disease) similarly cluster (LoGiudice *et al.*, 2003).

The mosquito vectors of encephalitis virus also congregate in ecotones (Lothrop *et al.*, 2002). Yellow fever virus is maintained in apes in Africa in an autochthonous forest ecosystem in the forest canopy (Figure 7.11). The canopy-dwelling mosquito, *Aedes africanus*, transmits the virus between apes. The mosquito *A. simpsoni* bridges the interface between the autochthonous forest ecosystem and the anthropurgic cultivated savannahs. This mosquito therefore maintains a plantation cycle in which humans and apes may be infected. Finally, the urban mosquito, *A. aegypti*, can maintain an urban cycle in humans. People who enter forests also may contract the infection from *A. africanus*.

Recreational trails are commonly located along ecotones, and these areas may be associated with an increased risk of human infection with tick-transmitted diseases such as Rocky Mountain spotted fever because of congregation of the tick vectors in these areas (Burg, 2001).

Ecotones also may provide contact zones for the switching of parasites from one host species to another; for example, the infestation of muskoxen by

²¹ In some ecosystems (e.g., tropical rain forests) an ecological climax is determined exclusively by plant/arthropod relationships (Janzen, 1971; Way, 1977).

²² Other terms, including 'boundary' and 'edge', also have been applied to ecotones (Hufkens *et al.*, 2009).

²³ The tendency for increased variety and diversity at boundaries is termed the **edge effect**.

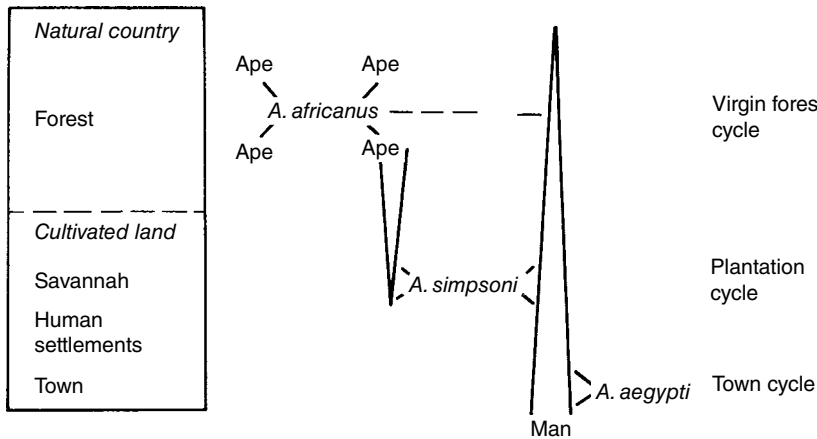


Fig. 7.11 The transmission of yellow fever between apes (primary hosts) and man (the secondary host). (Source: Sinnecker, 1976. Reproduced with permission of John Wiley and Sons, Ltd.)

nematodes in areas in Canada where the former overlap with sheep (Hoberg *et al.*, 2002). Additionally, some pathogens may concentrate in host populations at ecotones; for instance, Oropouche virus in Brazil, where banana and plantain plantations close to forests are suitable habitats for the vector, *Culicoide paraensis* (Yuill, 1986).

Several diseases can be propagated across the ecotone interface between wildlife ecosystems and cultivated land stocked with domesticated animals (Bengis *et al.*, 2002; Miller *et al.*, 2013; Wiethoelter *et al.*, 2015; see also Table 6.10). Sylvatic foci of infection may constitute refractory problems to the eradication of disease in livestock (e.g., tuberculosis in badgers: see Chapter 2), as well as increasing the frequency of disease in companion animals (e.g., epidemics of rabies in raccoons related to the increased occurrence of rabies in domestic cats: Gordon *et al.*, 2004).

Chronotones The term, 'chronotone' (Greek: *khronos* = 'time'), has been coined to refer to *temporal* (rather than just spatial) man-made changes in the use of ecosystems occurring over relatively short periods of time (Bradley, 2004). This can increase disease risk during the duration of change; for example, malarial risk increases during periods of deforestation.

Ecological mosaics

An ecological mosaic is a modified patch of vegetation, created by humans, within a biome that has reached a climax. Infection may spread from wild animals to humans in such circumstances. For example, clearing of the forest canopy encourages a close cover of weeds on the ground, creating conditions that are favourable for the incursion of field rats with mites infected with scrub typhus, which form mite islands and the resulting local areas of endemic scrub typhus (Audy, 1961).

Most non-human primates live in anthropurgic mosaics of farmland, human settlements, forest fragments and protected areas (Chapman and Peres, 2001). This brings them into closer contact both with humans, increasing the risk of disease transmission between the two groups (Table 7.5)²⁴, and with livestock, which may also be a source of infection (e.g., cattle as reservoirs of *Cryptosporidium parvum* and *Giardia duodenalis* for gorillas: Nizeyi *et al.*, 2002).

However, transmission does not always occur in the mosaics because suitable vectors may not be available. Thus, in Malaya, man lives unharmed in forests in mosaics with monkeys infected with a variety of species of *Plasmodium* that are pathogenic to man. Transmission to man from monkeys does not occur because vectors that bite both types of primate are not present in the ecosystem.

Landscape epidemiology

The study of diseases in relation to the ecosystems in which they are found is **landscape epidemiology**, a central tenet being that disease at the local level is influenced by the entire ecosystem. Terms conveying the same meaning are **medical ecology**, **horizontal epidemiology** (Ferris, 1967) and **medical geography**. Investigations are frequently qualitative, involving the study of the ecological factors that affect the occurrence, maintenance and, in the case of infectious agents, transmission of disease. This contrasts with the study of quantitative associations between specific diseases and hypothesized factors – sometimes termed

²⁴ Sanctuary apes may be treated for bacterial infections, and so their reintroduction into the wild poses the additional threat of the introduction of antibiotic-resistant bacteria into wild-ape populations (Schaumburg *et al.*, 2012).

Table 7.5 Infections exchanged between humans and non-human primates. (From Chapman *et al.*, 2005.)

Infection	Route of exchange	Direction of exchange
Herpes B	Animal bite	Non-human primate to human
Monkey pox	Animal bite	Non-human primate to human
Polio virus	Faecal–oral	Humans to non-human primate
Ebola	Hunting and butchering	Non-human primate to human
<i>Mycobacterium leprae</i>	Nasal secretion	Among primates
Tuberculosis	Respiratory droplet	Humans to non-human primate
Malaria	Vector	Both directions
Filariasis	Vector	Both directions
Yellow fever	Vector	Both directions
Dracunculiasis	Water-mediated	Humans to non-human primate
Schistosomiasis	Water-mediated	Non-human primate to human
SV 40	Vaccinations	Non-human primate to human
Gastrointestinal parasites	Faecal–oral	Both directions

‘vertical’ epidemiology – as described in Chapters 14, 15 and 22. Landscape epidemiology was developed by the Russian, Pavlovsky (1964)²⁵, and later expanded by Audy (1958, 1960, 1962) and Galuzo (1975); it involves application of the ecological concepts described to the study of disease.

Nidality

The Russian steppe biome was the home of the great plagues such as rinderpest. Many arthropod-transmitted infections present in the steppes also were limited to

distinct geographical areas. These foci were natural homes of these diseases and were called **nidi** (Latin: *nidus* = ‘nest’). The presence of a nidus depends on its limitation to particular ecosystems. An area that has ecological, social and environmental conditions that can support a disease is a **nosogenic territory** (Greek: *noso-* = ‘sickness’, ‘disease’; *gen-* = ‘to produce’, ‘to create’). A **nosoarea** is a nosogenic territory in which a particular disease is present. Thus, Britain is a nosogenic territory for rabies and foot-and-mouth disease, but is not a nosoarea for these diseases, because the microbes are prevented from entering the country by uncompromising importation procedures. Diseases that show strict geographical boundaries within an ecosystem or series of ecosystems are nidal because they are confined to a specific nidus. Salmonellosis is endemic in most parts of the world because virtually all vertebrates and some invertebrates (see Table 6.9) can act as hosts for the various species of *Salmonella*. Rabies, when maintained in foxes, is endemic in a large zone around the northern hemisphere because this large area supports a fox population of high density (Figure 7.12). The nosoarea for coccidioidomycosis was described earlier in this chapter (see Figure 7.2).

When diseases are vector transmitted, they are often restricted to more precise geographical boundaries than other infectious diseases. This is because the ecosystem has to satisfy the requirements of both the vertebrate host and the arthropod vector. Thus, Rocky Mountain spotted fever, a rickettsial disease of rodents transmitted by ticks, is essentially restricted to particular areas of North America, as the name of the disease suggests.

At the opposite end of the spectrum from diseases with a wide distribution are those that may be confined to relatively small areas within a town or on a farm. An isolated clump of trees that is used as a roost by starlings may be the only reservoir of infection for histoplasmosis within a large area. The faeces from these birds provide an ideal environment in which the fungal agent can survive and replicate (Di Salvo and Johnson, 1979). Even smaller nidi can be identified. For example, a focus of infestation with the tropical dog tick, *Rhipicephalus sanguineus*, was discovered in a house in London (Fox and Sykes, 1985), the warm conditions of the house providing a suitable environment for the tick. The affected dog had not been imported from abroad, but probably contracted the infestation from a quarantine kennel where it had been boarded.

Changes in ecosystems stemming from human activity can modify nosogenic patterns substantially, resulting in the emergence of infectious diseases (Table 7.6). These changes represent one class of factor that contributes to the introduction and spread of

²⁵ Pavlovsky’s thinking was based on the observation of ecotones, where the intrusion of humans into the Siberian taiga forests resulted in the occurrence of diseases at the interface between human settlement and virgin forest (Bradley, 2004). His main discipline was entomology, and his investigation of arthropod-borne diseases resulted in the term, ‘parasitocenosis’, to refer to the entire set of parasites, from microorganisms to multicellular organisms, living within, and on the surface of, the host’s body (Balashov, 2010). Consequently, the host was considered to be a biotope, which could be subdivided into smaller habitats, corresponding to different organs and tissues.

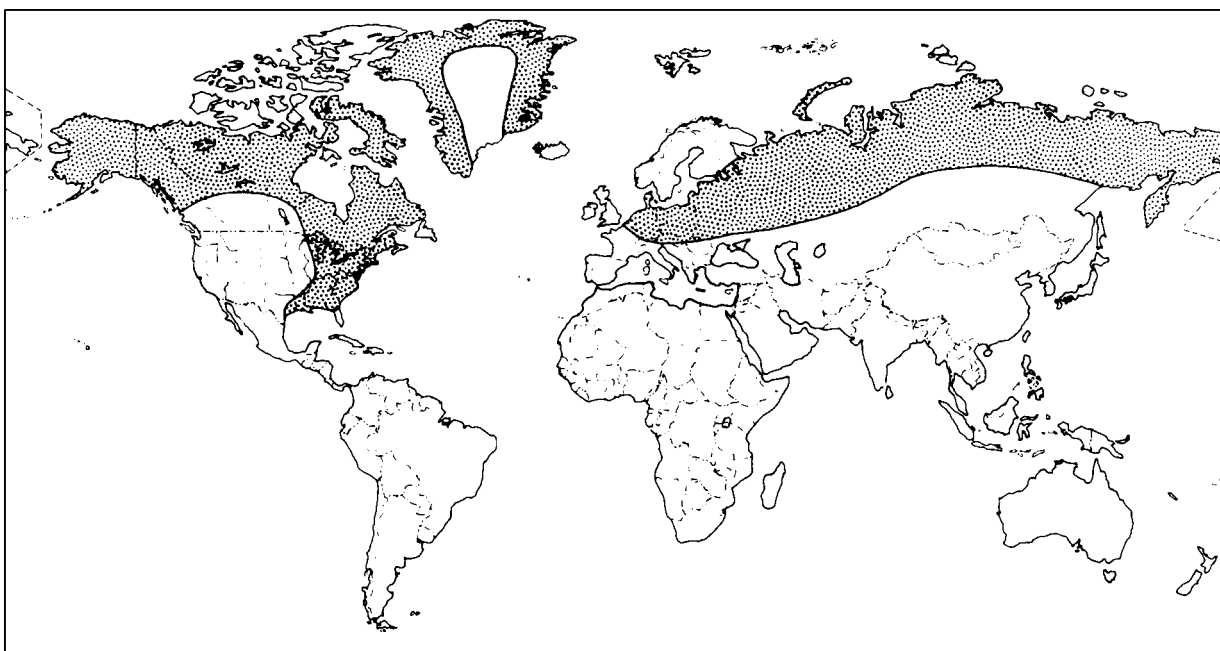


Fig. 7.12 Nosoarea (shaded) of endemic fox rabies. (Source: Winkler, 1975. Reproduced with permission of Elsevier.)

Table 7.6 Probable ecological factors responsible for emerging infectious diseases. (Compiled from Morse, 1995; Schrag and Weiner, 1995; and Williams *et al.*, 2002. Reproduced with permission from Elsevier.)

<i>Infection/agent</i>	<i>Factor(s) contributing to emergence</i>
Viruses	
Argentine, Bolivian haemorrhagic fever	Changes in agriculture favouring rodent host
Dengue	Urbanization, factors favouring mosquito hosts
Duck plague	Changes in lake water management
Hantaviruses	Ecological or environmental changes increasing contact with rodent hosts
Influenza (pandemic)	Possibly pig–duck agriculture, facilitating reassortment of avian and mammalian influenza viruses (see Chapter 5)
Lassa fever	Urbanization favouring rodent hosts, increasing exposure (usually in homes)
Rabies, US	Illegal translocation of raccoons to new areas
Rift Valley fever	Dam building, agriculture, irrigation; possibly change in virulence or pathogenicity of virus
Bacteria	
Mycoplasmal conjunctivitis of wild finches	Sale and transportation of birds
Lyme disease (<i>Borrelia burgdorferi</i>)	Reforestation around homes and other conditions favouring tick vector and deer (a secondary reservoir host)
Plague (<i>Yersinia</i> sp.)	Unsanitary urban environments
Parasites	
Schistosomiasis	Dam building

Table 7.7 Probable factors for the emergence of some infectious diseases. (Compiled from Morse, 1995; and Whittington, 2003.)

Factor	Examples of specific factors	Examples of diseases
Ecological changes (including those due to economic development and land use)	Agriculture; dams, changes in water ecosystems; deforestation/reforestation; flood/drought; famine; climate changes	Schistosomiasis (dams); Rift Valley fever (dams, irrigation); Argentine haemorrhagic fever (agriculture); Hantaan (Korean haemorrhagic fever) (agriculture); hantavirus pulmonary syndrome, southwestern US, 1993 (weather anomalies)
Human demographics, behaviour	Societal events: population growth and migration (movement from rural areas to cities); war or civil conflict; urban decay; sexual behaviour; intravenous drug use; use of high-density facilities	Introduction of HIV; spread of dengue; spread of HIV and other sexually-transmitted diseases
International travel and commerce	Worldwide movement of goods, animals and people; air travel	'Airport' malaria; dissemination of mosquito vectors; rat-borne hantaviruses; introduction of cholera into South America; dissemination of O139 <i>Vibrio cholerae</i>
Deliberate introduction	Introduction of natural or engineered agent in a legal or illegal control programme, or a terrorist act	Rabbit calicivirus
Technology and industry	Globalization of food supplies; changes in food processing and packaging; organ or tissue transportation; drugs causing immunosuppression; widespread use of antibiotics	Haemolytic uraemic syndrome (<i>Escherichia coli</i> contamination of hamburger meat); bovine spongiform encephalopathy; transfusion-associated hepatitis (hepatitis B, C), opportunistic infections in immunosuppressed patients; Creutzfeldt–Jakob disease from contaminated batches of human growth hormone (medical technology)
Microbial adaptation and change	Microbial evolution, response to selection in environment	Antibiotic-resistant bacteria; antigenic drift in influenza virus
Breakdown in public health measures	Curtailement or reduction in prevention programmes; inadequate sanitation and vector-control measures	Resurgence of tuberculosis in the US; cholera in refugee camps in Africa; resurgence of diphtheria in the former Soviet Union

such infections (Table 7.7), some of which can be related specifically to ecotones (Table 7.8) and changes in the relationships between humans, livestock and wildlife (Table 7.9).

Objectives of landscape epidemiology

Landscape epidemiology is founded on the concept that, if the nidality of diseases is based on ecological factors, then a study of ecosystems enables predictions to be made about the occurrence of disease and facilitates the development of appropriated control strategies. Three examples will illustrate this concept.

Leptospirosis

It is known that the prevalence of *Leptospira* serovar Ballum in the brown rat is density-dependent; therefore, estimation of the number of rats inhabiting an area enables a prediction of the prevalence of serovar

Ballum infections to be made (Figure 7.13; Blackmore and Hathaway, 1980). The number of rat burrows in an area is a good indicator of the number of rats, and these burrows are seldom more than 100 m from the major feeding ground, and are seldom more than 40 cm deep (Pisano and Storer, 1948). Thus, if inspection of the area reveals a large number of burrows and evidence of recent rat activity, it is likely that the area constitutes a reservoir of infection for serovar Ballum. Conversely, if the rubbish dump is well managed and the area surrounding it shows evidence of regular bulldozing and few inhabited burrows, then the rat population is likely to be small, and any rats present are unlikely to constitute a maintenance population for this leptospiral serovar.

Tularaemia

In 1966–1967 in Sweden, an epidemic of tularaemia, caused by *Francisella tularensis*, occurred with more

Table 7.8 Zoonotic emerging infectious diseases (EIDs) associated with ecotones. (From Despommier *et al.*, 2007.)

<i>EID</i>	<i>Reservoir</i>	<i>Transmission route</i>	<i>Ecotone involvement</i>	<i>Ecologically-similar EIDs (or pathogens causing them)</i>
Viruses				
Sin Nombre	Rodents	Contact with rodent excretions	Settlement–natural habitat	Andes, Junin, Laguna Negra, Lassa, Machupo, Puumala, Sabiá, Seoul, Guanarito, Hantaan
Yellow fever	Monkeys	Mosquito vector	Settlement–natural habitat	Dengue, Chikungunya, Zika, O'nyong-nyong
Nipah	Bats	Contact with bat excretions	Agriculture–natural habitat	Hendra, Menangle, Australian bat lyssavirus, SARS
Influenza	Birds, pigs, humans	Direct aerosolized contact	Settlement–agriculture–aquatic habitat	Japanese encephalitis
Rabies	Vertebrates	Direct contact	Settlement–natural habitat	Ebola, monkeypox, Marburg
Bacteria				
Lyme disease	Rodents, deer	Tick vector	Settlement–natural habitat	<i>Anaplasma phagocytophila</i> , <i>Ehrlichia chaffeensis</i> , <i>E. ewingii</i> , <i>Babesia microti</i> , <i>Rickettsia prowazekii</i> , <i>Vibrio parahaemolyticus</i> , <i>V. vulnificus</i>
Cholera	Humans, zooplankton, shellfish	Faecal–oral, contaminated seafood	Terrestrial–aquatic habitat	<i>Vibrio parahaemolyticus</i> , <i>V. vulnificus</i>
Leptospirosis	Vertebrates	Direct contact	Terrestrial–aquatic habitat	<i>Giardia duodenalis</i> , <i>Toxoplasma gondii</i> , <i>Cryptosporidium parvum</i> , <i>Campylobacter fetus</i> , <i>Francisella tularensis</i> , <i>Schistosoma</i> spp., <i>Escherichia coli</i>
Protozoa				
Malaria	Humans	Mosquito vector	Settlement–natural habitat	Chagas' disease, leishmaniasis, Loa loa, <i>Onchocerca volvulus</i> , Oropouche, Mayaro
Trypanosomiasis (sleeping sickness)	Vertebrates	Tsetse vector	Settlement–agriculture–natural habitat	<i>Echinococcus granulosus</i> , anthrax, Rift Valley fever, Crimean–Congo haemorrhagic fever, Kyasanur forest virus, <i>Mycobacterium bovis</i>

than 2000 human cases and a high mortality rate of hares (Borg and Hugoson, 1980). This epidemic was associated with the clearing of small areas of forest to create areas of grazing, and this synanthropic ecosystem led to a sudden increase in the population density of small rodents. Humans became infected by

sorting hay contaminated by dead infected voles (Dahlstrand *et al.*, 1971).

Kyasanur Forest disease

Kyasanur Forest disease is caused by an arbovirus. Symptoms in humans include headache, fever, back

Table 7.9 Conceptual framework of types of wildlife–livestock–human interface and their characteristics. (Adapted from Jones *et al.*, 2013. Reproduced with permission.)

<i>Type of wildlife–livestock–human interface</i>	<i>Level of biodiversity</i>	<i>Characteristics of livestock population</i>	<i>Connectedness between populations</i>	<i>Examples of zoonotic disease with altered dynamics</i>
‘Pristine’ (autochthonous) ecosystem with human incursion to harvest wildlife and other resources	High	No livestock	Very low; small populations and limited contact	Ebola, HIV, SARS, Nipah virus in Bangladesh and India
Ecotones and fragmentation* of autochthonous ecosystems: farming edges, human incursion to harvest natural resources	High but decreasing	Few livestock, multiple species, mostly extensive systems	Increasing contact between people, livestock and wild animals	Kyasanur Forest disease, bat rabies, inter-species transmission of <i>Escherichia coli</i> in Uganda, Nipah virus in Malaysia
Evolving landscape: rapid intensification of agriculture and livestock, coexisting with extensive and ‘backyard’ farming	Low, but increasing in wildlife living around human habitations	Many livestock, both intensive and genetically homogeneous, as well as extensive and genetically diverse livestock	High contacts between intensive and extensive livestock, people and wildlife living around human habitations; less with endangered wildlife	Avian influenza and Japanese encephalitis virus in Asia
Managed landscape: ‘islands’ of intensive farming, highly regulated; farmland converted to recreation and conservancy	Low, but increased number of some wildlife species living around human habitations	Many livestock, mainly intensive, genetically homogeneous and biosecure	Fewer contacts between livestock and people; increasing contacts with wildlife	Bat-associated viruses in Australia, West Nile virus and Lyme disease in the US

* **Fragmentation** is alteration of a habitat, resulting in spatial separation of smaller patches that are isolated from one another by a matrix of habitats unlike the original (Wilcove *et al.*, 1986). It may be natural (e.g., caused by spontaneous fires) or anthropogenic (e.g., agricultural-land conversion or deforestation). Such patches may be considered as ecotopes, if viewed spatially, rather than functionally (Forman and Godron, 1986). See also the earlier discussion on landscape patches.

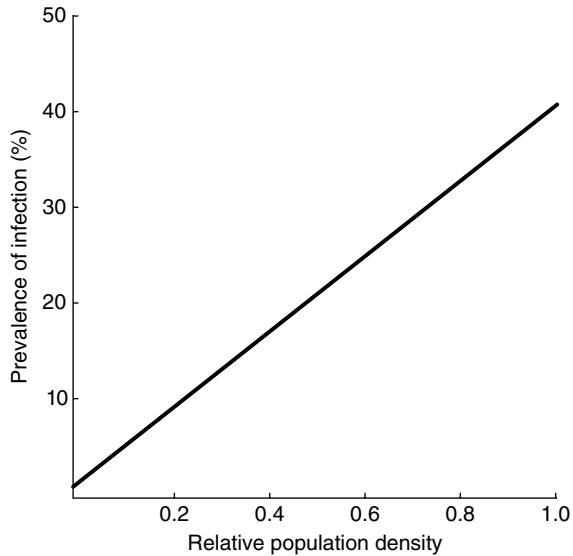


Fig. 7.13 Relationship between the relative population density and prevalence of *Leptospira* serovar Ballum, infection in the brown rat (*Rattus norvegicus*). (Adapted from Blackmore and Hathaway, 1980.)

and limb pains, vomiting, diarrhoea and intestinal bleeding (Work *et al.*, 1958). Death due to dehydration can occur in untreated cases. It is mainly restricted to an area 1550 km² in the Indian state of Karnataka (Pattnaik, 2006)²⁶. The virus endemically and inapparently infects some small mammals, including rats and shrews, in the local rain forest. The virus is transmitted by several species of tick (Singh *et al.*, 1964), only one of which, *Haemaphysalis spinigera*, will infest humans. The usual host of the tick is the ox. Thus, when humans create ecological mosaics by cultivating areas for rice, their cattle roam into the surrounding rain forest and may become infested with virus-infected ticks. Dense populations of ticks therefore build up around villages and, when infected, these ticks can transmit the infection to humans (Hoogstraal, 1966).

Landscape characteristics determining disease distribution

The ecological conditions that favour the localized distribution of diseases are not static, but involve changing spatial and temporal relationships involving several distinct characteristics (Lambin *et al.*, 2010):

- **landscape attributes** (e.g., the seroprevalence rates of West Nile virus in horses in Senegal decrease close to seawater and flooded banks because these are unfavourable locations for the mosquito vector: Chevalier *et al.*, 2010);

- **spatial relationships of critical habitats** (e.g., Lyme disease in Belgium is associated with large ecotones between settlements and forests in peri-urban areas: Linard *et al.*, 2007a);
- **connectivity of habitats** (e.g., the incidence of hantavirus in deer mice is higher in landscapes with higher levels of fragmentation: Langois *et al.*, 2001);
- **species associations** (e.g., the introduction of West Nile virus by migratory birds explains the spatial patterns of the virus' transmission: Tran *et al.*, 2007);
- **transmission pathways** (e.g., in addition to being transmitted by aerosol, influenza virus also may be transmitted to wild and domestic birds via contaminated water supplies: Markwell and Shortridge, 1982);
- **complex interaction of determinants** (e.g., in southwest France, spatial variations in the abundance of various species of sandfly vectors of canine leishmaniasis are determined differently by season and altitude: Hartemink *et al.*, 2011);
- **land use** (e.g., in the Camargue, water-management practices in rice fields influence the rate of contact between humans and potential malaria vectors: Linard *et al.*, 2009);
- **land ownership** (e.g., in Latvia, human cases of tick-borne fever are associated with state ownership of forests, because they are more accessible to the public than privately-owned forests, thus increasing access to vector habitats: Vanwambeke *et al.*, 2010);
- **human behaviour** (e.g., in Uganda, bovine trypanosomiasis control is constrained because not all farmers use acaricides that are effective against ticks and the tsetse vector of the disease: Bardosh *et al.*, 2013).

Various combinations of these factors influence disease distribution, with landscape attributes frequently being a common feature. Thus, the distribution of West Nile virus in Senegal is influenced by landscape attributes and species associations (Chevalier *et al.*, 2010), whereas the location of Puumala hantavirus in Belgium is affected by landscape attributes, species associations, spatial relationships, connectivity of habitats, transmission pathways, complex interaction of determinants, land use and human behaviour (Linard *et al.*, 2007b).

The dynamics of patches within landscapes also can affect not only pathogen transmission and maintenance but also the potential for host extinction (Table 7.10). However, there is no general rule that relates changes in prevalence in relation to either patch size or isolation. Thus, in prairie dogs, extinctions induced by plague are lowest in medium-sized colonies (Stapp *et al.*, 2004). This is not surprising in terms of the complexity of transmission dynamics: large patches should support larger host populations, but these will not necessarily be at a higher density.

²⁶ A variant, Alkhurma haemorrhagic fever virus, occurs in Saudi Arabia (Charrel *et al.*, 2001).

Table 7.10 Some studies of patch parameters and disease dynamics. (Reduced and modified from McCallum, 2008.)

Pathogen	Host	Conclusion
<i>Baylisascaris procyonis</i> (nematode)	Definitive: raccoon Intermediate: white-footed mouse	Both prevalence and intensity in mice are an increasing function of forest-patch area. Both are negatively related to the fraction of forested habitat nearby
Plague (<i>Yersinia</i> spp.)	Prairie dogs	Pathogen-induced extinction is lowest in intermediate-sized colonies. The fate of adjacent colonies is important, but no distance or isolation measures have proved significant
Sin Nombre (hantavirus)	Deer mice	Seroprevalence is lowest in patches with an intermediate proportion of preferred habitat within a 1 km radius. Seroprevalence is increased with fragmentation
Merthimids (nematodes)	Hawaiian spiders	Prevalence is increased with size of patch area
Lyme disease (<i>Borrelia burgdorferi</i>)	Vector: ticks (<i>Ixodes</i> spp.) Main reservoir: white-footed mouse	Prevalence in ticks declines linearly with size of forest-patch area

Further reading

- Aguirre, A.A., Ostfeld, R.S. and Daszak, P. (2012) *New Directions in Conservation Medicine*. Oxford University Press, Oxford. (A study of human and animal diseases in the context of One Health, ecosystem health and emerging diseases)
- Aguirre, A.A., Ostfeld, R.S., Tabor, G.M., House, C. and Pearl, M.C. (2002) *Conservation Medicine: Ecological Health in Practice*. Oxford University Press, Oxford. (A study of human and animal diseases in an ecological context)
- Altizer, S., Bartel, R. and Han, B.A. (2011) Animal migration and infectious disease risk. *Science*, **331**, 296–302. (A discussion of the consequences of long-distance migrations for the ecology of host–pathogen interactions)
- Bengis, R.G. (Coordinator) (2002) Infectious diseases of wildlife. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 1–402
- Bokma, B.H. and Blouin, E. (Eds) (2004) *Impact of Ecological Changes on Tropical Animal Health and Disease Control*. *Annals of the New York Academy of Sciences*, **1026**, 1–324
- Burnet, F.M. and White, D.O. (1962) *Natural History of Infectious Disease*. Cambridge University Press, Cambridge
- Cain, M.L., Bowman, W.D. and Hacker, S.L. (2011) *Ecology*, 2nd edn. Sinauer Associates, Sunderland. (A general ecology text, also including a discussion of parasitism from an ecological perspective)
- Chadwick, D.J. and Goode, J. (Eds) (1997) *Antibiotic Resistance: Origins, Evolution, Selection and Spread*. CIBA Foundation Symposium 207. John Wiley, Chichester
- Cherrett, J.M. (Ed.) (1989) *Ecological Concepts*. Blackwell Scientific Publications, Oxford. (A general ecology text, which also includes a discussion of predator/prey and host/pathogen interactions)
- Collinge, S.K. and Ray, C. (Eds) (2006) *Disease Ecology: Community Structure and Pathogen Dynamics*. Oxford University Press, Oxford
- Crawley, M.J. (Ed.) (1992) *Natural Enemies: The Population Biology of Predators, Parasites and Diseases*. Blackwell Scientific Publications, Oxford
- Cunningham, A.A., Dobson, A.P. and Hudson, P.J. (Eds) (2012) Disease invasion: impacts on biodiversity and human health. *Philosophical Transactions of the Royal Society, Series B*, **367**, 2803–2900
- Daszak, P., Cunningham, A.A. and Pepper, I.L. (1998) Emerging infectious diseases of wildlife – threats to biodiversity and human health. *Science*, **287**, 443–449
- Deem, S.L., Kilbourn, A.M., Wolfe, N.D., Cook, R.A. and Karesh, W.B. (2000) Conservation medicine. *Annals of the New York Academy of Sciences*, **916**, 370–377
- Delahay, R.J., Smith, G.C. and Hutchings, M.R. (Eds) (2009) *Management of Disease in Wild Animals*. Springer, Tokyo
- Desowitz, R.S. (1981) *New Guinea Tapeworms and Jewish Grandmothers. Tales of Parasites and People*. W. Norton and Company, New York and London. (Essays on ecological and anthropological aspects of some parasitic diseases)
- Dieckmann, U., Metz, A.J., Sabelis, M.W. and Sigmund, K. (Eds) (2002) *Adaptive Dynamics of Infectious Diseases: In Pursuit of Virulence Management*. Cambridge University Press, Cambridge. (A comprehensive presentation of the evolutionary ecology of infectious diseases)

- Edwards, M.A. and McDonnell, U. (Eds) (1982) *Animal Disease in Relation to Animal Conservation*. Symposia of the Zoological Society of London No. 50. Academic Press, London
- Emmanuel, N.N., Loha, N., Ojogba, O.K. and Ikenna, O.K. (2011) Landscape epidemiology: an emerging perspective in the mapping and modelling of disease and disease risk factors. *Asian Pacific Journal of Tropical Disease*, **1**, 247–250
- Ewald, P.W. (1994) *Evolution of Infectious Diseases*. Oxford University Press, Oxford
- Fayer, R. (1998) Global change and emerging infectious diseases. *Journal of Parasitology*, **86**, 1174–1181
- Galuzo, I.G. (1975) Landscape epidemiology (epizootiology). *Advances in Veterinary Science and Comparative Medicine*, **19**, 73–96
- Gibbs, E.P.J. and Bokma, B.H. (Eds) (2002) *The Domestic Animal/Wildlife Interface: Issues for Disease Control, Conservation, Sustainable Food Production, and Emerging Diseases*. *Annals of the New York Academy of Sciences*, Vol. **969**. New York Academy of Sciences, New York. (Includes discussions of disease transmission across ecological interfaces)
- Gilbert, A.T., Fooks, A.R., Hayman, D.T., Horton, F.L., Müller, T. *et al.* (2013) Deciphering serology to understand the ecology of infectious diseases in wildlife. *Ecohealth*, **10**, 298–313
- Goater, T.M., Goater, C.P. and Esch, G.W. (2014) *Parasitism: The Diversity and Ecology of Animal Parasites*, 2nd edn. Cambridge University Press, Cambridge
- Grenfell, B.T. and Dobson, A.P. (Eds) (1995) *Ecology of Infectious Diseases in Natural Populations*. Cambridge University Press, Cambridge
- Grenfell, B.T., Pybus, O.G., Gog, J.R., Wood, J.L.N., Daly, J.M. *et al.* (2004) Unifying the epidemiological and evolutionary dynamics of pathogens. *Science*, **305**, 327–332
- Hassell, J.M., Begon, M., Ward, M.J. and Fèvre, E.M. (2017) Urbanization and disease emergence: dynamics at the wildlife-livestock-human interface. *Trends in Ecology and Evolution*, **32**, 55–67
- Hastings, A. (2004) *Population Biology*, 2nd edn. Springer-Verlag, New York. (A basic text on population biology, with emphasis on simple mathematical models)
- Hawley, D.M. and Atlizer, S.M. (2011) Disease ecology meets ecological immunology: understanding the links between organismal immunity and infection dynamics in natural populations. *Functional Ecology*, **25**, 48–60. (An introduction to the link between variation in immune function in individuals or populations, and parasite spread and maintenance in populations)
- Huang, Y. and Rohani, P. (2005) The dynamical implications of disease interference: correlations and coexistence. *Theoretical Population Biology*, **68**, 205–215
- Hudson, P.J., Rizzoli, A., Grenfell, B.T., Heesterbeek, H. and Dobson, A.P. (Eds) (2002) *The Ecology of Wildlife Diseases*. Oxford University Press, Oxford
- Karesh, W.B., Dobson, A., Lloyd-Smith, J.O., Lubroth, J., Dixon, M.A. *et al.* (2012) Ecology of zoonoses: natural and unnatural histories. *The Lancet*, **380**, 1936–1945
- Keesing, F., Belden, L.K., Daszak, P., Dobson, A., Harvell, C.D. *et al.* (2010) Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature*, **468**, 647–652
- Kitron, U. (1998) Landscape ecology and epidemiology of vector-borne diseases: tools for spatial analysis. *Journal of Medical Entomology*, **35**, 435–445
- Learmonth, A. (1988) *Disease Ecology: An Introduction*. Basil Blackwell, Oxford. (A text focussing on human diseases, but also covering some zoonoses)
- Levin, B.R., Lipsitch, M.N. and Bonhoeffer, S. (1999) Population biology, evolution, and infectious disease: convergence and synthesis. *Science*, **283**, 806–809. (A discussion of population biology in the context of molecular epidemiology, pathogenesis, and disease control)
- Lindahl, J.F. and Grace, D. (2015) The consequences of human actions on risks for infectious diseases: a review. *Infection Ecology and Epidemiology*, **5**, 30048; doi 10.3402/iee.v5.30048
- Lord, R.D. (1973) Ecological strategies for the prevention and control of health problems. *Bulletin of the Pan American Health Organization*, **17**, 19–34
- Lower, G.M. (1984) The ecology of infectious and neoplastic disease: a conceptual unification. *Ecology of Disease*, **2**, 397–407
- Morse, S.S. (Ed.) (1993) *Emerging Viruses*. Oxford University Press, New York
- Norrgren, L. and Levengood, J.M. (Eds) (2012) *Ecology and Animal Health*. The Baltic University Programme, University of Uppsala, Uppsala
- Osofsky, S.A. (Ed.) (2005) *Conservation and Development Interventions at the Wildlife/Livestock Interface*. Occasional Paper of the IUCN, Species Survival Commission No. 30. IUCN, Gland and Cambridge. (Includes a discussion of diseases in wildlife and associated domestic species)
- Ostfield, R.S., Keesing, F. and Eviner, V. (Eds) (2008) *Infectious Disease Ecology*. Princeton University Press, Princeton and Oxford
- Pastoret, P.-P., Thiry, E., Brochier, B., Schwerts, A., Thomas, I. *et al.* (1988) Diseases of wild animals transmissible to domestic animals. *Revue Scientifique et Technique, Office International des Epizooties*, **7**, 705–736

- Pavlovsky, E.N. (1964) *Prirodnaya Ochagovost Transmissivnykh Boleznay v Svyazi s Landshoftnoy Epidemiologiyey Zooantroponozov*. Translated as *Natural Nidality of Transmissible Disease with Special Reference to the Landscape Epidemiology of Zooanthroponoses*. Plous, F.K. (translator), Levine, N. D. (Ed.) (1966). University of Illinois Press, Urbana
- Porter, J.W. (Ed.) (2001) *The Ecology and Etiology of Newly Emerging Marine Diseases*. Kluwer Academic Publishers, Dordrecht
- Rapport, D.J. (1999) Epidemiology and ecosystem health: natural bridges. *Ecosystem Health*, **5**, 174–180
- Rapport, D., Costanza, R., Epstein, P.R., Gaudet, C. and Levins, R. (Eds) (1993) *Ecosystem Health*. Blackwell Science, Oxford. (A discussion of the impact of environmental change on ecosystems, including human and animal disease)
- Reisen, W.K. (2010) Landscape epidemiology of vector-borne diseases. *Annual Review of Entomology*, **55**, 461–483
- Ricklefs, R.E. and Miller, G.L. (2000) *Ecology*, 4th edn. W. H. Freeman, New York. (A general ecology text, which also includes a discussion of parasitism and disease)
- Roche, B. and Guégan, J.-F. (2011) Ecosystem dynamics, biological diversity and emerging infectious diseases. *Comptes Rendus Biologies*, **334**, 385–392
- Schneider, R. (1991) Wildlife epidemiology. In: *Veterinary Epidemiology in the Real World: A Canadian Potpourri*. Ed. Waltner-Toews, D., pp. 41–47. Canadian Association of Veterinary Epidemiology and Preventive Medicine, Ontario Veterinary College, Guelph. (A concise discussion of the role of disease in regulating animal populations)
- Schrag, S.J. and Wiener, P. (1995) Emerging infectious diseases: what are the relative roles of ecology and evolution? *Trends in Ecology and Evolution*, **10**, 319–324
- Schwabe, C.E. (1984) *Veterinary Medicine and Human Health*, 3rd edn. Williams and Wilkins, Baltimore. (Includes a section on medical ecology)
- Siembieda, J.L., Kock, R.A., McCracken, T.A. and Newman, S.H. (2011) The role of wildlife in transboundary animal diseases. *Animal Health Research Reviews*, **12**, 95–111
- Singh, S.K. (Ed.) (2014) *Viral Infections and Global Change*. John Wiley and Sons, Hoboken. (Includes a discussion of the effects of ecological changes on the occurrence of virus infections)
- Sinnecker, H. (1976) *General Epidemiology*. Walker, N. (translator). John Wiley and Sons, London. (An ecological approach to epidemiology)
- Slingenbergh, J., Gilbert, M., de Balogh, K. and Wint, W. (2004) Ecological sources of zoonotic diseases. *Revue Scientifique et Technique, Office International des Epizooties*, **23**, 467–484
- Sterns, S.C. and Koella, J.C. (Eds) (2008) *Evolution in Health and Disease*, 2nd edn. Oxford University Press, Oxford
- Wiens, J.A., Moss, M.R., Turner, M.G. and Mladenoff, D. J. (Eds) (2007) *Foundation Papers in Landscape Ecology*. Columbia University Press, New York
- Wilcox, B.A. and Gubler, D.J. (2005) Disease ecology and the global emergence of zoonotic pathogens. *Environmental Health and Preventative Medicine*, **10**, 263–272
- Williams, E.S. and Barker, I.K. (Eds) (2008) *Infectious Diseases of Wild Mammals*, 3rd edn. Iowa State University Press, Ames
- Wilson, M.L. (2001) Ecology and infectious disease. In: *Ecosystem Change and Public Health: A Global Perspective*. Eds Aron, J.L. and Patz, J.A., pp. 283–324. Johns Hopkins University Press, Baltimore
- Wobeser, G.A. (2007) *Disease in Wild Animals: Investigation and Management*, 2nd edn. Springer-Verlag, Berlin and Heidelberg
- Wolfe, N. (2011) *The Viral Storm*. Allen Lane, London. (A popular introduction to the emergence of virus diseases from an ecological perspective)
- Wood, C.L., Lafferty, K.D., Deleo, G., Young, H.S., Hudson, P.J. et al. (2014) Does biodiversity protect humans against infectious disease? *Ecology*, **95**, 817–832

8

Patterns of disease

Methods of expressing the temporal and spatial distribution of disease were described in Chapter 4; this chapter discusses the various patterns of disease that can be detected when disease distribution is recorded. A considerable bulk of mathematical theory has been formulated to explain disease patterns (e.g., Bailey, 1975; Anderson and May, 1991; Keeling and Rohani, 2008). Most of this is beyond the scope of this book, but a brief introduction will be given in this chapter. Additionally, the application of mathematics to the development of predictive models, of practical value to disease control, is described in Chapter 23.

Epidemic curves

The representation of the number of new cases of a disease by a graph, with the number of new cases on the vertical axis and calendar time on the horizontal axis, is the most common means of expressing disease occurrence. The graph of an epidemic portrayed in this way produces an **epidemic curve**. *Figure 8.1* depicts the various parts of an epidemic curve, stylized to a symmetric shape for the purpose of illustration. Epidemic curves are given for foot-and-mouth disease in *Figure 4.1*, with the number of new outbreaks (see Appendix I) approximately indicating the number of new cases. Note that the culmination point (peak) is shifted to the left; that is, the curve is positively skewed.

Factors affecting the shape of the curve

The shape of the curve and the time scale depend on:

- the incubation period of the disease;
- the infectivity of the agent;
- the proportion of susceptible animals in the population;
- the distance between animals (i.e., animal density).

Thus, a highly infectious agent with a short incubation period infecting a population with a large proportion of susceptible animals at high density produces a curve

with a steep initial slope on a relatively small time scale, representing a rapid spread of infection among the population.

Kendall's Threshold Theorem

A minimum density of susceptible animals theoretically is required to allow a contact-transmitted epidemic to commence (i.e., for an infection to 'invade' a population). This is called the **threshold level**, and is defined mathematically by **Kendall's Threshold Theorem**¹ (in Discussion to Bartlett, 1957). *Figure 8.2* illustrates application of the theorem in relation to rabies in foxes. Above a certain density of susceptible animals, one infected fox can, on average, infect more than one susceptible fox, and an epidemic can occur; the greater the density, the steeper the slope of the progressive stage of the epidemic curve.

A second threshold, the **critical community size** (Bartlett, 1960; Swinton *et al.*, 1998), determines whether or not new susceptible individuals are recruited at a high enough rate for infection to *persist*. This is different from the invasion threshold, and is a population size, rather than a density. It is a complex function, which is affected by several factors, including infectiousness (see Chapter 6), birth rate, the basic reproductive number (see next section) and vaccination coverage (Metcalf *et al.*, 2013).

Although there is usually a positive relationship between host density and parasite abundance (Arneberg *et al.*, 1998), and host-density thresholds are theoretically predicted to be important components of host–parasite dynamics, empirical examples

¹ A theorem is a mathematical statement that has been proven (Clapham and Nicholson, 2009), and is essentially derived deductively (see Chapter 3) from previous theorems or axioms. This contrasts with a scientific theory, which is usually explored inductively (see Chapters 3 and 23). Proof of a theorem therefore is not necessarily vindication of the truth of the biological events to which the theorem relates; substantiation of these requires application of scientific method.

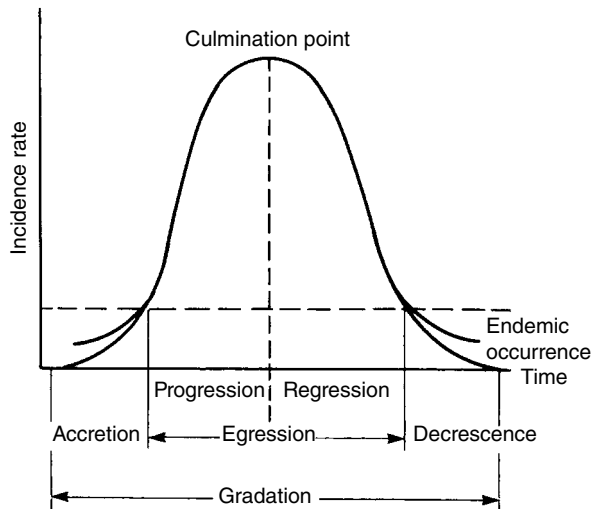


Fig. 8.1 Components of an epidemic curve (stylized to a symmetrical shape). The horizontal dashed line indicates the average number of new cases. (Source: Sinnecker, 1976. Reproduced with permission of John Wiley and Sons, Ltd.)

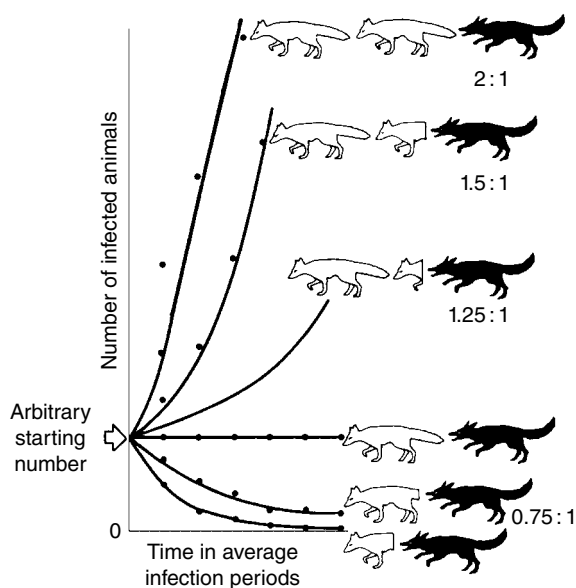


Fig. 8.2 Application of Kendall's Threshold Theorem to rabies in foxes: the theorem predicts that, if a rabid fox infects more than one other before it dies, the disease increases exponentially; if each animal infects less than one other, the disease decreases exponentially. White animals: susceptible; black animals: infected and infectious. Numbers refer to the **contact rate**: the number of susceptible animals that are infected by an infected animal. (Source: Macdonald and Bacon, 1980.)

of such thresholds are sparse (Lloyd-Smith *et al.*, 2005) and are usually confined to well-understood diseases for which there is a plethora of data; for example, human measles (Keeling and Grenfell, 1997), where

the critical community size also is estimated to be 250 000–300 000 (Black, 1966)². Consequently, few threshold values relating to animal diseases are known. Wierup (1983) has estimated that a minimum density of 12 dogs/km² is required before a canine parvovirus epidemic can occur. Threshold levels are not necessarily fixed for a particular host–parasite situation, and may be modified by factors that alter contact, such as host behaviour (Caley and Ramsay, 2001).

Basic reproductive number (R_0)

The ability of an infectious disease to spread in a population is frequently defined in terms of the **basic reproductive number (basic reproduction ratio; basic reproductive ratio; basic reproductive rate)**, R_0 , which is the average number of secondary cases caused by one typical infectious individual during its entire infectious period *in an entirely susceptible population* (Diekmann *et al.*, 1990). The basic reproductive number is a function of the number of secondary cases that occur per unit time and the amount of time that an individual is infectious. The number of secondary cases that occur per unit time is the **transmission rate**. It is equal to the product of the **transmission risk** (the probability of transmission per contact³) and the **contact rate** (the average number of contacts per unit time, where contact is defined as an event, regardless of whether or not transmission actually occurs). Confusingly, β is used by some authors to represent the transmission rate and by others to represent the transmission risk. Here, β is used to represent the transmission risk, and β_{rate} to symbolize the transmission rate.

Hence, for microparasitic diseases (see Chapter 7):

$$R_0 = \beta \times \kappa \times d,$$

where: β = risk of transmission per contact, κ = number of contacts per unit time, and d = duration of infectiousness.

As the *rate* of transmission is the product of the transmission risk and the contact rate ($\beta \times \kappa$), this also can be written:

$$R_0 = \beta_{rate} \times d.$$

² The rarity of empirical examples, however, is not evidence that host-density thresholds do not exist, and the theory is the basis of disease-control and eradication strategies in both wildlife and domestic animals (Grenfell and Dobson, 1995; Hudson *et al.*, 2001); for example, fertility control in badgers in the UK as a possible means of eradicating bovine tuberculosis (White *et al.*, 1997).

³ This is therefore a quantitative manifestation of 'effective contact' (see Chapter 6).

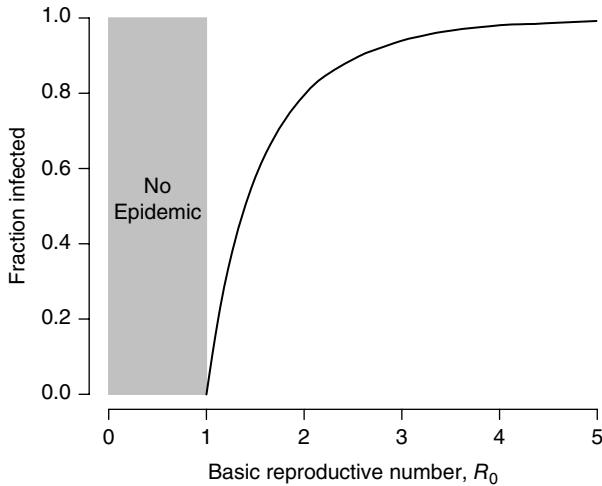


Fig. 8.3 Total fraction of the population infected as a function of the basic reproductive number, R_0 . (Adapted from Keeling and Rohani, 2008.)

If $R_0 > 1$, an infection will tend invade a population, and the estimate of the total fraction of the population that will eventually contract infection is directly related to its value (Figure 8.3). In contrast, if $R_0 < 1$, it cannot invade the population. Therefore, control strategies should aim to reduce R_0 to below 1. Hence, from the equation above, it is evident that control strategies should seek to reduce: (1) transmission, if contact occurs; (2) the contact rate; and (3) the duration of infectiousness; or a combination of these.

The probability of a single infected individual initiating an epidemic, P_{epidemic} , also can be calculated from R_0 (May *et al.*, 2001):

$$P_{\text{epidemic}} = 1 - (1/R_0).$$

For example, if $R_0 = 1.6$, then:

$$\begin{aligned} P_{\text{epidemic}} &= 1 - (1/1.6) \\ &= 1 - 0.625 \\ &= 0.375. \end{aligned}$$

The same numerical value also is the proportion of the population that needs to be protected from infection to prevent an epidemic (the 'herd immunity threshold'), assuming that protection is randomly distributed in the population (Fine *et al.*, 2011)⁴.

A benefit of defining R_0 in terms of β and κ (rather than using β_{rate}) is that it better delineates the

important effects of contact from those of transmission risk, and this aids consideration of control strategies. For example, isolation of animals is an important method of infection control. A veterinary hospital may have a policy of isolating dogs with parvovirus diarrhoea. The act of isolating the animals reduces (perhaps to zero) the rate of direct contact (κ) between those dogs and others. Nevertheless, the possibility of indirect contact, for example via movement of people, remains. However, such contacts may be kept to a minimum and be strategically timed (thus reducing κ for indirect contacts). Additionally, practising good hygiene, (e.g., hand washing and changing outer clothing) reduces the risk of transmission associated with each contact that does occur (β).

The basic reproductive number is not specific to a particular microbe. Rather, it is a characteristic of a particular microbial population in a particular host population at a specific time. For example, during a foot-and-mouth disease epidemic, contact rates (κ) in hill sheep are likely to be lower than in housed sheep⁵. Direct estimation of R_0 is therefore difficult (Dietz, 1993)⁶ and estimates are not necessarily transferable between contexts.

In the above equation, there is only a single contact rate (κ). Hence, the assumption is that all individuals make and receive the same number of contacts per unit time. This is unlikely to be the case because, continuing the example from above, it is known that some farms have many more 'contacts' than others (e.g., farms may have many visitors or few, be closed or open, have double or single fencing, be geographically isolated or in close proximity to other farms). This variation matters and can be measured as the **coefficient of variation** of the contact rate (CV) (May *et al.*, 2001). The CV is calculated as:

$$CV = \sigma/\mu,$$

where σ is the standard deviation and μ is the mean of the contact rate. Variation in contact rate can be incorporated in the calculation of R_0 as described by May and others (2001):

$$R_0 = b \times \kappa \times (1 + CV^2) \times d.$$

⁴ Protection commonly is by vaccination, but it can be any procedure that removes individuals from risk of infection (e.g., changes in behaviour in relation to human sexually-transmitted diseases).

⁵ Estimates of R_0 derived for smallpox have varied with time and place. Over the 1700s to 1900s, they tended to be in the range 3.5 to 6, but under particular circumstances (crowding and poorer socio-economic conditions in the 18th century) rose to nearer 10–12 (Gani and Leach 2001).

⁶ Indirect methods can be used when transmission is in equilibrium (e.g., when, on average, one infectious case produces one other infectious case); see Halloran (1998) for details.

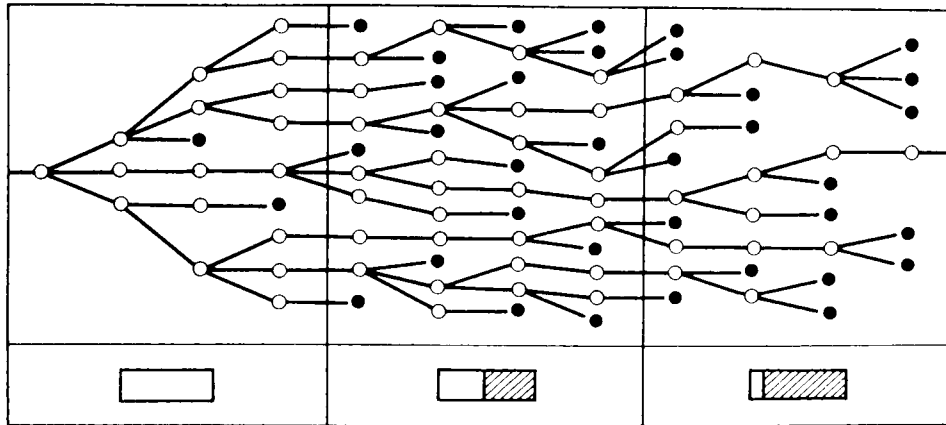


Fig. 8.4 The course of a typical epidemic caused by an infectious agent infecting a totally susceptible population. Each circle represents an infection, and the connecting lines indicate transfer from one case to the next. Black circles represent infected individuals who fail to infect others. Three periods are shown: the first when practically the whole population is susceptible; the second at the height of the epidemic; and the third at the close, when most individuals are immune. The proportions of susceptible (white) and immune (hatched) individuals are indicated in rectangles beneath the main diagram. (Source: Burnet and White, 1972. Reproduced with permission of Cambridge University Press.)

When there is no variation in contact rate between individuals, $\sigma = 0$ and hence $CV = 0$, and the equation reduces to $R_0 = \beta \times \kappa \times d$. In contrast, as the contact rate becomes more heterogeneous (some individuals have few contacts, others have many) the CV increases and R_0 also increases for given values of β , κ and d .

These equations include a single transmission risk per contact. They assume, therefore, that there is either a single type of contact, or that the transmission risk for a range of contact types can be adequately estimated in a single value. Whilst potentially useful, such assumptions are simplistic representations of the biology of most infectious diseases. More complex equations explicitly incorporating different contact types (with different values of β , κ and d for each) could be developed. For example, again considering foot-and-mouth disease, movement of vehicles and live animals are likely to result in different transmission risks and contacts rates between two farms. Similarly, the duration of infectiousness may vary depending on contact type; for example, the risk due to aerial transmission of foot-and-mouth disease may not be of the same duration as that associated with animal movements. Consideration of such complexity is unlikely to be helpful when attempting to estimate R_0 , but may be useful when extrapolating from such calculations to specific situations, such as the control of a particular epidemic.

The basic reproductive number also can be applied to macroparasitic diseases, where it refers to the average number of offspring produced throughout the lifespan of a mature parasite (Anderson and May, 1991); a parasite population will establish within

a host community only when $R_0 > 1$. It also can pertain to transmission between herds or flocks (between-herd/flock R_0 ; e.g., Barongo *et al.*, 2015) and to the evolution of modes of transmission (van Baalen, 2000).

As an epidemic proceeds, the number of susceptible animals is decreased, either as a result of the death of infected animals, or by increasing immunity following infection (Figure 8.4). Eventually, the epidemic cannot continue because there are insufficient susceptible animals available for infection. In the case of canine parvovirus, for instance, it has been estimated that an epidemic will stop when the density of susceptible dogs falls below 6/km² (Wierup, 1983). A period of time is then necessary to allow replacement of susceptible animals before another epidemic can commence. This effect is captured by the **effective reproductive number**, $R_{(t)}$ (Chowell *et al.*, 2007), which is the average number of secondary cases caused by one typical infectious individual during its entire infectious period at a particular time (i.e., when the population is not entirely susceptible). The effective reproductive number is related to R_0 by the susceptibility of the population:

$$R_{(t)} = R_0 \times S,$$

where S is the proportion of the population that is susceptible in calendar time, t . This explains the cyclicity of some epidemics, which occurs because of cyclical changes in S due to factors such as changes in population immunity, and birth of susceptible individuals. For example, influenza induces immunity in an exposed population, and so future outbreaks may occur in a population that is not entirely susceptible

(Ferguson *et al.*, 2003). Vaccination confers protection to various degrees (Boni *et al.*, 2004), and interference by other viruses also may complicate transmission (see Chapter 7). Thus, the proportion of the exposed population that is susceptible is likely to change from year to year, highlighting that $R_{(t)}$ is time-dependent.

Again, some of the parameters on which $R_{(t)}$ depends are difficult to quantify directly, and so estimation of $R_{(t)}$ commonly is indirect, often involving the fitting of mathematical models to real outbreak data (Cintrón-Arias *et al.*, 2009). An example of estimation of the effective reproductive number in relation to the epidemic of foot-and-mouth disease in the UK in 2001 is given by Haydon and others (2003); and Nishiura and Chowell (2009) discuss the parameter in detail.

Dissemination rate

In veterinary medicine, concern frequently focusses on the progress of an epidemic at herd level. The **dissemination rate (DR)** is the propensity of infection to spread to other herds (Miller, 1976)⁷. It represents the average number of uninfected herds (or premises) to which an infectious agent is delivered by each infected herd.

The DR depends on several factors:

- the environment (geography, herd density and sometimes weather);
- type of farming enterprise (species of livestock, opportunity for transmission by fomites);
- animal movements (e.g., marketing, movement to pasture from winter housing);
- behaviour of the farmer ('biosecurity', travel);
- disease-control strategies (quarantine, movement restrictions);
- host factors (immunity, concurrent disease, age, breed, pregnancy).

In epidemics involving farmed livestock (e.g., foot-and-mouth disease), infection usually is curtailed by a decline in the DR, rather than just by a lack of susceptible animals. The decline may be due to the implementation of disease-control strategies, increased awareness among livestock owners, and other factors initially favourable to dissemination but no longer

⁷ A herd-level parameter is valuable because herds are stationary, having only indirect contact with other herds (e.g., through fomites). The concept of 'effective contact' (see Chapter 6) occurring at random within a homogeneously mixed population, which forms the basis of simple epidemic models (e.g., the Reed–Frost model described later in this chapter), therefore is inappropriate.

acting (e.g., the mixing and crowding of animals in markets).

The complex of factors that generate a value of the DR renders precise prediction of the course of an epidemic impracticable. Therefore, during an epidemic, the **estimated dissemination rate (EDR)** is calculated from the observed outbreaks of disease. This represents the rate of growth of the epidemic. In foot-and-mouth disease it is often plotted using a seven-day interval: the number of outbreaks in a population over a seven-day period divided by the number of outbreaks in the same population in the previous seven-day period (e.g., Gibbens *et al.*, 2001b). An EDR >1 indicates that an epidemic is increasing, whereas an EDR <1 points to a decline. *Figure 8.5* plots the EDR of foot-and-mouth disease in Dumfries and Galloway, during the epidemic in the UK in 2001, plotted daily, using a seven-day interval. The EDR dropped below one on 21 March, and subsequently there were transient increases above one at the end of March and early in April. However, these last two peaks should not be interpreted as failures in disease control. They resulted from eruption of disease in new areas north-west of Dumfries and south and south-east of Dalbeattie, rather than recrudescence in the initial focus in the south-east of the region (see *Figure 11.11*). This highlights the importance of expressing disease occurrence both temporally and spatially, and recognizes the need to collect and analyse field data during an epidemic.

Common-source and propagating epidemics

A **common-source epidemic** is one in which all cases are infected from a source that is common to all individuals. If the period of exposure is brief, then a common-source epidemic is a **point-source** (or, more briefly, just a **point**) epidemic. A food-poisoning outbreak, in which a single batch of food is contaminated, is a typical point-source epidemic. *Figure 8.6* illustrates a point-source epidemic of human leptospirosis in the USSR in 1955 associated with the contamination of the water supply with the urine of infected dogs. An epidemic of leptospirosis was occurring in dogs, and contaminated urine was discharged on to fields. A cloud burst occurred on 28 June during a brief period of heavy rainfall. This washed off the topsoil. Some of the soil entered a water-pumping station inspection shaft, which was open for repair. Thus, the water supply was contaminated, and resulted in the human epidemic.

A **propagating epidemic** is an epidemic caused by an infectious agent in which initial (i.e., **primary**) cases excrete the agent, and thus infect susceptible individuals, which constitute **secondary** cases. Epidemics

Fig. 8.5 Daily estimated dissemination rate of foot-and-mouth disease in Dumfries and Galloway, UK, 2001. (Adapted from Thrusfield *et al.*, 2005b.)

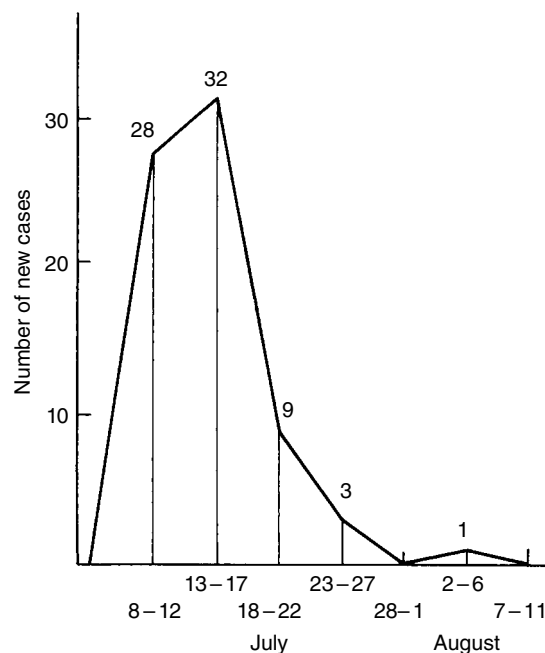
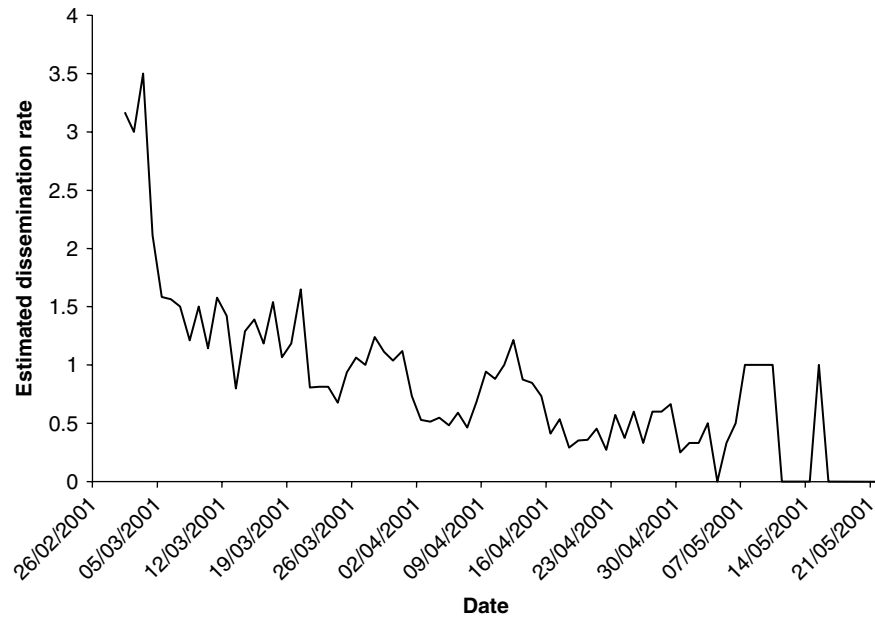


Fig. 8.6 A point-source epidemic: human leptospirosis associated with contaminated water supply, Rostov-on-Don, USSR, 1955. (Source: Ianovitch *et al.*, 1957.)

of foot-and-mouth disease are examples (see *Figure 4.1*). One of the primary cases is frequently the **index case**; that is, the first case to come to the attention of investigators.

The time interval between peaks of successive temporal clusters of cases, separating the primary from subsequent secondary cases, reflects the incubation

period of the infection. Typically, all cases of a point-source epidemic occur within one incubation period of the causal agent. Thus, if the period between subsequent peaks is less than the most common incubation period, then it is difficult to differentiate between a propagating epidemic and a series of point-source epidemics. Sartwell (1950, 1966) and Fine (2003) describe suitable techniques of differentiation, based on the statistical distribution of incubation periods.

The Reed–Frost model

The shape of the epidemic curve in a propagating epidemic in a defined population can be mathematically modelled (Bailey, 1975). One of the basic models is the **Reed–Frost model** (Abbey, 1952; Frost, 1976). In this model's classical simple form, the population is divided into three groups, comprising:

1. infected animals (cases);
2. susceptible animals;
3. immune animals.

The number of individuals in each group determines the shape of the epidemic curve and the pattern of immunity in the population.

Assuming that the period of infectiousness of infected animals is short, and the incubation period or latent period is constant, then, starting with a single case (or several simultaneously infected cases), new cases will occur in a series of stages. Cases occurring at each stage can be expected to have a binomial

distribution (see Chapter 12), depending on the number of susceptible and infectious animals at the previous stage. A **chain of binomial** distributions thus can be expected; this model is therefore termed a 'chain-binomial model'. The model also assumes that all infected animals develop disease, become infectious in the next stage, and then become immune.

The model is constructed using the formula:

$$C_{t+1} = S_t(1 - q^{C_t}),$$

where:

t = the time period: usually defined as the incubation period or latent period of the infectious agent (ideally, the serial interval of infection: see Chapter 6);

C_{t+1} = the number of infectious cases in time period, $t + 1$;

S_t = the number of susceptible animals in the time period, t ;

q = the probability of an individual *not* making effective contact.

The value, q , is given by $(1 - p)$, where p = the probability of a specific individual making effective contact (see Chapter 6) with another individual, which would result in infection if one were susceptible and the other were infectious. The term $(1 - q^{C_t})$ arises because it represents the probability that at least one of the C_t infectious cases makes effective contact. The magnitude of p is a matter of chance, and depends on a variety of factors, including those already described in Chapter 6. It is usually estimated empirically from real epidemics (Bailey, 1975)⁸.

If, at time t (the beginning of an epidemic), there are 100 susceptible animals, no immune animals, and one case, then $S_t = 100$ and $C_t = 1$.

If $p = 0.06$, then $q = (1 - 0.06) = 0.94$.

At time $t + 1$:

$$\begin{aligned} C_{t+1} &= 100(1 - 0.94^1) \\ &= 6, \end{aligned}$$

and

$$\begin{aligned} S_{t+1} &= 100 - 6 \\ &= 94. \end{aligned}$$

At time $t + 2$:

$$\begin{aligned} C_{t+2} &= 94(1 - 0.94^6) \\ &= 29, \end{aligned}$$

and

$$\begin{aligned} S_{t+2} &= 94 - 29 \\ &= 65. \end{aligned}$$

At time $t + 3$:

$$\begin{aligned} C_{t+3} &= 65(1 - 0.94^{29}) \\ &= 54, \end{aligned}$$

and

$$\begin{aligned} S_{t+3} &= 65 - 54 \\ &= 11, \end{aligned}$$

and so on.

The number of immune animals at any time period is the cumulative total of infected animals during the preceding time periods. Thus, at time $t + 1$, the number of immune animals $I_{t+1} = 1$ (the one case from time $t = 0$); at time $t + 2$, $I_{t+2} = 6 + 1 = 7$; at time $t + 3$, $I_{t+3} = 7 + 29 = 36$, and so on.

Table 8.1 presents the results of the Reed–Frost model, using the above parameters, for the complete course of the modelled epidemic. The results are also plotted in Figure 8.7. Note that an epidemic can only occur when $p \times S_t > 1$, and declines (or cannot initially occur) when $p \times S_t < 1$. The likelihood of an epidemic occurring, and the shape of the epidemic curve, are therefore functions of the probability of effective contact and the number of susceptible animals.

The *proportion* of the population that is susceptible often is used as a general guide to the likelihood of an infection spreading – traditionally quoted as at least 20–30% of the population; with the corollary that, if 70–80% of the population is immune, infection will not spread (but see Table 27.2)⁹. Although the latter level of protection will prevent a *major* epidemic, infection can spread with a relatively low proportion of susceptible animals if there are sufficient susceptible animals to render $p \times S_t$ greater than 1.

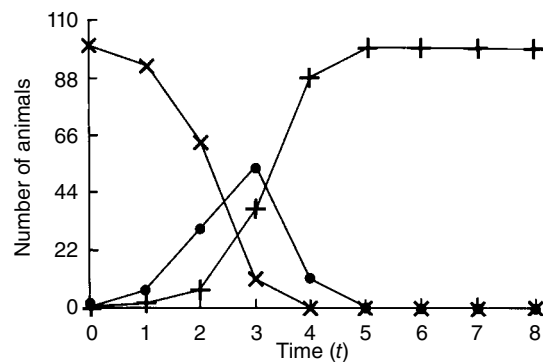
Figure 8.8 depicts epidemic curves that are simulated using various values for the number of susceptible and immune animals, and the parameter, p . A number of immune animals at the beginning of an

⁸ Simple epidemic models, such as the Reed–Frost model, assume that effective contact occurs at random, and that disease spreads as if infected and susceptible individuals are mixed homogeneously (the 'mean field assumption'). Understanding spatial aspects of disease occurrence requires assessment of the validity of this assumption (McCallum, 2008).

⁹ The value of 70% has been used as a general target for vaccine coverage, for example, in rabies vaccination programmes in foxes (Anderson *et al.*, 1981), but may require revision in specific rabies–fox systems (Eisinger and Thulke, 2005).

Table 8.1 Simulation of an epidemic using the classical Reed–Frost model.

Time (t)	Number of cases (C_t)	Number of susceptible animals (S_t)	Number of immune animals (I_t)	Totals	Probability of effective contact (p)	pS_t
0	1	100	0	101	0.06	6.00
1	6	94	1	101	0.06	5.64
2	29	65	7	101	0.06	3.90
3	54	11	36	101	0.06	0.66
4	11	0	90	101	0.06	0.00
5	0	0	101	101	0.06	0.00

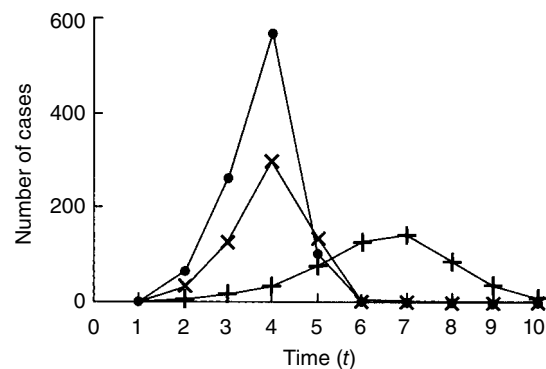
**Fig. 8.7** An epidemic curve, number of susceptible animals and number of immune animals simulated by the classical Reed–Frost model. —●— Cases; —+— immune animals; —X— susceptible animals. (Data from Table 8.1.)

epidemic can decrease the amplitude of the epidemic and delay its peak; a change in effective contact also can alter the amplitude.

The basic Reed–Frost model can be modified to include varying periods of infectiousness (Bailey, 1975), and control components, such as vaccination with a varying duration of immunity (Carpenter, 1988) and culling (Perez *et al.*, 2011)¹⁰.

Kendall's waves

Some epidemics – notably those caused by viruses – occur as a series of outbreaks, which can be considered as a series of epidemic waves: a **wave-train**. Three



	Initial number of susceptible animals	Initial number of immune animals	Probability of effective contact
—●—	1000	0	0.007
—+—	600	400	0.007
—X—	600	400	0.015

Fig. 8.8 Some epidemic curves simulated by the classical Reed–Frost model. Population size = 1000; a single case is introduced into the population.

types of wave, representing particular stages of a continuum, were identified by Kendall (1957); these are called **Kendall's waves** (Figure 8.9). There are three main differences between these waves (Cliff and Haggett, 1988):

1. **amplitude** – decreasing intensity from type 1 to type 3;
2. **peakedness** (concentration of cases) – also decreasing, from type 1 to type 3;
3. **skewness** – noticeable in type 1, but decreasing in succeeding types.

The shape of each wave in the wave-train at a given time or place in a population at risk of size S depends on the rate of transmission, β_{rate} , and the rate of removal, μ . Removal occurs when infected animals die, are isolated, or recover and become immune.

¹⁰ Some models of the age distribution of cancer also show remarkable similarities to the Reed–Frost model (Burch, 1966), reflecting underlying biological similarities. Contact between an infectious and a susceptible individual is similar to an environmental carcinogen effectively 'hitting' a cell; and the conversion of a susceptible individual to a case is similar to a mutation that converts a normal cell to a malignant one (see Chapter 5).

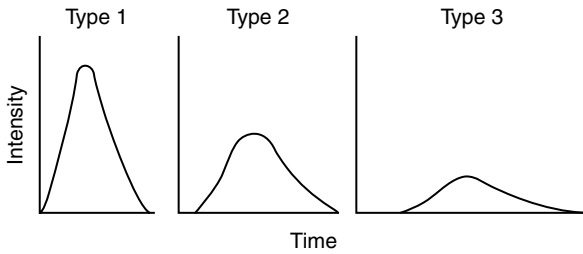


Fig. 8.9 Kendall's concept of changing wave shape over time. (Source: Cliff and Haggett, 1988.)

These two parameters are related to a third, S_c : the **relative removal rate**:

$$S_c = \mu / \beta_{\text{rate}}.$$

The relative removal rate defines a critical susceptible population threshold size whose magnitude compared with S determines the wave's shape (Smallman-Raynor and Cliff, 2012). When S is much

greater than S_c (S/S_c is greater than three), a type 1 wave occurs. Type 3 waves occur when S is only slightly greater than S_c (S/S_c is greater than one, but less than two); these waves are characterized by relatively lengthy outbreaks of low amplitude. Type 2 waves are intermediate to types 1 and 3 (S/S_c is greater than two, but less than three). (If S/S_c is less than one, there are only a few secondary cases, and there is no general epidemic.)

The shape of the waves changes as an epidemic spreads over time and space. This is exemplified by the epidemic of Newcastle disease that occurred in England and Wales in 1970–1971, and which is charted in *Figures 8.10* and *8.11*. The infection is spread by movement of live birds and other animals, fomites, poultry products and airborne transmission (Calnek, 1991). Although the disease is preventable by vaccination, the previous epidemic had occurred six years earlier and the subsequent casual attitude to vaccination resulted in a level of population

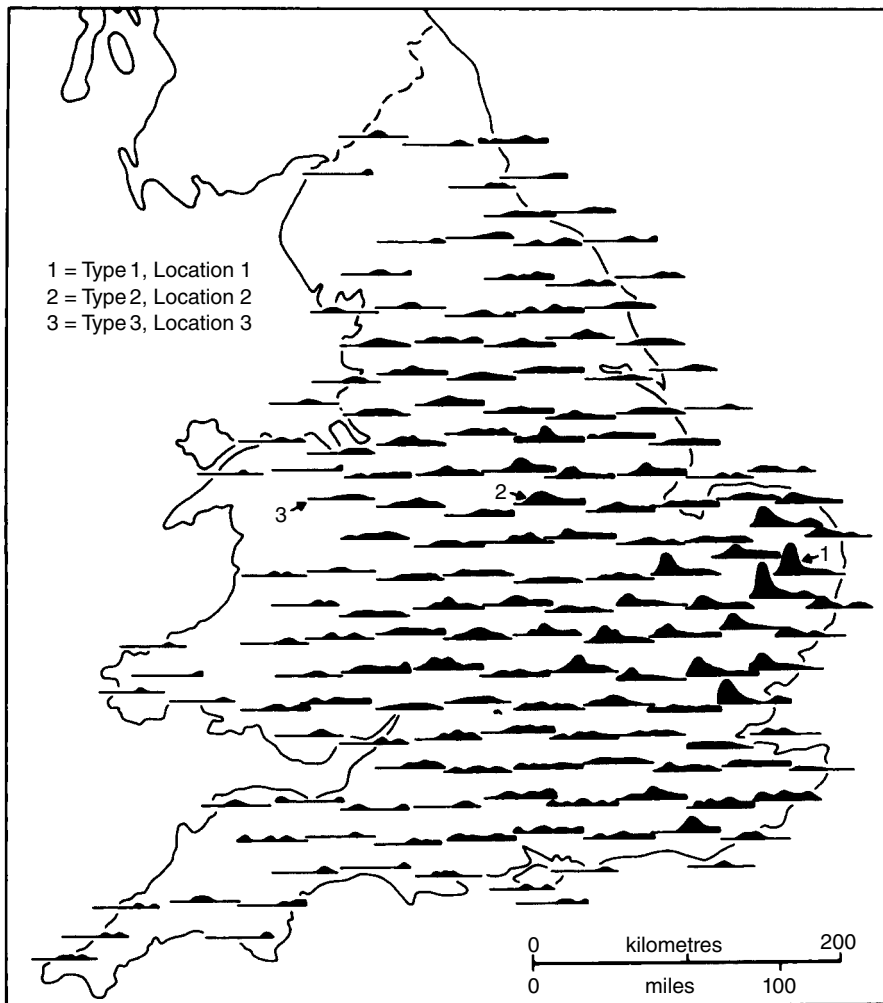
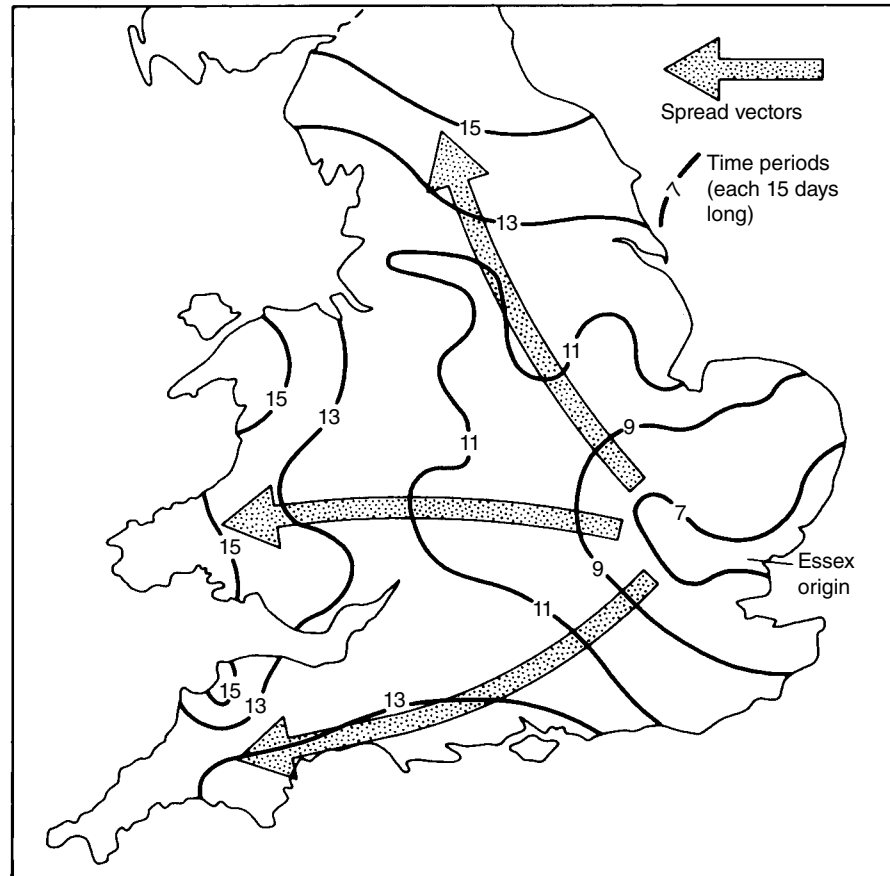


Fig. 8.10 Newcastle disease epidemic of 1970–1971 in England and Wales. (Source: Cliff and Haggett, 1988.)

Fig. 8.11 Progress of the Newcastle disease epidemic of 1970–1971 in England and Wales from its origins to the rest of the country. (Source: Cliff and Haggett, 1988.)



immunity well below the 75% required to prevent a major epidemic. The epidemic began in the east of England and spread westwards. Initially, the amplitude was great, but succeeding waves showed a transition over time and space from type 1 to type 3; locations 1, 2 and 3 in *Figure 8.10* corresponding to the wave types predicted by Kendall. The contours in *Figure 8.11* are time contours of 15-day periods (15 days is a rough multiple of the average incubation period, calculated from a range of 3–10 days). Thus, the contour with the value 9 marks the 135th day of the epidemic. These contours suggest that the ‘velocity’ of the epidemic temporarily decreased as the epidemic moved westwards.

The changes in the shape of these waves must result from a decrease in the value of S/S_c over time and space. This could occur either because S decreases and/or S_c increases. An increase in the latter could occur because of a change in the value of the rate of removal, μ , but this is unlikely for a specific disease. Alternatively, the rate of transmission, β_{rate} , could increase, but this is equally unlikely, necessitating a change in virulence or infectiousness of the agent during the course of the epidemic. Thus, the most

probable reason for the change in the wave shape is a reduction in S , which could plausibly be brought about by isolation of animals and vaccination. This is consistent with the pattern of the Newcastle disease epidemic, in which increased vaccinations and mandatory restrictions on poultry movement during the epidemic would have decreased the value of S .

Trends in the temporal distribution of disease

The temporal changes and fluctuations in disease occurrence can be classified into three major trends (*Figure 8.12*):

1. short-term;
2. cyclical (including seasonal);
3. long-term (secular).

Short-term trends

Short-term trends (*Figure 8.12b*) are typical epidemics, which already have been discussed.

Cyclical trends

Cyclical trends (Figure 8.12c) are associated with regular, periodic fluctuations in the level of disease occurrence. They are associated with periodic changes in the size of the susceptible host population and/or effective contact, and may produce **recurrent epidemics** or **endemic pulsations** (regular, *predictable* cyclical fluctuations). Thus, the three- to four-year cycle of foot-and-mouth disease in Paraguay (Figure 8.19), and the predicted four-year periodicity of fox rabies in Britain, with a contact rate of 1.9 (see Figure 23.4a), probably are related to the time taken for the susceptible population to reach the threshold level.

Seasonal trends

A seasonal trend is a special case of a cyclical trend, where the periodic fluctuations in disease incidence are related to particular seasons. Fluctuations may be caused by changes in host density, management practices, survival of infectious agents, vector dynamics and other ecological factors. Thus, before eradication,

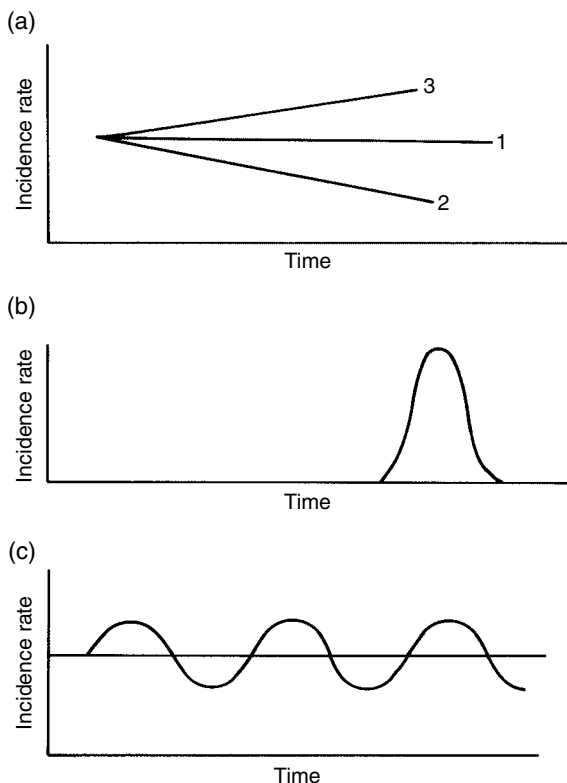


Fig. 8.12 Temporal trends in disease occurrence. (a) Long-term trend: 1, with equilibrium between infectious agent and host; 2, host/agent interaction biased to the host; 3, host/agent interaction biased to the agent. (b) Short-term trend. (c) Cyclical trend. (Source: Sinnecker, 1976. Reproduced with permission of John Wiley and Sons, Ltd.)

rinderpest occurred in Africa more commonly in the dry than the wet season because animals congregated at water holes, increasing the local animal density.

The prevalence of Lassa virus infection of the multimammate mouse (see Chapter 6) is related to density-dependent variations in mortality, competition with other rodents, and seasonal factors. The mouse may desert open fields and seek shelter indoors during the wet season, and this may explain the later peak in the incidence of human Lassa fever in the following dry season (Howard, 2009).

Rat plague demonstrates a seasonal incidence, being associated with climatically-determined fluctuations in the population size of certain fleas that are vectors of the disease. Additionally, the rat population increases during the inter-epidemic season, thereby exacerbating the seasonal trend (Pollitzer and Meyer, 1961).

Myxomatosis in lowland rabbits in the UK has a two-peaked annual cycle, with a main autumnal peak between August and January, and a subsidiary peak during February to April (Figure 8.13). This is the result of several ecological factors, including mass movement of fleas and seasonal fluctuations in the abundance of rabbits (Ross *et al.*, 1989). In Spain, clinical myxomatosis occurs in the winter and spring, corresponding to the recruitment of young susceptible rabbits (Calvete *et al.*, 2002). In France, the disease peaks in different seasons in different regions because of geographical variation in the distribution of the two main vectors: mosquitoes and fleas (Arthur and Louzis, 1988).

Leptospirosis is more common in the summer and early autumn than in the winter in temperate climates (Figure 8.14) because the warm, moist conditions during the summer predispose to survival of the pathogen (Diesch and Ellinghausen, 1975; Ward, 2002). The seasonality reported for some diseases of coral may be associated with increased sea temperature in the summer (Sato *et al.*, 2009; Zvuloni *et al.*, 2009).

In contrast, transmissible gastroenteritis of pigs is more common in winter than summer (Figure 8.15). This may be because the survival time of the virus is very short in summer because of the stronger ultraviolet light and higher temperatures then (Haelterman, 1963).

In the US, feline panleucopenia shows a seasonal peak in August and September (Reif, 1976). This is associated with a peak in the number of births in the cat population in June, which increases the number of susceptible cats in the population at risk. The kittens are protected passively by maternal antibody for approximately the first two months of life, therefore the peak 'herd' susceptibility occurs two months after the birth peak. Such seasonal fluctuations are less likely in canine than in feline populations because

Fig. 8.13 Monthly percentage of live-trapped farmland rabbits infected with myxomatosis, Hampshire, UK, 1971–1978. (Adapted from Ross *et al.*, 1989.)

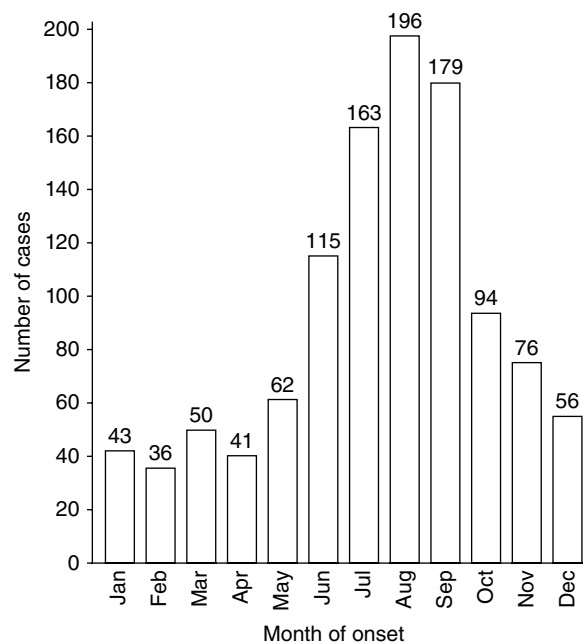
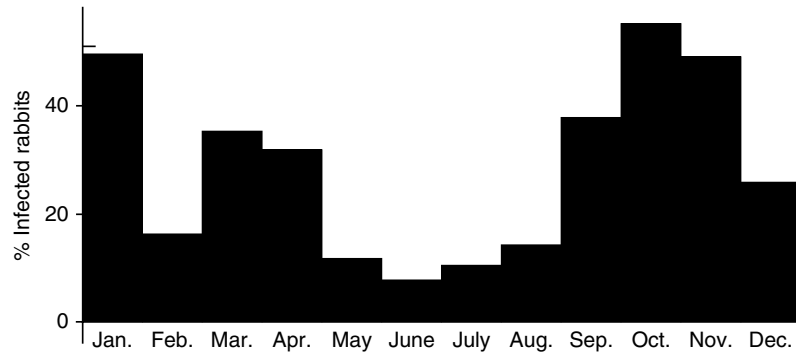


Fig. 8.14 Bar chart depicting the seasonal occurrence of human leptospirosis in the US. (Source: Diesch and Ellinghausen, 1975.)

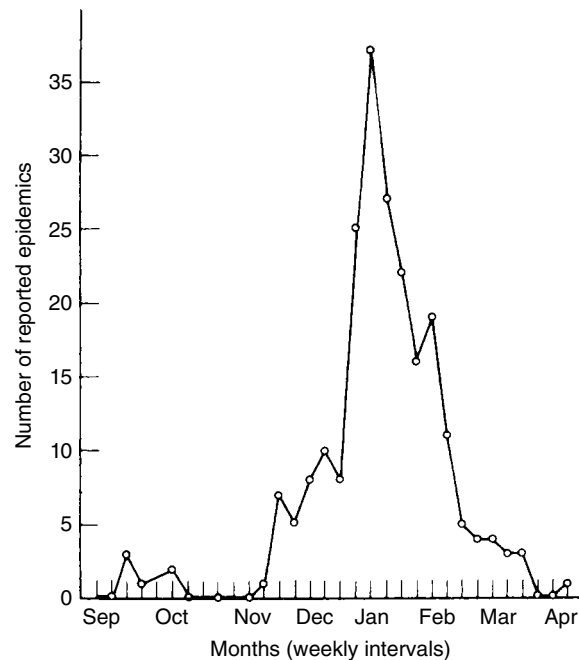


Fig. 8.15 Seasonal trend of transmissible gastroenteritis of pigs: reported epidemics in Illinois, 1968–1969. (Source: Ferris, 1971.)

births of puppies are distributed more evenly throughout the year than those of kittens (Tedor and Reif, 1978).

Some non-infectious diseases also may show seasonal trends. Thus, bovine hypomagnesaemia is common in spring and is associated, among other factors, with low levels of magnesium in rapidly growing pastures (see *Figure 3.7*).

Sometimes seasonal determinants may not be clearly identified. Human acute lymphoblastic leukaemia, for example, shows a peak in summer, presenting no obvious explanatory hypothesis; Ross and others (1999) suggest that it may be related to exposure to a seasonally-active infectious agent. The derivation of explanatory hypotheses, however, becomes intractable if there is conflicting evidence of seasonality.

For instance, there is inconsistent testimony for both the presence and pattern of seasonality of canine diabetes mellitus and human insulin-dependent diabetes (Fall *et al.*, 2007).

Long-term (secular) trends

Secular trends (*Figure 8.12a*) occur over a long period of time and represent a long-term interaction between host and parasite. If a balance occurs, then a stable, endemic level of disease is maintained (1 in *Figure 8.12a*); if the interaction is biased to the host, then there is a gradual decrease in disease occurrence (2); and if the interaction is biased to the parasite, there is a gradual increase in disease occurrence (3).

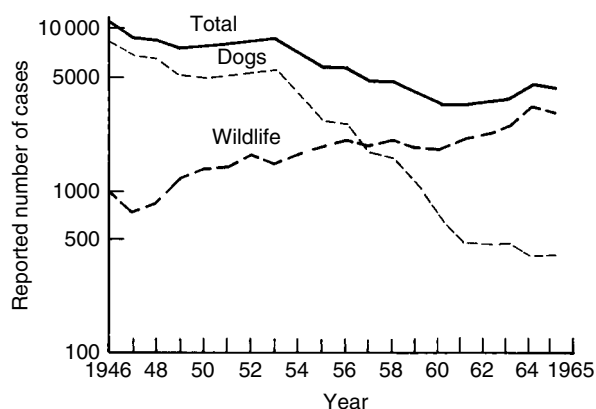


Fig. 8.16 An example of a secular trend: reported cases of rabies in the US, 1946–1965. (Source: West, 1972.)

Figure 8.16 illustrates a *reported* increasing long-term trend in the annual prevalence of rabies in wildlife in the US, whereas the prevalence in dogs is decreasing due to adequate control. ‘Reported’ is emphasized to stress that accurate estimation of trends is open to errors, some of which are described below.

Upward trends also may result from the intervention of people and changing human habits. Such trends occur with the so-called ‘diseases of civilization’ and ‘urbanization’ in man, (e.g., coronary heart disease) and the diseases of intensive production in animals. Secular decreases in morbidity may be the product of prophylaxis (e.g., vaccination). Mortality may show a secular decrease due to improved therapeutic techniques. The potential impact of climate change on long-term disease trends has been described in Chapter 5, and some long-term effects of ecological changes, including disease emergence, have been mentioned in Chapter 7.

True and false changes in morbidity and mortality

The temporal changes that occur in recorded morbidity and mortality rates may be either true or false. The recording of mortality rates is rarer in veterinary than in human medicine because recording death in animals is not compulsory. Thus, details of trends in mortality in animals usually are unavailable.

The common measures of morbidity – prevalence, cumulative incidence and incidence rate – comprise a numerator (number of cases) and a denominator (‘population at risk’ for the first two measures, and ‘animal-years at risk’, or a suitable approximation, for the third: see Chapter 4). Changes in either numerator or denominator induce changes in these measures that may be either true or false (Table

Table 8.2 Reasons for true and false temporal changes in incidence and prevalence according to changes in the numerator (cases) and denominator (animal-years at risk, etc./population at risk).

True changes

- Incidence: change in incidence
Prevalence: (a) change in incidence
(b) change in duration of disease

False changes

Prevalence and incidence:

1. Errors in the numerator:
 - (a) changes in the recognition of disease
 - (b) changes in the procedures for classifying disease
2. Errors in the denominator:
 - (a) errors in enumeration of the animal-years at risk, etc./population at risk

8.2). True changes in incidence can affect these recorded measures and prevalence; additionally, changes in disease duration affect prevalence (see Chapter 4).

A major cause of false changes is variation in the recognition and reporting of disease. Thus, the increasing secular trend in wildlife rabies in the US between 1946 and 1965 (Figure 8.16) may have resulted from increased recognition and reporting of affected animals, rather than a genuine increase in incidence. In the US, reports of feline heartworm disease have increased over the years (Guerrero *et al.*, 1992), but it is difficult to say if this is due to increased awareness, improved methods of diagnosis, or a true rise in the disease’s incidence. Similarly, apparent increases in parietal chronic pleuritis in Danish abattoirs can be explained by an increased frequency of detection resulting from increased diagnostic sensitivity (Enøe *et al.*, 2003).

The sampling of an animal population to record morbidity is also subject to inherent *variation* in the samples (see Chapter 12), thus appropriate statistical analysis should be undertaken (see Chapter 13).

Apparently changing patterns therefore should be interpreted with due regard to the possibility that they are artificial.

Detecting temporal trends: time series analysis

Short-term, seasonal and secular changes are temporal trends that can occur simultaneously, and may be mixed with random variation. In such circumstances, the various changes can be identified by statistical investigation. One method (originally applied in

Table 8.3 Percentage of sheep lungs condemned monthly because of pneumonia and/or pleurisy, and average monthly and yearly percentage condemnation rates (1979–1983) at a Scottish abattoir. (From Simmons and Cuthbertson, 1985.)

	Jan %	Feb %	Mar %	Apr %	May %	Jun %	Jul %	Aug %	Sep %	Oct %	Nov %	Dec %	Yearly % condemnation rate
1979	0.33	0.24	0.46	0.57	0.65	0.23	0.27	0.37	0.14	0.30	0.24	0.14	0.33
1980	0.40	0.38	0.39	0.65	0.58	0.49	0.49	0.19	0.27	0.34	0.30	0.44	0.41
1981	0.48	0.58	0.62	0.75	0.51	0.44	0.21	0.17	0.18	0.21	0.35	0.27	0.40
1982	0.72	0.71*	0.75*	0.85	0.45	0.34	0.26	0.43	0.95	0.60	1.41	0.63	0.68
1983	0.71	0.64	0.48	0.84	0.38	0.48	0.69	0.80	1.09	0.76	1.25	0.97	0.76
Average monthly % condemnation rate	0.53	0.51	0.54	0.73	0.51	0.40	0.38	0.39	0.53	0.44	0.71	0.49	

* Estimated.

commerce) that is used in epidemiology to detect temporal trends is **time series analysis**.

A time series is a record of events that occur over a period of time; cases of disease are typical events. The events are plotted as points on a graph, with 'time' along the horizontal axis. *Table 8.3*, for instance, records the percentage of sheep lungs condemned monthly because of pneumonia or pleurisy at a Scottish slaughterhouse. *Figure 8.17a* plots these monthly values. There is considerable variation in the location of the points, but, by eye, an annual cycle is suggested and there appears to be a slight secular trend of increased prevalence from 1979 to 1983. Trends in these data may be detected by several methods, including:

1. plotting;
2. calculation of rolling (moving) averages;
3. regression analysis;

the object being to identify, and, if required, to remove, random variation and seasonal and secular trends.

Plotting

Plotting time series data (usually using computer software) and joining the points by eye is an obvious, easy method of indicating a trend. However, it is susceptible to subjective interpretation and cannot counteract random variation readily. This is, however, a useful starting point, and plots can be supplemented with the results of rolling averages and/or regression analyses.

Calculation of rolling (moving) averages

A rolling average is the arithmetic average of consecutive groups of measurements. Thus, to construct a rolling three-month average of the monthly data in *Table 8.3*, sequential sets of three adjacent values are

averaged. For example, the rolling average for February 1979 is calculated by summing the monthly averages for January, February and March, and dividing by three:

$$\begin{aligned} &\text{three-month rolling average (February 1979)} \\ &= \frac{0.33 + 0.24 + 0.46}{3} = 0.34. \end{aligned}$$

The three-month rolling average for March 1979 likewise is calculated by summing the values for February, March and April 1979, and dividing by three. *Table 8.4* presents three-month rolling averages using the data in *Table 8.3*, and *Figure 8.17b* presents these averages graphically. This technique reduces random variation, and therefore may reveal underlying trends; an annual cyclical trend is more clearly visible in *Figure 8.17b*.

The seasonal trend in this example cannot be explained readily. However, it is known that the prevalence of atypical pneumonia increases as the stocking density increases and as the altitude at which sheep are reared decreases (Jones and Gilmour, 1983). Thus, lowland, intensively-reared sheep are more likely to develop pneumonia than hill sheep. Lambs slaughtered in the spring are most frequently from the lowlands, and lambs slaughtered in the late summer and autumn are most frequently from the hill; this policy therefore could explain the seasonal trend.

Two disadvantages of rolling averages are that the first and last elements of a data set cannot be averaged (January 1979 and December 1983 in *Table 8.4*), and the averages can be affected unduly by extreme values.

Regression analysis

Regression analysis is a statistical technique for investigating relationships between two or more variables.

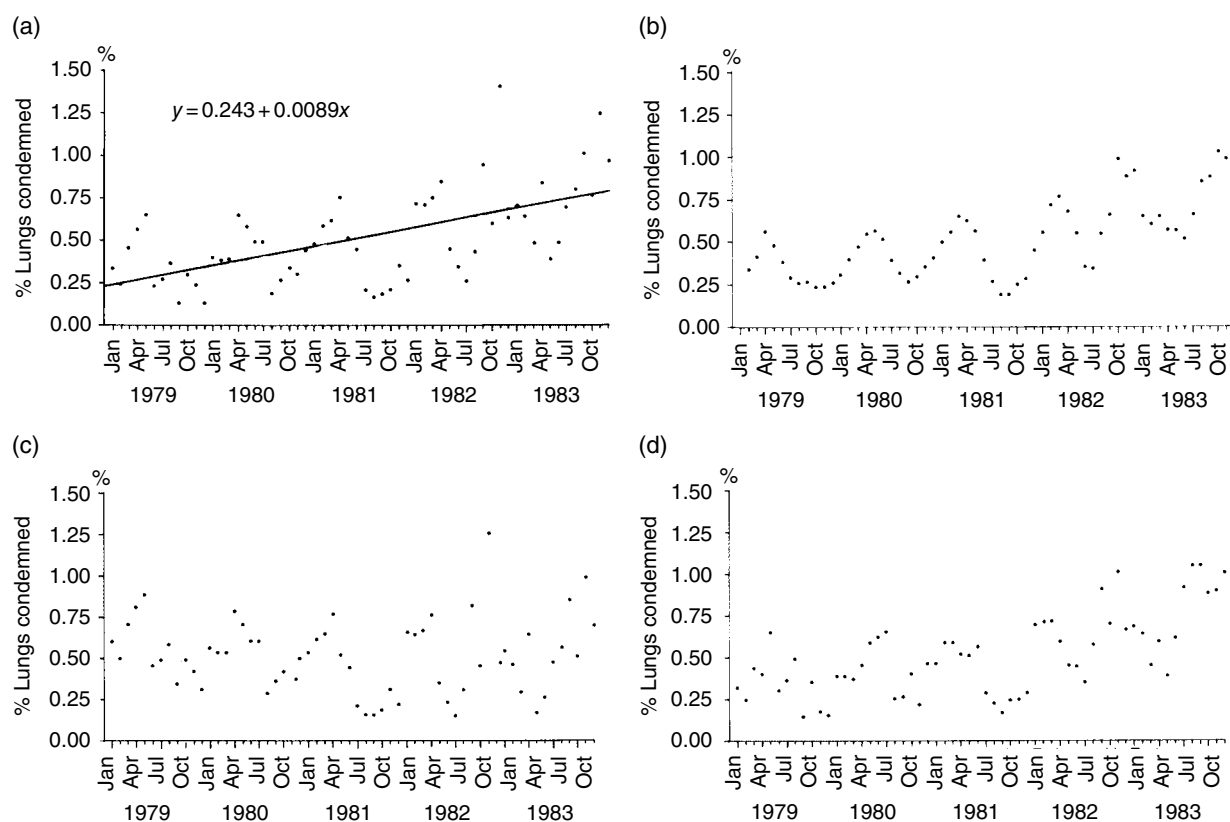


Fig. 8.17 Percentages of sheep lungs condemned monthly because of pneumonia and/or pleurisy, and average monthly and yearly percentage condemnation rates (1979–1983) at a Scottish abattoir. (a) Percentages of lungs condemned monthly because of pneumonia and/or pleurisy (data from Table 8.3) with regression line. (b) Three-month rolling average percentage lung condemnation rate because of pneumonia and/or pleurisy (data from Table 8.4). (c) Percentages of lungs condemned monthly (corrected to remove secular trend) because of pneumonia and/or pleurisy. (d) Percentages of lungs condemned monthly (corrected to remove seasonal trend) because of pneumonia and/or pleurisy. (Source: Simmons and Cuthbertson, 1985. Reproduced with permission.)

Table 8.4 Three-month rolling average percentage condemnation rates because of pneumonia and/or pleurisy (1979–1983) at a Scottish abattoir. (Calculated from the data in Table 8.3.)

	Jan %	Feb %	Mar %	Apr %	May %	Jun %	Jul %	Aug %	Sep %	Oct %	Nov %	Dec %
1979		0.34	0.42	0.56	0.48	0.38	0.29	0.26	0.27	0.23	0.23	0.26
1980	0.31	0.39	0.47	0.54	0.57	0.52	0.39	0.32	0.27	0.30	0.36	0.41
1981	0.50	0.56	0.65	0.63	0.57	0.39	0.27	0.19	0.19	0.25	0.28	0.45
1982	0.56	0.72	0.77	0.68	0.55	0.35	0.34	0.55	0.66	0.99	0.88	0.92
1983	0.66	0.61	0.65	0.57	0.57	0.52	0.66	0.86	0.88	1.03	0.99	

It requires knowledge of statistics and so the reader who is unfamiliar with basic statistics should read Chapters 12, 14 and 22 before proceeding further.

Regression (see also Chapter 22) and correlation (see Chapter 14) are related. However, there is one major difference: a correlation coefficient may be evaluated if both or all variables exhibit random variation.

Regression, however, involves the *selection* of individuals on the basis of one or more measurements (the explanatory variables) and then records the others (the response variables); therefore the explanatory variables should have no random variation. Discussion here will consider only one explanatory variable and one response variable.

When observations are made at defined intervals, these *selected* intervals of time represent the explanatory variable, x , which is why regression, not correlation, is applied to detecting an association between events and time. The technique can be applied to the data in Table 8.3.

A graph showing the variation of the mean of y in relation to x would show the relationship between y and x that is observed, in practice, with random variation. If it is assumed that the true values of y , for each value of x , lie in a straight line, then this line is known as the **regression line** of the linear regression of y on x . The slope of the line is termed the **regression coefficient** of y on x . This may be positive, negative, or, if x and y are unassociated, zero. The estimation of the regression coefficient and the intercept with the vertical axis, and the interpretation of the values of these estimates, is called **regression analysis**.

Assume that the equation of the true regression line is:

$$y = \alpha + \beta x,$$

where β is the regression coefficient, and α is the intercept defining the point of interception of the y axis by the regression line. A set of n points (x, y) of observations is available to estimate this line. The regression coefficient, β , is estimated by:

$$b = \frac{\sum(x - \bar{x})(y - \bar{y})}{\sum(x - \bar{x})^2} = \frac{\sum(xy) - (\sum x)(\sum y)/n}{\sum x^2 - (\sum x)^2/n}.$$

The intercept, α , is estimated by:

$$a = \bar{y} - b\bar{x}.$$

Using the data in Table 8.3, the values of x being integers from 1 to 60 (i.e., monthly intervals for five years) and of y being the respective monthly condemnation rates:

$$\begin{aligned}\sum x &= 1830.0 & \sum y &= 30.820 \\ (\sum x)^2 &= 3\,348\,900 & \sum x^2 &= 73\,810 \\ \sum(xy) &= 1100.0 & n &= 60 \\ \bar{x} &= 30.500 & \bar{y} &= 0.51367.\end{aligned}$$

Thus:

$$b = \frac{1100.0 - (1830.0 \times 30.820)/60}{73\,810 - 3\,348\,900/60} = 0.00889$$

and:

$$a = 0.51367 - (0.00889 \times 30.500) = 0.2425.$$

The regression line now can be plotted, substituting the values of x , from 1 to 60, in the formula for the regression line, to determine the respective values of y (Figure 8.17a).

Thus, when $x = 1$ (January 1979):

$$\begin{aligned}y &= 0.2425 + 0.00889 \times 1 \\ &= 0.2514;\end{aligned}$$

when $x = 2$ (February 1979):

$$\begin{aligned}y &= 0.2425 + 0.00889 \times 2 \\ &= 0.2603;\end{aligned}$$

and so on.

Note that, in this example, the relationship between x and y is linear.

The effect of the secular trend can be removed by subtracting $b(x - \bar{x})$ from each value of y . Thus, for July 1979, $x = 7$ and:

$$\begin{aligned}b(x - \bar{x}) &= 0.00889(7 - 30.500) \\ &= 0.00889 \times (-23.500) \\ &= -0.2089;\end{aligned}$$

and the value of y with the secular trend removed is:

$$0.27 - (-0.2089) = 0.4789.$$

The results for the 60-month period, with the secular trend removed, are depicted in Figure 8.17c.

The effect of seasonal variation can be removed by calculating a 'seasonal index'; in this example, for each month. The value of y for each month of a year is taken as a proportion of the total of y for that year; these proportions are averaged for a particular month over the period of study (five years in this instance) to give a seasonal index for each month of the year. The results are 'de-seasonalized' by dividing each value of y by the relevant monthly index multiplied by 12. Thus, for July 1979, the proportion of the total of y contributed by July is:

$$\begin{aligned}&0.27 / (0.33 + 0.24 + 0.46 + 0.57 + 0.65 + 0.23 \\ &\quad + 0.27 + 0.37 + 0.14 + 0.30 + 0.24 + 0.14) \\ &= \frac{0.27}{3.95} = 0.0684.\end{aligned}$$

The proportions for July 1980, 1981, 1982 and 1983 are 0.0995, 0.0440, 0.0320 and 0.0759, respectively. The July seasonal index therefore is:

$$\begin{aligned}&(0.0684 + 0.0995 + 0.0440 + 0.0320 + 0.0759) / 5 \\ &= 0.3199 / 5 = 0.0640,\end{aligned}$$

and the 'de-seasonalized' value for July 1979 is:

$$0.27 / (0.0640 \times 12) = 0.352.$$

The 'de-seasonalized' results for the period of study are shown in Figure 8.17d.

Note that a considerable amount of random variation remains when the secular and seasonal trends are removed (Figures 8.17c and d, respectively); this variation tends to obscure the seasonal trend in Figure 8.17c, and the secular trend in Figure 8.17d. In such circumstances, calculation of rolling averages provides a rapid means of reducing random variation. An increase in sample size also should reduce the effects of this variation. A formal significance test may be required when the effects of random variation are considerable. A description of a suitable test, and the estimation of the standard error and confidence limits for β , is given by Freund and Wilson (1998).

Time series data also can be analysed using **multi-variable regression methods** (see Chapter 22). Instead of only fitting a straight line to the data, repeating patterns (i.e., cyclical trends) also can be incorporated into the regression model; for example, using sine and cosine functions (Stolwijk *et al.*, 1999). Thus,

annual fluctuations can be incorporated using two additional terms:

$$y = \alpha + \beta_1 \times \sin\left(\frac{2\pi t}{T}\right) + \beta_2 \times \cos\left(\frac{2\pi t}{T}\right),$$

where t is the time period and T is the number of time periods per year. Hence, if the data were recorded monthly, t is the observation month, from 1 to n , where n = number of months of study (in this case, 60) and $T = 12$ (because there are 12 months in a year).

Biannual fluctuations can be incorporated into the model using two additional terms:

$$y = \alpha + \beta_1 \times \sin\left(\frac{2\pi t}{T}\right) + \beta_2 \times \cos\left(\frac{2\pi t}{T}\right) + \beta_3 \times \sin\left(2 \times \frac{2\pi t}{T}\right) + \beta_4 \times \cos\left(2 \times \frac{2\pi t}{T}\right),$$

and so on.

Figure 8.18a shows the data from Table 8.3 and Figure 8.17a together with a regression line estimated from a model including a linear term and sine and cosine functions for annual and biannual patterns. Although this regression appears to capture some of

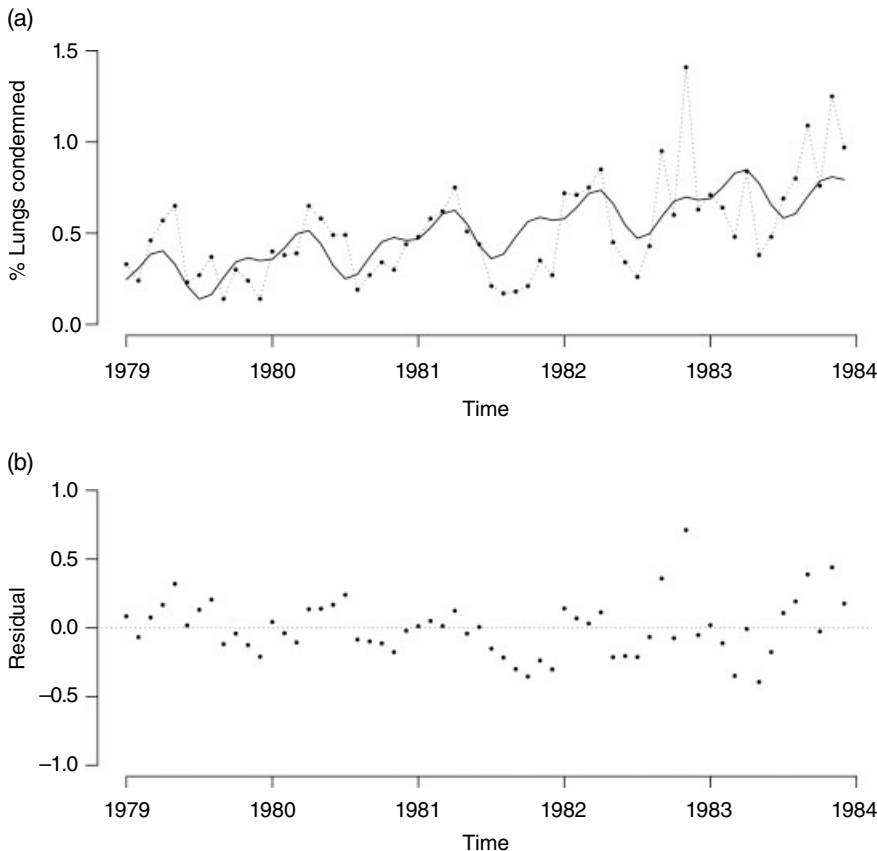


Fig. 8.18 Percentages of sheep lungs condemned monthly because of pneumonia and/or pleurisy, and average monthly and yearly percentage condemnation rates (1979–1983) at a Scottish abattoir. (a) Percentages of lungs condemned monthly because of pneumonia and/or pleurisy (data from Table 8.3 and illustrated in Figure 8.17a) with the prediction from a regression model including a linear term and sine and cosine function for annual and biannual patterns (solid line). (b) Residuals of the model depicted in Figure 8.18a.

the regular variation in the data, it is clear that considerable unexplained variation remains. This is evident in the residuals, which represent the difference between each observation and the predicted value for that observation, based on the regression model (see Chapter 22). *Figure 8.18b* shows the residual values over time; the residuals retain a cyclical pattern, suggesting further unexplained temporal effects. At least in part, this has occurred because the annual peaks are not exactly regular, but rather vary by up to a few months each year, possibly due to direct or indirect variations in, for example, weather patterns. The regression model could be extended to incorporate such data and test this hypothesis (assuming the data have been recorded). Fanshawe and others (2008) describe a method that enables cycles to be modelled as dynamic (i.e., varying somewhat from year to year) rather than as static (i.e., exactly repeating patterns each year, as in the model described previously).

Figure 8.19 shows the results of a time series analysis of foot-and-mouth disease in Paraguay (Peralta *et al.*, 1982). The disease shows a cycle with a periodicity of three to four years (peaks in 1972, 1975–1976 and 1979), due to type O virus. The small peak in 1974, which was not consistent with the cycle, was caused by a sporadic outbreak due to type C virus. The reason for the cyclicity may be the changes in the proportion of the susceptible cattle population, three to four years being necessary to increase again the number to the threshold level. Identification of this temporal pattern can indicate times when particular attention should be paid to control.

A similar time series analysis of rabies in Chile, comparing the disease's temporal pattern before and after implementation of a control campaign (Ernst and Fabrega, 1989), is depicted in *Figure 8.20*. There was a slight, but statistically significant, secular increase

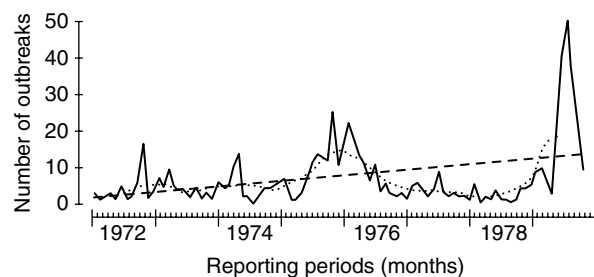


Fig. 8.19 Foot-and-mouth disease outbreaks reported by month, 12-month rolling average and trend, Paraguay, 1972–1979. — Raw data; 12-month rolling average; - - - trend. (Source: Peralta *et al.*, 1982. Reproduced with permission of Elsevier.)

in rabies between 1950 and 1960, attributable to cases in dogs, when no control was undertaken (*Figure 8.20a*). A national control programme, involving vaccination of humans and dogs, was instituted in 1961, and rabies declined between 1961 and 1970 (*Figure 8.20b*). The steady decline continued between 1971 and 1986 (*Figure 8.20c*). No cases were reported in 1982, but a previously undescribed sylvatic cycle was reported in insectivorous bats in 1985 and resulted in cases in dogs and cats (*Figure 8.20c*). This study also revealed a seasonal trend, and a five-year cyclical trend, possibly associated with fluctuations in the size of the susceptible dog population.

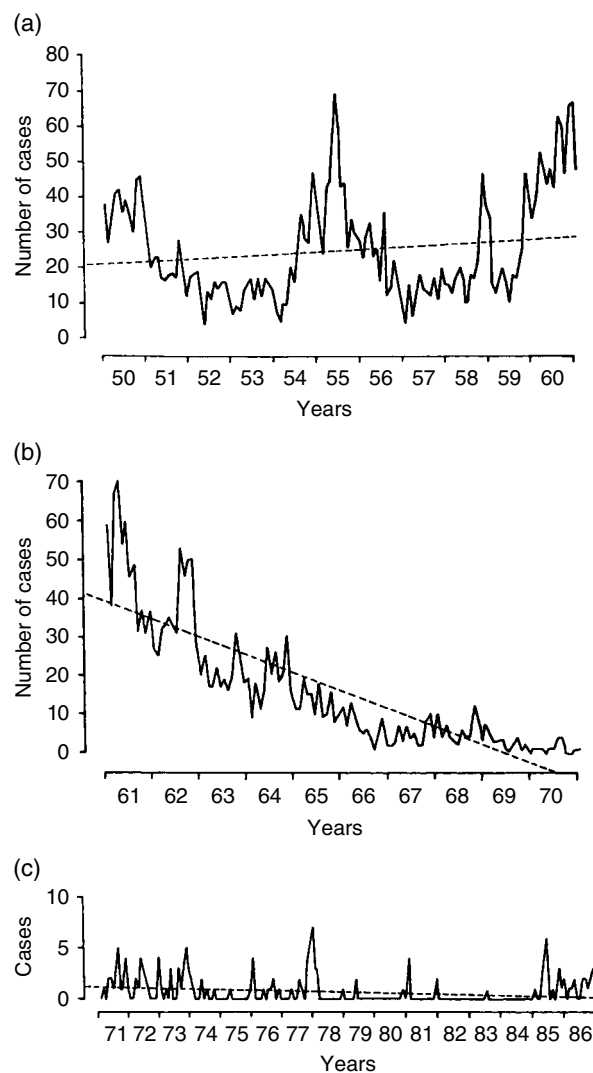


Fig. 8.20 Total number of laboratory-confirmed cases of rabies reported by month and trend, Chile, 1959–1986. (a) 1950–1960, (b) 1961–1970, (c) 1971–1986. — Raw data; - - - trend. (Source: Ernst and Fabrega, 1989. Reproduced with permission of Cambridge University Press.)

Regression is discussed further in Chapter 22 and in standard introductory statistics texts such as that by Petrie and Watson (2013) and, in the context of time series analysis, by Shumway and Stoffer (2011).

Trends in the spatial and temporal distribution of disease

Spatial trends in disease occurrence

An epidemic represents not only the clustering of cases over a period of time, but also the clustering of cases in a defined area. An infectious disease that propagates through a population results in a **contagious** or **clustered** spatial pattern, in contrast to sporadic outbreaks that are distributed **randomly**. These two patterns can be compared with **regular** spatial occurrence (Figure 8.21). 'Contagious' also can be applied, in a general sense, to the spatial clustering of disease, whether or not it is infectious (ecologists sometimes use 'over-dispersion' to refer to this type of spatial clustering in animal populations, with 'under-dispersion' referring to more regular spacing).

A variety of statistical distributions (see Chapter 12) serve as models for the spatial distribution of events (Southwood, 1978). The Poisson distribution has commonly been applied. The goodness of fit of a set of data to the Poisson distribution can be tested by performing a χ^2 test on the observed and expected values; standard statistical texts, such as Bland (2015), give details. If the variance is less than the mean, it implies the occurrence of a more regular distribution than is described by a Poisson series. If the variance is greater than the

mean, it implies that a 'contagious' distribution is present.

The identification of spatial clustering can assist in the identification of the cause of disease (Rothman *et al.*, 2008), its value lying in establishing that causal factors cluster (Law, 2005). Thus, the clustering of feline leukaemia among genetically unrelated cats provided early evidence of horizontal transmission and therefore of the disease's infectious nature (Brodey *et al.*, 1970)¹¹. It also may indicate areas for further investigation. For example, equine motor neuron disease is a degenerative condition, which occurs in North America and parts of South America, Europe and Asia. Its cause is unknown, but, in the US, vitamin E deficiency appears to play a role (Polack *et al.*, 2000; Mohammed *et al.*, 2007). It also shows distinct spatial clustering in the US, with the highest incidence in the north-east (Figure 8.22). Variation in the geographical distribution of reported risk factors (age and breed) and other potentially confounding factors (sex and month of diagnosis) does not explain the clustering (de la Rúa-Domènech *et al.*, 1995), suggesting that exploration of regional variations in management and husbandry practices may give clues to the cause of the disease.

The methods for identifying different spatial patterns are reviewed by Pfeiffer (2000) and are described in detail by Cliff and Ord (1981), Pfeiffer and others (2008) and Diggle (2013); and a sceptical critique of cluster investigation is presented by Rothman (1990).

Space–time clustering

Space–time clustering is an interaction between the places of onset and the times of onset of a disease; cases that are close in space tending to be close in time. The Poisson distribution sometimes can be applied to this type of interaction – particularly for large samples (David and Barton, 1966). Techniques for detecting space–time clustering are beyond the scope of this book. They are described by Knox (1964), David and Barton (1966), Mantel (1967) and Pike and Smith (1968). They are reviewed by Williams (1984), Schukken and others (1990), Pfeiffer (2000), Ward and Carpenter (2000a,b), Carpenter (2001), Pfeiffer and others (2008) and Diggle (2013).

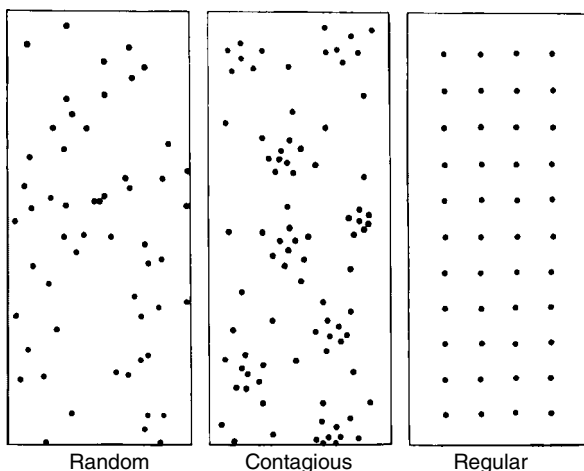


Fig. 8.21 Patterns of the spatial distribution of disease. (Source: Southwood, 1978.)

¹¹ Clustering due to infection is generally only apparent when the incubation period is short or the infection is rare or affects only a small number of susceptible individuals (McNally and Eden, 2004).

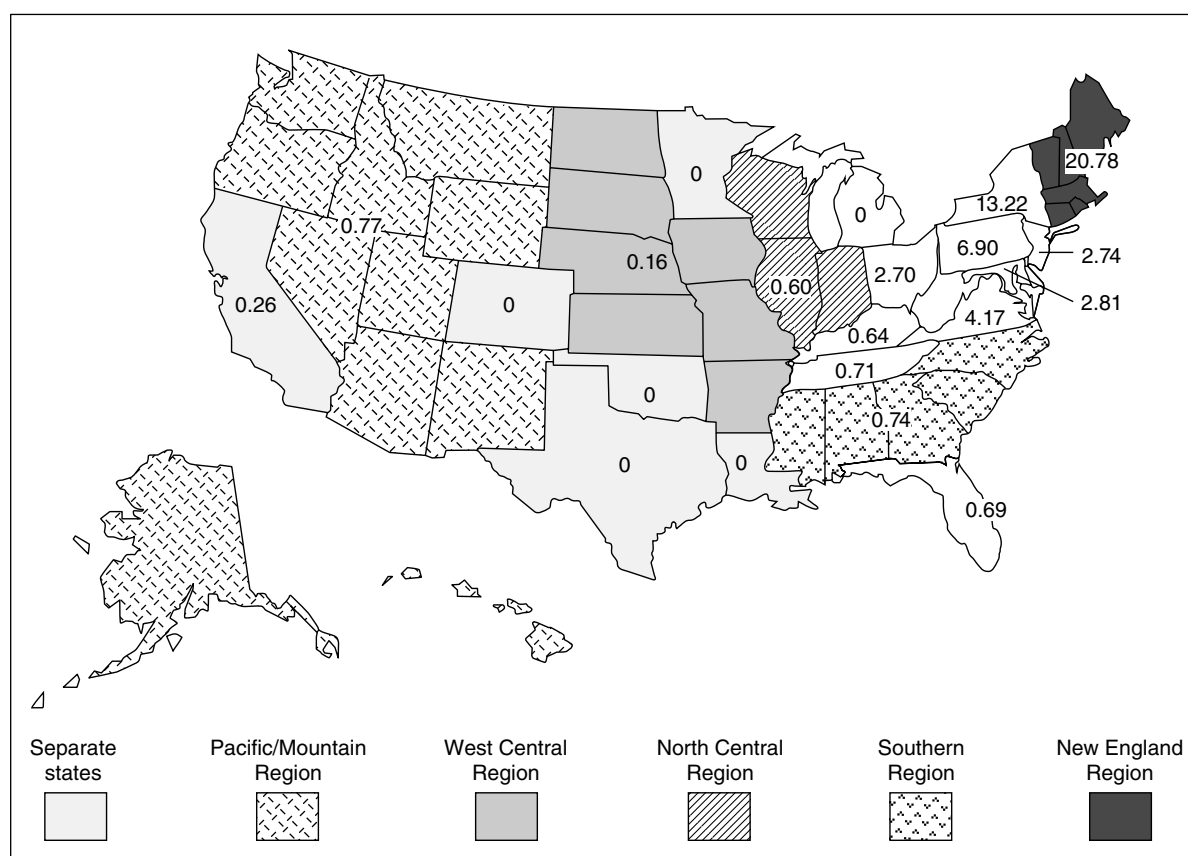


Fig. 8.22 State-specific or region-specific incidence rates of equine motor neuron disease (cases per 1 000 000 horse-years at risk) in 21 geographical units in the US, January 1985–January 1995. (Source: de la Rúa-Domènech *et al.*, 1995.)

Further reading

- Bailey, N.T.J. (1975) *The Mathematical Theory of Infectious Diseases*, 2nd edn. Charles Griffin and Company, London and High Wycombe
- Cliff, A.D. and Haggett, P. (1988) *Atlas of Disease Distributions: Analytic Approaches to Epidemiological Data*. Basil Blackwell, Oxford
- de Jong, M.C.M. and Diekmann, O. (1992) A method to calculate – for computer-simulated infections – the threshold value, R_0 , that predicts whether or not infection will spread. *Preventive Veterinary Medicine*, **12**, 269–285
- Devesa, S.S., Donaldson, J. and Fears, T. (1995) Graphical presentation of trends in rates. *American Journal of Epidemiology*, **141**, 300–304. (Guidelines on depicting trends in disease occurrence)
- Diggle, P.J. (2013) *Statistical Analysis of Spatial and Spatio-Temporal Point Patterns*, 3rd edn. CRC Press, Boca Raton
- Durr, P.A. and Gatrell, A.C. (Eds) (2004) *GIS and Spatial Analysis in Veterinary Science*. CABI Publishing, Wallingford
- Halloran, M.E., Longini, I.M. and Struchiner, C.J. (2010) *Design and Analysis of Vaccine Studies*. Springer Science + Business Media, New York. (Includes a comprehensive introduction to the uses of R_0)
- Halpin, B. (1975) *Patterns of Animal Disease*. Baillière Tindall, London
- Heffernan, J.M., Smith, R.J. and Wahl, L.M. (2005) Perspectives on the basic reproductive ratio. *Journal of the Royal Society Interface*, **2**, 281–293
- Mollison, D. (Ed.) (1995) *Epidemic Models: Their Structure and Relation to Data*. Cambridge University Press, New York
- Pfeiffer, D.U., Robinson, T.P., Stevenson, M., Stevens, K. B., Rogers, D.J. *et al.* (2008) *Spatial Analysis in Epidemiology*. Oxford University Press, Oxford

- Roberts, M.G. (2007) The pluses and minuses of R_0 . *Journal of the Royal Society Interface*, **4**, 949–961
- Sinnecker, H. (1976) *General Epidemiology*. Walker, N. (translator). John Wiley and Sons, London
- Smallman-Raynor, M. and Cliff, A. (2012) *Atlas of Epidemic Britain: A Twentieth Century Picture*. Oxford University Press, Oxford. (A general introduction to mapping epidemics, with descriptions and maps of major epidemics in Britain in the 20th century, including some animal diseases)
- Wei, W.W.S. (2013) Time series analysis. In: *The Oxford Handbook of Quantitative Methods. Volume 2: Statistical Analysis*. Ed. Little T.D., pp. 458–485. Oxford University Press, New York
- Wobeser, G.A. (2007) *Diseases in Wild Animals: Investigation and Management*. 2nd edn. Springer-Verlag, Berlin and Heidelberg. (Includes application of threshold theory to the control of diseases in wildlife)

9

Comparative epidemiology

Investigations of disease in one species of animal can provide valuable insights into the cause, pathogenesis and treatment of disease in another, and are an important part of **comparative medicine**. These investigations often use animals as biological models of diseases in humans.

Types of biological model

There are five types of biological model (Frenkel, 1969; Wilson, 1996):

1. experimental (induced);
2. negative (non-reactive);
3. orphan;
4. transgenic (gene-modified);
5. spontaneous (natural).

Experimental (induced) models

Many models are attempts to reproduce experimentally, in one species of animal, diseases, pathological conditions and impaired functions that occur in other species, notably in humans. For example, cigarette smoke has been administered via nasal catheters to donkeys to study the effects of long-term smoking on tracheobronchial mucociliary activity (Albert *et al.*, 1971); sheep have been used to explore the respiratory distress syndrome provoked by massive inhalation of smoke and hot air (Palmieri *et al.*, 2006; De Las Heras Guillamón and Clau, 2010) and responses to new therapies for post-menopausal osteoporosis (Turner, 2002); and marmosets have been studied to investigate the causes of inflammatory bowel disease (Elson *et al.*, 1995). Additionally, pharmacological models have been developed to improve extrapolation from animals to humans (Travis, 1987), from laboratory animals to different species of domestic animal (Nebbia, 2001), and from one domesticated species to another (Toutain *et al.*, 2010).

This approach is particularly meaningful when causal factors can be manipulated easily; examples

include nutritional deficiencies and excesses, endocrine disorders, and some microbial diseases, for which there are guidelines for therapeutic trials (Reeve-Johnson, 1998; Zak and Sande, 1999). However, if pathogenesis depends on several factors, the causal effect is 'weak', or there are fundamental biological differences between the diseases in animal and humans, then results may differ between animal species and humans. For instance, teratogenic defects may depend on maternal susceptibility to virus infection or absorption of chemicals during a brief but critical period of pregnancy, and many animal species may need to be tested before a suitable model is found. This has led to the conclusion that the value of experimental-animal models in predicting carcinogenic, therapeutic and other effects in humans is limited (Perel *et al.*, 2007; Shanks *et al.*, 2009)¹.

Negative (non-reactive) models

A negative model represents the counterpart to an induced model, and can be useful in studying why disease does not occur. Thus, normal mink may be studied to discover why they are more resistant to the pathogenic effects of the virus that causes severe disease in Aleutian mink (Henson *et al.*, 1976). Such 'non-models' are relatively rare, but are becoming increasingly important with the development of transgenic technology (see 'Transgenic (gene-modified) models' following). For example, transgenic mice have been generated to study the lack of development of autoimmune thyroiditis (Yan *et al.*, 2001).

Orphan models

Orphan models are natural diseases in animals that have no known analogue in humans, but which are distinguished when a similar human disease is later identified. They may then subsequently prove to be

¹ Validity of comparisons also may be affected by factors other than the intrinsic characteristics of diseases; for example, poor study design (van der Worp *et al.*, 2010).

valuable in changing thinking on human disease. An historical example is Rous sarcoma virus of chickens (see Chapter 1), which revolutionized thinking on the cause of cancer. More recent examples include Marek's disease, bovine spongiform encephalopathy, maedi-visna and feline leukaemia virus. If humans are subsequently shown to suffer from a disease similar to one that has already been described in animals, then the established veterinary literature may be very useful.

Transgenic (gene-modified) models

A transgenic animal carries a foreign gene that has been deliberately inserted into its genome, commonly by either transforming embryonic stem cells growing in tissue culture or injecting the desired gene into fertilized ova. Transgenic animals (particularly mice²) have been used as models of human diseases (Petters and Sommer, 2000; Sharma *et al.*, 2015). Examples include mouse models of retinal degeneration (Nir *et al.*, 2000), cystic fibrosis (Grubb and Boucher, 1999) and chronic hypertension (Didion *et al.*, 2000). Some mouse models do not replicate important disease signs and pathology, but other species (e.g., pigs) are considered to mimic some human diseases more closely (e.g., cardiovascular, neurological and diabetic disorders: Fan and Lai, 2013).

The initial goal of transgenic models was to study the genetic basis of disease, but future developments are likely to include investigation of gene interactions, and interactions between genes and the environment. A related aspect is **gene therapy**, which involves modification of genes for therapeutic gain (Wilson, 1996). For example, the Portuguese water dog is a suitable candidate for exploring the efficacy of gene therapy to correct abnormal metabolic processes related to human cardiomyopathy because a similar, rapidly progressive cardiomyopathy occurs in juveniles of that breed (Sleeper *et al.*, 2009). Similarly, mice, cattle, horses, dogs and cats have been identified as suitable surrogates for humans for a range of human genetic haematopoietic diseases with which affected animals share similarities, and for which gene therapy may be appropriate (Bauer *et al.*, 2009).

Spontaneous (natural) models

Spontaneous models utilize the natural occurrence of disease in animals in order to increase understanding of human diseases (Orkin, 1967; Andrews *et al.*, 1979). For example, comparative radiological, histological and serological investigations of sheep with and

without periodontitis have revealed features similar to those of rapidly destructive forms of human periodontal disease, suggesting that the disease in sheep may be a suitable model for similar diseases in man (Ismail *et al.*, 1989). Similarly, the genetic disease, muscular dystrophy, has been identified in mice as a suitable animal model for human Duchenne muscular dystrophy (Bulfield *et al.*, 1984; Wells and Wells, 2002). Spontaneous models also may be based on epidemiological investigations (notably, observational studies of diseases of companion animals) and constitute **comparative epidemiology**, which is the subject of this chapter.

Spontaneous versus experimental models

Companion-animal spontaneous-disease models have several advantages over experimental models. First, companion animals share similar environments with man, rather than living in the 'protected' environment of the laboratory (Backer *et al.*, 2001). Secondly, their diseases occur in natural circumstances, where interactions between a variety of causal factors may occur (Paoloni and Chanda, 2007); experimental induction of disease may not accommodate such interactions. Thirdly, companion animals are phylogenetically more closely related to humans than are the species commonly used in the laboratory (rats, mice, etc.³), evidenced by the high homology and close similarity between the canine and human genome (Kirkness *et al.*, 2003; Lindblad-Toh *et al.*, 2005); this is reflected in several naturally-occurring canine diseases having mutations in a homologous human gene associated with a similar disease (Ostrander *et al.*, 2000). Fourthly, companion animals are more likely than inbred laboratory animals to display the heterogeneity of response to some causal factors (e.g., toxic agents and carcinogens) that is characteristic of humans (Calabrese, 1986; Backer *et al.*, 2001). Finally, the ethical objection to animal experimentation cannot be levelled against studies of spontaneously-occurring diseases.

The value of comparative epidemiological studies may be either the coincidental product of investigations directed mainly at improving the health of animals, or may stem directly from the use of animals as surrogates for humans (Schwabe, 1984). Several areas of interest have developed, frequently using the surrogate approach.

² Transgenic mice have several advantages (Gordon and Khanna, 2010), including their small size, propensity to breed in captivity, and short average lifespan (approximately two years).

³ However, approximately 80% of the mouse genome is organized similarly to the human genome, which therefore still renders the mouse a valuable animal for genetic research; notably, for the location of DNA segments that influence human traits (Copeland *et al.*, 1993); alcoholism is an example (Grisel, 2000).

Cancer

A major field of comparative epidemiology has been the study of cancer. Cancers in dogs and cats are more akin to human cancers than laboratory-animal tumours (MacEwen, 1990; Zappulli *et al.*, 2005), in which tumour development and responses may not predict the progress of human tumours with the same histology (Schuh, 2004). In contrast, cancer in companion animals develops naturally in the context of an intact immune system, sharing many clinical, biological, histological, prognostic and genetic features with similar human tumours (Hellmén *et al.*, 1993; Waters *et al.*, 1996; Chang *et al.*, 2005b; Pinho *et al.*, 2012), and several companion-animal tumours have been identified as appropriate comparative models, based on similarity of tumour histotypes between humans and animals (Porrello *et al.*, 2006; Brønden *et al.*, 2009). Additionally, there are similarities in cancer size relative to patient size and cell kinetics; and the weight and surface area of dogs are sufficient to allow extrapolation to humans, with modest adjustment, in therapeutic trials (Gillette, 1982; Hahn *et al.*, 1994)⁴. Moreover, companion animals live long enough to determine potential delayed effects of therapy (Withrow, 2001).

Established veterinary databases, especially the Veterinary Medical Databases (VMDB: see Chapter 11) and the California Animal Neoplasm Registry, have provided much of the data used in comparative epidemiological studies, and the value of these databases to the studies was realized shortly after the databases were developed (Tjalma, 1968). The incidence of spontaneous tumours (notably cancers) is sufficiently high – particularly in the dog – to compile a large series of cases for study (Table 9.1 and Owen, 1979; Gobar *et al.*, 1998; Vail and MacEwen, 2000).

Monitoring environmental carcinogens

The ageing process in dogs and other animals is more rapid than in humans (Kirkwood, 1985). Tumours in dogs are seen most frequently between the ages of nine and 11 years (Dorn *et al.*, 1968) – much earlier than in humans. Thus, it is possible to assess the effects of

Table 9.1 Crude estimated annual rates for all cancers in the human, ox, horse, cat and dog, and lifespan-adjusted equivalents* (data from the US). (Simplified from Dorn and Priester, 1987.)

Species	Estimated rate of malignancy/100 000/year	
	Crude	Lifespan-adjusted
Man	287.3†	287.3†
Ox	177.2	53.2
Horse	256.3	117.9
Cat	257.4	72.1
Dog	828.3	165.7

* Method of adjustment outlined in Table 9.5.

† All cancers except those of skin (other than melanoma).

Approximately 150 non-melanoma skin cancers/100 000 should be added to make a comparison with other species more accurate.

possible environmental carcinogens more quickly in dogs than in humans. For example, the latent period of bladder cancer is as little as four years in the dog (Hayes, 1976) in contrast to at least 20 years in humans (Hoover and Cole, 1973). There is an association between canine bladder cancer and exposure to insecticides (Glickman *et al.*, 1989) and herbicides (Glickman *et al.*, 2004). There is also a significant positive correlation between the overall level of industrial activity and both canine bladder cancer morbidity (Figure 9.1a) and human bladder cancer mortality rates (Figure 9.1b). These correlations suggest a causal relationship (reasoning by the ‘method of concomitant variations’; see Chapter 3). Moreover, the proportion of canine oropharyngeal cancers that are tonsillar squamous cell carcinomas is much higher in polluted industrial locations than in non-industrial areas (Reif and Cohen, 1971; also Table 9.2). Similarly, there is an excess of non-Hodgkin’s lymphoma cases in dogs kept outdoors in São Paulo, Brazil (a city with five million cars and a million motorcycles) and these lymphomas show a similar excess in both dogs and humans in the most heavily polluted areas of the city (Kimura *et al.*, 2013). These results suggest that carcinogens, probably airborne, may be present in industrial areas, and that dogs and their tumours can act as **sentinels** (see Chapter 21), facilitating early identification of environmental carcinogens.

Negative findings also can be useful. For instance, increased incidence rates of neoplasia have not been demonstrated in dogs environmentally exposed to uranium products following their use in the building industry (Reif *et al.*, 1983). This provides evidence that uranium waste does not constitute a health hazard to dogs, and probably not to humans either.

⁴ Early radiologists used spontaneous tumours in animals to provide guidelines for human radiation therapy (Gillette, 1979), and canine tumours have been used to evaluate whole-body irradiation for lymphomas (Johnson *et al.*, 1968a,b). More recently, interest has focussed on osteosarcoma, mammary carcinoma, oral melanoma, oral squamous cell carcinoma, nasal tumours, pulmonary carcinoma and soft tissue sarcomas, with lymphomas receiving continued attention (MacEwen, 1990); whereas leukaemia, lymphoma and mammary tumours have been the subjects of investigation in cats (Jeglum, 1982).

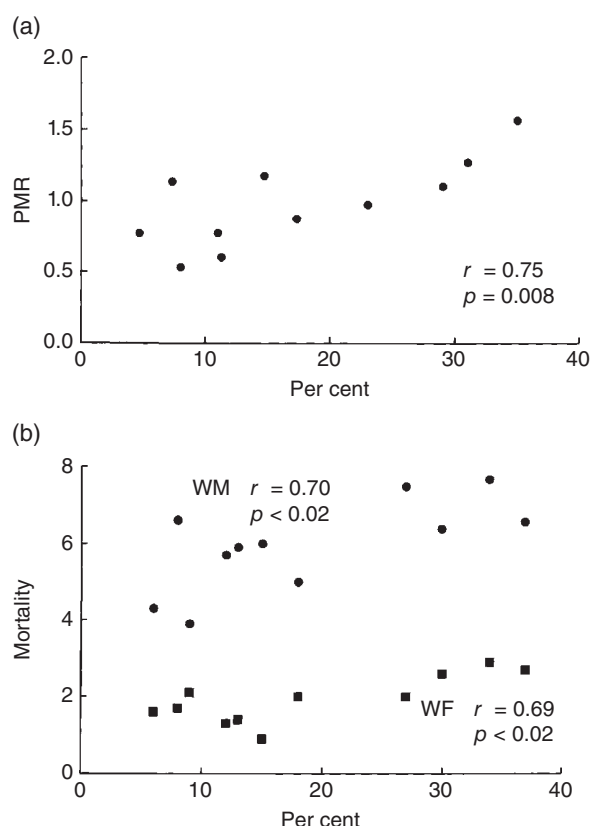


Fig. 9.1 (a) Proportional morbidity ratios (PMRs) of bladder cancer among dogs living in a 25-mile radius of their veterinary clinic in the US, plotted against the percentage of men employed in manufacturing industries in the counties with the veterinary clinic. (b) Human age-adjusted mortality/100 000 for bladder cancer (1950–1969) in white men (WM) and white women (WF) living in the counties with the surveyed veterinary clinics, plotted against the percentage of men employed in manufacturing industries in 1970. r = correlation coefficient; p = level of statistical significance (see Chapter 14). PMRs are standardized ratios based on proportional morbidity rates (Hayes *et al.*, 1981). (Source: Hayes *et al.*, 1981. Reproduced with permission of Oxford University Press.)

Identifying causes

Veterinary observational studies can provide clues to the causes of human cancer. For example, an association has been found between canine mesothelioma and exposure to both asbestos (which is a risk factor for human mesothelioma) and pesticides, notably flea preparations (Table 9.3). Some of the latter contain asbestos-like compounds, which could thus be risk factors for human mesothelioma and other tumours. Similar compounds are found in talc, and an association has been demonstrated between ovarian cancer in women and use of cosmetic talc (Cramer *et al.*, 1982). The latent period of human mesothelioma following occupational exposure to asbestos is long, nearly always greater than 20 years, with most cases occurring after 30 years (Selikoff *et al.*, 1979). In contrast, the greatest risk of development of canine mesothelioma is between five and nine years of age. Thus, mesothelioma in dogs may be a useful sentinel for unrecognized human exposure to asbestos.

Comparison of site-specific cancer rates also can offer clues to aetiology. Human cancer rates are at least three times greater than canine and feline rates for six major cancer sites: the digestive, respiratory and urinary systems, and the uterus, ovary and prostate gland (Schneider, 1976). These disparities provide indirect evidence of possible causes. For instance, the high rate of human ovarian cancer may be related to the regular menstrual cycle. Female cats have a similar oestrus cycle to women (cats are seasonally polyoestrous; women are polyoestrous; each with cycles of three to four weeks) but have a much lower rate of ovarian cancer, even with compensation for the proportion of neutered cats. However, pregnancy is much more frequent in cats than in women. Thus, pregnancy may protect against the cancer, implying that hormonal status may be a determinant.

Table 9.2 Proportions of canine oropharyngeal cancers that are tonsillar squamous cell carcinomas, by geographical location. (Adapted from Bostock and Curtis, 1984.)

Location	Year	Proportion (%)	Number of cases	Totals (all malignant oropharyngeal neoplasms)
London	1950–1953	46	35	76
US (non-industrial)	1959	6	8	124
	1964–1974	7	31	469
Philadelphia	1952–1958	22	29	130
South-east England	1978–1981	13	19	152
Melbourne	1978–1981	3	2	75

Table 9.3 Odds ratios and associated 95% confidence intervals for two risk factors for canine mesothelioma. (Simplified from Glickman *et al.*, 1983.)

Risk factor	Estimated odds ratio ($\hat{\psi}$)	95% confidence interval for ψ
Probable exposure of owner to asbestos by occupation or hobby	8.0	1.4, 10.6
Exposure to pesticide	11.0	1.5, 82.1

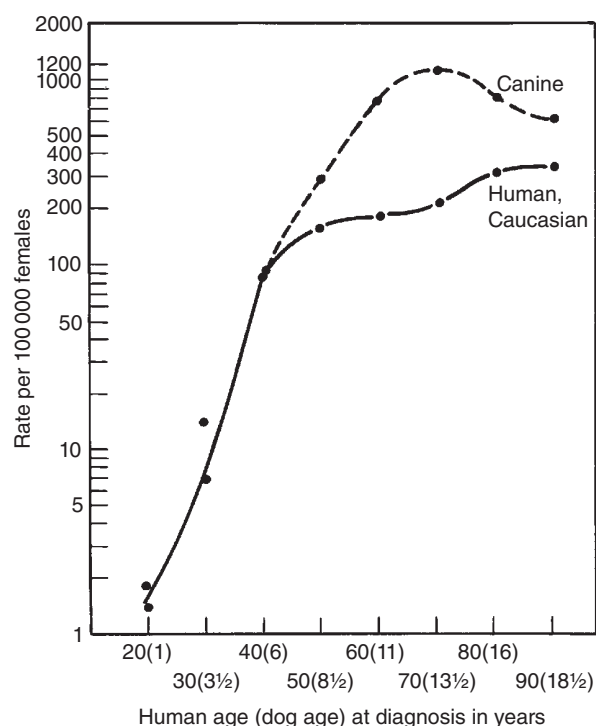


Fig. 9.2 Annual female human (Caucasian) and canine age-specific incidence rates for mammary cancer. (Adapted from Schneider, 1970.)

The age-specific incidence rates of mammary cancer in bitches and women are compared in Figure 9.2, after calculating a canine equivalent of human age (see 'Comparing ages' following). The rates are similar until the human menopause, when ovarian activity decreases and the human rate tends to stabilize, suggesting an hormonal determinant. This suggestion is supported by the demonstration of receptors for steroid hormones in the cytosol fractions from canine mammary carcinomas (Martin *et al.*, 1984), and also by the reduction in their growth rate by the anti-

oestrogen, tamoxifen, or by ovariectomy, after transplantation into nude mice (Pierrepont *et al.*, 1984)⁵.

Epidemiological studies in both dogs and cats (Misdorp, 1991) have demonstrated a dose-dependent relationship between progestagen therapy and the development of mammary tumours; and some human studies have identified a similar relationship associated with oral contraceptives (McPherson *et al.*, 1987; Miller *et al.*, 1989; Olsson *et al.*, 1989; UK National Case-control Study Group, 1989). This suggests that similar causal mechanisms are operating in companion animals and humans, and therefore that the former may be appropriate models for further study.

Animals are not exposed to some factors that may act as confounders in human studies. Investigations in animal populations, therefore, can avoid these potential confounders. For instance, smoking is a risk factor for human mesothelioma. The significant association between canine mesothelioma and exposure to asbestos, described above, is demonstrated in the absence of active smoking, and therefore is not confounded by it.

Potential risk factors also may be easier to quantify accurately in animals than in humans. The diet of dogs, for example, is relatively constant, particularly when proprietary foods are consumed. An investigation of the role of diet in the development of canine mammary cancer has not demonstrated an association between a high-fat diet and the disease, although similar human studies, with the attendant difficulties in estimating fat intake precisely, have produced conflicting results (Sonnenschein *et al.*, 1991).

Comparing ages

The lifespans of animals are different from that of humans (Figure 9.3). Thus, as a calendar and a 'biological' year are the same for humans, a correction should be made for the majority of species that have several 'biological' years during one calendar year, so that more meaningful comparisons of morbidity and mortality can be made. Two techniques are used (Kirkwood, 1985). First, anatomical and physiological characteristics that correlate with age can be sought (e.g., adult brain weight, onset of puberty and reproductive senescence). Secondly, the survival pattern of different species can be compared mathematically using an equation based on Gompertz's law

⁵ In contrast, there is a lower morbidity of lymphoma in entire female dogs compared with other gender groups, a finding in common with the disease in humans, where female gender and intact reproductive status also appear to be protective (Henry *et al.*, 2005).

(Olshansky, 2010), which defines a relationship between probability of death and age. The two techniques are frequently combined (Table 9.4).

The lifespan-adjustment method of Lebeau (1953) also amalgamates these two approaches and is illustrated in Figure 9.4. Lebeau argued that, once maturity is reached, ageing occurs at a constant rate in dogs and

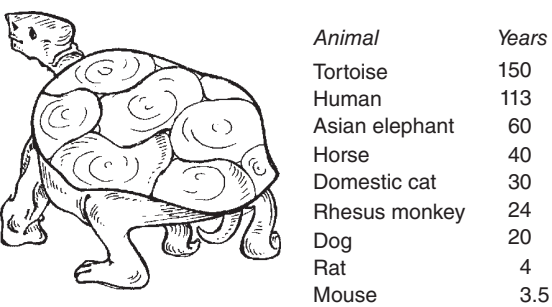


Fig. 9.3 The maximum verifiable lifespans of several animals.

Table 9.4 Examples of comparative ages between humans, dogs and cats. (From Schneider, 1976.)

Human age (years)	Canine age equivalent (years)*	Feline age equivalent (years)*
24	2	1–1.5
32–35	4	4
52–55	9	10
72–75	14	16
>91	>18	>21

* Based on the age when maturity is reached and on survival that excludes euthanasia of animals because of human-related reasons (e.g., if animals are unwanted).

humans. A one-year-old dog is equivalent in age to a 15-year-old person; a two-year-old dog to a 24-year-old person; and, above the age of two years, each year of a dog's life is equivalent to four human years. Thus, a 10-year-old dog is equivalent to a 56-year-old person (24 years, plus four years for each canine year from three years old onwards). The coefficients necessary to calculate human age equivalents for various ages of dog are listed in Table 9.5. Lebeau's technique is utilized in Figure 9.2. However, Reif (1983), Michell (1999), Bonnett and Egenvall (2010) and Kraus and others (2013) highlight the problems associated with such a conversion because the lengths of life of different breeds vary.

Other criteria for age equivalence are based on the mean age of populations and correction factors related to recorded maximum lifespans such as those presented in Figure 9.3. Thus, using the correction factors in Table 9.6, the estimated bovine rate of malignancy in Table 9.1 (177.2/100 000/year) is lifespan-adjusted by multiplying the value by 0.30, giving a lifespan-adjusted value of 53.2/100 000/year.

Another correction, in addition to lifespan adjustment, also should be made when comparing human and companion-animal populations because of the different proportions of individuals of various ages in each population. If canine populations (see Figures 5.4a and 5.4b) are compared with human populations, it can be noted that the shape of the canine population pyramid is similar to that of human populations with high fecundity and a low proportion of individuals surviving to old age; for example, 19th-century European and North American populations and contemporary populations in less-economically-developed countries (Figure 9.5a). It contrasts with the pyramid of human populations with low fecundity and a high proportion

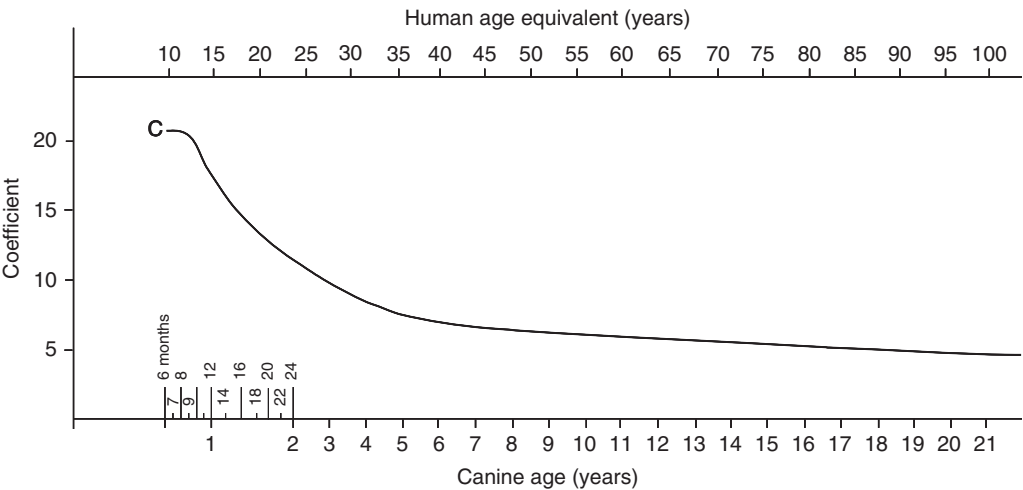


Fig. 9.4 Conversion of canine age to its human age equivalent. C = coefficient curve. (Adapted from Anon., 1954; after Lebeau, 1953.)

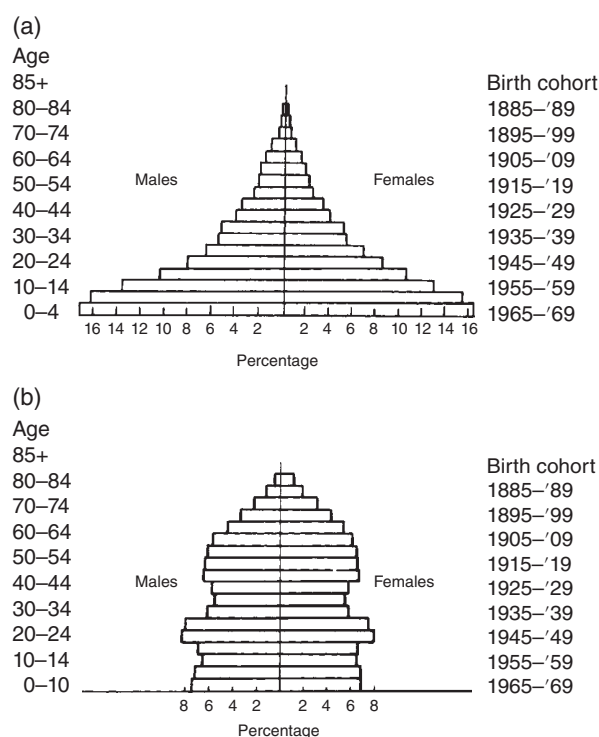
Table 9.5 Canine age, equivalence coefficient, and human age equivalent. (From Anon, 1954.)

Canine age	Coefficient	Human age
6 months	20.0	10 years
7 months	20.0	11 years 8 months
8 months	19.0	12 years 8 months
9 months	18.0	13 years 6 months
10 months	17.0	14 years 2 months
11 months	16.0	14 years 8 months
12 months (1 year)	15.0	15 years
14 months	14.5	16 years 11 months
16 months	14.0	18 years 8 months
18 months	13.5	20 years 3 months
20 months	13.0	21 years 8 months
22 months	12.5	22 years 9 months
24 months (2 years)	12.0	24 years
3 years	9.3	28 years
4 years	8.0	32 years
5 years	7.2	36 years
6 years	6.6	40 years
7 years	6.3	44 years
8 years	6.0	48 years
9 years	5.8	52 years
10 years	5.6	56 years
11 years	5.4	60 years
12 years	5.3	64 years
13 years	5.2	68 years
14 years	5.1	72 years
15 years	5.06	76 years
16 years	5.0	80 years
17 years	4.9	84 years
18 years	4.87	88 years
19 years	4.84	92 years
20 years	4.80	96 years
21 years	4.76	100 years

Table 9.6 Lifespan correction factors derived from the lifespan of several species. (From Dorn and Priester, 1987.)

Species	Recorded maximum lifespan (RML)	Correction factor (RML/100)
Man	100*	1
Ox	30	0.30
Horse	46	0.46
Pig	26	0.27
Sheep	20	0.20
Dog	20	0.20
Cat	28	0.28

* Arbitrary.

**Fig. 9.5** Human population pyramids. (a) Mexico, 1970; (b) Sweden, 1970. (Source: Ewbank and Wray, 1980.)

of individuals surviving to old age; for example, in more-economically-developed countries (Figure 9.5b). Most comparative studies are undertaken in the latter, and so the lifespan-adjusted rates in the populations that are being compared should also be age-adjusted (see Chapter 4).

Figure 9.6 presents crude rates, and age- and life-span-adjusted rates, for mammary cancer in bitches

and women, indicating the difference between the crude rates and the rates with two levels of adjustment. The crude rates suggest that cancer incidence is greater in bitches than in women. However, the age- and lifespan-adjusted rates, which reflect the comparative incidence best, demonstrate that women are more prone to the cancer than either bitches or cats.

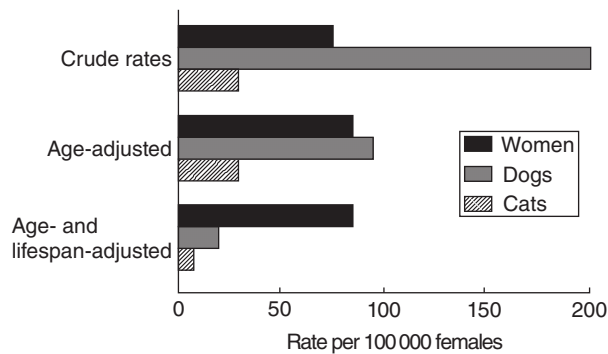


Fig. 9.6 Estimated annual incidence rates of mammary cancer in women, dogs and cats: crude rates and two levels of rate adjustment (data from the US). (Source: Dorn and Priester, 1987.)

Some other diseases

Diseases with a major genetic component

Comparative studies also provide evidence for genetic causal mechanisms. Thus, there is evidence, derived from epidemiological and family studies, of the importance of genetic factors in the aetiology of human congenital heart disease. Limited success has been achieved in identifying the anatomical defects that have significant genetic causal factors, and in determining the nature of the underlying genetic abnormality and the pathway from genetic defect to structural abnormality. However, studies on dogs have provided valuable insights into this type of disease. The inheritance of patent ductus arteriosus (PDA) in dogs is more complex than a simple autosomal dominant mode (Patterson *et al.*, 1971). There is evidence that inheritance of the condition is multifactorial (see Chapter 5), and that there are different thresholds for expression of the less severe ductus diverticulum and the fully patent ductus arteriosus (Figure 9.7).

Histological studies on litters of poodles genetically predisposed to PDA subsequently demonstrated that PDA is the result of a genetically-determined extension of the non-contractile wall of the aorta into the ductus arteriosus. The role of environmental factors (e.g., diet and season) in the cause of this disease is unclear, but such factors are known to influence other threshold traits (e.g., cleft palate in laboratory animals). Epidemiological studies in humans, based on comparison of disease rates in both siblings and offspring of affected individuals with rates in the general population (Figure 9.8), suggest a similar mode of inheritance to that in the dog (Zetterquist, 1972).

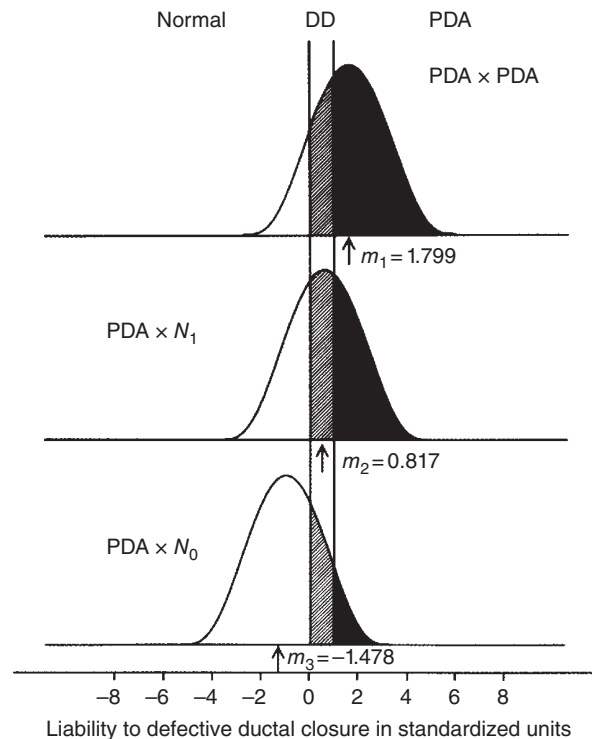


Fig. 9.7 Two-threshold model of hereditary patent ductus arteriosus (PDA) in dogs. N_1 : normal first-degree relatives; N_0 : normal unrelated dogs; m : mean liability in offspring of crosses, expressed as standardized units for likely incidence of the condition (Falconer and MacKay, 1996). Liability to defective ductal closure is depicted as a continuous variable, phenotypic discontinuities occurring at critical thresholds on the underlying scale. Below the lower threshold, the ductus closes throughout its length. Between the upper and lower thresholds, the ductus closes at the end nearest the pulmonary artery, but remains open over the rest of its length, producing a ductus diverticulum (DD). Beyond the upper threshold, the ductus remains open over its entire length, and PDA is present. The areas under the curves defined by the thresholds represent proportions of offspring observed in each phenotypic class. Mean liability of offspring from outcrosses of PDA-affected dogs to normal unrelated dogs (m_3) lies below, but overlaps, both thresholds. Mean liability in crosses between two dogs with PDA (m_1) lies above both thresholds. Crosses between PDA-affected dogs and normal first-degree relatives of PDA-affected dogs result in a distribution of offspring with a mean liability to defective ductal closure (m_2) approximately halfway between the other two matings. (Adapted from Patterson *et al.*, 1971.)

Other useful companion-animal models of genetic diseases are discussed by Patterson *et al.* (1982); they include those of lysosomal storage diseases and disorders of the immune system. The availability of high-quality genome sequences for the dog extends the prospects for its use as a model of single-gene and genetically-complex disorders in humans (Lequarré *et al.*, 2011).

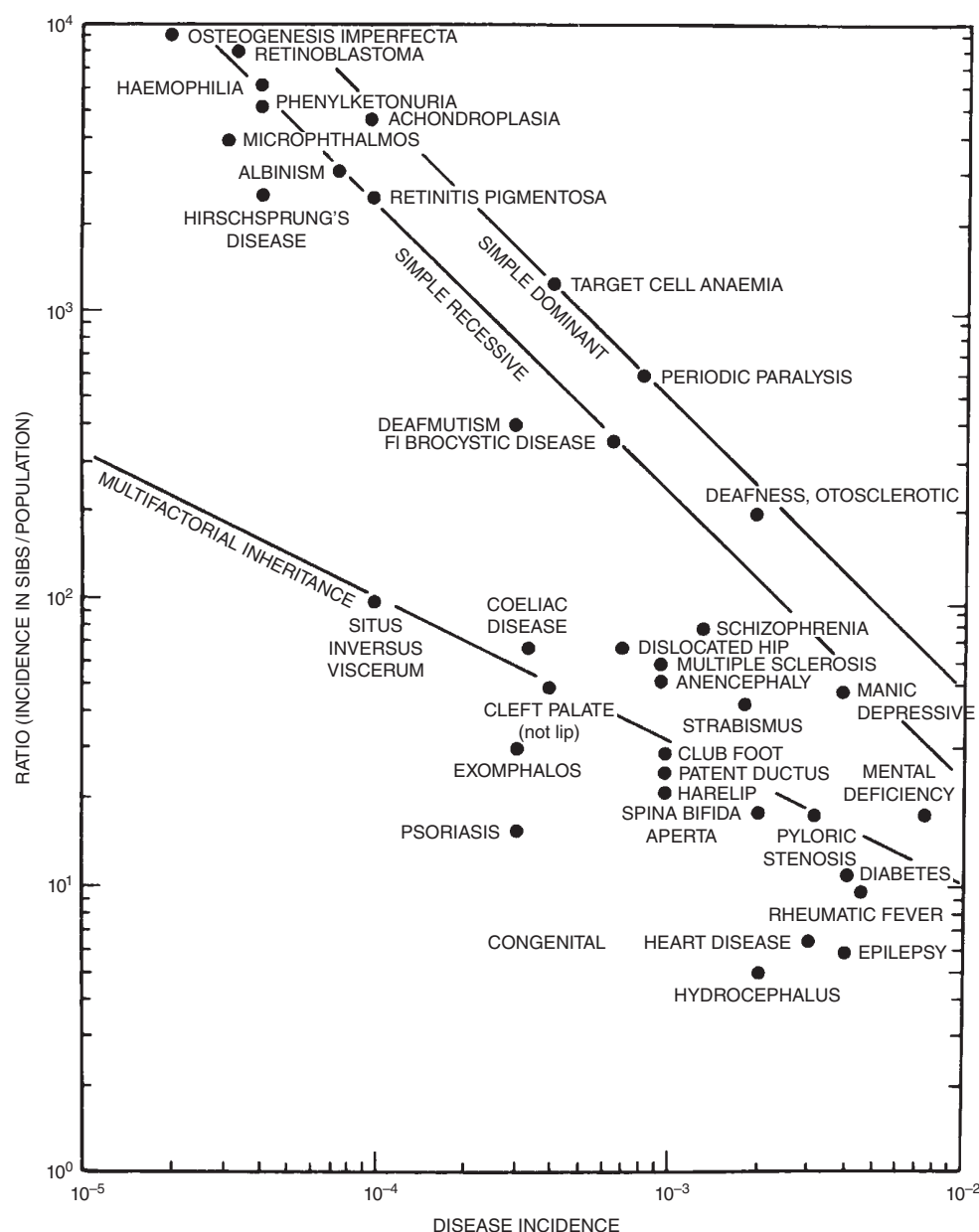


Fig. 9.8 Comparison of the risk in siblings of human patients for single-gene traits with the risk in siblings of patients for multifactorial traits. On a $\log_{10}$ scale, the incidence of each disease is plotted against the ratio of the incidence of the disease in siblings to the incidence in the general population. Single-gene and multifactorial conditions fall into two distinct clusters. The two-cluster effect is simply due to the reduced chance of individuals inheriting *all* necessary genes to show disease in multifactorial conditions. (Source: Newcombe, 1964.)

Some non-infectious diseases

Congenital defects, which may have known genetic determinants, are common in humans. Studies in companion animals can support or refute associated causal hypotheses. Hypospadias (a developmental abnormality of the urethral meatus) is relatively common in humans but its cause is unclear. It often occurs

with other developmental defects, is commoner in males than females, and has familial and seasonal associations. These characteristics suggest both genetic and environmental determinants. Using VMDB data, a breed predisposition has been demonstrated in Boston terriers (Hayes and Wilson, 1986), adding credence to genes as determinants.

Diseases associated with environmental pollution

Several environmental factors, including air pollution and occupational hazards, have been implicated as causes of various types of non-neoplastic chronic pulmonary disease (e.g., bronchitis and emphysema) in humans. Chronic pulmonary lesions also occur in the dog. These include interstitial fibrosis and chronic inflammation, emphysema and pleural fibrosis – collectively termed ‘chronic pulmonary disease’ (CPD).

The association between the prevalence of CPD, assessed radiographically, and age and environment (urban versus rural) has been studied in the US (Reif and Cohen, 1979). Results relating to Ithaca (rural) and Boston and Philadelphia (urban) showed a higher prevalence in dogs living in urban areas than in rural areas, but the difference was only identified in dogs over seven years of age, indicating that, in the dog, this may be the latent period of the cumulative effects of environmental hazards. In Philadelphia, an urban/rural prevalence difference also has been identified (Figure 9.9a), and this correlated approximately with atmospheric air pollution (Figure 9.9b).

Air pollution also is associated with neuroinflammation in children and dogs in Mexico City (Calderón-Garcidueñas *et al.*, 2008). Cerebral lesions are similar in both species and, in children, are associated with cognitive deficits (Calderón-Garcidueñas *et al.*, 2011).

The effects of environmental toxic agents also have been first identified in animals (Buck, 1979). These include trichlorethylene (in cattle), chlorinated naphthalenes (in cattle), aflatoxins (in dogs, cattle and pigs) and organophosphorus nerve agents (in sheep).

Animals also can be effective sentinels of environmental toxins because toxin levels in biological samples from animals represent an integrated measure of exposure to ambient environmental contamination, as in humans (Anon., 1991)⁶. For example, beef cattle (Salman *et al.*, 1990) and dogs (Backer, *et al.*, 2001) are useful sentinels of chlorinated hydrocarbon pesticides. Similarly, dogs scavenging on shorelines are sentinels of cyanobacterial toxins occurring in littoral bacterial scum (Codd *et al.*, 1992), possibly because they are attracted by the taste and odour of the bacteria. Dogs also are good predictors of levels of lead in the blood of children exposed to a lead-contaminated environment (Ostrowski, 1990).

Reif (1986, 2011), Rabinowitz and others (2005) and Schmidt (2009) provide detailed discussions of animals as environmental sentinels. The ‘Canary Database’ (Wiley *et al.*, 2005) collates studies of domestic animals and wildlife exposed to environmental hazards, and indicates their potential as sentinels.

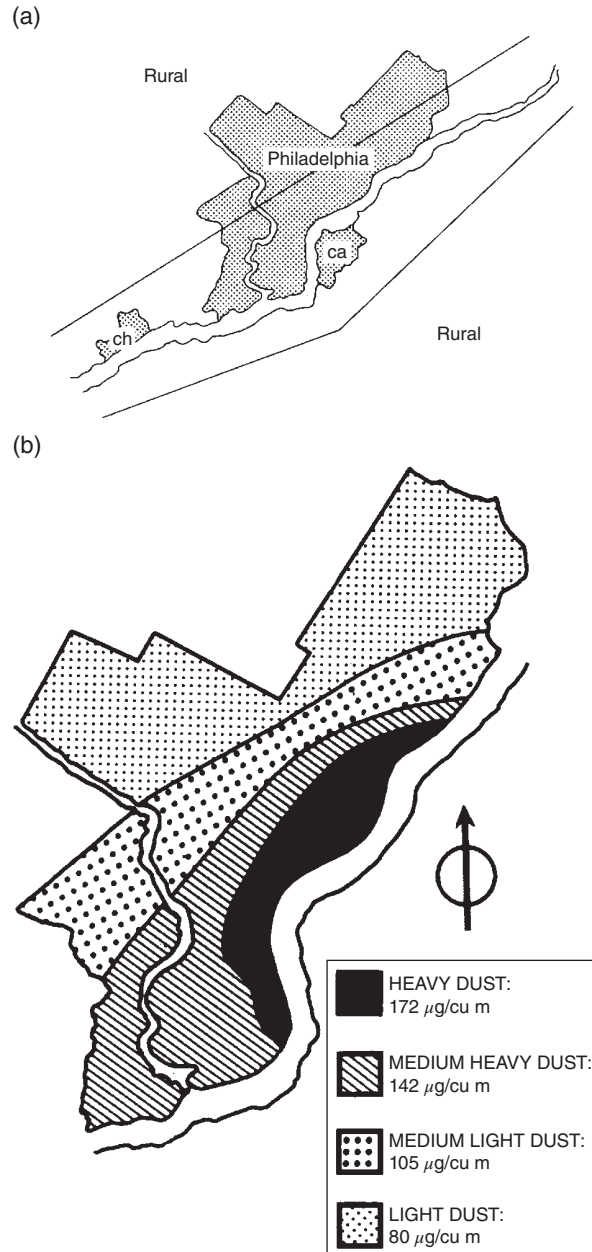


Fig. 9.9 The relationship between the prevalence of canine chronic pulmonary disease (CPD) and location, Philadelphia, US. (a) Relative prevalence of CPD in dogs 7–12 years of age: the prevalence is high in the area between the two lines, and low outside these lines. ca = Cambden; ch = Chester. (b) Atmospheric dust concentration. (Source: Reif and Cohen, 1970. Reproduced with permission of Taylor and Francis.)

⁶ The concept of using animals as sentinels for adverse human health events is not new, dating back to the canary as a sentinel for carbon monoxide in coal mines (Burrell and Seibert, 1914).

Reasoning in comparative studies

Comparative studies involve reasoning by analogy, and the inferences can be wrong, exemplified by Snow's conclusion that cholera and yellow fever are transmitted by contaminated water because they both occur in insanitary conditions (see Chapter 3). Such conclusions therefore should be treated with caution.

Inferences also should be tempered by the knowledge that many diseases are multifactorial, either because several factors are required to induce a single disease, or because several factors cause disease manifestations that cannot be distinguished at present (MacMahon, 1972). For instance, canine epileptiform seizures appear to be genetically determined in some circumstances, but environmentally induced in others (De Risio and Platt, 2014); and there is evidence that diabetes mellitus is caused by chemicals, autoimmune reactions and virus infection (Coppeters *et al.*, 2012).

Inference is less reliable between species, because similar manifestations in different species may be the result of different pathological processes. For example, a variety of species are subject to epileptiform and experimental seizures (Pitkänen *et al.*, 2017), but the relationship of these conditions to human epilepsy

is unclear (Uriarte and Saiz, 2016). Part of the problem is taxonomic: diseases named by clinical signs (e.g., epilepsy) are more likely to be multifactorial than those named by a specific lesion, because the former may be produced by several lesions, each of which may have distinct sufficient causes (see Figure 10.3). Thus, the inferences drawn from comparative studies are strengthened when the studies are undertaken on specific structures with similar lesions (e.g., the studies of bladder cancer described earlier), on the plausible assumption that similar lesions in identical structures are likely to have similar causes.

An important criterion for producing a convincing model is its ability to work in more than one situation (Davidson *et al.*, 1987). There are similarities in the modes of inheritance of several genetic diseases (e.g., canine and human haemophilia), and there are close similarities between the causes and, consequently, treatment of tumours in animals and humans. Such similarities suggest that animal models of human genetic and neoplastic diseases are sound, and that there is practical value in using them, particularly when the diseases are much more common in animals than in humans (e.g., canine osteosarcoma: Brodey, 1979; Rand, 2008).

Further reading

- Anon. (1991) *Animals as Sentinels of Environmental Health Hazards*. Committee on Animals as Monitors of Environmental Hazards, Board of Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council. National Academy Press, Washington DC
- Baba, A.I. and Cătoi, C. (2007) *Comparative Oncology*. The Publishing House of the Romanian Academy, Bucharest
- Bukowski, J.A. and Wartenberg, D. (1997) An alternative approach for investigating the carcinogenicity of indoor air pollution: pets as sentinels of environmental cancer risk. *Environmental Health Perspectives*, **105**, 1312–1319
- Bustad, L.K., Hegreberg, G.A. and Padgett, G.A. (1975) *Naturally Occurring Animal Models of Human Disease: A Bibliography*. Institute of Laboratory Animal Resources, National Academy of Sciences, Washington DC
- Calabrese, E.J. (1991) *Principles of Animal Extrapolation*. Lewis Publishers, Chelsea
- Conn, P.M. (Ed.) (2017) *Animal Models for the Study of Human Disease*. Academic Press, London
- Dorn, C.R. and Priester, W.A. (1987) Epidemiology. In: *Veterinary Cancer Medicine*, 2nd edn. Eds Theilen, G. H. and Madewell, B.R., pp. 27–52. Lea and Febiger, Philadelphia. (Includes a discussion of comparative oncology)
- Glickman, L.T. and Domanski, L.M. (1986) An alternative to laboratory animal experimentation for human health risk assessment: epidemiological studies of pet animals. *Alternatives to Laboratory Animals*, **13**, 267–285
- Hansen, K. and Khanna, C. (2004) Spontaneous and genetically engineered animal models: use in precancer drug development. *European Journal of Cancer*, **40**, 858–880
- Hau, J. (2008) Animal models of human diseases: an overview. In: *Sourcebook of Models for Biomedical Research*. Ed. Conn, P.M., pp. 3–8. Humana Press, Totowa
- Hayes, H.M. (1978) The comparative epidemiology of selected neoplasms between dogs, cats and humans. A review. *European Journal of Cancer*, **14**, 1299–1308
- Kelsey, J.L., Moore, A.S. and Glickman, L.T. (1998) Epidemiologic studies of risk factors for cancer in pet dogs. *Epidemiologic Reviews*, **20**, 204–217

- Lewis, S.M. and Carraway, J.H. (1992) Large animal models of human disease. *Lab Animal*, **21**, 22–29. (A discussion of species, excluding laboratory animals)
- Momanyi, N.K., Korir, R.A. and Mutiga, R.E. (2016) One Health and cancer: a comparative study of human and canine cancers in Nairobi. *International Journal of One Health*, **2**, 42–57
- Reif, J.S. (2011) Animal sentinels for environmental and public health. *Public Health Reports*, **126**, Suppl. 1, 50–57
- Richardson, R.C. (1983) Spontaneous canine and feline neoplasms as models of cancer in man. *Kal Kan Forum*, **2**, 89–94
- Rowell, J.L., McCarthy, D.O. and Alvarez, C.E. (2011) Dog models of naturally occurring cancer. *Trends in Molecular Medicine*, **17**, 380–388
- Scarpelli, D.G. (1997) Animal models of disease: utility and limitations. In: *Biological Aspects of Disease: Contributions from Animal Models*. Eds Iannaccone, P. M. and Scarpelli, D.G., pp. 1–13. Harwood Academic Publishers, Amsterdam
- Withrow, S.J., Vail, D.M. and Page, R.L. (2013) Why worry about cancer in pets? In: *Withrow & MacEwan's Small Animal Clinical Oncology*, 5th edn. Eds Withrow, S.J., Vail, D.M. and Page, R.L., pp. xv–xvi. Elsevier Saunders, St Louis. (Includes a brief discussion of comparative oncology)

10

The nature of data

The epidemiologist investigates the frequency and distribution of disease (and sometimes other characteristics, such as performance) in groups of animals. This involves the collection and analysis of **data** (singular: datum): '*facts, especially numerical facts, collected together for reference or information*' (*Oxford English Dictionary*, 1971). These data may relate, for example, to clinical signs, therapy, and post-mortem and laboratory examinations. If an investigation is undertaken prospectively, the epidemiologist has to decide what data should be collected. If an investigation is undertaken retrospectively, the epidemiologist may use data that have been collected by veterinary practices, abattoirs, laboratories, clinics and other organizations (see Chapter 21). Therefore, it is necessary to know whether the types of data that are collected are suitable for a particular investigation.

Inferences about the cause of disease involve categorizing individuals into a group that has a disease and a group that does not have the disease, the object being to decide whether these two groups differ with respect to possible determinants. This is the basis of observational studies (outlined in Chapter 2 and detailed in Chapters 15 and 16). Animals are put into the diseased category because they possess certain attributes, such as clinical signs and lesions, that are used to define, and sometimes to name, the disease. Knowledge of the various features that are pertinent to the classification of disease is therefore necessary.

Some data are observations; for example, the recording of diarrhoea. Other data are interpretations of observations; for instance, a diagnosis, which represents an interpretation of a set of clinical signs, lesions and laboratory results. The interpretation, and therefore the diagnosis, may be incorrect; this could result in animals being erroneously categorized as having a particular disease. Any inferences on association between possible causal factors and disease, drawn from a study of such animals, therefore may be erroneous.

Additionally, many data that epidemiologists use nowadays are stored in computers as codes.

Classification of data

Data can be broadly classified into **qualitative** and **quantitative** (Figure 10.1).

Qualitative data describe a property of an animal; that is, its membership of a group or class. Such data therefore are termed **categorical**. Examples are the breed and sex of an animal.

Quantitative data relate to **amounts**, rather than just indicating classes. Examples are prevalence, incidence, body weight, milk yield, temperature and antibody titre. These data may be further divided into **discrete** and **continuous**.

Discrete data can have only one of a specified set of values, such as whole numbers (1, 2, 7, 9, etc.); for example, the number of teats on a sow. Discrete data generate **counts**. Thus, aggregates of qualitative (categorical) data are counted (e.g., the total number of dogs in a kennels or cows on a farm).

Continuous data can have any value within a defined range (though the range can be infinite). Examples are the girth of a cow and its body weight. Continuous data are usually quantified by comparison with a fixed unit; that is, they are measured. Continuous data therefore generate **measurements**.

Scales (levels) of measurement

Although measurement can be applied strictly only to quantitative, continuous data, 'scale of measurement' is also conventionally used to describe the 'strength' of values that can be attached to both qualitative and quantitative data. There are four main scales (levels) of measurement (Stevens, 1946): **nominal**,

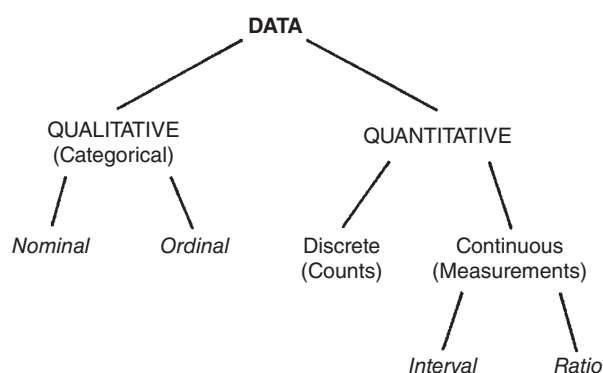


Fig. 10.1 A broad classification of data. Scales (levels) of measurement are italicized.

ordinal, interval and ratio (*Figure 10.1*); and a **visual analogue scale** also is sometimes used.

The nominal (classificatory) scale

This scale involves the use of numbers (or other symbols) to classify objects. Thus, male and female can be coded 0 and 1, respectively. Non-veterinary examples are the use of numbers or letters on aircraft, and car number plates to indicate their origin. **Dichotomous (binary)** data are a special form of nominal data in which the scale only has two possible categories (e.g., individuals classified as either 'diseased' or 'not diseased'). The property of a nominal scale is **equivalence**: members in a class must possess the same property.

The only legitimate operation that can be performed on a nominal scale is transformation of the symbols. For example, if the disease, diabetes, was numerically coded as 671, then this value could be transformed or changed to 932 for *all* diabetics. This scale is a 'weak' form of 'measurement'.

The ordinal (ranking) scale

The ordinal scale allows groups to be related to other groups. Most commonly, the relation can be expressed in terms of equal to (=), greater than (>) or less than (<). Examples are the use of body condition scores for sheep (Russel, 1991), cattle (DEFRA, 2001), horses (Henneke *et al.*, 1983) and dogs and cats (Lund *et al.*, 1999), and grading of severity of disease, such as nasal disease (Furtado and Constantino-Casas, 2013), oesophagogastric ulceration (Kopinski and McKenzie, 2007) and lameness (*Table 10.1*).

The difference between the nominal and the ordinal scale therefore is that the ordinal includes not only equivalence but also the 'greater than' and 'less than' property.

In the ordinal scale, any transformation must preserve the order. It does not matter what number is attached to a class, as long as the relationship with other classes is consistent. Thus, a carcass condition score scale can include 5 = 'good' and 1 = 'poor', and equally 1 = 'good' and 5 = 'poor', as long as the numbers between 1 and 5 maintain the same order of ranking. Although 'stronger' than the nominal scale, the ordinal scale is still a relatively 'weak' form of 'measurement'.

Ordinal scales sometimes are termed **numerical rating scales**.

The interval scale

In an interval scale, the distance between the ranked values is known with some accuracy. A good example is body temperature. Two thermal interval scales commonly are used – Celsius and Fahrenheit – each containing the same amount of information. The ratios of the intervals (temperature differences in this example) are independent of the zero point (0 °C = 32 °F) and are equal to the ratios of the differences on the other interval scales. For example:

37 °C = 99 °F (approximately),
22 °C = 72 °F (approximately),
6 °C = 43 °F (approximately).

Thus (°C)

$$\frac{37 - 22}{22 - 6} = \frac{15}{16} = 0.9375,$$

and (°F)

$$\frac{99 - 72}{72 - 43} = \frac{27}{29} = 0.9310344 \dots,$$

that is, the ratios are approximately the same (0.9).

The interval scale therefore includes equivalence, 'greater than' relationships, and ratios of intervals. Because the ratios are independent of the zero point, arithmetic calculations can be performed only on differences between numbers. The interval scale is a relatively 'strong' form of (actual) measurement.

The ratio scale

The ratio scale is an interval scale with a true zero point. Weight, for example, is a ratio scale. The various weight ratio scales may have units of kilograms, grams, pounds or ounces, but they all start from the same zero point. This means that arithmetic operations can be performed not only on the ratios of differences but also on the numbers themselves. Note that a ratio scale is not necessarily associated with ratios, many of which are ratios of counts (e.g., maternal mortality ratio: see *Table 4.8*). Weight is a ratio scale that is not a ratio.

Table 10.1 A scoring scheme for lameness in pigs*. (From Main *et al.*, 2000.)

Lameness score	Initial response to human presence	Pig's response after opening gate	Behaviour of individual within the group	Standing posture	Gait
0	Bright, alert and responsive (pigs rise immediately and approach inquisitively)	Inquisitive, will tentatively leave pen	Freely participates in group activity	Pig stands squarely on all four legs	Even strides. Caudal body sways slightly while walking. Pig is able to accelerate and change direction rapidly
1	As for score 0	As for score 0	As for score 0	As for score 0	Abnormal stride length (not easily identified) Movements no longer fluent. Pig appears stiff but still able to accelerate and change direction
2	As for score 0	As for score 0	May show mild apprehension to boisterous pigs	Uneven posture	Shortened stride. Lameness detected. Swagger of caudal body while walking. No hindrance in pig's agility
3	Bright but less response (may still remain down, or 'dog-sitting', before eventually rising)	Often last to leave pen	Apprehensive to boisterous pigs (usually remains separate from group activity)	Uneven posture. Will not bear weight on affected limb (appears to be standing on toes)	Shortened stride. Minimum weight-bearing on affected limb. Swagger of caudal body while walking. Will still trot and gallop
4	May be dull (only rises when strongly motivated)	Unwilling to leave familiar environment	Will try to remain separate from others within the group	Affected limb elevated off floor. Pig appears visibly distressed	Pig may not place affected limb on the floor while moving
5	Dull and unresponsive	No response	May appear distressed by other pigs in the group but may be unable to respond	Will not stand unaided	Does not move

* **Bold type** identifies the defining criteria that must be present to assign a score. Normal type shows supporting criteria that are useful for assigning a score.

**Fig. 10.2** An example of a visual analogue scale: severity of lameness in sheep (reduced). (Source: Welsh *et al.*, 1993.)

The visual analogue scale

The visual analogue scale (VAS) uses a straight line, usually 10 cm long, the extreme limits of which are marked with perpendicular lines. Both ends have a verbal description of each extreme of the variable that is being measured; *Figure 10.2* is an example of a VAS for recording severity of lameness in sheep. The observer marks the line in a position corresponding to the 'severity' of the variable that is being evaluated, and its position from the zero point is measured with a ruler (usually to a precision of 1 mm). The VAS is commonly used in human medicine to assess pain, and has been similarly applied in veterinary medicine

(Reid and Nolan, 1991; Lascelles *et al.*, 1997; Holton *et al.*, 1998; Thornton and Waterson-Pearson, 1999; Kent *et al.*, 2004; Matičić *et al.*, 2010)¹.

Ordinal (numerical rating) and visual analogue scales are relatively simple, non-invasive ways of recording the severity of lesions and clinical signs, and so are attractive options for recording changes

¹ The assessment of pain is complex, and there are differences between the veterinary and human fields (for veterinary and human definitions of pain, see Molony (1997) and IASP (2012), respectively). In the former, assessment is, of necessity, based on observations, whereas, in the latter, it includes subjective assessment and emotional responses by the affected individual (Melzack, 1983, 1987; Bennett, 2001). There are, however, similarities between assessment in infants and animals, where, in the absence of speech, physiological and behavioural changes may be used as indicators (Anand and Craig, 1996; Hardie, 2000; Holton *et al.*, 2001). For concise discussions of scales for measuring pain in animals, see Ashley and others (2005), Bufalari and others (2007) and Landa (2012).

in patients' status in clinical trials (see Chapter 17). However, both scales are subjective. For example, the lameness scoring system for pigs (*Table 10.1*), and similar schemes for lameness in poultry (Garner *et al.*, 2002), cattle (Sprecher *et al.*, 1997; Thomsen, 2008) and sheep (Welsh *et al.*, 1993; Angell *et al.*, 2015), are based on individuals' observations and subsequent classification of them (e.g., 'even strides' and 'movements no longer fluent' in *Table 10.1*). Similarly, scoring of gastric lesions in horses may involve 'mild hyperkeratosis' or 'moderate hyperkeratosis' (Murray *et al.*, 1996) and be described as 'superficial' or 'deeper' (MacAllister *et al.*, 1997). Some scoring schemes may be particularly complex; for example, canine hip dysplasia (Dennis, 2012) and stress (María *et al.*, 2004). Different observers therefore may put a different interpretation on what they see. Consequently, the accuracy and repeatability (see later in this chapter) of these scales are complex, and may be influenced by the experience of the observer (Kent *et al.*, 2001). Repeatability can vary along the length of both a VAS and an ordinal scale, with consistency logically more likely at the extremes (Plant, 2007). Welsh and others (1993) demonstrated good correlation² between the two scales, and between repeated measurements on the same animals, when assessing lameness in sheep. Main and others (2000) also found the repeatability of the ordinal scoring scheme given in *Table 10.1* to be high, when undertaken by observers who were familiar with it. However, repeatability may be poor with untrained observers such as uninstructed owners of animals (Hielm-Björkman *et al.*, 2011). Additionally, when less-subjective systems for assessing lesions are available, they have been shown to be superior to numerical rating scales and VASs (Quinn *et al.*, 2007).

These scales of measurement have been considered in detail because of their importance in determining appropriate statistical methods. Most statistical tests can be used with interval and ratio scales. However, not all tests can be applied to nominal, ordinal and visual analogue scales. This point will be expanded in Chapter 14.

Composite measurement scales

The measurements described so far either comprise objective, single values (e.g., weight) or single

² Note, however, that *correlation* is not the same as *agreement* (see Chapter 20).

Table 10.2 REQ* scoring for assessing pain in lambs. (Source: Molony, *et al.*, 2002. Reproduced with permission of Elsevier.)

Behaviour	Description
Restlessness	Number of times a lamb stood up and lay down; each unit scored included both the act of rising and lying down. Instances of the lamb rising as far as its knees were included in the count
Rolling	Lamb rolled from lying on one side, to the other without getting up. Half rolls where the lamb rolled on its back and then returned to lying on the same side were included
Jump	Lamb moved forward using 'bunny hops' with its hindlimbs
Foot stamping and kicking	One action was recorded when either a frontlimb or hindlimb (usually hindlimb) was lifted and forcefully placed on the ground while standing or was used to kick while standing or lying
Easing quarters	One action was recorded each time a frontlimb or hindlimb, including the shoulder and hindquarters, was moved in a less forceful manner than stamping or kicking, or the whole body was shifted or eased without moving from the place of rest; tensing of leg muscles was also included. Stretching with the forelegs forward and with the hindlimbs back was recorded separately and included in this category

* REQ = Restlessness...Easing Quarters.

subjective values based on several qualitative criteria (e.g., the lameness scoring scheme given in *Table 10.1*). Scales also may be devised by numerically combining nominal, ordinal, and interval and ratio data; these are termed **composite measurement scales** (CMSs).

Table 10.2, for example, summarizes the components of a CMS for acute pain in lambs following castration and tail-docking. The scale – termed REQ scoring (named from the initial letters of its first and last components) – sums the frequency of observation of various behavioural characteristics over a period of time (usually 30–60 minutes) after the procedures were undertaken.

Hielm-Björkman and her colleagues (2003, 2009) describe a CMS for assessing pain associated with chronic osteoarthritis in dogs (the 'Helsinki chronic pain index'), based on behavioural characteristics (e.g., 'mood', tail wagging, aggressiveness) and locomotor attributes (e.g., walking, jumping, movement after rest).

There is a danger that CMSs may be constructed somewhat arbitrarily, without evaluation of their properties – notably: (1) **content validity**: do they capture all aspects of the characteristic that they purport to assess (e.g., pain); (2) **construct validity**: do they adequately reflect the qualities of the characteristic³; and (3) **sensitivity to change**: do they reflect changes over time in response to clinical interventions? (Streiner and Norman, 2008)⁴.

Assessment of these characteristics of a CMS and reliability is complex (Coste *et al.*, 1995). The validity of the REQ CMS, for example, was judged by assessing the extent to which its scores correctly allocated lambs to different treatment groups intuitively ranked according to severity (e.g., bilateral castration and tail-docking with rubber rings was assumed to induce more pain than bilateral castration alone). The validity was deemed to be high, allowing this CMS to be used subsequently in a clinical trial of different techniques of castration and tail-docking (Kent *et al.*, 2004). If such CMSs for pain, based on observed behavioural changes, are valid, they offer advantages over VASs, many of which require the development of considerable assessment skills (Firth and Haldane, 1999) and have not had their reliability assessed (Holton *et al.*, 1998).

The variety of measurement scales that can be used may make it difficult to make comparisons between various studies addressing the same issue; notably, in meta-analysis (see Chapters 17 and 19). *Table 10.3*, for example, lists the scales of measurement that have been used to assess efficacy of vaccination against mycoplasmal pneumonia in pigs, where lung lesions were used as the main indicators of resistance following challenge with the microbe (either in the field or experimentally). It is difficult to match an ordinal score, both with a percentage (i.e., from 0 to 100) and a nominal categorization, all of which are used as scales of measurement (either at the

Table 10.3 Scales for recording lung lesions in some vaccination trials against *Mycoplasma hyopneumoniae*.

Measurement scale	Source
Ordinal: lung-lesion score	Dawson <i>et al.</i> (2002)
Continuous: percentage of pigs with maximum lung-lesion scores	Petersen <i>et al.</i> (1992)
Nominal: 'ongoing', 'healing' or 'healthy'	Wallgren <i>et al.</i> (1998)
Continuous: percentage consolidation of lungs	Ristow <i>et al.</i> (2002)
Continuous: percentage of pigs with pneumonic lesions	Pommier <i>et al.</i> (2000)

individual-animal or herd level) in the various studies listed in *Table 10.3*.

Data elements

Nomenclature and classification of disease

Nominal data relating to disease frequently include the names of diseases. The name given to a disease is closely associated with the way in which a disease is classified. Diseases are defined at three levels (*Figure 10.3a*) in relation to:

1. specific causes;
2. lesions or deranged functions;
3. presenting problems.

Diseases are generally named according to their allocation to one of these three levels; for example, parvovirus infection (specific cause), hepatitis (lesion) and ataxia (presenting problem). A fourth method of naming involves the use of eponyms; for example, Rubarth's disease and Newcastle disease.

The situation is frequently more complicated: a presenting problem may have more than one set of lesions and specific causes (*Figure 10.3b* and *c*). Similarly, one specific cause may produce more than one lesion and therefore more than one presenting sign (*Figure 10.3d*).

Veterinarians usually define (and therefore record) disease as a diagnosis in terms of a combination of specific cause, lesion and presenting problem. Sometimes one or more levels may be missing; thus, the dilated pupil syndrome in cats (feline dysautonomia) is defined in terms of lesions and presenting problem, although the specific cause is unknown (Barone,

³ These terms are more formally defined: (1) **content validity** – the extent to which a measurement incorporates the domain of the phenomenon under study; and (2) **construct validity** – the extent to which a measurement corresponds to the theoretical concepts (constructs) concerning the phenomenon under study (Porta, 2014). Some authors (e.g., Keszei *et al.*, 2010) additionally describe **face validity** – the extent to which a scale is subjectively viewed by experts (e.g., veterinarians) as covering the domain that is being investigated; it is therefore similar to content validity.

⁴ Validity, sensitivity to change, and repeatability are key components of the **psychometric** approach to measuring phenomena. This originated in psychology and psychiatry to measure abstract concepts such as intelligence and personality (Coaley, 2010), and now is used widely in measuring chronic pain in humans and animals (Reid *et al.*, 2013).

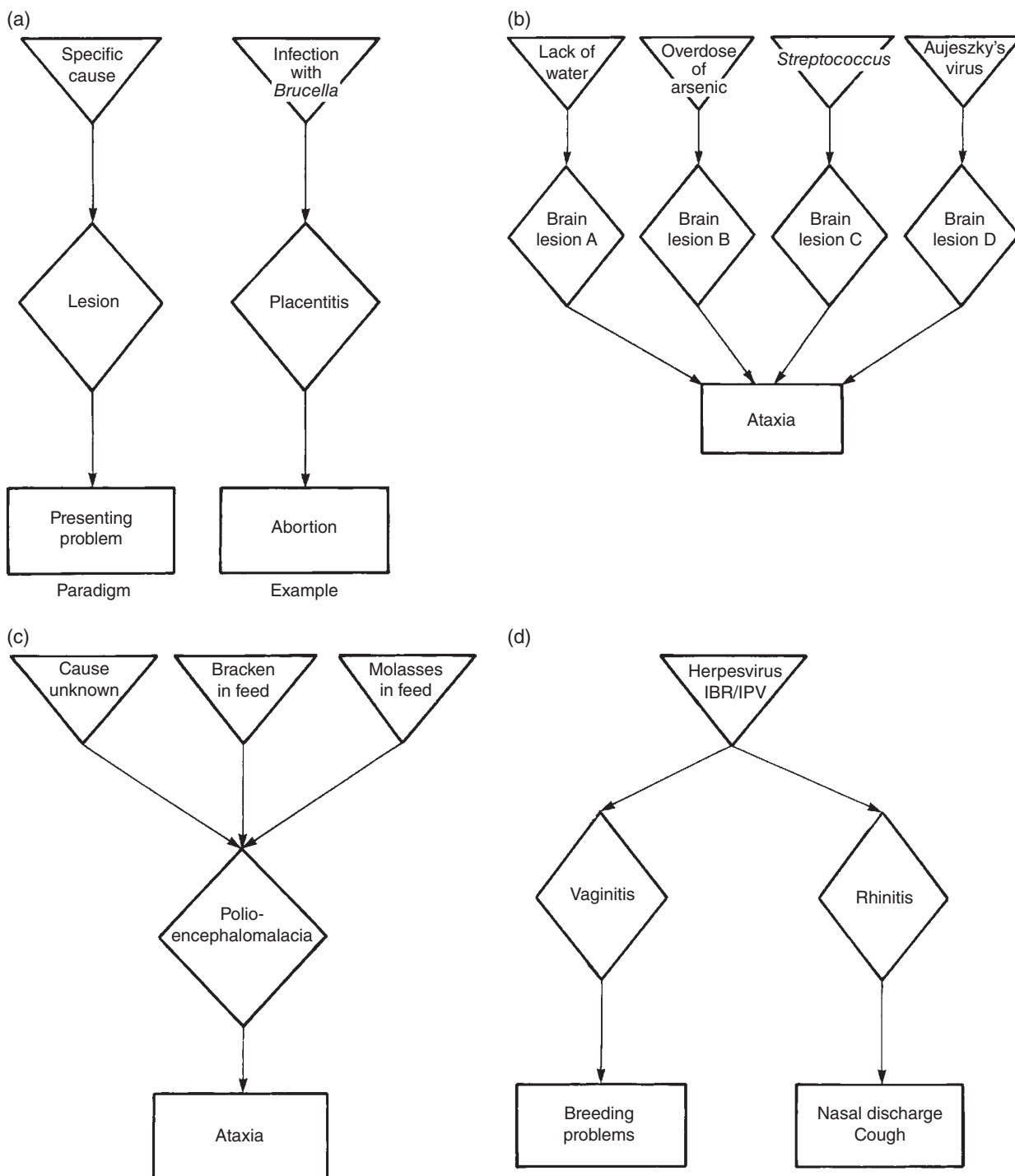


Fig. 10.3 (a) The three levels of disease classification. (b) A presenting problem common to multiple sets of lesions and specific causes. (c) A presenting problem and a lesion common to multiple specific causes. (d) A specific cause with divergent sets of lesions and presenting problems. In terms of causal model 1 (see Chapter 3), a specific cause represents a major component of a sufficient cause. ((a) Modified from Hall, 1978. (b) (c) and (d) From Hall, 1978.)

2012). Initially this disease was eponymously named the 'Key–Gaskell syndrome' (Gaskell, 1983).

The significance of the nomenclature and classification of disease in epidemiological investigations

Different sectors of the veterinary profession require different types of information for their work. Pathologists have a major interest in lesions. When notifiable diseases occur, administrators act according to sets of rules that are defined by legislation; the classification of these diseases is of little consequence to the action that is taken. Similarly, epidemiologists who undertake surveys of morbidity that do not involve the testing of a causal hypothesis are concerned only with identification of a disease, irrespective of the way in which it is classified or named. However, the method of classifying disease is important in causal investigations.

The classification shown in *Figure 10.3* can be simplified to two methods:

1. by **manifestations**, namely signs and lesions;
2. by **cause**.

An epidemiological causal investigation attempts to detect associations between causal factors and disease. The investigation therefore is conducted because one or more component causes are not known. In such a case, the disease that is being studied is frequently named according to manifestations. For example, considering *Figure 10.3b*, if the disease being studied were defined as ataxia (a presenting sign which is a manifestation), then ataxic animals would be heterogeneous with respect to cause: four separate sufficient causes, with different component causes (see Chapter 3), could be involved. This would make causal inference more difficult than if there were only a single sufficient cause that was common to all animals classified as diseased. Similarly, in *Figure 10.3c*, if the disease was defined as polioencephalomalacia (a lesion, which is a manifestation), then the animals with the lesion also could be heterogeneous with respect to cause. Classification by cause in *Figure 10.3a* assists causal inference, because a common sufficient cause may be demonstrated in all cases of animals that are classified as diseased.

Frequently, only suboptimal data relating just to manifestations (often just signs) are available, and they must be used with due regard to their defects, or as the basis of more detailed investigations. Russell and others (1982), for example, were aware that lameness (a datum defining disease in terms of presenting signs, and frequently involving several lesions and causes) was a problem in British dairy herds, and used this as the starting point for an investigation in which they

attempted to elucidate the underlying lesions and causes, by means of a questionnaire, before trying to develop suitable control strategies.

Diagnostic criteria

Disease can be diagnosed using one or more of four criteria:

1. clinical signs and symptoms⁵;
2. detection of specific agents;
3. reactions to diagnostic tests;
4. identification of lesions.

Observational and interpretive data

Records relating to the four diagnostic criteria may be of either **observations** or **interpretations** (Leech, 1980). The terms overlap in that observations usually imply a comparison with the normal, which itself is often a matter of judgement. For example, to observe and record that a cow is diarrhoeic implies that its faeces are less firm than those of its peers, and must also take into account that cows at peak yield, receiving a lot of concentrate, usually have loose faeces.

A clinical sign may represent an observation; for example, nasal discharge. A diagnosis represents an interpretation of one or several observations. Distemper, for example, is a diagnosis that implicitly involves the interpretation of several signs, including, perhaps, nasal discharge, diarrhoea and coughing.

Specific agents may be observed; for instance, species of *Babesia* may be identified in a blood smear. Alternatively, the presence of agents may be recorded interpretatively; for example, the presence of *Escherichia coli* in the faeces of an animal with diarrhoea may be recorded as being 'not clinically significant'.

Test reactions may be observed; for example, the recording of antibody titres in serological tests. Results also may be interpreted; for example, the result of an intradermal tuberculin test may be recorded as 'inconclusive'.

Pathological lesions may be observed; for example, an histological description of a skin tumour may be given. This description may then be interpreted as a diagnosis; for example, defining a skin tumour as equine sarcoid.

Interpretation involves the use of currently acceptable diagnostic criteria. The recording of 'distemper', mentioned above, assumes a causal association between a particular virus and several signs. This

⁵ A sign is an abnormal feature of the patient that is observed; a symptom is a subjective abnormal feature that is described by the patient himself in human medicine.

assumption, based upon previous experimental work and many field observations, has been acceptable for many years. Similarly, the recording of *E. coli* as being 'not clinically significant' in some diarrhoea cases uses a criterion based upon current knowledge of commensalism.

Sometimes, however, diagnostic criteria may be difficult to define; for example, when the histological appearance of a tumour can vary considerably (e.g., equine sarcoid: Ragland *et al.*, 1970). Additionally, criteria may be complex and subject to change. Thus, navicular disease has historically had several criteria for its diagnosis, including heel pain, soundness following palmar digital nerve block, and radiological evidence of enlarged vascular foramina, cystic formations, thinning of the flexor cortex, marginal osteophytosis and remodelling of the normal shape of the bone (McClure, 1988). However, many conditions causing heel pain can be blocked by palmar digital nerve block, and studies suggest that there is no difference between the frequency of radiographically identified lesions of navicular disease in clinically affected animals and animals with no history of lameness (Turner *et al.*, 1987).

An observational datum is easily recorded in full. However, it is difficult to record an interpretative datum fully; this would require not only the interpretation (e.g., a diagnosis) but also a record of the criteria used in the interpretation. In many cases, the criteria are implicit, as in the case of distemper, and so are not recorded explicitly. In others, where diagnostic criteria are complex (e.g., navicular disease), the criteria should be listed, otherwise comparison between cases (past, present and future) is impossible.

Observational and interpretative data are applied in different ways. Veterinary administrators, organizing national disease-control campaigns, frequently use interpretative data to make decisions. For example, an animal's future may depend upon whether it reacts 'negatively', 'inconclusively' or 'positively' to screening tests.

Epidemiological investigations, particularly of diseases of unknown aetiology, require data – such as details of diet, exposure to possible causal agents, and the various stages of a disease's pathogenesis – that are unambiguous. In this context, interpretative data may be misleading. Thus, when investigating obesity, it is better to have an approximate estimate of an animal's body weight and food intake, than to have a subjective (interpretative) impression of its weight as 'heavy' or 'light', and its food intake as 'a little' or 'a lot', because these terms may represent different weights and amounts of food to different people. Observational studies also require an unambiguous,

consistent definition of cases, and so the diagnostic criteria used to classify animals as diseased and healthy should be specified.

Sensitivity and specificity

Events may be recorded as being true when, actually, they are not. Thus, a dog may be diagnosed as having diabetes mellitus when it does not. This constitutes a **false-positive** record, and renders the diagnosis inaccurate. The error in this case may have resulted from an improper inference based upon only a few clinical signs such as polyphagia and polydipsia, with no supporting biochemical evidence. Alternatively, diabetes may not be diagnosed when it is actually present. This constitutes a **false-negative** record. Such errors inevitably lead to misclassification of 'diseased' and 'non-diseased' animals. These errors can occur when using clinical signs, detection of specific agents and reactions to diagnostic tests as diagnostic criteria.

These errors can be quantified by comparing results obtained by the diagnostic method with those obtained from an independent valid criterion. For example (Table 10.4), the validity of a faecal centrifugation/flotation technique in diagnosing equine cestodiasis can be determined by comparing the results of the test with those obtained from post-mortem examination of the intestinal tract to identify tapeworms (the independent valid criterion).

The **sensitivity** of a diagnostic method is the proportion of true positives that are detected by the method. The **specificity** of the method is the proportion of true negatives that are detected. Sensitivity and specificity can be quoted either as a probability between zero and one, or as a percentage. Thus, the sensitivity and specificity of the centrifugation/flotation technique are 0.61 and 0.98, respectively; that is, 61% and 98%, respectively.

Table 10.4 Possible results of a diagnostic test exemplified by application of a centrifugation/flotation technique to horses of known tapeworm status. (From Proudman and Edwards, 1992.)

Test status	True status		Totals
	Positive	Negative	
Flotation-positive	22(a)	1(b)	23
Flotation-negative	14(c)	43(d)	57
Totals	36	44	
Sensitivity = $a/(a + c) = 22/36 = 0.61$ (61%)			
Specificity = $d/(b + d) = 43/44 = 0.98$ (98%)			

The sensitivity and specificity of a diagnostic test are important in deciding the value of the test in disease surveillance (see Chapter 21), control campaigns (see Chapter 27) and in categorizing animals in observational studies (see Chapter 15). Diagnostic tests are considered in more detail in Chapter 20.

Accuracy, refinement, precision, reliability and validity

These terms can be used in relation to qualitative data (e.g., the description of a disease) and to quantitative measures (e.g., of prevalence and weight).

Accuracy

Accuracy is an indication of the extent to which an investigation or measurement conforms to the truth. Thus, if a set of scales records an animal's weight at 15 kg and this is the actual weight of the animal, then the measurement is accurate.

Refinement

The degree of detail in a datum is its **refinement**. Thus, 13 and 13.781 kg may both represent the accurate weight of an animal, but the second record is more refined than the first. Similarly, 'orthopaedic surgery' is a less refined description of a surgical technique than is 'bone plating'. In *Figure 10.4*, 'otitis externa' is a less refined diagnosis than either 'otitis externa caused by bacteria' or 'otitis externa caused by *Pseudomonas* spp.'. The less refined definition may be described as 'coarse grained', and the more refined definitions as 'fine grained' by analogy with photographic film (Cimino, 1998). Specificity is sometimes used synonymously with refinement.

Increasing the refinement of descriptive diagnostic data may improve their epidemiological value because it usually moves towards a definition of disease in terms of particular sufficient causes, whereas a less refined definition in terms of manifestations can include several sufficient causes (*Figure 10.3c*).

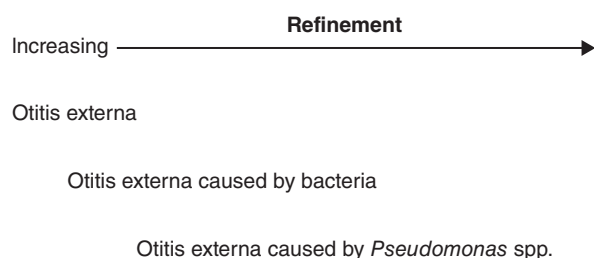


Fig. 10.4 Example of increasing refinement of a diagnosis.

Auxiliary tests frequently are required to increase the refinement of a diagnosis. Thus, physical examination may facilitate the diagnosis of otitis externa, but a more refined diagnosis of otitis externa involving *Pseudomonas* spp. infection would require the use of microbiological techniques. The refinement of auxiliary techniques also may vary. For example, the complement fixation test will detect types of influenza A virus, but will not detect subtypes. Identification of the latter requires more refined tests; for example, haemagglutination inhibition.

Precision

Precision can be used in two senses. First, it can be used as a synonym for refinement. Secondly, it can be used statistically to indicate the consistency of a series of measurements. Thus, repeated sampling of a population may allow estimation of a prevalence value of, say, $40\% \pm 2\%$. Alternatively, the value may be estimated as $40\% \pm 5\%$. The first estimate is more precise than the second. Precision, in the second sense, is discussed in more detail in Chapter 12.

Reliability

A diagnostic technique is **reliable** if it produces similar results when it is repeated. Thus **repeatability** is a characteristic of a reliable technique. This is defined in terms of the degree of agreement between sets of observations made on the same animals by the same observer (see Chapter 20). This contrasts with **reproducibility**, which can be defined in terms of agreement between sets of observations made on the same animals by **different** observers: BSI (1979)⁶.

Validity

If a diagnostic technique measures what it purports to measure, it is **valid**. Validity is a long-term characteristic of a technique, of which sensitivity and specificity are indicators. The validity of a technique depends

⁶ Everitt (1995) gives a fuller definition of repeatability as 'The closeness of results obtained in the same test material by the same observer or technician using the same equipment, apparatus and/or reagents over reasonably short intervals of time'; and reproducibility as 'The closeness of results obtained in the same test material under changes of reagents, conditions, technicians, apparatus, laboratories, and so on'. However, the terminology in this area is subject to variation in usage; Porta (2014), for example, draws no distinction between repeatability and reproducibility. The terms **ruggedness** and **robustness** also are encountered. Most opinions define robustness as a measure of the resistance of a test to being affected by physical factors, and ruggedness as a measure of how well a test performs under a wide variety of operational conditions (Crowther *et al.*, 2006).

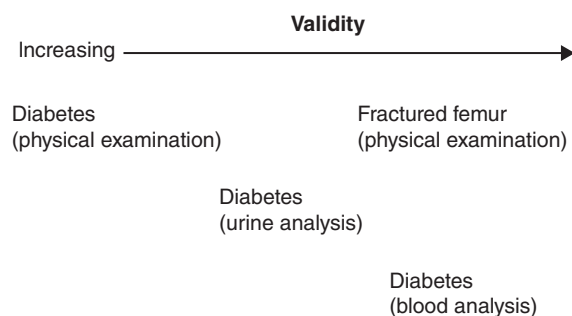


Fig. 10.5 Validity of diagnostic techniques in relation to the disease being studied.

upon the disease that is being investigated and the method of diagnosis. A midshaft femoral fracture, for example, may be diagnosed very accurately when using only physical examination (*Figure 10.5*): the lesion is rarely misdiagnosed; thus physical examination, as a diagnostic technique, is highly valid in this instance. However, physical examination alone may not be considered sufficiently error-free when used to diagnose diabetes mellitus. Biochemical examination, in this case urine analysis, may be used to decrease error, and fasting blood sugar estimation to decrease it further. The use of auxiliary diagnostic aids, such as biochemical, radiological and microbiological investigation, is simply a means of increasing the accuracy of a diagnosis by selecting diagnostic techniques of higher validity.

The value of a diagnostic technique is judged in terms of its **reliability** and **validity**. This can be exemplified using target shooting as an analogy (*Figure 10.6*). In target A, each shot is accurate (i.e., close to the bull's-eye, which represents the true value); therefore validity and repeatability are high. In target B, none of the shots is accurate, but the result is repeatable. In target D, none of the shots is accurate, and reliability is low because the shots are inconsistent with one another. Target C illustrates that, *on average*, it is possible to obtain high validity when the accuracy of individual shots is low. Thus, high validity corresponds to shots, on average, hitting the bull's-eye, whereas high reliability corresponds to shots being tightly packed.

Although there has been ambiguity in the use of these five terms (Porta, 2014), accuracy is best considered as the property of a single diagnosis (i.e., of one shot at the bull's-eye) whereas validity should be considered as a long-term characteristic of a diagnostic technique (i.e., the average result of several shots).

Sensitivity, specificity and reliability are discussed further in Chapter 20.

Bias

The shots fired at target B in *Figure 10.6* are not accurate, but they are repeatable. Thus, the results are reliable, but of low validity. In this example, the results may have occurred because the sights on the gun were **biased** to the right of the centre of the target. A similar bias can occur in diagnostic tests and epidemiological studies. Bias is any systematic (as opposed to random) error in the design, conduct or analysis of a study that renders results invalid.

Several types of bias can be identified (Porta, 2014). Major ones are:

- **bias due to confounding** (outlined in Chapter 3; detailed in Chapters 15 and 18);
- **information (observational) bias**, involving misclassification of animals as diseased and non-diseased, or exposed and not exposed to a factor, when comparing groups;
- **interviewer bias**, where an interviewer's opinions may affect accurate reporting of data;
- **measurement bias**, involving systematic errors in measurement;
- **selection bias**, where animals selected for study have systematically different characteristics from those that are not selected for study; for example, animals selected from an abattoir are unlikely to have clinical disease, whereas the general population will include some clinically diseased individuals.

Bias is a *long-run* effect. Any individual shot in target B could have been one of those in targets C or D. Only when all of the shots have been fired can the bias be detected, because it requires demonstration of reliability. Thus, an individual observation from a population cannot be detected as being biased. Similarly, if many samples produce repeatable inaccurate results, then bias is present in the *population* from which the sample was drawn.

Bias can be corrected if its extent is known. Thus, the sights on the gun can be adjusted to compensate for the bias; shots will then be accurate. Similarly, biased estimates of prevalence, resulting from a test's specificity and sensitivity being less than 100%, can be corrected if the sensitivity and specificity are known (see Chapter 20).

Representation of data: coding

Data are usually represented by both words and numbers. An alternative type of notation involves **coding**. This is a means of representing text and numerals in a standardized, usually abbreviated, form. The coding of

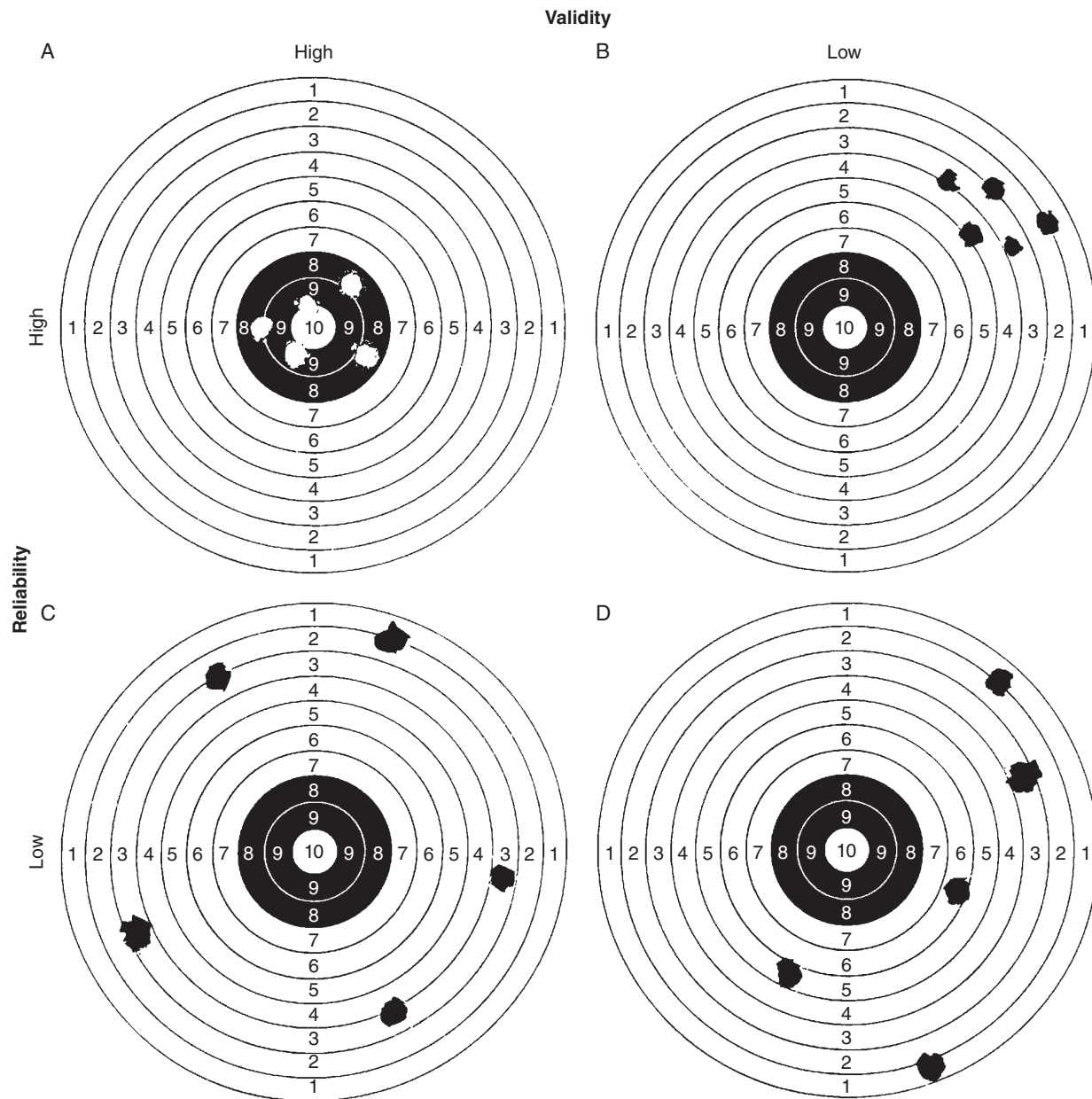


Fig. 10.6 Reliability and validity of diagnostic tests presented as analogues of target shooting.

medical information has spanned over two centuries, having its origins in the 18th-century disease classification system of John Graunt (see Chapter 1), the *Nosologia Methodica* of Francois Bossier de Lacrois, and the *Synopsis Nosologiae Methodicae* of William Cullen (Gantner, 1977). Momentum increased in the 1960s and 1970s when computers became available. Codes are easier and more economic to handle by a computer than is plain text and so offer advantages to computerized recording systems (see Chapter 11). Thus, it is easier and quicker to record the code number 274 than to write its possible textual equivalent,

'contagious bovine pleuropneumonia'. More recently, advances in software and computer storage capacity have made the efficiency of codes less critical than hitherto. However, coded data remain of value; for example, in statistical packages that require manipulation of numerically coded values.

Code structure

Data about animals divide into two groups; any system of coding must cope with both groups. The first includes categories that relate to **permanent**

(time-invariant) data such as details of species, breed, date of birth and sex. These data have been termed 'tombstone data' because they remain unchanged during the whole of an animal's life. The second group comprises data relating to events that **vary** during life, such as date of occurrence, lesions, test results, signs and diagnoses. The various categories sometimes are called **descriptors** or **specifier types**. Other data can be derived from these categories; for example, the animal's age at the onset of disease.

Components

The data that define disease can comprise a single component, called an **axis**; for example, 'bronchiolitis'. Alternatively, the definition can be broken down into constituent parts. In the case of a lesion, two convenient axes are the underlying pathological process and its site (topography). Using this **biaxial** system (two axes), bronchiolitis can be recorded as 'inflammation' and 'bronchiole'. Similarly, surgical procedures can be coded biaxially, in terms of procedure and site; for example, 'intramedullary pinning' and 'femur'. It therefore is possible to build up disease definitions from basic components in various axes.

Numeric codes

Numeric codes represent text by numbers; for example, seborrhoea = 6327. Most of the early veterinary and medical coding systems were numeric. An early veterinary system, developed in North America, was the *Standard Nomenclature of Veterinary Diseases and Operations* (SNVDO: Priester, 1964, 1971), which was based on the medical *Standard Nomenclature of Diseases and Operations* (SNDO: Thompson and Hayden, 1961). This coded diseases and operations (treatments) biaxially: diseases by topography and either aetiology or lesion, and operations by topography and procedure. Examples are:

diagnosis: 3530 3900.0 = bronchiolitis due to allergy
treatment: 723 52 = ureteric anastomosis.

In these examples, the diagnosis includes a topographical part (3530 = bronchiole) and an aetiological part (3900.0 = allergy). Similarly, treatment is defined in terms of topography (723) and procedure (52).

This system became largely obsolescent in the early 2000s.

Consecutive and hierarchic codes

A **consecutive** code is one in which consecutive numbers are drawn up to represent data; for example, 001 = distemper, 002 = infectious hepatitis, 003 = acute cystitis.

Table 10.5 An example of hierarchic numeric codes.

	Code	Meaning
Treatment	100	General medical therapy
	110	Antibiotic
	112	Oxytetracycline
	120	Parasiticide
	122	Thiabendazole
Species and breed	100	Horse
	110	Pony
	111	Welsh mountain pony
	120	Warm-blooded
	121	English thoroughbred
	200	Dog
	300	Cat
	400	Cow
	410	Friesian

It also is possible to draw up a list of codes with a **hierarchic** structure; that is, with initial digits representing broad categories, and succeeding digits indicating more refined categories; the more digits used, the more refined the definition. An example is given in *Table 10.5*, and its hierarchic structure, resembling the roots of a tree, is illustrated in *Figure 10.7*. The use of initial digits alone produces 'coarse grain' definitions; use of additional succeeding digits produces 'fine grain' (i.e., more detailed) definitions.

There are three advantages to hierarchic codes. First, if accurate auxiliary diagnostic techniques are not available and therefore refined diagnoses are not definable, then a coarse grain code may be used without succumbing to the temptation of offering a more refined diagnosis than can be substantiated. Secondly, the individual interests of data collectors can be accommodated. For example, someone with a specialist interest in chemotherapy may be able to record the use of a particular antibiotic, whereas those who are content with recording just that an antibiotic has been used may record in the coarser grain. Thirdly, most computerized recording systems allow flexible querying at various levels of a hierarchy using a 'wild card' facility. This uses a character which, when positioned on the right of specified codes, indicates that the codes specified on the left of it, and *all* codes on its right, are to be selected. For instance, if '%' is the wild card character, and a query is conducted on the treatment codes in *Table 10.5*, thus:

'select 1%',

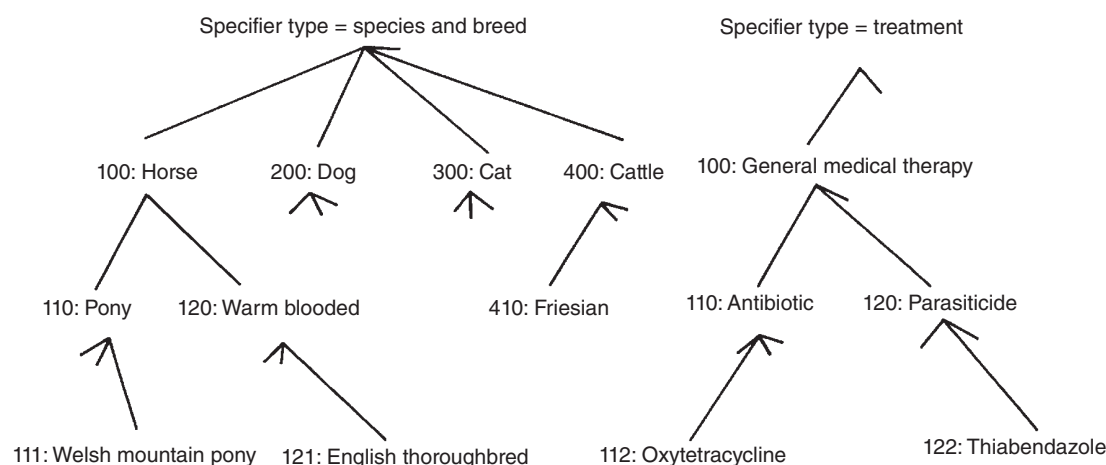


Fig. 10.7 Diagrammatic representation of a hierarchic code (code listed in Table 10.5).

then all records of medical therapy at all levels of the hierarchy would be selected (all antibiotics, all parasiticides, etc). In contrast, if '12%' were specified, only all parasiticides would be selected.

In addition to recording qualitative data, it is possible to use codes to record quantitative data such as test results. There is some loss of refinement compared with the use of the results themselves because coding often involves the grouping of individual values into ranges of values (blocks). Table 10.6, for example, illustrates the numeric codes for ranges of numbers of helminth eggs isolated from faeces. Some test results are already blocked, although the blocking is often concealed by the mode of expression. Thus, a serological test quoted as positive at a dilution of 1:1024 implies that it was negative at 1:1025, whereas the next dilution tested that

yielded a negative result was probably 1:2048. Blocking converts interval and ratio data to ordinal data, and this must be considered when selecting statistical methods for analysing the coded data (see Chapter 14).

Alpha codes

Alpha codes represent plain text by alphabetical abbreviations or acronyms, for example: FN = female neuter, M = male.

Table 10.7 lists some components of a hierarchic alpha coding system for diagnoses. It has two axes: location and abnormality. Thus, displaced abomasum is coded DA PD, retained placenta is coded GL PR, and hepatitis is coded DH B.

Table 10.6 An example of numeric coding of quantitative data: frequency codes for numbers of eggs per gram of faeces (epg) for use on a parasitology data report form. (From Slocombe, 1975.)

Code	epg	Code	epg
004	1–500	014	5001–5500
005	501–1000	015	5501–6000
006	1001–1500	016	6001–6500
007	1501–2000	017	6501–7000
008	2001–2500	018	7001–7500
009	2501–3000	019	7501–8000
010	3001–3500	020	8001–8500
011	3501–4000	021	8501–9000
012	4001–4500	022	9001–9500
013	4501–5000	023	9501–10 000

Table 10.7 An example of hierarchic alpha diagnostic codes (two axes). (From Williams and Ward, 1989a.)

Axis 1 Location		Axis 2 Abnormality	
Code	Meaning	Code	Meaning
D	Digestive system	B	Inflammation
DA	Abomasum	E	Exudate
DE	Oesophagus	EBH	Haemorrhage
DH	Liver		
		P	Position
G	Reproductive system	PD	Displaced
GO	Ovary	PL	Dislocation
GU	Uterus	PR	Retention
GL	Placenta	PP	Prolapse
GS	Scrotum		

Table 10.8 An example of alphanumeric diagnostic codes (three axes). (From Stephen, 1980.)

Code	Axis 1 Name of disease	Axis 2 Anatomical system involved	Axis 3 Aetiological agent or other indicated cause
ABO 10	Abomasitis, mycotic	UDIG (upper digestive)	MYCO (mycotic)
BRA 10	Brachygnathia	BOJOS (bones, joints, etc.)	CONGEN (congenital)
ENC 45	Encephalomyelitis, equine, Western	NERVO (nervous)	VIRO (virus)
EPI 10	Epidermitis, porcine, exudative	SKAP (skin and appendages)	BACTE (bacterial)
TOX 64	Seneciosis (including pyrrolizidine poisoning)	LIVBIP (liver and biliary)	TOXO (toxicosis)

Alphanumeric codes

Alphanumeric codes have evolved more recently than numeric codes. An example is given in *Table 10.8*, where the disease is located in three axes: the disease's name, its topography and its aetiology. The first axis classifies the disease according to its name. In the first example in the table, the first (alpha) part of the first axis relates to the broad disease category (abomasitis) and the second (numeric) component produces a 'finer grain' description (mycotic abomasitis); this therefore is a simple hierarchy. The second axis classifies the disease according to the anatomical system that it affects (UDIG = upper digestive system). The third axis classifies the disease according to cause (MYCO = mycosis).

Signs may also be coded alphanumerically. *Table 10.9* illustrates a hierarchic alphanumeric code of signs.

SNOMED

The most widely used multiaxial international coding system is *SNOMED* (*Systematized Nomenclature of Human and Veterinary Medicine*: SNOMED, 2004), which was introduced as an exclusively medical system for pathology reports, *SNOP* (*Systematized Nomenclature of Pathology*), in 1965 in the US. An early version was modified for veterinary use (Cordes *et al.*, 1981), and the *Systematized Nomenclature of Veterinary Medicine* (*SNOVET*), which used six axes – topography, morphology, aetiology, function, disease and procedure (Pilchard, 1985) – subsequently evolved. This was modified and combined with *SNOMED* to form *SNOMED III* (Côté *et al.*, 1993), which, despite shortcomings, is considered a sound base for coding veterinary findings, phrases, terms and concepts (Case, 1994; Klimczak, 1994). Examples of *SNOMED III* codes, which hierarchically occupy nine axes (topography; morphology; function; living organisms; chemicals, drugs and biological products; physical agents,

activities and forces; occupations; social context; and modifying linkages to modify contents of axes) are given in *Table 10.10*.

Diseases are given specificity by capturing the organ, pathological change, causal agent and functional disturbance, thus:

Tuberculosis:

Lung: T 28000

Granuloma: M 44060

Mycobacterium tuberculosis: E 2001

Fever: F 03003.

SNOMED RT (*SNOMED Reference Terminology*) was introduced in 2000 to provide an infrastructure for electronic health recording worldwide, and *SNVDO* codes also have been mapped to *SNOMED RT* (Folk *et al.*, 2002). The most recent version is *SNOMED CT* (*SNOMED Clinical Terms*), which was introduced in 2002 after international collaboration (IHTSDO, 2014). This includes several hierarchies (axes) and sub-hierarchies, and is very flexible, facilitating not only recording of clinically-relevant information but also statistical reporting, public health surveillance, health research and economic analysis. It is also broadly compatible with related systems such as medical and veterinary abstracting databases (Ruch *et al.*, 2008).

VeNom

VeNom is a set of codes for veterinary clinical terms for use in veterinary referral-hospital electronic patient records and first-opinion veterinary practice management systems (VeNom Coding Group, 2014). It was stimulated by interest in clinical coding in

Table 10.9 An example of hierarchic alphanumeric codes for signs. (From White and Vellake, 1980.)

Acoustic sense signs	
A00	Deafness
A01	Complete deafness
A02	Partial deafness
A10	Discharge from ear
A11	Bloody discharge from ear
A12	Purulent discharge from ear
A20	Excess wax/cerumen in ear
A30	Abnormal ear size
A40	Other signs referable to the ear
A41	Mite infestation of ear, parasite, dirt
A42	Odour bad ear
A43	Cold ears
Digestive system signs	
D00	Abnormal appetite
D01	Decreased appetite
D02	Polyphagia – excessive appetite
D03	Anorexia – loss of appetite
D04	Pica – depraved appetite
D10	Difficulty in prehension, cannot get food in mouth
D20	Chewing difficulty
D30	Signs of the jaw
D31	Weakness of jaw
D32	Inability to close jaw
D33	Inability to open jaw
D34	Malformation of jaw
D40	Odour from mouth
D50	Signs referable to the oral mucosa
D51	Bleeding oral mucosa
D52	Ecchymosis oral mucosa
D53	Petechia oral mucosa
D54	Dryness oral mucosa
D55	Cold oral mucosa

primary-care practices (Upjohn *et al.*, 2008). The numeric hierarchic codes were developed by the Royal Veterinary College, London, and the University of Glasgow, and include lists of diagnoses, presenting problems, and administrative tasks related to canine and feline practice. Terms initially were derived from *SNOMED* codes, but subsequently evolved independently because of the perceived complexity of *SNOMED* nomenclature. The main aims of the VeNom Coding Group are to promote and facilitate coding in veterinary practices and other sectors of the veterinary profession.

Symbols

Symbols can be used as codes. Thus, ↑ and ↓ are frequently used to represent elevated and lowered body temperature, respectively. Symbols may be combined with letters such as D+ and D– to indicate the presence and absence, respectively, of diarrhoea. *Table 10.11* lists some examples of symbolic codes used in the international veterinary disease reporting system of the *Office International des Epizooties* (see Chapter 11). These may appear to represent very vague descriptions. However, they reflect the level of knowledge of morbidity in many countries, where reliable, accurate, quantitative data are unavailable. The value of such general terms was noted in the 19th century by a notable medical epidemiologist William Farr: ‘*The refusal to recognize terms that express imperfect knowledge has an obvious tendency to encourage reckless conjecture*’ (see Chapters 1 and 3).

Choosing a code

The choice of a code is partly subjective. Some people find numbers easier to handle; others are more content with letters. There are, however, several definable advantages and disadvantages to numeric and alpha codes.

If acronyms or abbreviations are used (e.g., ABO for abomasum) they may be easier to relate to the plain text data and to remember than numeric codes (Williams and Ward, 1989a).

In some cases, alpha codes, in order to be acronymic, may require more characters than their numeric counterparts, even though 26 alternatives are available for each alpha character, and 36 for alphanumeric, compared with only 10 for numeric systems. This has been an important space consideration in computerized systems, which, in any case, can pack numeric data more tightly than either alpha or alphanumeric strings. The recent expansion in computer storage volume, however, has rendered this less critical than hitherto.

Numeric codes can be entered into computers more quickly than alpha codes. Most computer keyboards have a numeric keypad which can be depressed more quickly than the alphabetic typewriter-style keyboard, especially if the user cannot type.

If a consecutive numeric code is used, care should be taken to ensure that it is long enough to accommodate all definitions of a specifier type. A four-digit code (e.g., 0000 to 9999), can accommodate 10 000 definitions (1000, if the last digit is a ‘check digit’ – see later in this chapter), and then would be ‘full’. If more than 10 000 definitions were required, a five-digit code would have to be used.

Table 10.10 Examples of *SNOMED* codes (topography, morphology and living organisms axes). (From Côté *et al.*, 1993.)

<i>Axis</i>	<i>First-level code</i>	<i>Second-level code</i>	<i>Third-level code</i>
Topography (T)	T01 Skin and appendages	T010–012 Skin appendages	T01100 Epidermis
			T01110 Stratum corneum
			T01120 Stratum lucidum
		T013 Skin appendages	
		T017 Animal skin structures and appendages	
			T01705V Comb T01706V Dewlap
Morphology (M)		M017–018 Eruptions	
			M01720 Maculopapular
			M01780 Erythema
			M01790 Erythematous plaque
Living organisms (L)	L1–2 Bacteria and rickettsiae	L107 <i>Actinomyces</i>	
			L10801 <i>A. bovis</i>
			L10807 <i>A. suis</i>

Table 10.11 An example of symbolic codes. (From FAO/WHO/OIE, 1997.)

<i>Code</i>	<i>Disease occurrence</i>
–	Not reported
?	Suspected but not confirmed
(+)	Exceptional occurrence
+	Low sporadic occurrence
++	Endemic
+++	High occurrence
+?	Serological evidence and/or isolation of causative agent, no clinical disease
+..	Disease exists, distribution and co-occurrence unknown
()	Confined to certain regions
)(	Ubiquitous
!	Recognized in country for the first time
<=	Only in imported animals (quarantine)
...	No information available

If there is a requirement for a variable ‘grain’ in the definition, then a hierarchic code is the most suitable. Alpha codes can be used, although numeric codes are more common. Numeric codes can have 10 categories (0–9) at each level of the hierarchy. Alpha codes, based on the Roman alphabet, can have 26 categories (A–Z) at each level of the hierarchy.

Numeric codes are not subject to language differences. Alpha codes, however, if acronymic, may need to vary according to the language of the user.

When all of these merits are considered, subjective assessment is still a major factor. This is a reason for the considerable range of codes that are available and for the lack of a universally acceptable one.

Error detection

Provision must be made for verifying data during the process of combining them with an existing collection of data. In general, as many checks as possible should be made on each datum, and the criteria used for checking should remain a permanent characteristic of the data that have satisfied the criteria. This is important when combining data from different

sources and when combining data that have satisfied different, perhaps incompatible, sets of criteria.

Consistency

Initially, checks should be made that the data are recognizable, and then that they are internally consistent. Thus, it is illogical to record an elevated body temperature and a lowered body temperature at the same time. Placing the event in the context of what else is known about the animal's life history may reveal other inappropriate data. For instance, the recording of 'dystocia' becomes unacceptable when the animal's existing record shows it to be either male, or juvenile, or to have undergone an ovariohysterectomy some time ago. All of these inconsistencies would suggest that the animal may have been misidentified or that some other data entry error has occurred.

The initial check that the code is recognizable is simple if consecutive codes are employed: the check is that the code that is offered is neither less than the lowest nor greater than the highest code that is in use. Alpha-numeric codes may need to be checked against a list held within the computer containing all permissible codes. Some codes (e.g., *Table 10.8*) use combinations of code elements taken from different lists; for example, representing organs, pathological processes and causes. Each element must be identified and checked against the appropriate list; further checks are required to ensure that any particular combination of organ, pathological process and cause is feasible and, for quantitative data, that extreme values are plausible.

'Finger trouble'

Data that are entered via keyboards (a common form of data entry in computerized systems: see Chapter 11) are subject to four types of error, collectively and colloquially termed 'finger trouble'. These errors are:

1. **insertion**, where extra characters are erroneously added;
2. **deletion**, where characters are omitted;
3. **substitution**, where the wrong character is typed;

4. **transposition**, where the correct characters are typed in the wrong order.

The danger is that the incorrect version may be an allowable code, which means something totally different from the intended code. If the codes have the same number of characters, then insertion and deletion are easily detected because the incorrectly entered code is the wrong length. The likelihood of entry of an incorrect code can be reduced by increasing the 'redundancy' of the code; that is, by adding more characters to the code. Unfortunately, highly redundant codes with many characters require many keystrokes, which itself increases the chance of error. A balance must be found between these conflicting requirements.

Check digits

These are mathematical functions of the preceding digits of the code, for example:

$$5029 = \text{anaemia.}$$

The first three digits (502) are a consecutively ordered code, which relates to anaemia, and the fourth digit (9) is a check digit, which is a function of the code numbers. The check digit, in this case, may have been generated using the formula:

$$(1\text{st digit} \times 2) + \text{second digit} - \frac{\text{third digit}}{2} \\ = \text{check digit.}$$

The computer will not accept 5028 or 5020 because it recognizes that the last digit is not the correct function of 502. Similarly it will not accept 5019 or 5039, which represent two totally different diagnoses. This is therefore a useful validation check against corruption to incorrect codes. (Of course, in this example, 4229 and 4109 would be consistent with the formula used to produce the check digit, but these are so unlike 5028 and 5020 that it is very improbable that they would be entered.)

Further reading

- Abramson, J.H. and Abramson, Z.H. (2008) *Research Methods in Community Medicine*, 6th edn. John Wiley and Sons, Chichester. (Includes guidelines on construction of measurement scales)
- Bowling, A. (2001) *Measuring Disease*, 2nd edn. Open University Press, Buckingham. (A comprehensive review of measurement scales in human medicine, containing useful general principles)

- Cimino, J.J. (1995) Coding systems in health care. In: *Yearbook of Medical Informatics*. Eds van Bommel, J.H. and McCray, A.T., pp. 71–85. International Medical Informatics Association, Rotterdam
- Cimino, J.J. (1996) Review paper: coding systems in health care. *Methods of Information in Medicine*, **35**, 273–284. (A review of coding schemes for patient care, clinical research, decision support, and reporting)

- Cimino, J.J. (2006) In defense of the Desiderata. *Journal of Biomedical Informatics*, **39**, 299–306. (A critique of medical terminology, with particular reference to computerized data recording and retrieval)
- Cornet, R. and de Keizer, N. (2008) Forty years of SNOMED: a literature review. *BMC Medical Informatics and Decision Making*, **8**, Suppl. 1, S2; doi 10.1186/1472-6947-8-S1-S2
- Hall, S.A. (1978) Farm animal disease data banks. *Advances in Veterinary Science and Comparative Medicine*, **22**, 265–286. (Includes a discussion of disease nomenclature)
- Keszei, A.P., Novak, M. and Streiner, D.L. (2010) Introduction to health measurement scales. *Journal of Psychosomatic Research*, **68**, 319–323
- Leech, F.B. (1980) Relations between objectives and observations in epidemiological studies. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 7–11 May 1979. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 254–257. Australian Government Publishing Services, Canberra
- McDowell, I. (2006) *Measuring Health: A Guide to Rating Scales and Questionnaires*, 3rd edn. Oxford University Press, Oxford
- Stanfill, M.H., Williams, M., Fenton, S.H., Jenders, R.A. and R Hersh, W.R. (2010) A systematic literature review of automated clinical coding and classification systems. *Journal of the American Medical Informatics Association*, **17**, 646–651. (A review of reviews of medical coding systems)
- Streiner, D.L. and Norman, G.R. (2008) *Health Measurement Scales: A Practical Guide to their Development and Use*, 4th edn. Oxford University Press, Oxford. (Includes a discussion of the ‘philosophy’ of validity and reliability)

11

Data collection and management

This chapter focusses on some principles and methods of data collection, storage and distribution as useful information¹. Emphasis will be placed on the suitability of the techniques to epidemiological studies, although some of the standard methods of clinical case recording will be outlined. Finally, some examples of veterinary information systems will be given. The collection of veterinary data in the context of disease surveillance is discussed in Chapter 21.

Data collection

Methods of data collection

Data are collected in three main ways, by:

1. **observation** (e.g., clinical examination, diagnostic imaging and post-mortem examination);
2. completing **questionnaires** (either directly or by interview);
3. use of **documentary sources** (e.g., clinical records, and records of diagnostic laboratory results), with an increasing use of data sets generated by other workers².

Data derived from the first two methods are **primary**, whereas data obtained by the third method are **secondary**.

Observation is central to the practice of clinical veterinary medicine, and is also important in many epidemiological investigations (e.g., outbreak investigation: see Chapter 27). Additionally, surveillance (see Chapter 21), surveys (see Chapter 13) and observational studies (see Chapter 15) may use secondary data. However, there are occasions when the appropriate

information is not readily available, in which case it must be collected using questionnaires.

Questionnaires

A questionnaire is a set of written questions. The person who answers the questionnaire is termed the **respondent**.

Structure of a questionnaire

The questionnaire is designed to record information:

- in a standard format;
- with a means of checking and editing recorded data;
- by a standardized method of questioning;
- with a means of coding.

Questions may be either **open-ended** or **closed**.

Open-ended questions These allow the respondent freedom to answer in his or her own words; for example, '*What is your opinion of intramammary preparation X?*' The chief advantage of the open question is the freedom of expression that it permits: the respondent is allowed to comment, pass opinions and discuss other events that are related to the question's topic. The disadvantages are, first, that open questions can increase the length of time taken to complete a questionnaire and, secondly, that the answers cannot be coded (see Chapter 10) when the questionnaire is designed, because the full range of answers is not known. A range of answers may be difficult to categorize and code. Continuous variables can be grouped into intervals (e.g., 0.0–1.9 kg, 2.0–2.9 kg) for coding.

Closed questions Closed questions have a fixed number of options of answers. **Numerical** formats require the respondent to enter an appropriate number; for example, '*What is the age of your dog (to the nearest year)?*' A **dichotomous** format requires one of only two possible answers, such as '*Do you use intramammary tube X for dry cow therapy (answer yes or no)?*'

¹ Methods of structuring, physically storing and ordering records have been described in detail elsewhere (Duppong and Ettinger, 1983; Thrusfield, 1985b; Nelson and White, 1990; van Sluijs and van Nes, 2009).

² Donnellan and Lucas (2013) discuss the analysis of existing data collected by others.

Multiple choice questions may require either a single answer; for example *'When did your dog last have a litter? – Within the last 3 months, 4–6 months, 7–11 months, 1–5 years, more than 5 years, never?'* or multiple answers, for example, *'Which of the following types of animals do you currently own (Please tick all that apply) – dog, cat, horse, rabbit, bird?'*

Ranking questions are similar to multiple-answer questions, but enable collection of information on the relative preference, merit or importance of the responses; for example, *'Please indicate, in rank order, what you consider to be the most important traits in a pet dog from the following list – purebred, obedient, size, good with children, does not shed hair, healthy, intelligent.'* Weights of the ranked positions are applied in reverse. Thus, the first choice is ranked 7, and the seventh choice is ranked 1. This method of weighting ensures that, if data are presented on a chart, the preferred choices are clear. Ranked questions may require either all options to be ranked (as in the previous example) or a limited number of those that are available.

Rating scale questions similarly allow calculation of a weighted average based on the weight assigned to each answer choice. A common rating scale is the **Likert scale**³ (Likert, 1932), where questions involve a series of statements from which respondents may choose in order to rate their response to evaluative questions such as *'Strongly agree', 'Agree', 'Undecided', 'Disagree', 'Strongly disagree'*, which may then be numerically scored (in this example: 5, 4, 3, 2, 1)⁴.

Numerical rating questions attach integer numbers to the strength or intensity of responses; for example, *'No pain = 0' to 'Worst possible pain = 10'*. Alternatively, responses can be recorded diagrammatically; for example, by using a visual analogue scale (see Chapter 10).

The **semantic differential** question (Osgood, 1953; Osgood *et al.*, 1957) does not label each rating point with an individual description, like a Likert scale. Instead, it places one statement on the far left of the scale and the opposite of that statement on the far right; that is, the extremities of the scale are labelled with opposing, polar adjectives. Examples of such adjective pairs include: *'best–worst'*; *'good–bad'*; *'important–*

unimportant'; *'effective–ineffective'*⁵. Responses can be documented either by numerical rating or diagrammatically. If numerical rating is used, an odd number of points frequently is presented (often seven), but other numbers of points are equally applicable. The adjectival pairs tend to be very general properties and so can be applied to virtually any issue; this contrasts with Likert scales, which are usually topic-specific (Fabrigar *et al.*, 2005).

The advantages of the closed question are ease of analysis and coding because of the limited, fixed response that is allowed. Codes can be allocated when the questionnaire is designed. The closed question is also quick to answer. A major disadvantage is that, because the options of answers are fixed, the options made available may not cover the full range of responses that the respondents wish to provide, and answers may not reveal related information that may be important.

Coding

It is now unthinkable not to store the results of a questionnaire in a computerized database (described later in this chapter). Therefore, questionnaires are frequently coded to facilitate transcription to such databases. Each question and each possible answer is coded. For example, in the question:

26. What is the sex of your animal?

Enter 1 if male and 2 if female,

the question is coded as number 26, and the options to the answers to the dichotomous question are coded as either 1 or 2. Alternatively, coding may take place at the time of data entry; for example, into a spreadsheet program. The name of the respondent may be coded if confidentiality is required. It also is desirable to justify numerical answers to the right for transcription to computerized systems. For example, the date 8 March 2014 is coded as:

0	8	0	3	1	4

not:

8	3	4			

to allow for 31 days in the month, 12 months in the year, and 100 years (00–99) in a century.

³ Strictly, Likert scales and rating scales are not entirely synonymous (Carifio and Perla, 2007).

⁴ This strongly implies that Likert values are ordinal, but much research treats them as interval values (see Chapter 10). See Svensson (2001), Jamieson (2004), Norman (2010) and Brown (2011) for a full discussion of the analysis of Likert responses and ordinal scales.

⁵ A key characteristic of the semantic differential question is that it explores the cultural or emotional association that the adjective pairs connote, one adjective being viewed as 'positive' (e.g., *'good'*) and the other as 'negative' (e.g., *'bad'*).

Computer programs (e.g., *TeleForm*⁶) can be used to automate or semi-automate data entry and coding from questionnaires. These are able to detect checked boxes and other marks used for completing closed questionnaires and can read text and numbers, particularly when constrained print fields⁷ are used. Free text and drawings can be collected and stored as images, and subsequently can be displayed on the computer screen in order to manually enter text or describe the drawing. Such software has a validation function that displays uncertain responses, such as unclear letters in text or incorrectly completed check boxes (e.g., where more than one response appears to have been given to a question requiring a single answer).

Designing a questionnaire

The success of a questionnaire depends on careful design. Ideally, everyone who is issued with a questionnaire should complete it. The proportion of those who respond is the **response rate** (usually expressed as a percentage). The **non-response rate** is therefore 100 – response rate (%); for example, a response rate of 70% represents a non-response rate of 30%. Non-response from a respondent may be either total, in which the questionnaire is not returned, or partial, in which some questions are not answered but the partially answered questionnaire is returned. Good questionnaire design decreases both types of non-response.

Initial presentation The title of the questionnaire should be brief and accurate. A polite letter, explaining the reason for producing the questionnaire, and the value of the results deriving from its completion, should be enclosed (*Figure 11.1*). The inclusion of a reply-paid envelope should increase the response rate. It is also useful to indicate the estimated time that it will take to complete the questionnaire. Initial questions should be particularly interesting and easy to answer, to generate immediate enthusiasm and encourage the respondent to continue answering the questions.

Wording Wording should be unambiguous, brief, polite, unemotional and non-technical. If technical terms are used, then they should be defined simply. Common ambiguous terms include 'often', 'occasionally', 'severe', 'mild', 'heavy' and 'light'. Double

negatives should be avoided. Each question should contain only one idea. Sensitive, emotive and emotional questions should be avoided⁸. The questionnaire should be as short as possible, whilst obtaining the required information. Brevity is particularly important in less-economically-developed countries (Gill, 1993; Kumar, 2006), where translation also may require attention (Lee *et al.*, 1999).

Question sequence Related questions may need to be separated because the answer given to one question may influence that given to the succeeding question, producing the phenomenon termed 'carry over'. General questions should be presented first, and specific ones later. The questionnaire can be made more interesting by 'branching out' from one question to another; for example '*If you answer yes to question 2, then move to question 8*'. However, the number of questions should be as small as possible, while achieving the objectives of the survey.

Ideally the number of different types of questions used (see following) should be kept to a minimum, and similar formats should be grouped together in order to minimize confusion. Where rating-scale questions are used, consistent scales (for example, 1 = least, 5 = most) should be maintained.

Question structure Closed questions must be **mutually exclusive** and **exhaustive**. For example, the age of animals may be expressed as:

Age (please tick): under 6 months ☐
 6–12 months ☐
 over 12 months ☐

In this case, there is no overlap of ages between categories (they are mutually exclusive) and all possible ages are included (the options are exhaustive). To ensure exhaustiveness, it may be necessary to include **dumping categories**, which accommodate all possibilities remaining after the specified options are

⁶ <http://www.cardiff-teleform.com/> (accessed 27 July 2017).

⁷ Constrained print fields provide a dedicated space for each letter or number, bounded by lines, to guide the user.

⁸ Sensitive *subjects*, however, may be addressed. For example, Munro and Thrusfield (2001d) studied sexual abuse of animals (a sensitive issue) using a questionnaire (comprising both open-ended and closed questions) distributed to veterinary general practitioners in the UK. The questions did not specifically address the issue, but related to a purely factual description of the site and type of suspected non-accidental injuries. Only after the questionnaires had been returned were cases of a sexual nature identified, either from characteristics of the site and type of injury (in the completed closed questions) or from comments of the respondents (in open-ended responses).

This survey is supported by



and



FACULTY of VETERINARY MEDICINE
Royal (Dick) School of Veterinary Studies
The University of Edinburgh
Summerhall
Edinburgh EH19 1QH

Non-accidental injury in the dog and cat

Dear Colleague

Please help us with this survey.

What is this survey about?

The survey is about **non-accidental injury (NAI)** in the dog and cat. Non-accidental injury is also sometimes known as **physical abuse**. In children, you may also know it as the '**Battered Child Syndrome**'. In dogs and cats it therefore can be called the '**Battered Pet Syndrome**'.

Why study NAI?

First: although non-accidental injury is known to occur in companion animals, recognition and diagnosis can be very difficult for veterinary surgeons. There is no published comprehensive account of the circumstances, clinical signs and pathology of the 'battered pet'. In **child** physical abuse, which has been recognised for over 30 years, guidelines are available for recognising the condition and for helping to **differentiate** between **accidental** and **non-accidental** injury. For example, unexplained subdural haematomata and retinal haemorrhages are highly suggestive of NAI and a torn frenulum is virtually diagnostic.

The experiences of veterinary surgeons who complete this questionnaire will help to ascertain the extent of NAI in the dog and cat, as seen in clinical practice, and to formulate guidelines to differentiate between accidental and non-accidental injury.

Secondly: a diagnosis of NAI is important because there is evidence that the occurrence of physical abuse to animals may indicate that similar abuse is being directed against other family members, such as the children. It may also be an early indicator of future violent behaviour by the perpetrator[†].

Will your answers be anonymous?

Yes. Your name and address are not requested.



[†] Arkow, P. (1994) Child abuse, animal abuse and the veterinarian.
Journal of the American Veterinary Medical Association, **204**, 1004–1007

Fig. 11.1 A letter introducing a questionnaire on non-accidental injuries in dogs and cats. (Black-and-white reproduction of a three-colour original.) (Study reported in Munro and Thrusfield, 2002a,b,c,d).

considered. For example, 'other' is a dumping category in:

Against what infections has your dog been vaccinated (please tick):

distemper ☐ parvovirus ☐ hepatitis ☐
leptospirosis ☐ other ☐

Lack of confidentiality may increase the non-response rate. Total anonymity (e.g., *Figure 11.1*) ensures confidentiality but prevents the tracing back of information. A limited degree of confidentiality can be ensured by preventing identification of

respondents by the majority of the data-processing staff. Three ways of doing this are:

1. by having a separate sheet of paper for the respondent's name and address, with a code identifying the respondent on the questionnaire;
2. by using a coded strip down one side of the questionnaire, which can be detached;
3. by using carbon paper or carbonless copy paper, allowing duplicates to be produced, but with the respondent's name masked off most of the copies.

Figures 11.2 and 11.3 are two examples of questionnaires.

Practice
Vet
Farm

Management System

If 1. distance from pasture to parlour on grass
1 up to 100 yds
2 100–200 yds
3 200–400 yds
4 400–800 yds
5 over 800 yds

road
hardcore

Date of Visit
D
M
Y

Hours per day when feet are wet

No of cows on farm

Is cow
1 Dry, pregnancy status unknown
2 Dry, pregnant
3 Dry, non-pregnant
4 Lactating

Calving date
if 2, expected
if 4, last
D
M
Y

Heangirth (cm)

Age (yr)

Predominant breed
1 Friesian
2 Ayrshire
3 Shorthorn
4 Guernsey
5 Jersey
6 Other

Origin
1 Homebred
2 Bought

Leave blank

LEG

1
2
3
4
5
6
7
8
9

FOOT

01 – Sole ulcer
02 – White line separation
03 – White line abscess
04 – Punctured sole and pus
05 – Foreign body in sole
06 – Overworn sole
07 – Foul of the foot
08 – Interdigital hyperplasia
09 – Interdigital foreign body
10 – Under run heel
11 – Sandcrack
12 – Aseptic laminitis
13 – Deep sepsis
14 – Other

ARC I.R.A.D. 1977
LAMENESS SURVEY

1 Bone
2 Muscle
3 Joint and ligaments
4 Tendon, sheath and bursa
5 Vessels
6 Other

1 Infection
2 Trauma
3 Both infection and trauma
4 Other

Cause

Tissue most affected

Site above foot

1 RF
2 RH
3 LH
4 LF

Foot/Leg

1 Inner
2 Outer

Claw

Foot lesion

Site in claw

Condition of claw

1 Normal
2 Overgrown
3 Axial rotation
4 Unequal size

Cow

Anterior
Posterior
Abaxial
Axial

Code	Term	Description
01	Sole ulcer	Circumscribed pododermatitis at sole-heel junction, with or without protrusion of granulation tissue through horn defect
02	White line separation	Breakdown of integument of white line, usually abaxially, and impaction of foreign material into crack
03	White line abscess	Breakdown of integument of white line, usually abaxially, and occurrence of septic laminitis of wall
04	Punctured sole and pus	Traumatic penetration of solar horn with infection of laminae and pus production
05	Foreign body in sole	Self-explanatory
06	Overworn sole	Sole which is obviously flexible, horn usually not more than 3 mm thick, often discoloured laminae due to haemorrhagic bruising
07	Foul of the foot	Interdigital necrosis affecting skin and, or, subcutaneous tissues
08	Interdigital hyperplasia	Thick interdigital skin fold, often with localised areas of pressure necrosis or ulceration as secondary feature
09	Interdigital foreign body	Self-explanatory
10	Under run heel	Separation of heel horn axially towards sole-heel junction, usually with some exposure and infection of sensitive laminae; erosion of heel horn
11	Sandcrack	Vertical split in wall horn at coronet or lower down, involving sensitive tissues
12	Aseptic laminitis	Generalised digital pain and heat, often involving more than one foot, and without break in integument
13	Deep sepsis	Self-explanatory
14	Other	

Fig. 11.2 A questionnaire survey of lameness in cattle in Great Britain. Respondents were requested to indicate which categories were appropriate to their farm and cattle. (Source: Russell, *et al.*, 1982. Reproduced with permission of BMJ Publishing Group Ltd.)

In the first example (a lameness survey in cattle), the following concepts are illustrated:

- a dumping category (the inclusion of '6. Other' in description of breeds);
- confidential coding of the veterinary practice and veterinarian (2 digits) and farm (3 digits);
- a clear description and definition of terms that are used (sites and nature of lesions in this example).

INVESTIGATION OF JOHNE'S VACCINATED HERDS

Conducted by: The Epidemiology Unit, Central Veterinary Laboratory, Weybridge, Surrey.

PLEASE NOTE ALL INFORMATION WILL BE TREATED AS STRICTLY CONFIDENTIAL

Herd Owner _____ 1. Herd Code _____

Address _____

2. Present herd type: Dairy ☐ Beef ☐ Mixed ☐

3. Replacement policy. Entirely home reared. Please write YES or NO _____

4. % breed composition of present adult herd _____

5. Present adult herd size _____

6. Present system of winter housing: Cowshed ☐ Cubicles ☐ Loose ☐ Outwintered ☐7. Method of pasture utilization: Intensive ☐ Extensive ☐

8. Date of approval for use _____/_____/19____

9. Year in which vaccination started. 19____

10. If vaccination has been intermittent please give the years in which either vaccine was not used or in which only some of the animals retained for breeding were vaccinated _____

11. Year in which vaccination ceased. 19____ Write NA if not applicable _____

12. Average number of clinical cases of Johne's disease per year prior to vaccination _____ cases

13. Adult herd size at start of vaccination _____

14. Number of clinical cases occurring in each year since vaccination started:

Years since start of vaccination	Number of clinical cases	Years since start of vaccination	Number of clinical cases
1		5	
2		6	
3		7	
4		8	

If clinical cases occurred 9 years or more after start of vaccination please specify which year(s)

15. Have there been any major changes in breed since vaccination started? Please write YES or NO _____

If 'YES', please specify change with approximate date from _____ to _____ in 19____

16. Have there been any changes in herd type since vaccination started? Please write YES or NO _____

If 'YES', please specify change with approximate date from _____ to _____ in 19____

17. Have any attempts been made to identify latently infected animals? Please write YES or NO _____

If 'YES', please specify diagnostic methods used: CFT ☐ Johnin ☐ Microsc. Exam ☐ Faeces Culture ☐and whether positive animals were called: Always ☐ Sometimes ☐ Never ☐

18. Please indicate which of the following control measures are practised on the farm:

Please write YES or NO

(a) prompt removal of confirmed clinical cases _____

(b) only 'bucket' rearing of calves (in dairy herds) _____

(c) adequate separation of calves from adults (in dairy herds) _____

(d) removal from herd of calves born of infected dams _____

- (e) adequate hygiene of food and water supplies to housed animals _____
- (f) piped water supplies to all cattle at pasture _____
- (g) prevention of access to ponds and ditches etc. _____
- (h) calves grazed on pastures not used by adult cattle _____
19. Have any other changes in management been instituted to control the disease?
- _____
- _____
- _____
20. Do you think the vaccine has had a valuable part to play in the control of Johne's disease in this herd? Please write YES or NO
- _____

GENERAL COMMENTS

THANK YOU FOR YOUR CO-OPERATION IN THIS INVESTIGATION.

The completed questionnaire should be sent to:

J. W. Wilesmith, B.V.Sc., M.R.C.V.S.
Epidemiology Unit,
Central Veterinary Laboratory,
New Haw,
Weybridge,
Surrey KT14 3NB.

Fig. 11.3 A questionnaire survey of Johne's disease (paratuberculosis) in vaccinated cattle in Great Britain. (A questionnaire produced by John Wilesmith of the Central Veterinary Laboratory, UK, 1977.)

In the second example (a survey of Johne's disease in vaccinated herds), these additional concepts are demonstrated:

- closed questions (e.g., No. 2);
- open-ended questions (e.g., No. 19);
- mutual exclusiveness and exhaustiveness (e.g., No. 17);
- a clear title, with a statement of confidentiality;
- polite presentation (e.g., the last portion of the questionnaire that thanks the respondent for his or her co-operation).

Questionnaires can be either completed by respondents after they have been delivered to them (e.g., by mail or email), or completed by an interviewer who presents the questions, verbally, either in person or by telephone (Lavrakas, 2009), to the respondents. Respondents also can complete questionnaires directly via the Internet (e.g., Gobar *et al.*, 1998; Schonlau *et al.*, 2002; Gosling *et al.*, 2004), in which circumstance the results may be automatically stored directly in a computer, thereby preventing transcription errors (Best and Harrison, 2009).

Mailed and self-completed questionnaires

Requirements The main requirements for a mailed or self-completed questionnaire are:

- a list of respondents to act as the sampling frame (see Chapter 13) (e.g., a register of veterinarians);
- great clarity: no one will be present to cope with any difficulties that may arise;
- a covering letter, politely explaining the reason for sending the questionnaire (e.g., *Figure 11.1*).

Advantages The main advantages of this type of questionnaire are that:

- it is relatively cheap with potential for wide coverage;
- it is quick and easy to organize;
- it avoids interviewer bias (see following section);
- it allows a highly motivated respondent to 'check the facts' over a period of time;
- respondents have the opportunity to reply anonymously, which may produce a high response rate.

Disadvantages The disadvantages of this type of questionnaire are that:

- clarity of question may be difficult to achieve;
- the questionnaire cannot prevent reviewing of questions, which may lead to undesirable modification of previously answered questions.

Response rate In mailed and self-completed questionnaires, response rates tend to be low – 50% is

not uncommon, and the rate can be as low as 10% (Edwards, 1990). The issuing of reminders or more questionnaires usually does not increase the response rate much (Thrusfield *et al.*, 1998; Barnett, 2003). The issuing of pens with questionnaires, similarly, also does not appear to increase the response rate (Clark *et al.*, 2001).

Factors influencing the response rate are:

- the sponsor – a respected sponsor should enhance the likelihood of obtaining a high response rate (e.g., *Figure 11.1*);
- the nature of the respondent population – a well-educated group should produce a higher response rate than a poorly-educated group; similarly, a group with a specialist interest in the subject of the questionnaire should generate a high response rate;
- the subject and aim of the study – if the study is of obvious direct relevance and value to the respondent, then he or she is likely to reply (e.g., the response of wool-growers to mailed questionnaires on blowfly strike: Ward, 2000);
- appearance and length of the questionnaire – if it is attractive and concise, then response should be good;
- confidentiality.

The results of questionnaires should be treated with caution when response rates are lower than about 70% because there may be systematic differences between those who do, and those who do not, respond (**response bias**). For example, in a questionnaire survey of scrapie in the UK, the average sheep flock size was larger on farms whose owners responded than on those whose owners did not respond (Wooldridge *et al.*, 1992).

Interviews

A personal interview can overcome some of the disadvantages of mailed and self-completed questionnaires, and is particularly useful if many of the questions are open-ended, and where illiteracy of the respondent is a problem. The tone of the interviewer's voice may, however, bias the respondent's answer, by implying a desirable answer (**interviewer bias**). Questionnaires can be longer than self-completed ones, and response rates of 90% sometimes can be achieved (Edwards, 1990). However, personal interviews can be costly to organize, involving training, payment and travelling expenses of interviewers.

Telephone interviews Interviews also can be conducted over the telephone, rather than face-to-face (Groves *et al.*, 1988; Frey, 1989; Barriball *et al.*, 1996; Carr and Worth, 2001). Telephone interviews

generally have high response rates⁹, and can produce results more quickly and cheaply than personal interviews and mailed questionnaires. Additionally, they can readily reach geographically-dispersed respondents, enhance the safety of interviewers, and facilitate oversight of interviewers (Kempf and Remington, 2007). However, questions need to be short to reduce conversation time to a minimum. The absence of visual or non-verbal cues also may be a drawback, particularly in qualitative research (Novick, 2008).

Telephone interviews also can be used to follow-up non-responders in surveys that are initially based on mailed and self-completed questionnaires, in an attempt to reduce (and assess) response bias.

Computer-assisted interviews The use of computers for face-to-face interviews, rather than using 'pencil and paper', is increasing in popularity, and computers also can be used for telephone interviews and self-completed questionnaires (Saris, 1991; Couper *et al.*, 1998; Abramson and Abramson, 2008). Interviewers carry 'laptop' computers from which questions are read out and into which responses are entered.

Advantages include:

- cost savings (central data entry is obviated, results can be obtained quickly, and printing costs are avoided);
- improvement of questionnaires ('branching out' can be built into the program and increased in complexity; data can be checked and corrected during the interview);
- increased efficiency (coding and typing responses is relatively quick; data are available for analysis very soon after being recorded).

Disadvantages include:

- inappropriateness for small-scale studies (the price of computers and associated software can be high);
- increased preparation time (including time for training of interviewers, computer programming and coding of questions);
- problems encountered by interviewers (respondents may be wary of the computer, the computer may malfunction or become drained of power, the interview process may be slow if the interviewer is slow at typing);
- potential difficulty in subsequently deciphering questionnaires and results on paper (leading to increased volume of documentation);

- slightly increased time in conducting interviews, compared with 'pen-and-paper' methods;
- potential for typing errors.

A general introduction to interviewing technique is provided by Oppenheim (1992). Guest and others (2003) and Kvale and Brinkmann (2009) explore interview sample size. The use of veterinary interviews is discussed by Ruppanner (1972) and, in less-economically-developed countries, by Perry and McCauley (1984). Examples of their use include surveys of schistosomiasis in the Sudan (McCauley *et al.*, 1983b), swine fever in Honduras (McCauley, 1985), bovine health and productivity in Zambia (Perry *et al.*, 1984) and sheep diseases in New Zealand (Simpson and Wright, 1980). The advantages and disadvantages of various interview techniques are discussed by Opendenakker (2006).

Testing questionnaires

Several drafts of a questionnaire are usually required following testing. There are normally two stages to testing: **informal** and **formal**.

Informal testing Informal testing is carried out on colleagues who can detect trivia, ambiguities and defects in questionnaire design.

Formal testing Formal testing is undertaken on a small random sample of the population on which the full survey will be conducted. This testing is called a **pilot survey**. The size of the sample is chosen using the guidelines for sample size determination in surveys (see Chapter 13). The pilot survey exposes further defects in questionnaire design. This survey should never be used as part of the full survey, and respondents used in the pilot survey should never be used again in the full one.

Criteria for success of questionnaires

The two main criteria for the success of a questionnaire are **reliability** and **validity** (see Chapter 10).

Reliability A questionnaire, like a diagnostic test, is reliable if it produces consistent results (see *Figure 10.6*). Reliability can be assessed by issuing the same questions to the same respondents more than once (e.g., Reeves *et al.*, 1996b; Tiira and Lohi, 2014) and assessing the agreement between the responses. The method of assessment depends on the scale of measurement (see Chapters 10 and 20). Slater (1997) reviews measures of reliability for questionnaires.

Validity Validity is a measure of the degree to which answers, on average, reflect the truth (see *Figure 10.6*).

⁹ The factors affecting response rate to telephone interviews are complex, ranging from the 'authority' of the interviewer to the perception that domestic privacy is being invaded (Groves, 1990).

Validity therefore is achieved by comparing the results of the questionnaire with an independent reliable criterion. For example, Selby and others (1973, 1976) compared the results of a mailed questionnaire with information derived from farm records and diaries, when investigating congenital abnormalities in pigs; Perry and colleagues (1983), in a survey of several animal diseases in Zambia, compared the results obtained from an owner interview survey with those obtained from an investigation of sentinel herds; the results of a telephone interview on diet and exercise in dogs were compared with subsequent written dietary records (Slater *et al.*, 1991); the results of a personal interview on pig production were compared with validated production records (Deen *et al.*, 1995); and farmers' diagnoses of Johne's disease were compared with veterinary and diagnostic-laboratory diagnoses (Çetinkaya *et al.*, 1998). Sensitivity and specificity (see Chapters 10 and 20) can be calculated for questions with either a positive or negative ('yes'/'no') outcome; continuous and ordinal data require other techniques (Maclure and Willett, 1987). Early validation is desirable, to allow questionnaire alterations to be instigated as quickly as possible, and to minimize the delay between respondents answering a questionnaire and their answers being validated. It therefore should be the final step of questionnaire design, undertaken as soon as sufficient data are available (Pugh *et al.*, 2015).

Unreliability often means invalidity. However, reliable questions may not always be valid (see Figure 10.6b).

Further details of veterinary questionnaires can be found in Waltner-Toews (1983), Edwards (1990) and Vaillancourt and others (1991). Pfeiffer (1996) discusses the use of veterinary questionnaires and interviews in less-economically-developed countries.

Quality control of data

'Quality control' ('quality assurance') refers to the checks that are conducted during and after the collection of data, to ensure that data are accurate and suitable for their intended purpose (Biemer and Lyberg, 2003).

Sources of data

There are several ways of maintaining the quality of primary sources. If observations are recorded by the data analyst, then he or she should ensure that they are carefully documented. If observations are recorded by another person, or if the data are recorded on a self-completed questionnaire or by an interview, then the criteria for assessing the success of a questionnaire, above, provide the quality-control guidelines.

Secondary data may be harder to assess (Sørensen *et al.*, 1996). If documentation is recent, then the data may be cross-checked by issuing a questionnaire, or by

interview. For example, during the 2001 foot-and-mouth disease epidemic in the UK, details of stock location were recorded by the veterinarian visiting each infected farm. Sometimes details were inadequate, and it was also noted that the data were recorded at a stressful time. Therefore, a postal questionnaire was distributed to owners of affected farms with deficient data, and remaining deficiencies corrected by personal interview, followed up by telephone interview, if necessary (Thrusfield *et al.*, 2005b). If several sources are used (particularly for research), then it is necessary to assess if there is agreement between them, both in terms of **completeness** and **correctness** (Mörk *et al.*, 2010)¹⁰.

Documentary sources dating back many years may be hard or impossible to validate.

Data entry

Ideally, two people should be involved in data entry, to reduce the likelihood of transcription errors. Coding of data also should allow software to detect inconsistencies or 'illegal' values, and detect 'finger trouble' (see Chapter 10), and is therefore particularly useful if only one person is available for data entry. Some quantitative values may appear to be extreme in the context of other values in the data set, and it may not be possible to verify their authenticity. Armitage *et al.* (2002) describe how to explore and manage such values.

Missing values

It is not uncommon for data to be incomplete; for example, if there is partial non-response in a questionnaire. There are two ways of tackling this issue. First, attempts can be made to complete that data (e.g., by telephoning respondents); this is therefore a quality-control issue. Secondly, the data can be recorded in an incomplete form, and the problem addressed at the stage of data analysis. There are then two approaches. First, all records with incomplete data are excluded from data analysis, and therefore only complete records included: this is **complete-subject (complete-case) analysis** (Pigott, 2001). This is a simple approach, but is only valid if the records with complete data are a random sample of the records of all subjects in a study, or of subjects within complete categories (Little and Rubin, 2002; He, 2010). However, data are lost (particularly when many records are incomplete) and analysis is therefore less efficient than if the data were complete. Alternatively, incomplete records can be included in analysis. The two main methods involve: (1) predicting missing values based

¹⁰ Agreement is considered to be 'high' at 90%, 'fair' at 80–89%, and 'poor' at ≤70% (Egenvall *et al.*, 1998; Jordan *et al.*, 2004; Penell *et al.*, 2007).

on the patterns of values in complete records; or (2) assigning weights to missing data (Little and Rubin, 2002; He, 2010). Note, however, that creating categories for missing variables, for subsequent analysis, generally is inadvisable (Vach and Blettner, 1991; Greenland and Finkle, 1995)¹¹.

Allison (2002), Acock (2005), Horton and Kleinman (2007), McKnight and others (2007), Graham (2012), Baraldi and Enders (2013) and MISSINGDATA (2015) review methods for dealing with missing data.

Data storage

After data have been collected, they should be saved in a **database**; that is, a structured collection of data, which is the basis of an organized data storage and retrieval system.

Database models

A database containing animal records includes different types of data that comprise different components of the records. The data consist of several categories (**specifier types**); for example, breed, sex, age and clinical signs, that are attributes of the animal. Some of these are permanent attributes and therefore are **case-specific**; for example, breed and date of birth. Others, such as diagnoses and signs, change from one consultation (and therefore record of consultation) to another, thus being **record-specific**.

The association between the various components of a record can be viewed in several ways, depending on the way in which the data are stored, producing five models of a database.

The 'record' model

The **record** model is the traditional way of structuring data. The central component is the individual record, which contains case- and record-specific data. This is a useful approach for the clinician concerned mainly with individual patient care. However, it is difficult to correlate specifier types between records. Correlation is very useful in epidemiological studies; for example, correlation of breed, age and sex with disease.

The 'flat' model

A **flat-file** database is designed around a single table, and often is used in **spreadsheet** programs. Flat-file data consist of nothing but data and, if necessary, delimiters (characters denoting the beginning or end of a

character string, word, or item of data) with no structural relationships between the records and fields (i.e., rows and columns). Common delimiters include tabs, commas and spaces; in cases where each field has a fixed length no delimiter is necessary. Flat-file data usually contain a single record per row.

Flat-file data often contain duplicate data. For example, in *Table 11.1*, two animals each appear twice, separately, because they contribute data from two lactations, and the information on breed is repeated in each of the rows. This duplication has little effect in this example, but can cause problems due to the additional effort required to enter duplicate data and due to increased file size when the data set is large and contains much duplicate information, which is prone to data corruption.

Note that in *Table 11.1* categorical data have been assigned numeric codes. Non-numeric codes can be used, and may facilitate data entry and interpretation (such as using M and F for male and female, respectively), but some statistical programs have problems dealing with non-numerical data.

Common mistakes in construction of flat files (illustrated in *Table 11.2*) include:

- use of multiple codes as indicators for the same data categories; this may inadvertently occur if the person or people entering the data switch between using *M* and *Male* as the indicator for male sex; alternatively, this error may occur due to data entry errors ('finger trouble'), such as the misspelling of *Female* as *Femal*; these problems may be more likely to occur if non-numeric and long codes are used;
- inclusion of non-numerical information within columns containing numerical data (e.g., inclusion of *kg* or *ml* to indicate units of measurement);
- inclusion of numerical data measured in different units within the same column (e.g., insertion of weights measured in either kilograms and pounds);
- inclusion of multiple pieces of information in a single column (e.g., incorporation of multiple presenting problems in a single patient in a single column, such as the animal in the second row: *pyrexia* and *vomiting*); a better method (also shown in *Table 11.2*) is to include distinct columns for each presenting problem; if required, columns also can be used to indicate the presence of specific combinations of presenting problems.

If data are to be merged between two flat files, the data must be copied and 'pasted' from one file to another, and changing information in one file has no bearing on other files (cf. 'The relational model' following). However, flat files are easy to design, relative to relational databases, and also can be shared easily over the Internet and between computers.

¹¹ There is, however, merit in understanding the pattern of 'missingness' (MISSINGDATA, 2015).

Table 11.1 The structure of data components in a simple flat-file database.

Unique ID	Animal ID	Year	Parity	Breed	Milk fever	Date of calving	Calving to conception interval (days)
1	1	1	1	2	0	23/11/2012	89
2	1	2	2	2	0	01/12/2013	101
3	2	1	2	1	1	11/06/2012	117
4	2	2	3	1	0	18/07/2013	62
5	3	1	3	3	0	11/04/2013	90
6	4	1	3	3	1	09/05/2013	85

Table 11.2 An example of common errors in the construction of flat files.

Unique ID	Record number	Sex	Weight	Presenting problem	Vomiting	Pyrexia	Alopecia	Pruritus	Overweight
1	3804	M	6.4kg	Vomiting	1	0	0	0	0
2	3769	F	19.2kg	Pyrexia, vomiting	1	1	0	0	0
3	5896	Male	13.2kg	Alopecia	0	0	1	0	0
4	7609	Femal	19.8lb	Pruritus and alopecia	0	0	1	1	0
5	8039	Female	8.3kg	Overweight	0	0	0	0	1

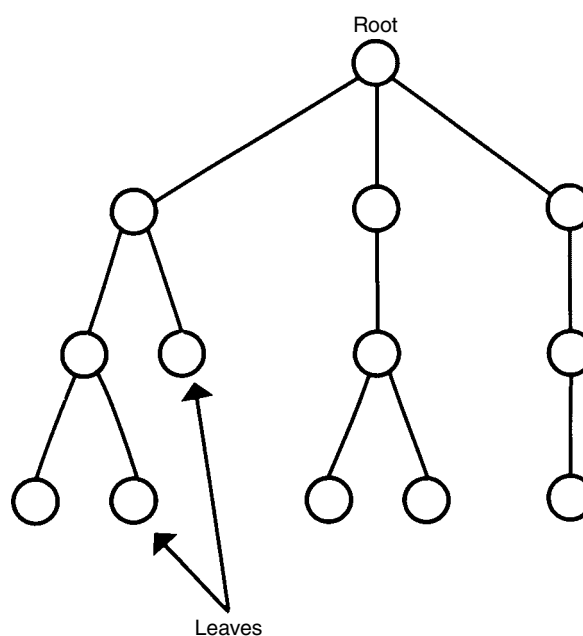
The 'hierarchic' model

The **hierarchic** model and the two that follow are true database models and are more complex than flat files. These models enable multiple flat files to be linked to one other in order to prevent or reduce the need to duplicate data and to enable rapid interrogation and retrieval of the data or subsets of the data. In this model, data components are stored in **nodes**, which are arranged in a tree-like structure (Figure 11.4). The uppermost level of the hierarchy has only one node; it is called a **root**. With the exception of the root, every node has one node related to it at a higher level; the latter is called the former's **parent**. No component can have more than one parent. Each component can have one or more components related to it at a lower level; these are called **children**. Components at the end of the branches (i.e., with no children) are called **leaves**. Figure 11.4 shows a hierarchy with four levels. An example of a hierarchy with only two levels would be one with 'veterinary practice' (the root) and 'veterinary surgeons in the practice' (the children).

The 'network' model¹²

Using the terminology of tree structures, if a child in a data relationship has more than one parent, then the

¹² The term 'network' model, in this context, should not be confused with mathematical network models of infectious disease, described in Chapter 23.

**Fig. 11.4** The 'tree' structure of a hierarchical database.

relationship cannot be a strict hierarchical one. In these circumstances, the structure is described as a **network**. The network model therefore includes and extends the hierarchic model. An example is

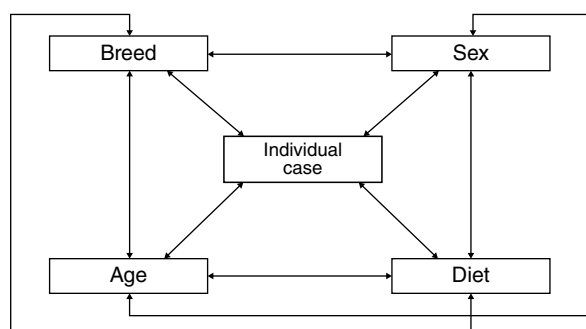


Fig. 11.5 An example of some possible relations in a simple 'network' database. (Source: Thrushfield, 1983a.)

illustrated in *Figure 11.5*. This model allows relationships to occur between many data components and is therefore of considerable epidemiological value because it allows correlation of determinants (e.g., age and sex) with disease if the determinants and diseases are stored as components of the network.

The 'relational' model

In the **relational** model, all data are represented by two-dimensional tables, which have the following properties:

- entries in the table are single-valued; neither repeating groups nor arrays are allowable;
- the entries in any one column must all be of the same kind (e.g., one column might contain animals' sexes, and another contain animals' ages);
- each column has a unique name and the order of columns is immaterial;
- each row of the table is unique and the order of the rows is immaterial.

An example is given in *Table 11.3*.

The relational approach is different from that of the hierarchic and network models. The principal difference is that, in the hierarchic and network models, relationships are expressed explicitly and are predefined. In the relational model, the basic data structure is predefined but record relationships are not defined until they are used. This ability to dynamically reflect relationships, combined with the simplicity of the relational model, makes it potentially more flexible than the other models, which it can also represent. The main disadvantage of the relational model is its inefficiency relative to the other two, because, although the model is simple, it is complex to implement on computers. However, the increasing sophistication of computer technology has now made this model the basis of most computerized databases, enabling data to be

Table 11.3 The structure of data components in a simple 'relational' database.

<i>Animal's name</i>	<i>Age</i>	<i>Sex</i>
Patch	7	M
Sally	5	F
Thor	13	M
.		
.		
.		
Arthur	4	M
Liz	15	F
Brenda	9	F
.		
.		
.		

shared over the Internet and between other computers and software systems.

Non-computerized recording techniques

Longhand recording techniques

The recording of data in longhand is still used in some general veterinary practices, although computerized storage methods are now widespread.

Day books The day book is a long-established type of longhand record. It is an 'open' record of cases examined during the day; that is, it allows any data to be recorded in any form, usually as a narrative description. This type of record is of limited epidemiological value because it is bulky and does not allow either an animal's previous consultations to be located rapidly or correlation of components of different records.

Record cards Cards, stored in drawers or boxes, were a common means of storing clinical records, and are still used in some veterinary practices. They use a 'record' model of a database, each card often referring to one animal. They are usually stored alphabetically according to owners' names. The record-specific data are ordered chronologically on each card, providing a complete history of the patient. Cards may be blank, in which case they can be used to provide an 'open' record. Alternatively, they can be printed as a 'closed' record, that is, with a fixed number of options (see 'Questionnaires' earlier in this chapter). These options commonly include details of specifier types such as

breed, sex and date of birth. They are usually partially closed, consisting of a closed section, but with a blank area for the 'open' recording of additional details.

Pro-formas A pro-forma is an extension of the partially-closed record. It is a mainly closed record, comprising a check list of many features (*Figure 11.6*). This can produce a very detailed record of observational and interpretative data. However, the merits of producing a comprehensive record must be weighed against the unwillingness of busy clinicians to complete them, and against the value of the data. Completion of all components of the record, especially if a 'mandatory' part of the running of a clinic or laboratory, can rapidly lead to rejection of the system (Darke, 1982). Pro-formas should always have an open section to accommodate any salient details that are not included in the closed part. The closed section can be coded and therefore readily entered into a computer.

Computerized recording techniques

Computers are now an efficient and commonplace – rapidly approaching universal – means of storing, analysing and retrieving data, in addition to acting as complex calculators. The invention and subsequent development of silicon microchips has decreased the size and cost of computers, thereby making them readily available to a wide range of users including general veterinary practitioners. Although the initial reason for a practitioner to acquire a computer may be as an aid to practice management (e.g., Pinney, 1981), the machine also can manipulate clinical data very efficiently.

A wide range of software is available for data storage, manipulation and analysis. Spreadsheet and database programs have become a mainstay of data handling and are useful for basic analyses. Appendix III lists some useful packages for data storage and analysis in epidemiological studies.

The veterinary profession has benefited generally from the Internet; for example, in the development of computer-based learning (Short, 2002), which compares favourably with traditional teaching methods (e.g., Abutarbush *et al.*, 2006), and the general sharing of professional information that the Internet facilitates. Additionally and specifically, veterinary epidemiologists can now share data, statistical software, and the results of research and disease surveillance (e.g., the *Office International des Epizooties* reporting system: see *Table 11.4*).

The locations (Uniform Resource Locators: URLs) of some Internet resources of value to veterinary epidemiology are listed in Appendix IV.

Veterinary recording schemes

Scales of recording

The types of veterinary recording schemes were classified over 40 years ago by Hugh-Jones (1975):

- microscale schemes (*Figure 11.7a*) concerned with internal disease problems in separated populations such as those on farms and research institutes;
- mesoscale schemes (*Figure 11.7b*) involved with more widely-distributed disease problems; for example, data collection at abattoirs, diagnostic laboratories and clinics;
- macroscale schemes (*Figure 11.7c*) designed to collect data with the purpose of gaining an international or national view of disease.

Microscale schemes

In the microscale scheme, information is transferred from the farm to the practice or consulting organization where it is analysed and the results are necessarily fed back to the farm to effect health improvement. With an 'on-farm' system, data may be manipulated and analysed without leaving the farm's confines. Herd health and productivity schemes are typically microscale. Essentially, they record individual and herd performance and productivity and sometimes disease occurrence, and relate productivity to disease in an attempt to improve the former. These will be considered in greater detail in Chapter 26.

Mesoscale schemes

In the mesoscale scheme, data are transmitted from their source to the organizations responsible for analysis. The data are usually of more direct value to the analysing institution than to their source. Therefore, feedback of data is a less important component of the system (although still desirable). Small-animal practice records are an example of this type of recording system.

Macroscale schemes

Macroscale schemes are designed to gain a global, globally regional, national or nationally regional picture, and therefore are not designed primarily to help specific animal units. There is thus little flow of data back to their origin. National and international monitoring and surveillance programmes are typically macroscale.

Several contemporary recording projects now have various combinations of macroscale, mesoscale and microscale elements. For example, the Canadian

ROYAL (DICK) SCHOOL OF VETERINARY STUDIES

UNIVERSITY OF EDINBURGH

100,006

OWNER	Surname Mr. Mrs. Miss CAMPBELL, Mrs.		Initials H.		Address 42, Glenayre Place	
	Phone home business		clinician A.B.Jones		student	animals name Petra
ANIMAL	species Dog	breed Shetland Collie	sex F.	age 4yrs.	colour Champagne/White	weight 9kg
ATTENDANCE	date 1/6/77 hour		ADMITTANCE ☐		DISCHARGE ☐	date hour
PRIMARY COMPLAINT	Pruritus					
REFERRED BY	Name		reply sent		admitting clinician	
	Address		phone No.		attending clinician	
MEDICAL HISTORY						
IMMUNISATION : state age, none or ? Initial : C.D. C.V.H.						
Repeats : C.D. C.V.H.						
Initial : Lep. can/ict. Other						
Repeats : Lep. can/ict.						
PREVIOUS ILLNESS, injury or surgery (specify +age, or none)						
DURATION OF ILLNESS 1 Month Length of time owned 4 Years						
APPETITE : anorexia variable fair good ☒ excessive depraved						duration
THIRST : normal ☒ decreased increased excessive						
RESPIRATION : normal ☒ shallow dyspnoeic abdominal painful						
SNEEZING : Nasal discharge (nature) Salivation (nature)						
COUGH : none dry soft persistent paroxysmal						
VOMITION : none occasional frequent persistent haematemesis retching only						
Vomitus : character and when occurs						
FAECES : none normal ☒ soft (not formed) fluid blood mucus other						
Defaecation : normal ☒ difficult painful frequency colour						
URINATION : none normal ☒ frequent difficult painful incontinent haematuria						
Volume : normal ☒ decreased increased colour :						
TEMPERAMENT : normal listless depressed cantankerous restless excitable ☒ neurotic						
NERVOUS : normal ☒ excitement hysteria convulsions chorea nystagmus ataxia paraplegia						
Paralysis (define)						
SKIN : normal inflamed pruritus ☒ loss of hair alopecia pigmentation hyperkeratosis Licking rubbing						
Biting (site) Ectoparasites :						
Wounds (site)						
Tumour (site) Other lesions :						
MUSCULO-SKELETAL : normal ☒ Lameness (limb) Injuries (site) :						
Swellings (site) Deformities (site)						
SEXUAL : Fertility : proven apparently infertile unknown						
Libido : normal none excessive unknown						
OESTRUS : age of onset last oestrus normal abnormal (define)						
intervals false preg Last litter No of litters						
Any discharge (define)						
EYES : normal ☒ discharge (specify) Blindness Other lesions						
EARS : normal ☒ rubbing ear head shaking smell discharge other						
DIET : Daily amount Meat (specify) Fish						
Carbohydrate Vegetables Other						
OTHER RELEVANT INFORMATION Synthetic fibre bedding						
MEDICAL HISTORY						

Fig. 11.6 An example of a clinical case record pro-forma. (Source: Thrusfield, 1983a.)

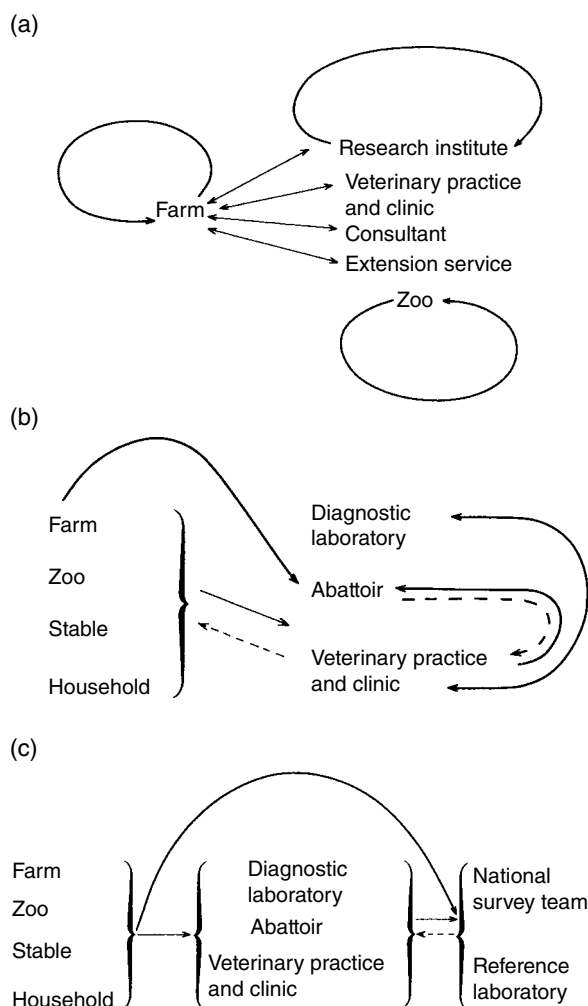


Fig. 11.7 Veterinary data recording schemes defined according to flow of data: (a) microscale schemes; (b) mesoscale schemes; (c) macroscale schemes. Solid arrows indicate main paths of data flow. Broken arrows indicate paths of limited or no data flow. (Source: Thrusfield, 1983b; after Hugh-Jones, 1975.)

Animal Productivity and Health Information Network, APHIN (see Table 11.4), includes on-farm microcomputers that record herd health and productivity (microscale), but these are also linked to a central database, which can provide a regional view of animal health (macroscale). This triple classification, however, still provides a useful focus on the broad objectives, flow of data and general geographical scope of a recording scheme.

Veterinary information systems

Databases themselves only have limited value. Maximum benefit from the data is obtained only when they are converted to **information**. Data and information are not synonymous, and, when they are treated as such,

confusingly similar terms like 'information processing' and 'data processing' arise. Information comprises data that have been processed and organized for some purpose so that someone can extract **meaning** from them. A **system** is generally defined as an entity comprising at least two related components (Ackoff, 1971). However, a precise definition of an **information system** is difficult to achieve (Callaos and Callaos, 2002; Alter, 2008) but, in a practical veterinary context, may be considered as collections of disease-related data that are integrated to satisfy the informational requirements of its users (e.g., farmers, veterinary practitioners, epidemiologists and administrators), together with the people and computers that process the information. The borderline between a simple database and an information system also is indistinct because stored data, even before they are processed, can have value as information. However, an important characteristic of an information system is its ability to deal with large, complex issues (e.g., the national control of epidemics). Figure 11.8 is a general outline of a national veterinary information system, in which data flow from their source on farms to the scientific (i.e., veterinary) community and to other salient sectors of society. During the transmission of data down the information system's 'conveyor belt', data are 'distilled' to generate useful information.

An extension of the information system is the **decision support system** (McNurlin *et al.*, 2009). Its two key components are:

1. a focus on decision makers – at both policy and implementation levels – rather than on the system itself;
2. 'seamless integration' of all components of the system so that all features can be accessed via a single user interface without regard to which component of the system is providing the answers; this represents a logical development from the simple database where data analysis may be handled quite separately from data entry and storage – often not even on the same computer.

Decision support systems have these characteristics (Morris *et al.*, 1993):

- they tend to be directed at relatively unstructured, poorly specified problems;
- they combine models and analytical techniques with traditional data storage and retrieval functions;
- they focus on features that make them easy to use by people whose computer 'literacy' is low;
- they emphasize flexibility and adaptability to accommodate changing circumstances (e.g., changes in approaches to decision making) and therefore can

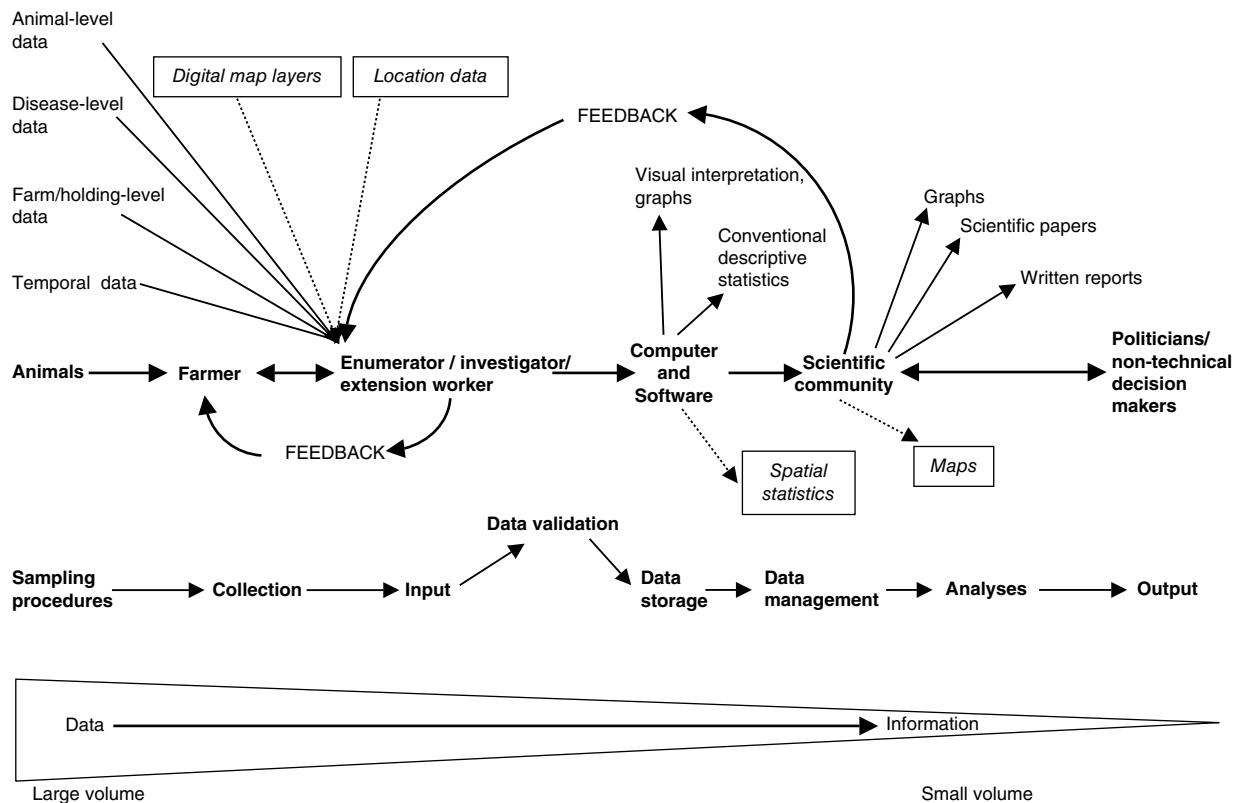


Fig. 11.8 The structure of a national veterinary information system. Input from geographical information systems is indicated by dotted lines and italicized text in boxes. (From an idea by Andrew Paterson, Department for Environment, Food and Rural Affairs, London.)

be valuable parts of a national animal health information system.

Decision support systems do not, themselves, produce unequivocal answers, but offer guidance to policy makers, who may need to enlist other views. They also provide a useful audit of policies.

Data collection

Data may be collected either **passively** or **actively** (see Chapter 21), and the passively-collected data are prone to selection bias (see Chapters 10, 18 and 21). However, the use of passively-collected data involves little extra cost. Actively-collected data, in contrast, are gathered specifically to fulfil the requirements of the information system, are not available from pre-existing sets of data, and therefore have a cost attached to them. Therefore, the technical and economic feasibility of active collection needs to be considered.

Many epidemiological studies require details of the population at risk, as well as diseased animals (e.g., for calculation of morbidity and mortality values). Thus, census data also may be an important part of

an information system. These data are collected routinely in many countries and therefore are passively available for use in information systems. However, the accuracy of these data needs to be assessed before they are used.

The value of collected data

The value of collected data needs to be judged in terms of the objectives of the information system. Some pitfalls associated with inappropriate data collection are summarized by **Finagle's 'Law'** (Opit, 1987):

- the information one **has** is not what one **wants**;
- the information one **wants** is not what one **needs**;
- the information one **needs** is not what one **can get**;
- the information one **can get** costs more than one **wants to pay**.

Thus, the objectives of an information system should be clearly defined *before* the system is designed, and *before* active data collection begins. This necessitates discussion with *all* potential users of the system *before* it is designed.

Objectives

There are several objectives of a comprehensive information system:

- surveillance of endemic diseases;
- fulfilling international reporting needs;
- monitoring productivity;
- identifying new syndromes;
- supporting and monitoring the technical efficacy of control programmes;
- managing laboratory data;
- providing information for the economic assessment of disease and its control.

These objectives can be fulfilled at three levels: **farm**, **national** and **international**, and the information required at each level therefore needs to be identified. The **flow** of information in the information system can then be defined.

Farm level This includes individual producers, producer groups (e.g., farm cooperatives), and product processors. Individual producers generally need records of diseases on their farms, and details of productivity (e.g., milk production and reproductive efficiency). Producer groups require similar information on productivity, although it may be prudent to keep details of an individual farm's disease problems confidential. Product processors need details of hazards such as zoonotic diseases and residues (e.g., for milk processors, details of milk-borne infections and antibiotic contamination).

National level At this level, several organizations have different information requirements. Departments of agriculture utilize aggregated data on farm productivity. Veterinary services need a national view of animal disease, including the monitoring of zoonoses, and a log of veterinary activity. The agricultural industry needs details of product sales. National banks may require information on productivity potential before awarding loans; similar information also may be needed by insurance companies.

International level Various regional and global agencies utilize information on animal health and productivity. Examples of the former are the European Union, the *Pan-American Health Organization (PAHO)*, the *South Asian Association for Regional Cooperation (SAARC)* and the *Animal Production and Health Commission for Asia (APHCA)*. The latter include the *World Health Organization (WHO)* and the *Food and Agriculture Organization (FAO)* of the United Nations. The Internet URLs of several of these are listed in Appendix IV.

The United Nations also has several regional and global agencies that are particularly concerned with funding agricultural projects in less-economically-developed countries, and for which information on animal health and productivity therefore is valuable. These include the *World Bank*, the *International Fund for Agricultural Development (IFAD)*, the *Economic Commission for Latin America and the Caribbean (ECLAC)*, the *United Nations Development Programme (UNDP)* and the *Inter-American Development Bank (IDB)*.

Information system implementation

Implementation of an information system is undertaken in several stages:

- definition of the data requirements and flow of information;
- identification of areas where passive and active data collection are appropriate;
- identification of areas where computerization is desirable, and of standards for exchange of data between other integrated systems;
- construction of a work plan, including any incremental steps in computerization (e.g., national centres first, followed by local centres) and in fulfilling information requirements (e.g., international needs may need to be supplied before national or local needs);
- training;
- assessment of progress;
- modification of the system, if necessary.

Each stage must be considered in the context of the availability of data generated by existing data-collection procedures, and the circumstances of the country or region in which the system is to be implemented. For example, a less-economically-developed country may have greater restrictions on manpower and financial and technical resources than a more-economically-developed one.

Attention needs to be given to the selection of appropriate hardware and software. Servicing and maintenance of the former should be available locally, and it should have sufficient processor power and auxiliary storage to run the software and store the amount of data that is anticipated. Additionally, in less-economically-developed countries, an uninterrupted power supply unit (UPS) is desirable to prevent damage to hardware and loss of data during power fluctuations and interruptions.

Various types of software may be required, and should be well supported by suppliers. A database management system is suitable for storing, handling

and extracting data, and for generating reports. Statistical packages also may be required for the analysis of data (see Appendix III); and a word-processing package is invaluable for producing reports and other communications. A geographical information system (see later in this chapter) also may be useful. The portability of files between packages is an important consideration, therefore packages should not be selected in isolation but in relation to one another. Ideally, all components should be 'seamlessly integrated'. Finally, data should be regularly 'backed-up' (i.e., extra copies stored on auxiliary storage or servers).

Some examples of veterinary databases and information systems

Computerized veterinary information systems have a history dating back to the mid-1960s (Tjalma *et al.*, 1964; Hutton and Seaton, 1966; Griner and Hutton, 1968; Hutton, 1969). *Table 11.4* lists some examples of veterinary databases and information systems. A brief outline of some of them will indicate their scope and the techniques employed in updating and using them. Dupuy and others (2013) summarize 27 European veterinary syndromic surveillance (see Chapter 21) databases.

The Office International des Epizooties (World Organisation for Animal Health) international disease-reporting system

The Paris-based *Office International des Epizooties* (OIE: *World Organisation for Animal Health*) is an international organization concerned with the global control of animal disease. It was founded in Paris in 1924 following an outbreak of rinderpest in Belgium in 1920, and has a macroscale reporting system that records data on the major infectious epidemic diseases of animals throughout the world.

The objectives of OIE are to:

- ensure transparency in the global animal disease situation;
- collect, analyse and disseminate veterinary scientific information;
- encourage international solidarity in the control of animal diseases;
- safeguard world trade by publishing health standards for international trade in animals and animal products;
- improve the legal framework and resources of national veterinary services;
- provide a better guarantee of food of animal origin and to promote animal welfare.

It is therefore necessary to know the natural history of diseases relevant to trade, the risk of transmission, and the biological and economic consequences of introduction of a pathogen. Until 2004, two categories of disease were defined (OIE, 1987):

1. 'List A': communicable diseases which have the potential for very serious and rapid spread, irrespective of national borders, which are of serious socio-economic or public health consequence, and which are of major importance in the international trade of animals and animal products (e.g., African horse sickness, contagious bovine pleuropneumonia, foot-and-mouth disease, highly pathogenic avian influenza, Newcastle disease, sheep pox and swine vesicular disease);
2. 'List B': communicable diseases which are considered to be of socio-economic and/or public health importance within countries and which are significant in the international trade of animals and animal products (e.g., anthrax, contagious equine metritis, fowl pox, glanders, Johne's disease (paratuberculosis), leptospirosis and rabies).

However, in May 2004 these lists were replaced by a single list of notifiable terrestrial and aquatic animal diseases (*Table 11.5*). The single list is used in order to be in line with the terminology of the Sanitary and Phytosanitary Agreement of the World Trade Organization, by classifying diseases as specific hazards and giving all listed diseases the same degree of importance in international trade. The list is reviewed annually and new lists come into force on 1 January of the following year.

The *World Animal Health Information Database* (WAHID) provides access to all data held within OIE's *World Animal Health Information System* (WAHIS), which is a secure website that allows users from Member Countries to submit standard notification reports directly to OIE, including: **immediate notification reports** of disease, infection or unusual epidemiological events, **follow-up reports** to the immediate notifications, and **final reports** when outbreaks have ended or the disease has become endemic; as well as **six-monthly reports** (which include monthly breakdowns of listed diseases, infections and infestations present in the country) and **annual reports**.

NADIS

NADIS (*National Animal Disease Information Service*) is a collaborative macroscale system, formed in 1995 to monitor diseases in cattle, sheep and pigs across the UK (NADIS, 2015). Sixty veterinary practices and

Table 11.4 Some veterinary databases and information systems, past and present.

<i>Macroscale</i>	<i>Mesoscale</i>	<i>Microscale*†</i>
African Union Inter-African Bureau for Animal Resources animal resources information system (Chibeu, 2011)	Australian slaughter check scheme (Pointon and Hueston, 1990)	APHIN [cattle, pigs] (Dohoo, 1988, 1992; APHIN, 2014)
<i>African VETLAB Network</i> (FAO, 2015)	British pig health schemes abattoir-lesion monitoring programme (Sanchez-Vazquez <i>et al.</i> , 2011)	Australian slaughter check scheme [pigs] (Pointon and Hueston, 1990)
ANADIS (Australia) (Roe, 1980; Andrews, 1988; Cannon, 1993)	Danish pig health and production surveillance system (Christensen <i>et al.</i> , 1994)	Bristol sheep health and productivity scheme [sheep] (Morgan and Tuppen, 1988)
APHIN (Canada) (Dohoo, 1988, 1992; APHIN, 2014)	Danish swine slaughter inspection bank (Willeberg, 1979)	<i>Checkmate</i> [dairy cattle] (Booth and Warren, 1984)
Australian State animal disease information systems (Andrews, 1988)	Edinburgh SAPTU clinical record summary system (Stone and Thrusfield, 1989)	<i>CHESS</i> [pigs] (Huirne and Dijkhuizen, 1994)
<i>BENCHMARK</i> (Canada) (Martin <i>et al.</i> , 1990)	<i>FAHRMX</i> (Bartlett <i>et al.</i> , 1982, 1985, 1986; FAHRMX, 1984)	<i>COSREEL</i> [cattle, sheep, pigs] (Russell and Rowlands, 1983)
<i>CAHN</i> (Canada) (Kloeze <i>et al.</i> , 2010)	Florida teaching hospital data retrieval system (Burridge and McCarthy, 1979)	<i>DairyCHAMP</i> [dairy cattle] (Udomprasert and Williamson, 1990; Marsh <i>et al.</i> , 1993; AIM, 2014)
Californian turkey flock monitoring system (Hird and Christiansen, 1991)	Liverpool clinical recording and herd health system (Williams and Ward, 1989b)	<i>DairyComp 305</i> [dairy cattle] (VAS, 2017)
Chilean livestock information system (Sotomayor, 2011)	Minnesota disease-recording system (Diesch, 1979)	<i>DAISY</i> [dairy cattle] (Pharo, 1983; Esslemont <i>et al.</i> , 1991; Esslemont, 1993b)
Danish pig health and production surveillance system (Christensen <i>et al.</i> , 1994)	<i>NCASP</i> (Glickman <i>et al.</i> , 2006)	Danish pig efficiency control system [pigs] (Herløv and Vedel, 1992)
Danish pig health scheme (Willeberg <i>et al.</i> , 1984; Mousing, 1988; Willeberg, 1992)	Parasitology diagnostic data program (Slocombe, 1975)	Danish pig health and production surveillance system [pigs] (Christensen <i>et al.</i> , 1994)
Danish swine slaughter inspection data bank (Willeberg, 1979; Willeberg <i>et al.</i> , 1984)	Queensland veterinary diagnostic-data-recording system (Elder, 1976)	<i>Edinburgh DHHPS</i> [dairy cattle] (Kelly and Whitaker, 2001; DHHPS, 2014)
<i>EID2</i> (McIntyre <i>et al.</i> , 2013; Wardeh <i>et al.</i> , 2015)	<i>SAVSNET</i> (Radford <i>et al.</i> , 2010, 2011; Sánchez-Vizcaino <i>et al.</i> , 2015) [§]	<i>FAHRMX</i> [dairy cattle] (Bartlett <i>et al.</i> , 1982, 1985, 1986; FAHRMX, 1984)
<i>FAO EMPRESS-i Asia</i> : regional animal disease information system for Asia (Jia, 2011)	Slovakian Veterinary Service management system (Haladej and Hurcik, 1988)	<i>InterHerd</i> [dairy and beef cattle, and mixed enterprises] (NMR, 2014)
German national animal disease-reporting system (Staubach <i>et al.</i> , 2011)	<i>VetCompass</i> (VetCompass, 2014) [§]	Liverpool clinical recording and herd health system [dairy cattle] (Williams and Ward, 1989b)
Indonesian animal health information systems (Soegiarto, 2011)	<i>Veterinary Recording of Zoo Animals</i> (Roth <i>et al.</i> , 1973)	Ontario dairy monitoring and analysis program [dairy cattle] (Kelton <i>et al.</i> , 1992)
Japanese disease-reporting system (Tanaka, 1992)	<i>VIDA</i> (Hall <i>et al.</i> , 1980; VIDA, 2013)	<i>PigCHAMP</i> [pigs] (Stein, 1988; PigCHAMP, 2017)
Management and disease information retrieval system for broiler chickens in Northern Ireland (McIlroy <i>et al.</i> , 1988; Goodall <i>et al.</i> , 1997)	<i>VMDB/VMDP</i> (Priester, 1975; Warble, 1994; VMDB, 2014)	<i>SIRO</i> [dairy cattle] (Goodall <i>et al.</i> , 1984)
Management and disease information retrieval system for farmed Atlantic salmon in Northern Ireland (Menzies <i>et al.</i> , 1992)		<i>Totalvet</i> [dairy cattle] (QMMS, 2017).
<i>NADIS</i> (UK) (NADIS, 2015)		<i>VAMP</i> [dairy cattle] (Noordhuizen and Buurman, 1984)
<i>NAHIS</i> (Australia) (Morley, 1988; Garner and Nunn, 1995; Garner, 2011; NAHIS, 2014)		<i>VIRUS</i> [dairy cattle] (Martin <i>et al.</i> , 1982)
<i>NAHMS</i> (US) (King, 1985; Glosser, 1988; Curtis and Farrar, 1990; Bush and Gardner, 1995; Wineland and Dargatz, 1998; NAHMS, 2014)		

(Continued)

Table 11.4 (Continued)

Macroscale	Mesoscale	Microscale*†
Namibian veterinary information system (Biggs and Hare, 1994; Hare and Biggs, 1996)		
New South Wales animal disease information system (Rolfe, 1986)		
New Zealand laboratory management and disease surveillance information system (Christiansen, 1980)		
New Zealand national livestock database (Ryan and Yates, 1994)		
Nigerian animal health information system (Ogundipe <i>et al.</i> , 1989)		
<i>Northern Ireland Animal and Public Health Information System/Northern Ireland Food Animal Information System</i> (Houston, 2001; DARD, 2014)		
OIE International disease-reporting system/World Animal Health Information system (Blajan and Chillaud, 1991; Jebara, 2011; Jebara <i>et al.</i> , 2012; OIE, 2015b)		
Ontario dairy monitoring and analysis program (Kelton <i>et al.</i> , 1992)		
<i>RADAR (Rapid Analysis and Detection of Animal-related Risks)</i> (UK) (Lysons <i>et al.</i> , 2007; Paiba <i>et al.</i> , 2007)		
Serbian veterinary information management system (Plavšić <i>et al.</i> , 2009)		
Slovakian Veterinary Service management system (Haladej and Hurcik, 1988)		
Swedish dairy-cow disease-recording system (Mörk <i>et al.</i> , 2010)		
Swiss national animal health information system (Riggenbach, 1988)		
Taiwanese disease-reporting system (Sung, 1992)		

* These systems are health and productivity schemes. They may also function to varying degrees as mesoscale and macroscale systems.

† The type of livestock recorded in each system is in square brackets.

§ These also function as macroscale systems.

the farm animal units from six veterinary colleges take part in scanning surveillance (see Chapter 21), recording all the diseases that they encounter on a daily basis. This information is loaded onto a central database every week, and monthly reports are produced, so that disease trends can be monitored and early variations from normal trends detected. It also publishes a monthly parasite forecast for farmers, based on detailed Meteorological Office data, and monthly disease alerts, highlighting diseases that are particularly prevalent.

NAHMS

NAHMS (*National Animal Health Monitoring System*) is a macroscale system, designed to measure the incidence, prevalence and cost of health-related events in livestock in the US, and to identify determinants of disease in modern production systems. It has evolved in response to calls in the US for a national surveillance system for endemic conditions of livestock, which began in the 1920s and continued during the 1960s and 1970s (Poppensiek *et al.*, 1966; Hutton and Halvorson, 1974).

Table 11.5 OIE listed diseases, infections and infestations in force in 2014. (From OIE, 2014.)

Multiple species diseases, infections and infestations	
Anthrax	Porcine cysticercosis
Bluetongue	Porcine reproductive and respiratory syndrome
Brucellosis (<i>Brucella abortus</i>)	Swine vesicular disease
Brucellosis (<i>Brucella melitensis</i>)	Transmissible gastroenteritis
Brucellosis (<i>Brucella suis</i>)	Lagomorph diseases and infections
Crimean Congo haemorrhagic fever	Myxomatosis
Epizootic haemorrhagic disease	Rabbit haemorrhagic disease
Equine encephalomyelitis (Eastern)	Fish diseases
Foot-and-mouth disease	Epizootic haematopoietic necrosis
Heartwater	Infection with <i>Aphanomyces invadans</i> (epizootic ulcerative syndrome)
Infection with Aujeszky's disease virus	Infection with <i>Gyrodactylus salaris</i>
Infection with <i>Echinococcus granulosus</i>	Infection with HPR-deleted or HPR0 infectious salmon anaemia virus
Infection with <i>Echinococcus multilocularis</i>	Infection with salmonid alphavirus
Infection with rabies virus	Infectious haematopoietic necrosis
Infection with rinderpest virus	Koi herpesvirus disease
Infection with <i>Trichinella</i> spp.	Red sea bream iridoviral disease
Japanese encephalitis	Spring viraemia of carp
New world screwworm (<i>Cochliomyia hominivorax</i>)	Viral haemorrhagic septicaemia
Old world screwworm (<i>Chrysomya bezziana</i>)	Crustacean diseases
Paratuberculosis	Crayfish plague (<i>Aphanomyces astaci</i>)
Q fever	Infectious hypodermal and haematopoietic necrosis
Rift Valley fever	Infectious myonecrosis
Rinderpest	Necrotising hepatopancreatitis
Surra (<i>Trypanosoma evansi</i>)	Taura syndrome
Tularemia	White spot disease
Vesicular stomatitis	White tail disease
West Nile fever	Yellowhead disease
Sheep and goat diseases and infections	Cattle diseases and infections
Caprine arthritis/encephalitis	Bovine anaplasmosis
Contagious agalactia	Bovine babesiosis
Contagious caprine pleuropneumonia	Bovine genital campylobacteriosis
Infection with <i>Chlamydophila abortus</i> (enzootic abortion of ewes, ovine chlamydiosis)	Bovine spongiform encephalopathy
Infection with peste des petits ruminants virus	Bovine tuberculosis
Maedi-visna	Bovine viral diarrhoea
Nairobi sheep disease	Enzootic bovine leukosis
Ovine epididymitis (<i>Brucella ovis</i>)	Haemorrhagic septicaemia
Salmonellosis (<i>S. abortusovis</i>)	Infection with <i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> SC (contagious bovine pleuropneumonia)
Scrapie	Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis
Sheep pox and goat pox	Lumpy skin disease
Swine diseases and infections	Theileriosis
African swine fever	Trichomonosis
Infection with classical swine fever	Trypanosomosis (tsetse-transmitted)
Nipah virus encephalitis	

(Continued)

Table 11.5 (Continued)

Equine diseases and infections	
Contagious equine metritis	
Dourine	
Equine encephalomyelitis (Western)	
Equine infectious anaemia	
Equine influenza	
Equine piroplasmosis	
Glanders	
Infection with African horse sickness virus	
Infection with equid herpesvirus 1 (EHV-1)	
Infection with equine arteritis virus	
Venezuelan equine encephalomyelitis	
Avian diseases and infections	
Avian chlamydiosis	
Avian infectious bronchitis	
Avian infectious laryngotracheitis	
Avian mycoplasmosis (<i>Mycoplasma gallisepticum</i>)	
Avian mycoplasmosis (<i>Mycoplasma synoviae</i>)	
Duck virus hepatitis	
Fowl typhoid	
Infection with avian influenza viruses and infection with influenza A viruses of high pathogenicity in birds other than poultry including wild birds	
Infectious bursal disease (Gumboro disease)	
Newcastle disease	
Pullorum disease	
	Turkey rhinotracheitis
Bee diseases, infections and infestations	
	Infection of honey bees with <i>Melissococcus plutonius</i> (European foulbrood)
	Infection of honey bees with <i>Paenibacillus larvae</i> (American foulbrood)
	Infestation of honey bees with <i>Acarapis woodi</i>
	Infestation of honey bees with <i>Aethina tumida</i> (small hive beetle)
	Infestation of honey bees with <i>Tropilaelaps</i> spp.
	Infestation of honey bees with <i>Varroa</i> spp. (varroosis)
Mollusc diseases	
	Infection with abalone herpesvirus
	Infection with <i>Bonamia exitiosa</i>
	Infection with <i>Bonamia ostreae</i>
	Infection with <i>Marteilia refringens</i>
	Infection with ostreid herpesvirus 1 microvariant
	Infection with <i>Perkinsus marinus</i>
	Infection with <i>Perkinsus olseni</i>
	Infection with <i>Xenohaliotis californiensis</i>
Amphibians	
	Infection with <i>Batrachochytrium dendrobatidis</i>
	Infection with ranavirus
Other diseases and infections	
	Camelpox
	Leishmaniosis

An important requirement is that farm enterprises are randomly selected so that representative, unbiased results are obtained (see Chapter 13). Following early random selection schemes (e.g., the Minnesota disease recording system: Table 11.4), a pilot project involving pig producers, called *NADS* (*National Animal Disease Surveillance System*), began in the States of Ohio and Tennessee in 1983, and *NAHMS* now extends over the country to cover the full range of livestock species. Data relating to disease occurrence, demography and costs (e.g., of preventive measures) are collected from the selected farms. Samples of blood, faeces, feed and water are collected from some participating farms to validate clinical diagnoses and producers' observations. Following local data collection and analysis, records are forwarded to a national coordinating centre for aggregation and regional and national analysis. Individual-producer and state summary reports are also produced.

VMDB

The *VMDB* (*Veterinary Medical Databases*) is a collaborative mesoscale database involving most veterinary schools in North America. It is located at the University of Missouri, and has four subsidiaries: *Equine Eye Registration Foundation* (a registry and research database of horses unaffected by the major heritable eye diseases); *Canine Eye Registration Foundation* (a registry and research database of purebred dogs unaffected by the major heritable eye diseases); *DNA* (a registry of Irish setters that have passed a DNA test for progressive retinal atrophy or canine leukocyte adhesion deficiency); and *Health Information Managers* (providing information for managers). Data were coded using *SNVDO* codes, but *SNOMED* codes (see Chapter 10) are now used (Folk *et al.*, 2002). The *VMDB* data sources, like many of the others (see Chapter 21), demonstrate selection

bias, but the database (which dates back to 1964, and contains several million records) has provided a considerable amount of material for epidemiological investigations, including observational studies.

VIDA

VIDA (*Veterinary Investigation Diagnosis Analysis*) is a mesoscale database comprising records of specimen submissions to the eight regional laboratories of the Animal and Plant Health Agency in England and Wales, and the eight laboratories attached to Scotland's Rural College (Hall *et al.*, 1980; VIDA, 2013). These submissions are voluntary and constitute only a proportion of all post-mortem and laboratory specimens. The individual laboratories code diagnostic data, which are then submitted electronically to a central server for storage in a relational database. Annual summaries are produced, and there is also an *ad hoc* data-retrieval service.

The bias in the data is likely to be influenced by several factors, including the particular clinical presentation of a suspected disease, the level of awareness of a disease and its perceived importance, thus requiring caution in interpreting both individual summary figures and trends.

Herd management software

Numerous computer software programs have been developed to assist daily animal management, herd performance monitoring, and problem analysis. Typically these are microscale schemes. Current and past dairy management systems include: *Dairy-CHAMP*; *DairyCOMP 305* (Rapnicki *et al.*, 2012; VAS, 2017); *Interherd* (Hanks, 2009); and *Totalvet* (QMMS, 2017). Events related to individual cows are recorded in relation to reproductive and lactation cycles and health records (e.g., records of service, pregnancy diagnosis, and mastitis). These systems are able to generate a range of reports to support management, monitor performance, and identify and analyse problems, such as excessive calving to conception intervals, and are therefore important components of health and productivity schemes (see Chapter 26).

Pig herd health and productivity software programs include: *PigCHAMP*, a decision support system for pig-breeding enterprises (Stein, 1988; *PigCHAMP*, 2017); *PigORACLE*, an economic expert system for assessing breeding herd performance; *CHESS* (*Computerized Herd Evaluation System for Sows*: Huirne *et al.*, 1992); and an expert system for evaluating herd culling

and replacement policy, *PorkCHOP* (Dijkhuizen *et al.*, 1986; Huirne *et al.*, 1991).

Cattle Tracing System

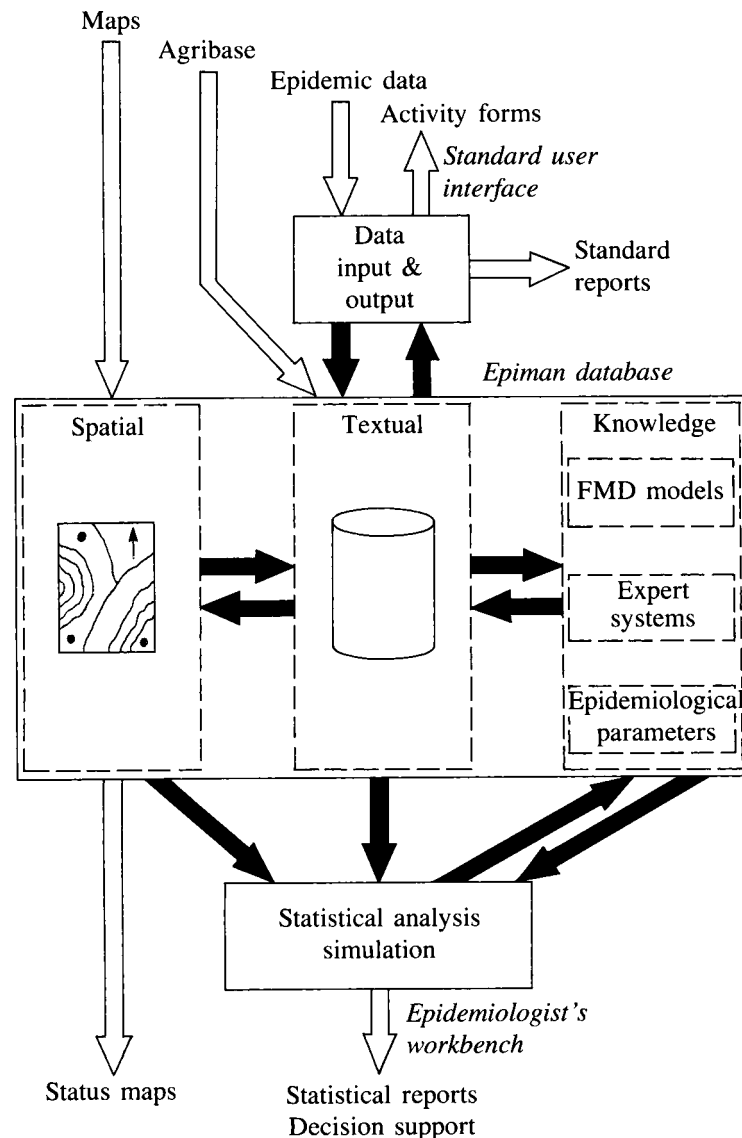
The British Cattle Tracing System (CTS) began in 1998 and records demographic and movement data of cattle in Great Britain. The database collects information from cattle registrations (including date of birth, sex, breed and date of death or export), animal holdings (including location data for farms, livestock markets, common land and so on) and movement notifications. The database makes it possible to check which animals are registered as being present on a farm and track where animals have been during their lifetime. As well as being used to ensure traceability of animals, the data held in this system are available to researchers and have been used for a wide range of purposes, including investigation and modelling of infectious disease spread (e.g., Robinson *et al.*, 2007; Carrique-Mas *et al.*, 2008; Kiss *et al.*, 2008) and investigation of the characteristics of the British cattle movement population and movement between farms (e.g., Mitchell *et al.*, 2005; Robinson and Christley, 2007). Bowling and others (2008) summarize systems of identifying and tracing cattle in a number of countries, including Australia, New Zealand, Namibia, Botswana, Japan, South Korea and Brazil.

EpiMAN

EpiMAN (Morris *et al.*, 1992, 1993; Stärk *et al.*, 1998; Sanson *et al.*, 1999) is a decision support system for the control of diseases that require national control or eradication procedures. It was developed in New Zealand, initially for the control of exotic diseases. The system is mounted on microcomputers that can be linked by communications networks, and is portable so that it can be moved easily to required locations. For example, it was used in the UK during the 2001 foot-and-mouth disease epidemic (Morris *et al.*, 2001).

The main components of *EpiMAN*, applied to foot-and-mouth disease control, are illustrated in Figure 11.9. Spatial and textual data relating to infected areas are stored in a relational database management system. The spatial data are handled by a geographical information system. There is provision to store information on all farms in a country in which the system is used (the 'Agribase': Sanson and Pearson, 1997). A Monte Carlo model (see Chapter 23) simulates spread of infection on individual farms; airborne transmission is modelled using the Gaussian plume dispersion model (see Chapter 6); and a third model predicts spread between farms, using parameters such as the size of infected areas, contact slaughter and 'ring'

Fig. 11.9 The structure of the EpiMAN decision support system. (Adapted from Morris *et al.*, 1993.)



vaccination (vaccinating animals in a ring around an infected area: see Chapter 27). Finally, expert systems assign priorities to tracing animals and people who moved on to or off infected premises prior to diagnosis of the disease, and rate the risk of infection on unaffected farms so that daily patrol schedules can be planned. Keeling (2005), however, notes that the complexity of this approach has been recognized as a limitation because of the level of detail required for many parameters in the model.

EID2

EID2 (ENHanCED Infectious Diseases database) is an automated and systematically generated open-access database of pathogens of humans and animals (particularly domestic species), their hosts and vector species, and their global distribution (McIntyre *et al.*, 2013;

Wardeh *et al.*, 2015). The database comprises pathogen–location and host–pathogen relationships identified from associations between organisms recorded in metadata¹³ of the National Center for Biotechnology Information (NCBI, 2017a) and *PubMed* publication abstracts (NCBI, 2017b), using semi-automated procedures. Algorithms are used to extract and analyse material contained in these sources, storing results in the database. Although originally set up as a tool for use within the ENHanCED project, which focusses on identifying diseases of highest impact that are influenced by climate (McIntyre

¹³ Metadata comprise structured information that describes, explains, locates, or otherwise makes it easier to retrieve, use or manage an information resource (NISO, 2004). Metadata are often called 'data about data' or 'information about information'.

et al., 2010), EID2's large-scale and semi-automated nature means that it is now used widely in research projects; for example, to generate lists of hosts by pathogen, pathogens by host, and host–pathogen systems within countries. The database also contains information on climatic variables and demographic data. By linking these data with pathogen data, the current global distribution of pathogens can be explored, and future distribution modelled (Ward *et al.*, 2009).

NCASP

The NCASP (*National Companion Animal Surveillance Program*) was established at Purdue University to monitor clinical syndromes and diseases using the medical records of companion animals visiting over 500 veterinary hospitals owned by the largest provider of companion-animal health care in the US (Glickman *et al.*, 2006). It was originally developed for syndromic surveillance (see Chapter 21) of agents of bioterrorism (e.g., anthrax, botulism, plague and tularaemia). Surveillance was expanded by inclusion of electronic reports from a chain of diagnostic laboratories serving over 18 000 private practices. The program displays temporal and spatial patterns of disease in dogs, cats and other companion animals. It can detect unusual clusters of potential or emerging zoonotic infections, and monitors the activity of fleas and ticks.

SAVSNET

The SAVSNET (*Small Animal Veterinary Surveillance Network*) was set up in 2008 by the University of Liverpool to meet a perceived deficiency in companion-animal surveillance (Radford *et al.*, 2010). In 2012, it became a partnership between the University of Liverpool and the British Small Animal Veterinary Association, with the goal of capturing data and making them available to a wide scientific community.

SAVSNET collects data from two sources: (1) veterinary diagnostic laboratories, publishing quarterly reports of diagnostic activity online; and (2) data collected from practice management software during consultations by veterinarians in private practice. The latter includes signalment (i.e., an animal's age, sex and breed), postcode, free text and treatments, and is augmented by a short questionnaire at the end of each consultation.

Three major opportunities will exist to utilize SAVSNET. First, members of the public will be able to access basic statistics. Secondly, contributing veterinarians will receive information allowing them to compare their clinical data with those of other contributors. Thirdly, researchers will be able to apply for access to existing data and to post their queries. It is

anticipated that the volume of data collected will facilitate effective analysis of conditions with rare risk factors and low prevalence. To date, the data have been used to provide new insights into the use of antibacterial preparations in practice (Radford *et al.*, 2011), syndromic surveillance of gastrointestinal disease (Jones *et al.*, 2014) and laboratory-based surveillance of canine parvovirus (Sánchez-Vizcaíno *et al.*, 2015).

VetCompass

VetCompass (*Veterinary Companion Animal Surveillance System*) is a companion-animal health surveillance system (see Chapter 21), developed by the Royal Veterinary College, London, in collaboration with the University of Sydney, which collates electronic patient records from veterinary practices across the UK (VetCompass, 2014). It was created to meet a call for improved epidemiological understanding of disorders affecting pedigree dogs (Bateson, 2010).

Practitioners record summary diagnostic terms during consultations, using a VeNom Code list (see Chapter 10) embedded within practice management systems (PMSs). Electronic patient record data are extracted from PMSs using clinical queries and transferred automatically to a secure *VetCompass* database. Demographic data (animal, owner and clinic identification, species, breed, sex, date of birth, neutering status, insurance status, microchip status and weight) and clinical data (clinical notes, summary diagnostic terms and treatment) are recorded.

VetCompass studies have examined systemic glucocorticoid pharmacotherapy (O'Neill *et al.*, 2012), and the epidemiology of canine epilepsy (Kearsley-Fleet *et al.*, 2013) and chronic kidney disease (O'Neill *et al.*, 2013). *VetCompass* collaborators within the UK include the University of Cambridge, Queen's University Belfast, the Animal Health Trust and the Kennel Club; and the *VetCompass* model has been extended internationally to Australia, Spain and Germany.

Geographical information systems

Geographical (geospatial) information systems (GIS) are systems for collecting, storing, managing, interrogating and displaying spatial data in an integrated environment, which nowadays is usually digital computing (Longley *et al.*, 2005). They have a range of powerful functions in addition to simple mapping (see Chapter 4); these include graphical analysis based on spatial location, statistical analysis and modelling.

Structure of GIS

Data that are input may be **cartographic data**, describing the location of features; and textual

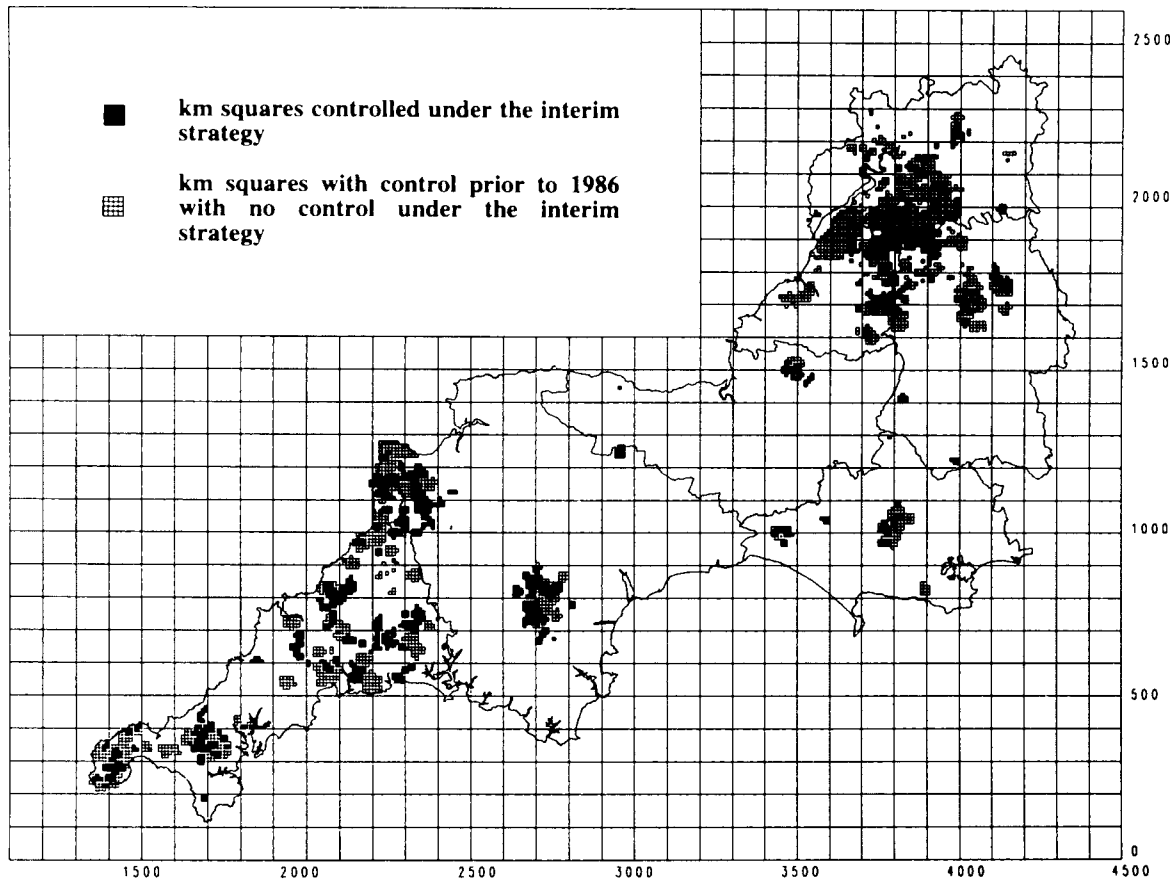


Fig. 11.10 Areas of statutory badger control in south-west England: an example of a grid-based map generated by a GIS. Badgers are reservoirs of infection with *Mycobacterium tuberculosis* in this region. Statutory badger removal to control bovine tuberculosis started in 1975 and was based on areas delineated by physical barriers such as roads, so that the instigating herd of cattle and nearby herds were included. This strategy was costly and, in 1986, was replaced by an 'interim' strategy in which badgers were removed only from land used by infected herds in which infection had been unequivocally associated with badgers. Further analysis with the GIS identified areas where pre-emptive badger control could be applied. (Source: Clifton-Hadley, 1993.)

attribute data, describing characteristics of the features. These types of data may be **primary** or **secondary**. Primary data may be **directly sensed** from field sketching, interviews and measurements. Alternatively, they may be **remotely sensed** (Hay *et al.*, 2000; Rinaldi *et al.*, 2006); that is, collected by a device not in direct contact with the object that is being sensed (e.g., a photographic camera). Meteorological satellites also have been used to detect habitats of ticks, mosquitoes, trematodes (Hugh-Jones, 1989; Marechal *et al.*, 2008) and tsetse flies (Hendrickx *et al.*, 2000; Rogers, 2000).

The GIS then store these **geographically referenced data** in a database management system in a form that can be graphically queried and summarized.

Cartographic data must be stored in **digital form** on computers. The digital maps are stored in two basic formats: **grid-based (raster-based)** and

vector-based. In grid-based systems, information is stored uniformly in relation to each cell that forms the grid (Figure 11.10). In vector-based systems, points and lines (arcs) are used to represent geographical features, the lines being composed of their respective straight-line segments. Areas enclosed by lines (e.g., farms) are termed polygons. A **digitizing tablet** is used to convert maps to a digital format for vector-based storage. This is an electronic board and pointer that accurately transcribes a map to digital format. A **scanner** is used for raster-based storage.

Grid-based systems store and manipulate regional and remotely-sensed data conveniently, but data processing is relatively slow if high resolution is required. In contrast, vector-based systems have inherently high resolution but are complex to implement. Many current systems can analyse both vector and raster data.

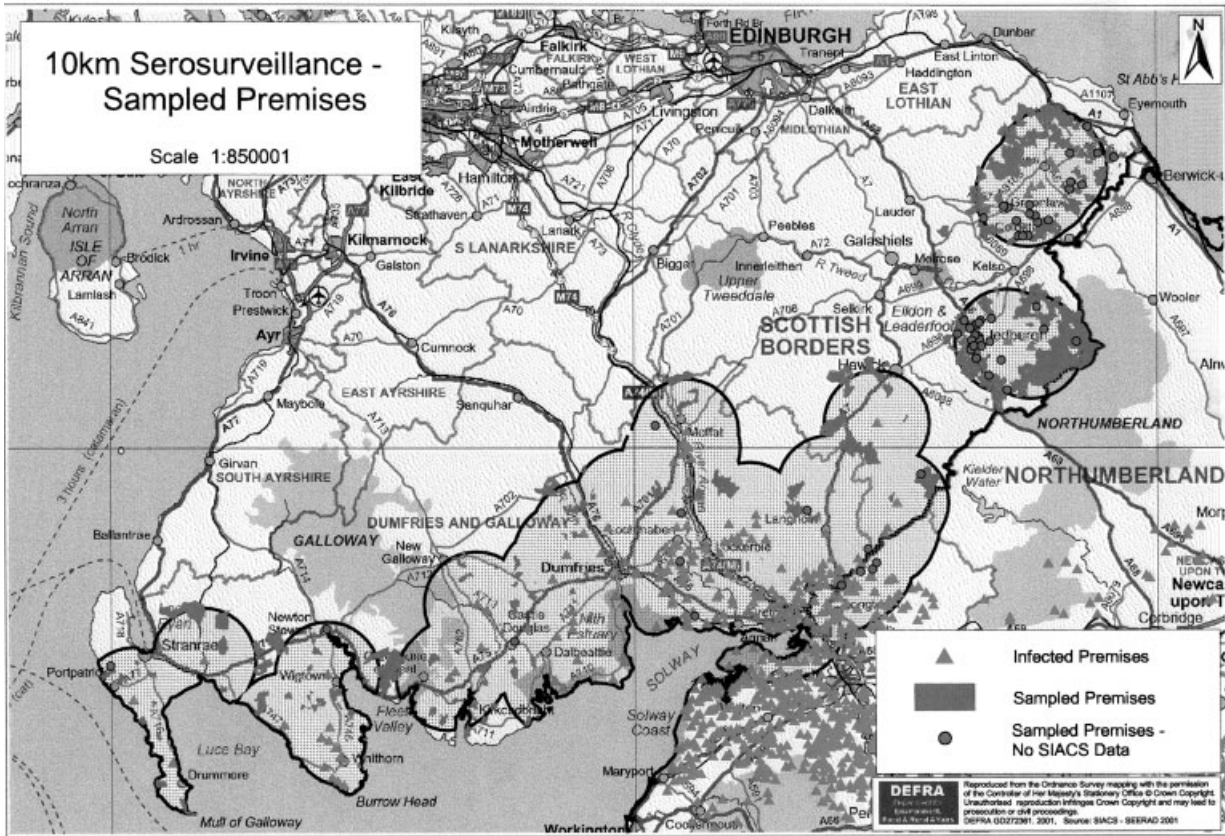


Fig. 11.11 Identification of all small-ruminant holdings within a 10-km radius of premises with confirmed foot-and-mouth disease infection (the 'sampling frame'), preparatory to selecting a sample to demonstrate freedom from infection: an example of neighbourhood analysis using a GIS. Few stocked holdings were within a 3-km radius because of prior pre-emptive culling. (Source: Stringer and Thrusfield, 2001.)

Applications of GIS

Applications of GIS (Sanson *et al.*, 1991a) include:

- **cartography**, with the advantage over traditional techniques that special-purpose maps can be produced and updated rapidly;
- **neighbourhood analysis** which allows an investigator to list all features that meet specified criteria (e.g., in the foot-and-mouth disease epidemic that occurred in the UK in 2001, in some regions all premises within a 3-km radius of an infected farm were identified, and sheep on these premises were pre-emptively culled (see Chapter 27) to reduce the perceived risk of spread of the disease, after which serosurveillance was conducted on small ruminants within a 10-km radius of each infected premises: *Figure 11.11*);
- **buffer generation** around or along certain features (e.g., definition of all properties at risk of infection within a given distance of an infected farm, or along a road that has been used by infected animals);
- **overlay analysis** in which two or more data sets are superimposed on top of one another and areas of intersection (overlay) of features identified (e.g., overlaying land-form, vegetation and watering-point

locations to identify areas where animals are difficult to muster for tuberculin testing: Laut, 1986);

- **network analysis** permitting optimal routing along networks of linear features;
- **three-dimensional surface modelling** (e.g., construction of isoplethic maps with height proportional to disease incidence or other characteristics: *Figure 11.12*).

The ability of GIS to link graphic and non-graphic data facilitates powerful analysis of the spatial distribution of disease and related factors. These systems are being increasingly applied to animal disease control as integral parts of veterinary decision support systems. Some GIS and remote-sensing applications are listed in *Table 11.6*, and the value of GIS and remote sensing are discussed by Hugh-Jones (1991a,b), Hay and others (2000), Cringoli and others (2005), Rinaldi and others (2006), Bergquist (2011) and Ferrè and others (2011). Paterson (1995) outlines some of the problems associated with the practical implementation of GIS in veterinary epidemiology – particularly for the new user.

Fig. 11.12 Spatial distribution of the number of tuberculin test reactors in cattle in New Zealand, 1988–1989: an example of an isoplethic map (see Chapter 4) generated by a GIS. The heights of the peaks represent numbers of tuberculin test reactors; the bases represent geographical areas of the country. (From *OIE Scientific and Technical Review*, 10 (1), March 1991.)

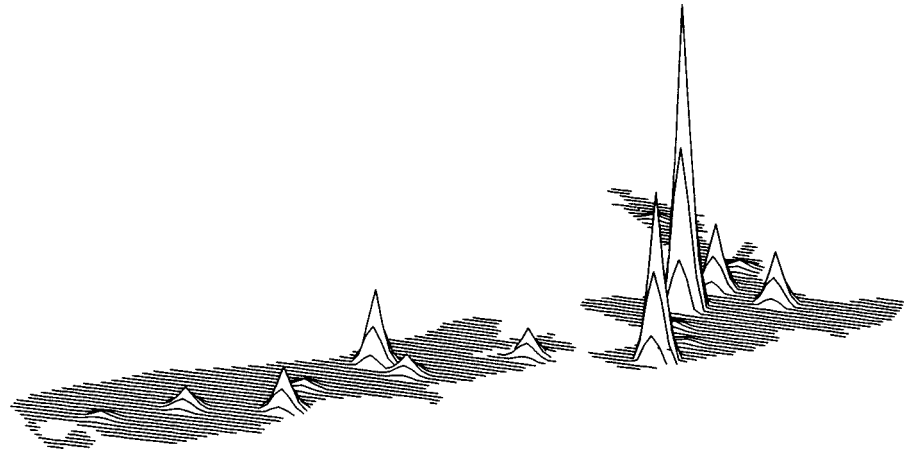


Table 11.6 Some applications of geographical information systems and remote sensing in veterinary and related fields.

Area of application	Country/continent	Source
Animal disease surveillance, monitoring and disease control	Global	Amin <i>et al.</i> (2012); Dhama <i>et al.</i> (2013)
Antibiotic resistance	US	Singer <i>et al.</i> (1998)
Aujeszky's disease	US	Marsh <i>et al.</i> (1991)
	Hungary	Solymosi <i>et al.</i> (2004)
Avian influenza (H5N1)	India	Dhingra <i>et al.</i> (2014)
	Nigeria	Ekong <i>et al.</i> (2012)
Bovine tuberculosis	Ireland	Hammond and Lynch (1992)
	New Zealand	Sanson <i>et al.</i> (1991b)
	UK	Clifton-Hadley (1993)
Blowfly strike	Australia	Ward and Armstrong (2000)
Canine cancer distribution	US	O'Brien <i>et al.</i> (1999)
<i>Corynebacterium pseudotuberculosis</i> infection (equine)	US	Doherr <i>et al.</i> (1999)
Disease control	Africa	Paterson <i>et al.</i> (2000)
Distribution of <i>Culicoides imicola</i> (vector of bluetongue)	Iberia	Rawlings <i>et al.</i> (1997)
	Morocco	Baylis and Rawlings (1998)
	Southern Africa	Baylis <i>et al.</i> (1999)
	Mediterranean	Baylis <i>et al.</i> (2001)
	Europe and North Africa	Tatem <i>et al.</i> (2003)
Distribution of <i>Echinococcus multilocularis</i> in foxes	Germany	Staubach <i>et al.</i> (1998); Berke (2001)
Dracunculiasis	West Africa	Clarke <i>et al.</i> (1991)
Ecology of bovine neosporidiosis	Italy	Rinaldi <i>et al.</i> (2005)
Ecology of ovine paramphistomosis	Italy	Cringoli <i>et al.</i> (2004)
Emergency rabies vaccination of foxes	Italy	Mulatti <i>et al.</i> (2011)
Equine motor neuron disease	US	de la Rúa-Domènech <i>et al.</i> (1995)
Fascioliasis	Belgium	Charlier <i>et al.</i> (2011)
	Ethiopia	Yilma and Malone (1998)
	Germany	Kuerpick <i>et al.</i> (2013)

(Continued)

Table 11.6 (Continued)

Area of application	Country/continent	Source
Foot-and-mouth disease	Brazil New Zealand South Africa Taiwan UK	Arámbulo and Astudillo (1991) Sanson <i>et al.</i> (1991a) Dion <i>et al.</i> (2011) Yamane <i>et al.</i> (1997) Lawson and Zhou (2005)
Gastric cancer	UK	Matthews (1989)
Geographical referencing of farms	UK	Durr and Froggatt (2002)
Habitat of the snail <i>Fossaria bulimoides</i> (intermediate host of <i>Fasciola hepatica</i>)	US	Zukowski <i>et al.</i> (1991)
Handling epidemiological data	US	Campbell (1989)
Human population growth in relation to tsetse fly extinction	Africa	Reid <i>et al.</i> (2000)
Leptospirosis	US	Hennebelle <i>et al.</i> (2013)
Livestock production and health atlas	Global	Clements <i>et al.</i> (2002); GLiPHA (2014)
Mosquito population dynamics	US	Wood <i>et al.</i> (1991)
Prediction of outbreaks of <i>Oestrus ovis</i>	Namibia	Flasse <i>et al.</i> (1998)
Prediction of outbreaks of Rift Valley fever	Africa	Linthicum <i>et al.</i> (1991)
Q fever	The Netherlands	Schimmer <i>et al.</i> (2010)
Site selection for fish farming	Ghana	Kapetsky <i>et al.</i> (1991)
<i>Rickettsia conorii</i>	Italy	Mannelli <i>et al.</i> (2001)
Theileriosis	Africa	Lessard <i>et al.</i> (1988, 1990)
Tick and flea activity	Europe	Beugnet <i>et al.</i> (2009)
Tick habitats: <i>Amblyomma variegatum</i>	St Lucia Guadeloupe	Hugh-Jones (1991c) Hugh-Jones and O'Neil (1986) Hugh-Jones <i>et al.</i> (1992)
Lyme disease tick distribution	US	Kitron <i>et al.</i> (1991); Nicholson and Mather (1996)
<i>Rhipicephalus appendiculatus</i>	Czechoslovakia Africa	Daniel and Kolár (1990) Lessard <i>et al.</i> (1990) Perry <i>et al.</i> (1990) Randolph (1994)
Trypanosomiasis	Burkina Faso West Africa	Michel <i>et al.</i> (2002) de La Rocque <i>et al.</i> (2005)
Tsetse fly control	Botswana Ethiopia Zambia	Allsopp (1998) Sciarretta <i>et al.</i> (2005) Symeonakis <i>et al.</i> (2007)
Vector-borne diseases and vector distribution	Global	DeGroote <i>et al.</i> (2012)
<i>Vibrio vulnificus</i> (model for cholera)	US	Hugh-Jones <i>et al.</i> (1996)
Wildlife disease management	Africa, New Zealand, UK	Pfeiffer and Hugh-Jones (2002)

Further reading

Abramson, J.H. and Abramson, Z.H. (2008) *Research Methods in Community Medicine*, 6th edn. John Wiley and Sons, Chichester. (Includes guidelines on interviews and self-administered questionnaires)

Albert, D.P., Gesler, W.M. and Levergood, B. (Eds) (2000) *Spatial Analysis, GIS, and Remote Sensing Applications in the Health Sciences*. Sleeping Bear Press, Chelsea

- Association for Veterinary Informatics Newsletter.
<http://www.avinformatics.org> (accessed 31 July 2017)
- Bennett, A.E. and Ritchie, K. (1975) *Questionnaires in Medicine*. Nuffield Trust, London. <http://www.nuffieldtrust.org.uk/publications/questionnaires-medicine> (accessed 31 July 2017)
- Blood, D.C. and Brightling, P. (1988) *Veterinary Information Management*. Baillière Tindall, London
- Boynton, P.M. (2004) Administering, analyzing, and reporting your questionnaire. *British Medical Journal*, **328**, 1372–1375
- Casley, D.J. and Lury, D.A. (1982) *Data Collection in Developing Countries*. Clarendon Press, Oxford
- Cimino, J.J. (2006) In defense of the Desiderata. *Journal of Biomedical Informatics*, **39**, 299–306. (A critique of medical terminology, with particular reference to computerized data recording and retrieval)
- Clements, A.C.A., Pfeiffer, D.U., Otte, M.J., Morteo, K. and Chen, L. (2002) A global livestock production and health atlas (GLiPHA) for interactive presentation, integration and analysis of livestock data. *Preventive Veterinary Medicine*, **56**, 19–32
- Dillman, D.A., Smyth, J.D. and Christian, L.M. (2014) *Internet, Phone, Mail and Mixed-Mode Surveys: The Tailored Design Method*, 4th edn. John Wiley and Sons, Hoboken.
- Dohoo, I.R. (1994) Specific issues concerning the application of information systems in developing countries. *World Animal Review*, **3**, 19–24
- Durr, P.A. and Gatrell, A.C. (Eds) (2004) *GIS and Spatial Analysis in Veterinary Science*. CABI Publishing, Wallingford
- Ellis, P.R. (1993) Information systems in disease control programs. In: *Diagnosis and Epidemiology of Foot-and-Mouth Disease in Southeast Asia*. Eds Copland, J.W., Gleeson, L.J. and Chamnanpood, C., pp. 111–115. Australian Centre for International Agricultural Research, Canberra
- FAO (1999) *Manual on Livestock Disease Surveillance and Information Systems*. FAO Animal Health Manual No. 8. Food and Agriculture Organization of the United Nations, Rome
- FAO (2011) *Challenges of Animal Health Information Systems and Surveillance for Animal Diseases and Zoonoses*. Proceedings of the International Workshop organized by FAO, 23–26 November 2010, Rome, Italy. Animal Production and Health Proceedings No. 14. Food and Agriculture Organization of the United Nations, Rome
- Featherston, R.H. (1997) Benefits of a national database for patient medical records. *Journal of the American Veterinary Medical Association*, **210**, 173–174
- Fowler, F.J. and Cosenza, C. (2009) Design and evaluation of survey questions. In: *The SAGE Handbook of Applied Social Research Methods*, 2nd edn. Eds Bickman, L. and Rog, D.J., pp. 375–412. Sage Publications, Thousand Oaks
- Frerichs, R.R. and Selwyn, B.J. (2002) Microcomputers, the Internet, and epidemiology. In: *Oxford Textbook of Public Health*, Vol. 2, 4th edn. Eds Detels, R., McEwen, J., Beaglehole, R. and Tanaka, H., pp. 745–758. Oxford University Press, Oxford
- Grosh, M. and Glewwe, P. (Eds) (2000) *Designing Household Survey Questionnaires for Developing Countries: Lessons from 15 Years of the Living Standards Measurement Study*. Oxford University Press, New York
- Gwartney, P.A. (2007) *The Telephone Interviewer's Handbook*. Jossey-Bas, San Francisco
- Hay, S.I., Graham, A.J. and Rogers, D.J. (Eds) (2006) Global mapping of infectious diseases: methods, examples and emerging applications. *Advances in Parasitology*, **62**, 1–390
- Hay, S.I., Randolph, S.E. and Rogers, D.J. (Eds) (2000) Remote sensing and geographical information systems in epidemiology. *Advances in Parasitology*, **47**, 1–351
- Hernandez, M.J. (2013) *Database Design for Mere Mortals*, 3rd edn. Addison-Wesley, New Jersey
- Heske, L., Berendt, M., Jäderlund, K.H., Egenvall, A. and Nødtvedt, A. (2014) Validation of the diagnosis of canine epilepsy in a Swedish animal insurance database against practice records. *Preventive Veterinary Medicine*, **114**, 145–150. (A comparison of agreement between different sources of clinical data)
- Hugh-Jones, M. (Ed.) (1991) Applications of remote sensing to epidemiology and parasitology. *Preventive Veterinary Medicine*, **11**, 159–366
- Kelley, K., Clark, B., Brown, V. and Sitzia, J. (2003) Good practice in the conduct and reporting of survey research. *International Journal for Quality in Health Care*, **15**, 261–266
- Lavrakas, P.J. (Ed.) (2008) *Encyclopedia of Survey Research Methods*. Sage Publishing, Thousand Oaks
- Longley, P.A., Goodchild, M.F., Maguire, D.J. and Rhind, D.W. (Eds) (1999) *Geographical Information Systems*, 2nd edn. 2 vols. John Wiley and Sons, Chichester. (A reference work)
- Longley, P.A., Goodchild, M.F., Maguire, D.J. and Rhind, D.W. (2001) *Geographical Information Systems and Science*. John Wiley and Sons, Chichester. (An introductory text)
- Mangione, T.W. (2009) Mail surveys. In: *The SAGE Handbook of Applied Social Research Methods*, 2nd edn. Eds Bickman, L. and Rog, D.J., pp. 475–508. Sage Publications, Thousand Oaks
- McCrossan, L. (1991) *A Handbook for Interviewers*. HMSO, London

- Morris, R.S. (Ed.) (1991) Epidemiological information systems. *Revue Scientifique et Technique, Office International des Epizooties*, **10**, 1–231
- National Agricultural Library (1992) *International Directory of Animal Health and Disease Data Banks*. United States Department of Agriculture, Washington DC
- Penell, J.C., Egenvall, A., Bonnett, B.N. and Pringle, J. (2007) Validation of computerized Swedish horse insurance data against veterinary clinical records. *Preventive Veterinary Medicine*, **82**, 236–251. (*A comparison of agreement between different sources of clinical data*)
- Pfeiffer, D., Robinson, T., Stevenson, M., Rogers, D. and Clements, A. (2008) *Spatial Analysis in Epidemiology*, Oxford University Press, Oxford. (*Analysis and display of spatial data*)
- Pollari, F.L., Bonnett, B.N., Allen, D.G., Bamsey, S.C. and Martin, S.W. (1996) Quality of computerized medical record abstract data at a veterinary teaching hospital. *Preventive Veterinary Medicine*, **27**, 141–154
- Reddy, M.A. (2008) *Textbook of Remote Sensing and Geographical Information Systems*, 3rd edn. BS Publications, Hyderabad
- Revie, C.W., McKendrick, I., Irwin, T., Gu, Y., Reid, S.J.W. et al. (1996) The application of hybrid information systems to decision support in the veterinary and agricultural domains. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Glasgow, 27–29 March 1996. Eds Thrusfield, M.V. and Goodall, E.A., pp. 48–57
- Smith, R.D. (2003) The application of information technology to the teaching of veterinary epidemiology and public health. *Journal of Veterinary Medical Education*, **30**, 344–350
- United Nations (2005) *Household Sample Surveys in Developing and Transition Countries*. Department of Economic and Social Affairs, Studies in Methods, Series F, No. 96. United Nations, New York. https://unstats.un.org/unsd/hhsurveys/pdf/Household_surveys.pdf (accessed 31 July 2017)

12

Presenting numerical data

The epidemiologist makes inferences from data collected from groups of animals. The data are frequently quantitative, comprising numerical values. A fundamental characteristic of numerical biological data is their inherent **variability**. The weights of 100 Friesian cows, for example, will not be identical; there will be a range of values. If the 100 cows were a sample of a much larger group – say the national herd – then a different sample of 100, drawn from the same national herd, is almost certain to have a different set of weight values.

Variability is of importance to the epidemiologist in two circumstances: when a **sample** is taken and when different groups of animals are being **compared**. In the first circumstance, it is necessary to assess to what extent the sample's values are representative of those in the larger population from which the sample was drawn. In the second circumstance, it is often necessary to decide whether or not a difference between two groups can be attributed to a particular factor. In epidemiology, this frequently involves detecting an association between disease and hypothesized causal factors, and is discussed in Chapters 14 and 15. For example, if the effect of ketosis on milk yield were being investigated, then two groups of cows – one comprising cows with ketosis and one consisting of cows without ketosis – could be compared with respect to milk yield.

A detected difference in yield could be due to:

- the effect of ketosis;
- inherent natural variation in milk yield between the two groups;
- confounding variables (see Chapters 3, 15 and 18) such as breed: cows of different breeds may be present in different proportions in each group, when the different breeds produce different milk yields.

In this example, the second and third reasons for the difference can confuse the investigation by contributing to differences in the milk yields of the two groups.

Statistical methods exist to separate the effects of the factors that are being investigated from random variation and confounding. Essentially, these involve estimating the **probability** of an event taking place. Probability is a numerical measure taking values between zero and one. An event that is impossible has a probability of zero, whereas an event that is certain has a probability of one. Probability also may be thought of as the frequency of certain events relative to the total number of events that can occur. Thus, the probability of throwing a 'head' with an unbiased coin is $1/2$ (0.5). The probability of throwing either a 'head' or a 'tail' (i.e., the total probability) is one. Similarly, prevalence (see Chapter 4) is a measure of probability. A specific prevalence value is an estimation of **conditional probability**; a male sex-specific prevalence of 30% means that there is a probability of 0.3 of any one animal having a disease at a given point in time, conditional on its being male.

This chapter deals with the probability distributions of numerical data that are the basis of many statistical tests (some of which are described in Chapter 14) and with the methods of displaying numerical values. The statistical content of this book is not comprehensive; it is designed to give the reader a basic knowledge of some relevant concepts and techniques. The reader who is unfamiliar with elementary mathematical notation should first consult Appendix II.

Some basic definitions

Variable Any observable event that can vary. Variables may be either continuous or discrete (see Chapter 10). An example of a continuous variable is the weight of an animal. An example of a discrete variable is the number of cases of disease. In some circumstances, the numerical values of the variable are called **variates**.

Study variable Any variable that is being considered in an investigation.

Response and explanatory variables A response variable is one that is affected by another (explanatory) variable; for instance, an animal's weight may be a response variable and food intake an explanatory variable, because weight is assumed to depend on the amount of food consumed. In epidemiological investigations, disease often is considered as the response variable; for example, when studying the effects of dry cat food (the explanatory variable) on the incidence of feline urolithiasis. There also may be circumstances in which disease is considered as the explanatory variable; for instance, when studying the effect of disease on weight. Response variables often are called **dependent variables** or **outcome variables** and explanatory variables are called **independent variables** or **input variables**.

Parameter A quantity that can differ in different circumstances, but is constant in the case that is being considered. It may be a **constant** in a mathematical formula or model. For example, a survey may be designed to detect a minimum disease prevalence, such as 20%. Although prevalence can vary, the minimum detectable prevalence is defined for the objectives of the survey as a single unvarying value, and is therefore a parameter of the survey, which is incorporated in the appropriate formula to detect the specified minimum disease prevalence (see Chapter 13). A parameter also may be a **measurable characteristic** of a population such as the average milk yield of a herd of dairy cows.

Data set A collection of data.

Raw data The initial measurements that form the basis of analyses.

Some descriptive statistics

Table 12.1 lists sample weights of two groups (A and B) of piglets, when weaned at three weeks of age. These can be considered as random samples of a much larger group of piglets; namely, all piglets at three weeks of age. The inherent variability is obvious. The number of piglets with weights within defined intervals (i.e., the group **frequency distribution** of the weights) for Group B is recorded in Table 12.2 and depicted in Figure 12.1. This figure, which summarizes the data, is called a **histogram** (see below: 'Displaying

Table 12.1 Specimen three-week weaning weights (kg) of two groups (A and B) of piglets.

Group A				
4.2	5.3	5.6	6.0	6.4
4.6	5.3	5.7	6.0	6.4
4.7	5.4**	5.7	6.1	6.4
4.8	5.4	5.7	6.1	6.5
4.9	5.4	5.9*	6.1	6.5
5.1	5.4	5.9	6.1	6.5
5.2	5.4	5.9	6.1**	6.8
5.2	5.5	5.9	6.2	6.8
5.2	5.5	6.0	6.3	6.8
5.3	5.5	6.0	6.4	
$n = 49$; $\bar{x} = 5.76$ kg; $s = 0.60$ kg; $Q_1 = 5.4$ kg; $Q_2 = 5.9$ kg; $Q_3 = 6.1$ kg; $SIR = 0.35$ kg				
Group B				
2.6	4.3	4.6	4.8	5.3
3.4	4.3	4.6	5.0	5.5
3.6	4.3**	4.6	5.0	5.5
3.8	4.4	4.6	5.0	5.6
3.9	4.4	4.7*	5.0	5.6
4.0	4.4	4.7	5.1	5.6
4.0	4.4	4.7	5.1**	5.6
4.1	4.5	4.8	5.2	5.7
4.1	4.5	4.8	5.2	6.3
4.2	4.5	4.8	5.2	
$n = 49$; $\bar{x} = 4.69$ kg; $s = 0.67$ kg; $Q_1 = 4.3$ kg; $Q_2 = 4.7$ kg; $Q_3 = 5.1$ kg; $SIR = 0.40$ kg				

* Median.

** Quartiles.

Table 12.2 Grouped frequency distribution for the three-week weaning weights of piglets in Group B of Table 12.1.

Weight (kg)	Number of piglets
2.6–3.0	1
3.1–3.5	1
3.6–4.0	5
4.1–4.5	13
4.6–5.0	15
5.1–5.5	8
5.6–6.0	5
6.1–6.5	1

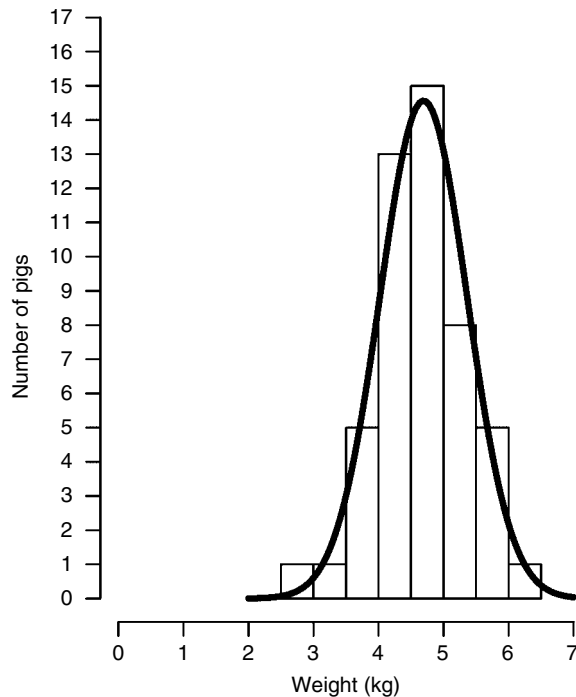


Fig. 12.1 Observed distribution of the weights of the 49 piglets in Group B depicted as a histogram (rectangles) and a fitted Normal curve (smooth curve). (Data from Table 12.1.)

quantitative data'). The intervals on the horizontal axis are 0.5 kg wide. The number of piglets within each interval is proportional to the area of the vertical bars. If the intervals on the horizontal axis are equal, as in this example, then the number of piglets within each interval is also proportional to the height of the bars. Alternatively, the vertical plots and the mid-points of the horizontal intervals can be joined, rather than constructing bars, in which case a **frequency polygon** is constructed. These data can be summarized further by the use of descriptive statistics that are measures of **position** and **spread** of the histogram.

Measures of position

A commonly adopted measure of position is the **mean** of the sample, denoted by $\bar{x}$ (pronounced 'x-bar'). It is calculated using:

$$\bar{x} = \frac{\sum x}{n},$$

where n is the number of values in the random sample. In Table 12.1, $n = 49$ in each group, and $\bar{x} = 5.76$ kg in Group A, and 4.69 kg in Group B.

Each sample has been assumed, implicitly, to have been drawn from a much larger population; thus,

the mean of the sample is only an **estimate** of the true population mean, μ . Only if all the population is investigated can the parameter μ be known. As the sample size increases, $\bar{x}$ will be a better estimator of μ ; that is, the **precision** of $\bar{x}$ as an estimator of μ will increase.

The **median** of the sample, sometimes denoted by Q_2 , is another measure of position. It is the value below which half, and therefore above which half, of the observations lie. It divides the distribution into equal, ordered subgroups and is termed a **quantile**. Quantiles that divide the distribution into hundredths are centiles (percentiles). The median therefore is the 50th centile (percentile).

The median values in Table 12.1 for Groups A and B, respectively, are marked with an asterisk, '*'. Again, the sample median is an estimator of the true population median.

The lower and upper **quartiles**, Q_1 and Q_3 , respectively, are defined as the two values that are mid-way between the lower and upper extreme values and the median. For Groups A and B they are marked with two asterisks, '**'. Thus, 25% of values fall below Q_1 , and 75% of values lie above it; Q_1 is therefore the 25th centile. Similarly, 75% of values fall below Q_3 and 25% of values lie above it; Q_3 is therefore the 75th centile.

Quartiles may be located between two values; notably, when there is an even number of observations. Interpolation is then required. If there are n observations, the first quartile (Q_1) is the observation at position $(n + 1)/4$; the second quartile (median: Q_2) is the observation at position $2(n + 1)/4$; and the third quartile (Q_3) is the observation at position $3(n + 1)/4$. For example, suppose $n = 10$. Then $(10 + 1)/4 = 2.75$, and Q_1 is between the second and third observations (call them x_2 and x_3), three-quarters of the way up. Thus, $Q_1 = x_2 + 0.75(x_3 - x_2)$. Similarly, $(10 + 1)/2 = 5.5$, and Q_2 is between the fifth and sixth observations, half-way up. Thus, $Q_2 = x_5 + 0.5(x_6 - x_5)$, where x_5 and x_6 are the fifth and sixth observations. Again, since $3(10 + 1)/4 = 8.25$, $Q_3 = x_8 + 0.25(x_9 - x_8)$, where x_8 and x_9 are the eighth and ninth observations.

Consider this data set, comprising six observations: 9, 12, 16, 22, 27, 31.

The first quartile (Q_1) is the observation at position $(n + 1)/4 = 7/4 = 1.75$. Q_1 is between the first and second observations, three-quarters of the way up:

$$9 + 0.75(12 - 9) = 9 + 2.25 = 11.25.$$

The median (Q_2) is the observation at position $2(n + 1)/4 = 14/4 = 3.5$. Q_2 is between the third and fourth observations, half of the way up:

$$16 + 0.5(22 - 16) = 16 + 3 = 19.$$

The third quartile (Q_3) is the observation at position $3(n + 1)/4 = 21/4 = 5.25$. Q_3 is between the fifth and sixth observations, one-quarter of the way up:

$$27 + 0.25(31 - 27) = 27 + 1 = 28.$$

Measures of spread

Measures of spread are a little more difficult to calculate than those of position. Two examples of simple measures of spread are the range and the mean of the absolute deviations of the individual values from the mean. However, these measures often do not distinguish different sets of data.

A commonly adopted measure is the **sample variance**, s^2 , which is calculated by:

$$s^2 = \frac{\sum (x - \bar{x})^2}{n - 1}.$$

This formula may be rewritten in a form that is more easily calculated with small calculators, namely:

$$s^2 = \frac{\sum x^2 - \{(\sum x)^2/n\}}{n - 1}.$$

The square root of the sample variance is called the sample **standard deviation**. Using the values from *Table 12.1*, Group B, and the formula for s^2 above, the sample standard deviation, s , is given by:

$$\begin{aligned} &= \sqrt{\frac{\sum x^2 - \{(\sum x)^2/n\}}{n - 1}} \\ &= \sqrt{\frac{1100.27 - (229.8^2/49)}{48}} \\ &= \sqrt{21.62/48} \\ &= 0.67 \text{ kg.} \end{aligned}$$

Just as the sample mean is an estimate of the population mean, so the sample variance and sample standard deviation are estimates of the **population variance**, σ^2 , and the **population standard deviation**, σ (sigma), respectively.

When summary statistics are presented, the sample standard deviation should be presented as well as the sample mean in order to indicate the variability within the population.

A measure of spread that sometimes accompanies the median is the **semi-interquartile range (SIR)**. This is half of the range between the quartiles, Q_1 and Q_3 (i.e., half of the **interquartile range, IQR**):

$$SIR = \frac{Q_3 - Q_1}{2}.$$

This is an estimator for the **population semi-interquartile range**.

Alternatively, and increasingly, a sample may be summarized by a **five-point summary** consisting of the minimum, lower quartile, median, upper quartile and maximum.

Statistical distributions

The Normal distribution

If *many* piglets were weighed, rather than just the 49 in the data set shown in *Table 12.1*, and if the intervals used in the histogram in *Figure 12.1* were reduced, then the bars would become narrower. Eventually, the corresponding frequency polygon would trace a smooth curve. One such curve has been fitted over the bars in *Figure 12.1*. The curve has one peak in the middle and is symmetrical. This bell shape is typical of a family of frequency distributions known as the **Normal** family of distributions. It is better spelled with an upper case N to avoid confusion with other meanings of the word. Another name for this distribution is the **Gaussian**¹ distribution. This distribution is described by two parameters: its mean, μ , and its standard deviation, σ .

The Normal curve can be used as a smooth approximation to a histogram based on a sample of values, as in *Figure 12.1*, or as the paradigm of the population distribution of a variable. The latter can be described mathematically as a **density function** (Samuels *et al.*, 2012), and plotted graphically as a **density curve**, which can be interpreted quantitatively in terms of areas under the curve (*Figure 12.2*). All Normal curves can be made equivalent with respect to areas under them by rescaling the horizontal axis. The rescaled variable is denoted by z , the **standardized Normal deviate**:

$$z = \frac{x - \mu}{\sigma}.$$

The values $z = 0, 1, 2, 3$ therefore correspond to $x = \mu, \mu + \sigma, \mu + 2\sigma$ and $\mu + 3\sigma$, respectively, derived thus:

$$\text{if } x = \mu, z = \frac{\mu - \mu}{\sigma} = 0;$$

$$\text{if } x = \mu + \sigma, z = \frac{\mu + \sigma - \mu}{\sigma} = \sigma/\sigma = 1;$$

$$\text{if } x = \mu + 2\sigma, z = \frac{\mu + 2\sigma - \mu}{\sigma} = 2\sigma/\sigma = 2;$$

$$\text{if } x = \mu + 3\sigma, z = \frac{\mu + 3\sigma - \mu}{\sigma} = 3\sigma/\sigma = 3.$$

¹ The German mathematician, Carl Friedrich Gauss, discussed the distribution in the early 19th century (Stahl, 2006).

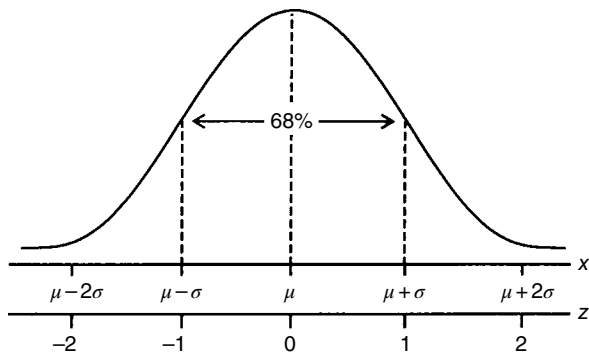


Fig. 12.2 A Normal density curve showing the relationship between μ , σ , z and the proportion of observations for Normally-distributed data.

The z scale can be used to ascertain the proportion of observations that fall within a specified range of values. Approximately 68% of all Normally-distributed values lie within one standard deviation of the mean of the population from which they were sampled ($\mu - \sigma$ to $\mu + \sigma$; $z = -1$ to $z = 1$), and 95% within approximately two standard deviations of the mean (precisely: $\mu - 1.96\sigma$ to $\mu + 1.96\sigma$; $z = -1.96$ to $z = 1.96$) (Figure 12.2).

In many cases, the Normal distribution provides a workable approximation to the distribution of biological variables; for this reason it is a very important distribution. However, this distribution cannot be applied to all variables. Measurements to which Normality does not apply (although it can do as an approximation for large samples) are counts and ordinal data that only have a small number of intervals on the scale (see Figure 10.1). Visual analogue measurements also may not be Normally distributed.

The binomial distribution

This distribution relates to discrete data when there are only two possible outcomes on each occasion; for instance, the sex of a calf at birth can only be either male or female. An example is given in Table 12.3. The two outcomes may be of any kind but, for convenience, here are termed 'success' and 'failure'. On n occasions, the probability $Pr(r)$ of r successes out of n trials is found to be:

$$Pr(r) = \frac{n!}{r!(n-r)!} p^r (1-p)^{n-r} \quad [r = 0, 1, 2 \dots n; 0 < p < 1],$$

where p = probability of success on a single occasion assuming *no association* between the outcomes occurring on different occasions. In this example, two males (outcomes, $r = 2$) may be born during three pregnancies (occasions, $n = 3$). If it is assumed that the sex of

Table 12.3 Possible series of calves born to a cow during three successive uniparous gestations (M = male; F = female).

First gestation	Second gestation	Third gestation	Total male	Total female
M	M	M	3	0
M	M	F	2	1
M	F	M	2	1
M	F	F	1	2
F	M	M	2	1
F	M	F	1	2
F	F	M	1	2
F	F	F	0	3

the first calf does not affect the sex of future calves, and $p = 0.52$, then the probability, $Pr(2)$, will be:

$$Pr(2) = \frac{3!}{2!1!} (0.52)^2 (0.48) \quad [\text{note : } n - r = 1] \\ = 0.39.$$

The value of p can vary considerably between 0 and 1; for example, in some genetically-determined diseases.

The Poisson distribution

The Poisson distribution² is concerned with counts. It is applicable when events occur randomly in space or time. Some commonly-quoted examples are the distribution of blood cells in a haemocytometer and the distribution of virus particles infecting cells in tissue culture. This distribution is important in epidemiology because it relates to the spatial and temporal distribution of disease. The random occurrence of cases of disease in unit time or in unit area can follow a Poisson distribution. A significant departure from this distribution therefore indicates temporal and geographical departures from randomness (see Chapter 8).

The distribution is characterized by one parameter, λ (lambda): the average count per unit area or per unit time.

The probability of counts of $r = 0, 1, 2, 3, 4$, and so on, is given by the formula:

$$Pr(r) = \frac{e^{-\lambda} \lambda^r}{r!} \quad [\lambda > 0, r = 0, 1, 2 \dots],$$

² The distribution is eponymously named after the 19th-century French mathematician, Siméon-Denis Poisson (see Chapter 1).

where e is a constant: the base of natural (Napierian) logarithms = 2.718 28. The value of $e^{-\lambda}$ can be found in published tables and is determined in a range of computer software and on many pocket calculators.

For example, suppose that a tissue culture monolayer is being infected with virus particles. If there are 1×10^6 cells to which are added 3×10^6 virus particles, then the average count/cell (λ) is 3. The proportion of cells expected to be infected with, for example, two particles can be calculated using the formula above, with $\lambda = 3$ and $r = 2$. Substituting in the formula:

$$Pr(2) = e^{-3} 3^2 / 2!$$

From tables, software or a calculator, $e^{-3} = 0.0498$.

Thus:

$$\begin{aligned} Pr(2) &= 0.0498 \times 3^2 / 2! \\ &= 0.2241. \end{aligned}$$

This means that the expected proportion of cells infected with two virus particles is 22.41%.

Other distributions

There are many other statistical distributions. Some deviate from Normality; some of these deviations are illustrated in *Figure 12.3*. The mean and median are equal when a variable is symmetrically distributed; and the mean and standard deviation provide good measures of position and spread. However, when frequency distributions deviate from Normality, this may not be true. Thus, with a positive skew, the mean is located to the right of the peak of the frequency distribution. In such cases, the median and quartiles (or semi-interquartile range) are better measures of position and location. Some distributions are neither Normal nor binomial nor Poisson. Two other distributions are compared with the Normal in *Figure 12.4*. If unusual distributions are suspected, then expert statistical advice should always be obtained.

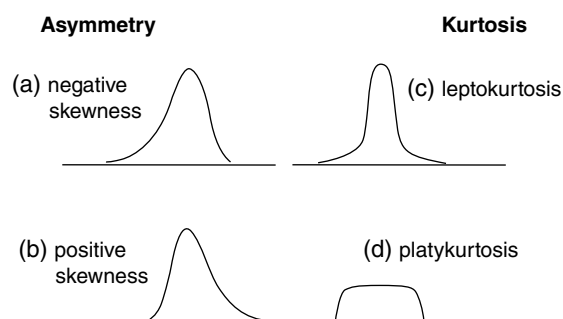


Fig. 12.3 Some deviations from the Normal distribution. (Source: Sard, 1979. Reproduced with permission of the British Veterinary Association.)

Transformations

Natural scales of measurement are not always the simplest to analyse and interpret because they may produce non-Normal distributions. However, it is sometimes possible to transform these distributions to approximate Normality by changing the way in which the variables are expressed – usually by raising the variables to a simple power or by converting them to logarithms (Zar, 2010). The distribution for which the logarithms of the data are Normally distributed is called the **lognormal distribution** (*Figure 12.4*). This distribution occurs in biology. It is characterized by a positive skew similar to, but not necessarily the same as, that depicted in *Figure 12.3b*. An example is given in *Figure 12.5*, which shows the frequency distribution of antibody titres to Newcastle disease virus in vaccinated birds, plotted on a logarithmic scale. In this case, a transformation was performed by diluting serum samples logarithmically before the titration was performed, initially to base 10 (1/10) and thereafter to base 2 (1/20, 1/40, 1/80, etc.). The application of logarithmic scales to serological investigations is discussed in Chapter 20. Epidemic curves (see Chapter 8) and incubation periods are often lognormal (Sartwell, 1950, 1966; Armenian and Lilienfeld, 1974,

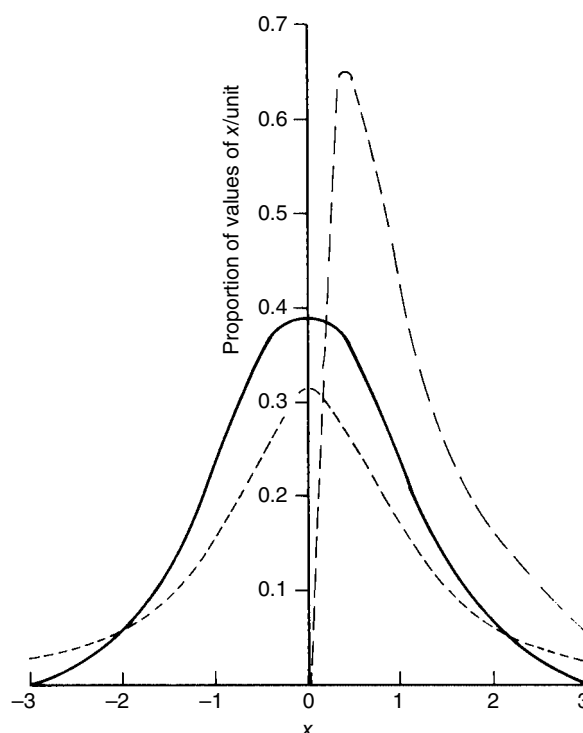


Fig. 12.4 Two non-Normal distributions compared with the Normal distribution. — Normal distribution; - - - t -distribution; — — — lognormal distribution.

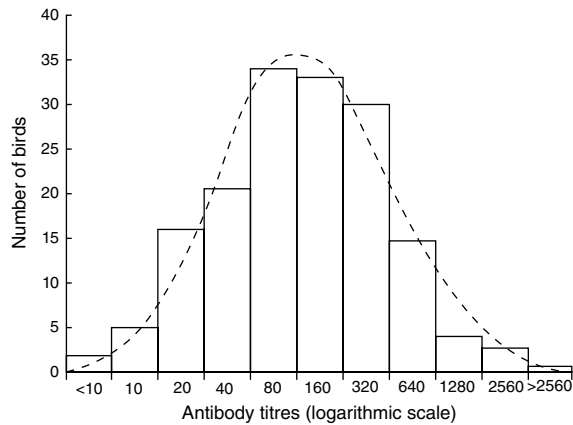


Fig. 12.5 An example of a transformed lognormal distribution. Distribution of the haemagglutination-inhibiting antibody titres of a group of 165 domestic fowls, six weeks after having been inoculated with a single dose of an inactivated Newcastle disease virus vaccine. The broken line shows a Normal curve fitted to these results. (Source: Herbert, 1970.)

1983). In the case of epidemic curves, the data (numbers of new cases) are discrete, but may be treated as continuous measurements when numbers are large.

Normal approximations to the binomial and Poisson distributions

When samples are large, and p is not too close to zero or one, the binomial distribution can be approximated to the Normal. The approximation is better when a 'continuity correction' is applied to binomial data, to allow for the discrete nature of the data, while the Normal distribution relates to continuous data. Similarly, when samples are large, the Normal distribution provides a good approximation to the Poisson distribution. Therefore, techniques that are used to analyse Normally-distributed data sometimes can be applied to the binomial and Poisson distributions. Such techniques, which become more accurate as sample size increases, are termed **asymptotic (large-sample) methods**.

Estimation of confidence intervals

The mean

The sample mean gives a single estimate for the population parameter, μ , and is therefore called a **point estimate**. Repeated sampling of the population will produce different sample means as estimates of the population mean. The sample mean has a distribution; the mean of this distribution is equal to the population

mean, but its variance is σ^2/n : the population variance divided by the sample size. The square root of the variance of the sample mean is termed the **standard error of the mean** (s.e.m.) to avoid confusion with the standard deviation of the individual values. It is given by:

$$\text{s.e.m.} = \sigma / \sqrt{n}.$$

This may be estimated by what is termed the **estimated standard error of the mean** (e.s.e.m.), which is obtained by replacing σ by s :

$$\text{e.s.e.m.} = s / \sqrt{n}.$$

The e.s.e.m. is an indication of the **precision** (see Chapter 10) of the sample's estimate of the population mean: the smaller the e.s.e.m., the greater the precision³.

It often is more useful to quote a range within which one is reasonably confident that the true mean will lie. This range is known as a **confidence interval**. It combines a measure of location (the mean) with a measure of spread (the s.e.m.). For example, on 95% of occasions that samples are taken from a population of Normally-distributed values, the confidence interval given by the sample mean $\bar{x} \pm 1.96 \text{ s.e.m.}$ will contain the true population mean. For a single sample, the interval $\bar{x} \pm 1.96$ is known as the **95% confidence interval**⁴. The upper and lower points of a confidence interval are **confidence limits**.

When the s.e.m. is estimated by the e.s.e.m. (which is nearly always the case), the multiplier 1.96 must be replaced by an appropriate value to take account of sampling variability. The value is obtained from tables (Student's⁵ t -distribution: Appendix V). It depends on the required level of confidence, the sample size and a related figure known as the **degrees of freedom**. If a sample consists of n **independent** observations (the value of one observation does not influence the value

³ Care should be taken when choosing whether to present the sample standard deviation (s) or the e.s.e.m. The sample standard deviation should be used when the aim is to describe the spread of the data around the mean, and hence to make an inference about the variability in the underlying population. The e.s.e.m. should be used when the aim is to express the precision of the estimate of the mean. It is not uncommon for these parameters to be used inappropriately, particularly the use of the e.s.e.m., rather than s , when attempting to indicate the variability in the population.

⁴ Other terms that are used in various contexts to convey similar concepts are 'fiducial interval' (Wang, 2000), 'security interval' (Borissavlievitch, 1958), 'credible interval' (Biller and Oser, 2015) and 'probability interval' (see Chapter 23 and Appendix I).

⁵ 'Student' is the pseudonym of William Gosset, a chemist at the Guinness Brewery in Dublin, who discovered the class of t -distributions in 1908. He considered himself a student, still learning statistics, and so authored papers under that pseudonym.

of another), then there are, in a sense, n independent 'pieces of information'. Calculating the mean uses one piece of information, leaving $(n - 1)$ for the variance. This is because knowledge of $(n - 1)$ observations and the mean fixes the value of the remaining observation. The number $(n - 1)$ is the number of degrees of freedom.

Using the values from Group B (Table 12.1):

$$\begin{aligned}\text{e.s.e.m.} &= s/\sqrt{n} \\ &= \frac{0.67}{\sqrt{49}} \\ &= 0.096.\end{aligned}$$

To select the value of the appropriate multiplier for a 95% confidence interval from Appendix V, the column headed '0.05' ($1 - 95/100$) is chosen. There are 48 degrees of freedom ($49 - 1$). This number is not represented in the table, and so the highest value, not greater than 48, which is represented in the table, is picked: 40. The appropriate multiplier is therefore 2.021, and the 95% confidence interval is:

$$\begin{aligned}\bar{x} \pm 2.02 \text{ e.s.e.m.} &= 4.69 \pm 0.194 \text{ kg} \\ &= 4.496, 4.884 \text{ kg},\end{aligned}$$

assuming that the three-week weaning weights are Normally distributed. (A comma conventionally separates the lower and upper limits of a confidence interval.)

Other confidence intervals can be estimated. To select the value of the appropriate multiplier for a 90% confidence interval from Appendix V, the column headed '0.10' ($1 - 90/100$) is chosen, and the appropriate multiplier is 1.684. The 90% confidence interval therefore is:

$$\begin{aligned}\bar{x} \pm 1.68 \text{ e.s.e.m.} &= 4.69 \pm 0.161 \text{ kg} \\ &= 4.529, 4.851 \text{ kg}.\end{aligned}$$

The appropriate multiplier for a 99% confidence interval is 2.704 (selected from the column headed '0.01': $1 - 99/100$).

Note that, with a given data set, greater confidence is associated with a wider confidence interval.

When samples are large, the Normal distribution provides a good approximation to Student's t -distribution, and techniques that are used to analyse Normally-distributed data can be applied. The multipliers used in calculating confidence intervals then correspond to those for the Normal distribution (e.g., 1.960 for 95%, 2.576 for 99%, as in Appendix VI). These correspond to the values given for Student's

t -distribution when the number of degrees of freedom is infinite (the last row in Appendix V).

The median

An approximate 95% confidence interval for the median is estimated by first calculating:

$$r = \frac{n}{2} - \left(1.96 \times \frac{\sqrt{n}}{2}\right) \text{ and } s = 1 + \frac{n}{2} + \left(1.96 \times \frac{\sqrt{n}}{2}\right),$$

where r = the ordered observation corresponding to the lower limit, s = the ordered observation corresponding to the upper limit, and n = the sample size (Altman *et al.*, 2000).

Using the values from Group B (Table 12.1), $n = 49$; thus:

$$r = \frac{49}{2} - \left(1.96 \times \frac{\sqrt{49}}{2}\right) \text{ and } s = 1 + \frac{49}{2} + \left(1.96 \times \frac{\sqrt{49}}{2}\right).$$

Thus, $r = 24.5 - 6.86$, and $s = 1 + 24.5 + 6.86$.

That is, $r = 17.6$, and $s = 32.4$.

The closest observation to 17.6 is observation 18; that is, 4.5 kg; whereas the closest observation to 32.4 is observation 32; that is, 5.0 kg. The approximate 95% confidence interval for the median is therefore 4.5, 5.0 kg. (Note the similarity to the 95% confidence interval for the mean, arising because the values in Group B are symmetrically distributed, and so the mean and median are similar.)

If other confidence intervals are required, the appropriate multipliers are obtained from Appendix VI.

This approximate method is acceptable for most sample sizes (Hill, 1987). The exact method is given by Altman and others (2000), who also describe interval estimation of other quantiles. The estimate of the confidence limits may be affected by 'ties' (i.e., observations with identical values) (Conover, 1999).

A proportion

Using the notation in the previous discussion of the binomial distribution, in a single sample the estimate of a proportion, p , is $\hat{p} = r/n$, where r is the observed number of successes. (Note the 'hat' on the letter p , as a reminder that it is an estimate of the true proportion.) This sample proportion has a distribution; the mean of the distribution is equal to p , and its variance to $p(1 - p)/n$. The standard deviation of the distribution is termed its **standard error**:

$$\sqrt{\frac{p(1-p)}{n}},$$

which may be estimated thus:

$$\sqrt{\frac{\frac{r}{n}\left(1-\frac{r}{n}\right)}{n}}.$$

Note that $p(1-p)$ is maximal when $p = 0.5$. Hence, for a given n , the variance, and therefore the standard error, will increase as the proportion tends towards 50%, and decrease as it tends towards 0% or 100%.

For large samples (say, $n > 30$), the distribution is approximately Normal, and so, if p also is not near to zero or one, an approximate 95% confidence for the true proportion, p , can be computed using **Wald's method** (Wald, 1943):

$$\frac{r}{n} \pm 1.96 \sqrt{\frac{\frac{r}{n}\left(1-\frac{r}{n}\right)}{n}},$$

and, if other confidence intervals are required, the appropriate multipliers again are obtained from Appendix VI.

This method is presented in most statistics textbooks, is widely accepted and applied, and is the default method in many statistical software packages, but is inaccurate for a range of values of n and p (Brown *et al.*, 2001).

The **Agresti–Coull method** (Agresti and Coull, 1998) gives more accurate results than the Wald method, and is recommended when $n > 40$ (Brown *et al.*, 2001). For a 95% interval:

$$\frac{\tilde{r}}{\tilde{n}} \pm 1.96 \sqrt{\frac{\frac{\tilde{r}}{\tilde{n}}\left(1-\frac{\tilde{r}}{\tilde{n}}\right)}{\tilde{n}}},$$

where: $\tilde{r} = r + (1.96^2)/2$, and $\tilde{n} = n + (1.96^2)$.

The **Wilson method** (Wilson, 1927) is appropriate for any value of n and is recommended over the Agresti–Coull method when $n < 40$. The Wilson 95% confidence interval is calculated thus:

$$\frac{\tilde{r}}{\tilde{n}} \pm \frac{1.96\sqrt{\tilde{n}}}{\tilde{n}} \times \sqrt{\frac{r}{n}\left(1-\frac{r}{n}\right) + \frac{(1.96)^2}{4n}},$$

noting that $\tilde{r}$, r , $\tilde{n}$ and n all appear in this equation.

An example of Wilson's method, using alternative notation, is given in Chapter 20.

Another alternative approach involves calculation of **exact** confidence intervals (Clopper and Pearson, 1934)⁶; these are provided in Appendix VII. Thus, if

five male calves, r , and seven female calves are born to 12 cows, n , the sample estimate of the probability of a calf being male, $\hat{p}$, is $5/12 = 0.4167$. Consulting Appendix VII (which defines r as x), with $x = 5$ and $n = 12$, and $n - x = 7$, the 95% confidence interval is 0.151, 0.723.

Using these data with the methods described above yields the following results:

Wald 95% confidence interval:	0.138, 0.696,
Agresti–Coull 95% confidence interval:	0.193, 0.681,
Wilson 95% confidence interval:	0.193, 0.681,
exact 95% confidence interval:	0.151, 0.723.

Note the wide intervals obtained with the Wald and exact methods, relative to those derived by the Agresti–Coull and Wilson methods.

A detailed description and critique of these and various other methods of calculating confidence intervals for proportions is given by Blaker (2000), Brown and others (2002) and Newcombe (2013). Kurshid and Ageel (2010) provide a useful bibliography on the topic.

The Poisson distribution

Table 12.4 lists the observed distribution of yeast cells in a sample, n , of 400 haemocytometer cells (column 2), and the expected frequencies according to the Poisson distribution (column 3). Using the notation in the previous discussion of the Poisson distribution, λ can

Table 12.4 Distribution of yeast cells in a haemocytometer. (From Bailey, 1995.)

Number of cells in a square (x)	Observed number of squares	Expected number of squares
0	0	3.7
1	20	17.4
2	43	40.6
3	53	63.4
4	86	74.2
5	70	69.4
6	54	54.2
7	37	36.2
8	18	21.2
9	10	11.0
10	5	5.2
11	2	2.2
12	2	0.9
>12	0	0.4
Totals (n)	400	400.00

⁶ Such methods are termed 'exact' because they are based directly on the binomial distribution rather than on any approximation to it. They are not necessarily 'better' than approximate methods (Krishnamoorthy and Peng, 2007; Zou, 2010).

be estimated by the mean of the observed frequencies in column 2, $\bar{x} = (1 \times 20 + 2 \times 43 + 3 \times 53 \dots + 12 \times 2) / n = 4.68$. When λ is large, the Poisson distribution tends to Normality, and, even when λ is not large, $\bar{x}$ may be approximately Normal, provided the sample size is large. If $n\bar{x} > 30$ (as in this example), a standard error can be estimated thus:

$$\sqrt{\frac{\bar{x}}{n}}.$$

An approximate 95% confidence interval for λ is then:

$$\bar{x} \pm 1.96 \sqrt{\frac{\bar{x}}{n}};$$

that is, $4.68 \pm 1.96 \sqrt{\frac{4.68}{400}} = 4.68 \pm 0.11 = 4.46, 4.90$,

and, if other confidence intervals are required, the appropriate multipliers are again obtained from Appendix VI.

If the sample size is small, confidence intervals should be obtained from statistical packages or tables (Appendix VIII).

Some epidemiological parameters

Proportions: prevalence, cumulative incidence, cumulative mortality and survival

Prevalence, cumulative incidence, cumulative mortality and survival (without censored observations) are all simple proportions; and the distribution of a proportion follows from the binomial distribution. Confidence intervals therefore can be calculated using methods appropriate to the binomial distribution. This topic is addressed in more detail in connection with field surveys (Chapter 13) and diagnostic testing (Chapter 20).

Multinomial proportions

Sometimes, a sample may contain several discrete classes, for example, different breeds of animal. In such circumstances, confidence intervals can be calculated for the proportion in each class, based on a **multinomial distribution**.

Table 12.5 lists the numbers of toy, small, medium, large and giant dogs in a veterinary clinic population over a two-year period; this is known to be representative of the general dog population in the UK (Thrusfield, 1989). There are five classes in this sample. To calculate confidence intervals for these classes, three values are required:

1. the number in each class (n);
2. the total number in all classes (N);
3. a suitable value of χ^2 (see Chapter 14).

The value of χ^2 is chosen, depending on the number of degrees of freedom (number of classes – 1, in this instance) and the desired *level of significance* (see Chapter 14). A 95% confidence interval corresponds to a level of significance of 5% ($P = 0.05$). A 90% confidence interval is equivalent to $P = 0.10$, and a 99% confidence interval is equivalent to $P = 0.01$, and so on.

For a 95% confidence interval, consulting Appendix IX, $P = 0.05$, degrees of freedom = 4 (i.e., 5 – 1): thus $\chi^2 = 9.488$.

Approximate upper and lower confidence limits then can be calculated using the equation of Gold (1963):

$$\frac{n_j}{N} \pm \chi \sqrt{\frac{\frac{n_j}{N} \left(1 - \frac{n_j}{N}\right)}{N}},$$

where n_j is the number in class j , and N is the number in all classes.

Table 12.5 Numbers of dogs of different sizes recorded in a small-animal practice, and 95% confidence limits for the proportion of dogs of different sizes in the population using the methods of Gold (1963), Quesenberry and Hurst (1964) and Sison and Glaz (1995). (Data extracted from Edinburgh University's Small Animal Practice Teaching Unit database: Stone and Thrusfield, 1989.)

Class of dog	n	Proportion in each class	95% confidence limits					
			Gold method		Quesenberry and Hurst method		Sison and Glaz method	
			Lower	Upper	Lower	Upper	Lower	Upper
Toy	295	0.0562	0.0464	0.0660	0.0472	0.0668	0.0417	0.0710
Small	1 634	0.3112	0.2916	0.3309	0.2919	0.3312	0.2968	0.3260
Medium	969	0.1846	0.1681	0.2011	0.1687	0.2016	0.1701	0.1994
Large	2 241	0.4269	0.4058	0.4479	0.4060	0.4480	0.4124	0.4416
Giant	111	0.0211	0.0150	0.0273	0.01584	0.0282	0.0067	0.0359
Total	5 250							

Thus, for small dogs, the limits are:

$$\begin{aligned} & \frac{1634}{5250} \pm \sqrt{9.488} \times \sqrt{\frac{\frac{1634}{5250} \left(1 - \frac{1634}{5250}\right)}{5250}} \\ &= 0.311 \pm 3.080 \sqrt{\frac{0.311 \times 0.689}{5250}} \\ &= 0.311 \pm 3.080 \times 0.006389 \\ &= 0.311 \pm 0.0197 \\ &= 0.2916, 0.3309. \end{aligned}$$

The 95% confidence interval for the percentage of small dogs in the general population therefore is 29.2%, 33.1%.

Alternative methods have been proposed by others, including Quesenberry and Hurst (1964) and Bailey (1980). Using the notation above, the method of Quesenberry and Hurst (1964) is:

$$\frac{\chi^2 + 2n_j \pm \chi \sqrt{\chi^2 + 4n_j \left(\frac{N-n_j}{N}\right)}}{2(N + \chi^2)}.$$

Therefore, for small dogs, the limits are:

$$\begin{aligned} & \frac{9.488 + 3268 \pm 3.080 \sqrt{9.488 + 6536 \left(\frac{5250 - 1634}{5250}\right)}}{2(5250 + 9.488)} \\ &= \frac{3277.488 \pm 3.080 \sqrt{9.488 + 4501.75}}{10518.98} \\ &= \frac{3277.488 \pm 3.080 \times 67.166}{10518.98} \\ &= 0.2919, 0.3312, \end{aligned}$$

and the 95% confidence interval is again 29.2%, 33.1%.

Sison and Glaz (1995) recommend a more accurate method than those described above, but it is best undertaken using statistical software.

Table 12.5 lists the 95% limits for all classes of dog, using the two methods exemplified here, and also, for comparison, presents the limits calculated using the method of Sison and Glaz (1995).

Incidence rate

Confidence intervals for incidence rates require a different approach, because the denominator comprises the complex unit, 'animal-time at risk'. A suitable method is based on the Poisson distribution. The numerator in the sample estimate of the incidence rate (i.e., the number of diseased animals), only, is used in the computation.

If the number of cases is greater than 100, a 95% interval is calculated thus:

$$\text{the lower limit, } \lambda_L = \left(\frac{1.96}{2} - \sqrt{x}\right)^2,$$

$$\text{and the upper limit, } \lambda_U = \left(\frac{1.96}{2} + \sqrt{x}\right)^2,$$

where x = the observed number of cases (Altman *et al.*, 2000).

For instance, if a sample generated an incidence rate of 108 cases per 757 animal-months at risk:

$$\begin{aligned} \lambda_L &= \left(\frac{1.96}{2} - \sqrt{108}\right)^2 \\ &= (0.98 - 10.39)^2 \\ &= 88.6; \\ \lambda_U &= \left(\frac{1.96}{2} + \sqrt{108}\right)^2 \\ &= (0.98 + 10.39)^2 \\ &= 129.3. \end{aligned}$$

The point estimate of the incidence rate per animal-year is therefore $(108/757) \times 12 = 1.71$ cases per animal-year. The 95% interval estimate is $(88.6/757) \times 12$, $(129.3/757) \times 12 = 1.41, 2.05$ cases per animal-year.

If other confidence intervals are required, the appropriate multipliers should be selected from Appendix VI.

If the number of cases is less than 100, computer packages or Appendix VIII should be used.

Other parameters

Confidence intervals can be estimated for many parameters whose point estimates have already been described in Chapter 4, including survival computed in survival analyses and adjusted (standardized) measures (Altman *et al.*, 2000). Calculation of confidence intervals for other parameters is discussed in the chapters in which they are encountered.

Bootstrap estimates

The standard methods for calculating confidence intervals for population parameters or attributes are based on assumptions about the distribution of samples selected from a population. Large samples generally guarantee that the distribution of the estimates is Normal. However, small samples may require other distributional assumptions; for example – when calculating a confidence interval for the mean, and when the standard deviation has to be calculated – that samples

from a population whose distribution is Normal will follow Student's *t*-distribution. However, this may not be the case, in which circumstance the estimate of the confidence interval for the mean will be inaccurate. This problem can be overcome by using computers to generate 'bootstrap'⁷ estimates.

The technique is based on the principle that a single sample provides information on the distribution of the population from which it was drawn. Software can repeatedly randomly select individual values from the sample, one at a time, replacing each value immediately after it is selected, so that it is in a position to be randomly selected again (so-called 'sampling with replacement'; see also Chapter 13), until a sample with the same number of observations as the original is produced. This procedure is then repeated many times (50–200 times for interval estimation of the mean, but more than 1000 repetitions may be required for some parameters, and, with modern computers, 100 000 or more iterations is commonplace). The variability between the many samples that are generated reflects the standard error that would be derived from sampling the population many times. Therefore, if a confidence interval for the mean was being estimated, and 200 repetitions were conducted, there would be 200 values for the sample mean⁸. The **percentile method** then involves sorting these values in order. A 95% confidence interval would then have the 2.5 centile (the fifth value if 200 repetitions are conducted) as its lower limit, and the 97.5 centile (the 195th value) as its upper limit. Similarly, a 90% confidence interval would then have the fifth centile (the 10th value) as its lower limit, and the 95th centile (the 190th value) as its upper limit. In practice, the percentile method is subject to bias, but this can be estimated and corrected.

The accuracy of a bootstrap estimate depends on the extent to which the distribution of values in the original sample reflects a variable's distribution in the population from which the sample was drawn. The number of bootstrap repetitions will not increase the accuracy of the method if the original sample is unrepresentative (Good, 2005).

Full details of appropriate bootstrap techniques are presented by Efron and Tibshirani (1993), Davison and Hinkley (1997) and Chernick (2008).

⁷ The term is derived from the saying 'pulling oneself up by one's bootstraps'.

⁸ Clearly, sampling *without* replacement would be of no value: it would merely generate the values in the original sample, but in a different order. Therefore, the mean and standard deviation would be the same for all repetitions.

Displaying numerical data

Clear presentation of data aids interpretation, analysis and communication. Methods of presentation include **tables, charts, graphs and plots**. Some people distinguish between charts, graphs and plots, but the use of these terms is inconsistent and so they will be used interchangeably here and collectively referred to as **figures**, a term that also includes photographs and drawings.

Tables and figures may reveal interesting facts about data and their distributions, but also can obscure or mislead if poorly executed. Figures should be constructed in order to convey one or more points, and the design of a figure should be sensitive to the need to communicate the points. For example, if comparisons are to be made between data using graphs placed side by side, the axes of the graphs should have the same lengths and limits. It is important that figures are not overly cluttered and that the scale is sufficient to allow important detail to be seen clearly. Careful use of colour can help interpretation, but consideration should be given to the impact on colour-blind readers and the effect of monochrome (grey-scale) copying. Furthermore, colour sometimes can conceal results and produce artifacts (Rogowitz and Treinish, 1998). Information in a figure may be obscured undesirably if:

- points lie underneath legends, annotations or the axes;
- data extend beyond the axes' scales (when this is done deliberately, it should be clearly communicated);
- data points or confidence intervals overlap and coalesce;
- symbols and lines closely resemble one another other in shape, style or colour.

Axis labels, annotations and other text should avoid codes. Figures and figure legends should be self-explanatory, and it is important to consider differences in 'visual literacy' (Avgerinou and Ericson, 1997), which may hamper their correct interpretation.

Displaying qualitative data

Qualitative (categorical) data and, sometimes, discrete data (see Chapter 10) can be displayed as frequencies (or relative frequencies). Common formats for display of frequencies include **tables, pie charts and bar charts**.

Tables

Tabulation is one of the commoner techniques of displaying qualitative and quantitative data. It involves

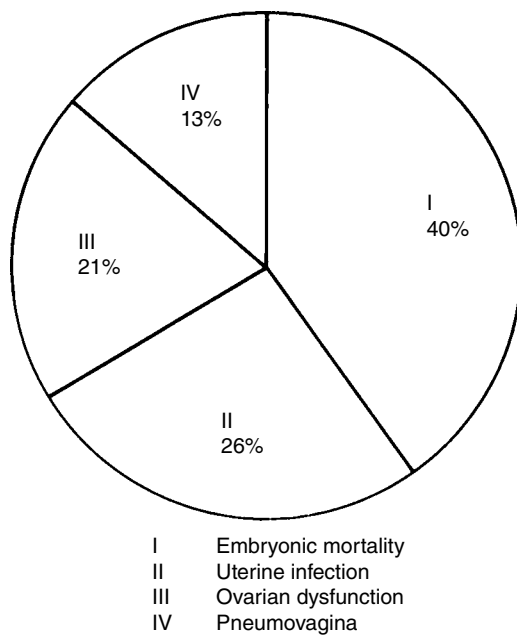


Fig. 12.6 An example of a pie chart: proportions of principal mare infertility detected in over 100 clinical cases reported and presented. (Source: Fraser, 1976.)

listing numerical values (and sometimes text) in rows and columns. Examples of tables can be found throughout this book. Quantitative data are often displayed as frequencies (e.g., *Table 1.8*) or relative frequencies (e.g., *Table 9.2*, column 3). One-way (simple) tabulation presents one characteristic, such as a table reporting the number of each breed of cattle present on a farm. Two-way (double) tabulation classifies the data according to two characteristics; for example, a table reporting the number of cattle on a farm by breed and sex. Complex tabulation involves classifying data by more than two characteristics; for example, a table reporting the number of cattle on a farm by breed, sex and age.

Pie charts

A pie chart is a circle (the 'pie') in which individual characteristics are represented as 'slices', the angle of a segment being proportional to the relative frequency of the corresponding characteristic. An example is given in *Figure 12.6*. Computer software facilitates depiction of the 'pies' with a three-dimensional appearance (*Figure 12.7*). However, the use of a three-dimensional appearance provides no additional information and may hinder interpretation. A balance between visual impact and clarity therefore is required.

Bar charts

Bar charts include a separate horizontal or vertical bar for each category of data, the length of which is proportional to the frequency or relative frequency of that category (*Figure 12.8*). Typically, gaps are included between the bars in order to indicate that the data are categorical or discrete (in contrast to histograms, described in the next section). The bars representing ordinal data usually are arranged in accordance with the ordinal scale, but the bars representing nominal data can be in any order. A **Pareto chart** is a bar chart in which the bars are plotted in decreasing order of relative frequency from left to right, and also may include a line showing the cumulative scores⁹.

Data about different sub-groups can be presented by grouping the bars. Each of the sub-groups is represented by a separate bar and the groupings usually are distinguished through the use of spacing and colour or shading.

Displaying quantitative data

Quantitative (discrete and continuous) data may be summarized in **tables**, **box plots**, **violin plots** and **histograms**. Alternatively, each observation (data point) may be displayed in **individual-value plots**, **scatter plots** and **time series plots**.

Box plots

Box plots (often also called **box-and-whisker plots**) display summary information about the frequency distribution of quantitative data (Tukey, 1977; Erickson and Nosanchuk, 1979). This often is based on a five-point summary, described earlier, particularly when displaying non-Normally-distributed data. An example is shown in *Figure 12.9a* using the data in *Table 12.1*. The central horizontal line indicates the median; the upper and lower extremities of the vertical lines (the 'whisker') mark the maximum and minimum values of the data set; the horizontal sides of the large rectangles (the boxes) represent the quartiles.

A common modification first calculates the value one-and-a-half times the interquartile range. This value is then added to, and subtracted from, the upper and lower quartiles, respectively. The whiskers are then extended to the most extreme observations still within this range (*Figure 12.9b*).

Other alternatives include extending the whiskers: (1) to one standard deviation above and below the mean (if using Normally-distributed data); (2) to the 10th and 90th percentiles; or (3) to the 2.5th and

⁹ The Pareto chart was originally applied to quality control, for which there are now modifications (Wilkinson, 2006).

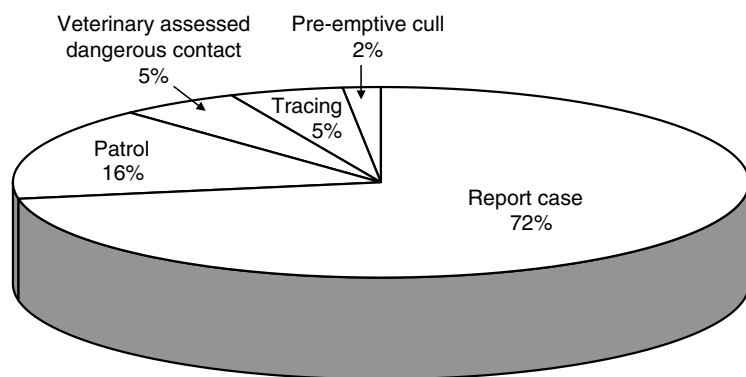


Fig. 12.7 An example of a 'three-dimensional' pie chart: methods of identification of infected premises in the foot-and-mouth disease epidemic in Dumfries and Galloway, UK, 2001. (Adapted from Thrusfield *et al.*, 2005a.)

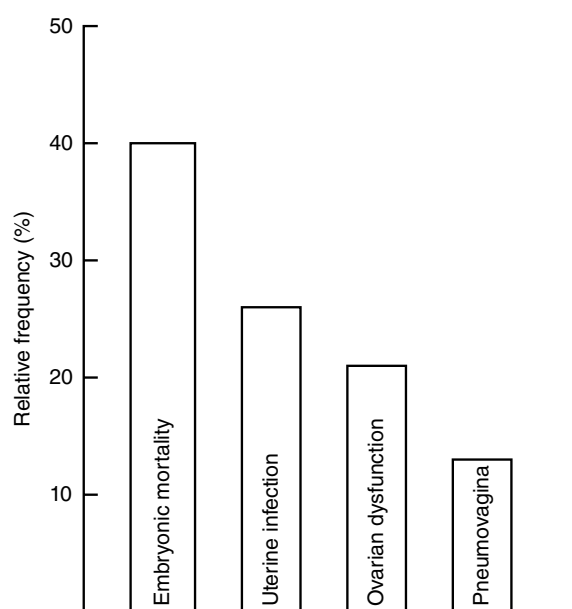


Fig. 12.8 An example of a bar chart: proportions of principal mare infertility conditions detected in over 100 clinical cases reported and presented. (Data from Figure 12.6.)

97.5th percentiles. Data outside the limits of the whiskers are considered to be **outliers** and are usually individually plotted (the small circles in Figure 12.9b).

Violin plots

Box plots summarize distributions using only five values, but this simplicity may hide important characteristics, such as the **modality** (number of most often occurring data values). This is particularly troublesome if there is no prior information about the distribution, because comparing distributions with differing modalities may not be appropriate. One solution to these types of problems is to add into the box plot indications of the **density** of the underlying distribution.

Violin plots (Figure 12.9c) are a modification of box plots that adds a **kernel density plot** (Hintze and Nelson, 1998) using appropriate statistical software. Typically, there is a marker for the median (a white dot in Figure 12.9c), and a box indicating the interquartile range, as in a standard box plot. Figure 12.9c also includes whiskers, in this case extending exactly 1.5 times the interquartile range above and below the box. The kernel density plot overlying the box plot illustrates the distributional characteristics of the data and provides important information that is not present in the box plot alone. It is evident that the data for groups A and B are unimodal (i.e., have only one maximum) and are approximately Normally distributed. Markedly different distributions, such as bimodal, could produce similar box plots to those in Figure 12.9a and 12.9b, but would be clearly distinguished by the violin plot¹⁰.

Individual-value plots

Individual-value plots (Figure 12.9d) provide a visual representation of each datum, rather than summary information. They can be used either to identify possible outliers or to compare groups. Individual-value plots usually provide an indication of the number of data values, but can become unclear with an increasing number of values.

Histograms

The histogram is comparable to a bar chart, but is used for continuous variables, whereas the bar chart is used for discrete data. Histograms provide a graphical representation of the distribution of data by plotting

¹⁰ There are several other methods, of various degrees of complexity, for expressing density. These include 'vase plots' (Benjamini, 1988), 'bean plots' (Kampstra, 2008), 'sectioned density plots' (Cohen and Cohen, 2006), 'summary plots' (Potter *et al.*, 2010) and 'density strips' (Jackson, 2008).

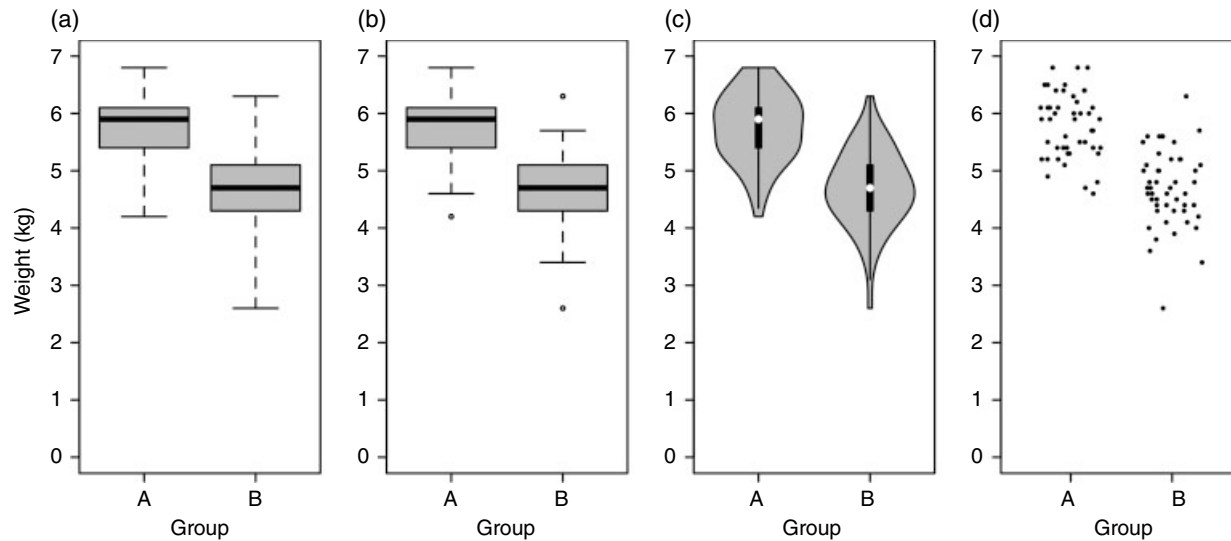


Fig. 12.9 Box plots (a and b), violin plot (c) and individual-value plot (d) of weaning weights of two groups (A and B) of piglets. See the text for explanations of each plot. (Data from Table 12.1.)

adjacent rectangles representing discrete intervals (bins) (see Figure 12.1). The width of a rectangle indicates the range of the interval and the total area of the rectangle represents the frequency (or relative frequency) of observations within that range. The bins are often selected to be of equal size, in which case the height of the rectangles indicates the frequency (or relative frequency). The number, and ranges, of bins should be selected in order to illustrate the main distributional patterns in the data. Unlike bar charts, histograms are usually drawn without gaps between the rectangles, in order to indicate clearly the continuous nature of the data.

Population pyramids (e.g., Figure 5.4 and Figure 9.5) usually are composed of two histograms (one for males, the other for females), with the horizontal axis depicting population size and the vertical axis indicating age.

Scatter plots

Scatter plots (Figure 12.10) are used to illustrate the relationship between two (and occasionally three) quantitative variables. As with the individual-value plot, each datum is included in the plot; but, in contrast to individual-value plots, the position of each point is determined by the value of two (or three) variables. For plots of two variables, one is selected to plot on the horizontal axis and is called the x variable, and the other is plotted on the vertical axis and is called the y variable. Where one variable is believed to depend on the other, the response (dependent) variable is

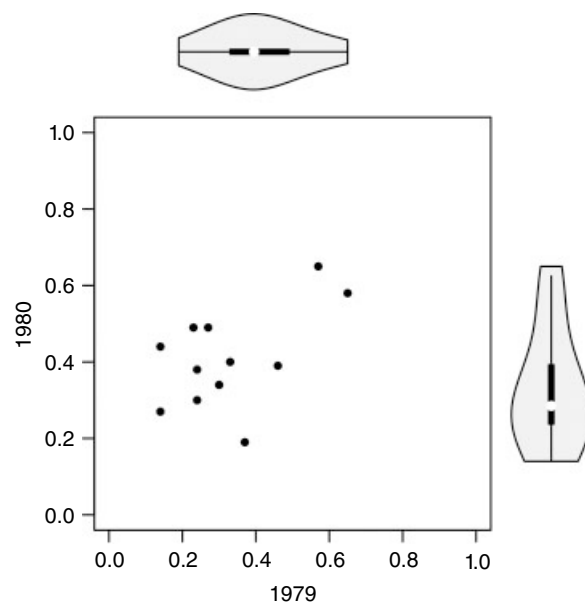


Fig. 12.10 Scatter plot of percentage of sheep lungs condemned monthly because of pneumonia and/or pleurisy at a Scottish abattoir (1979 vs 1980). (Data from Table 8.3.) Violin plots depicting summary information and the distribution of each variable supplement the scatter plot.

typically selected as the y variable, and the explanatory (independent) variable as the x variable.

Plotting time-related data

Time series (**time trend**) plots are particular forms of scatter plots in which the x variable represents time (Figure 12.11). The plotting of epidemics in this way

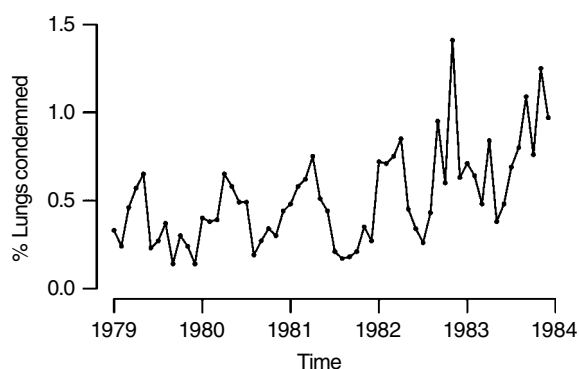


Fig. 12.11 Time series plot depicting percentage of sheep lungs condemned monthly because of pneumonia and/or pleurisy at a Scottish abattoir (1979–1983). (Data from *Table 8.3*.)

produces **epidemic curves**, in which the number of cases in each time period is often presented as vertical bars, rather than points (see *Figure 4.1*).

Time lines depict disease and related events (e.g., implementation of control procedures) in chronological order, along a horizontal line representing the passage of time. They are a simple and useful way of visualizing key incidents (e.g., *Figure 12.12*).

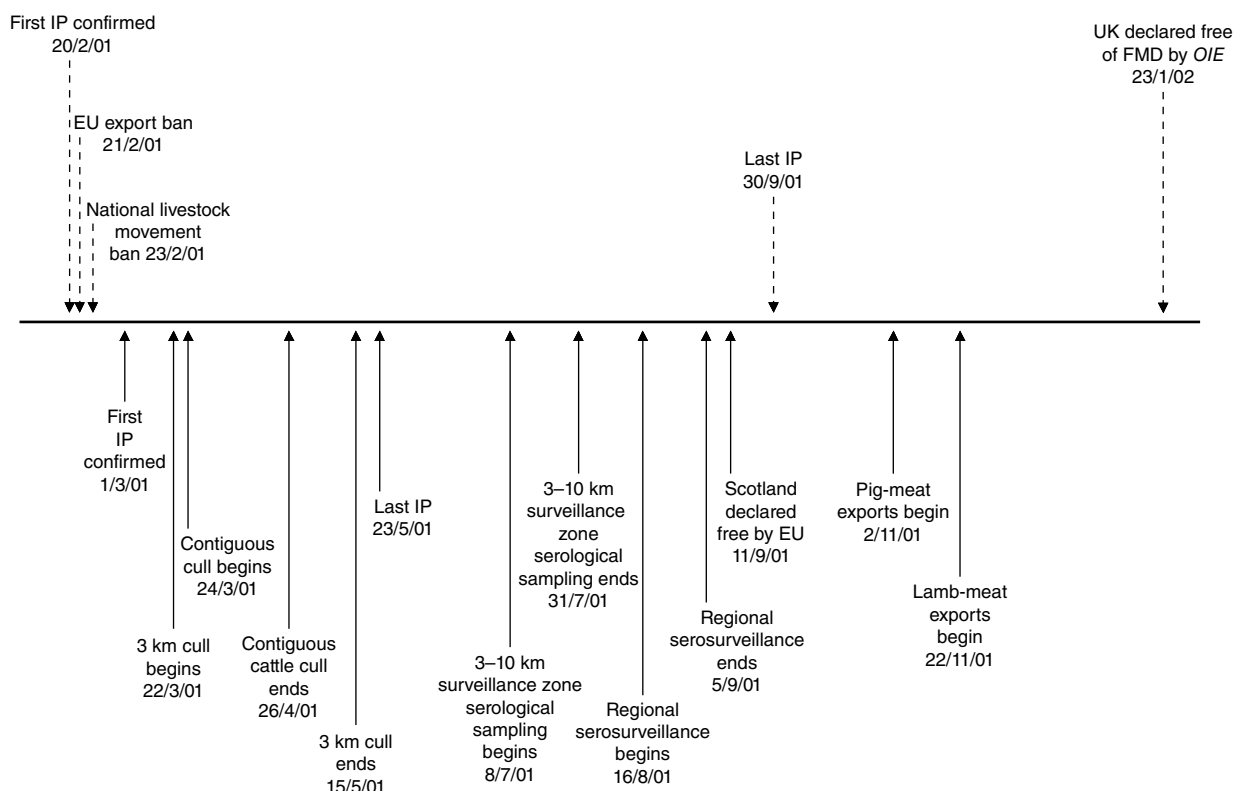


Fig. 12.12 Time line of the main events in the foot-and-mouth disease epidemic in Dumfries and Galloway, Scotland, 2001. IP: infected premise. (Source: Thrusfield *et al.*, 2005b.)

Time series plots can be annotated to include information about important incidents. This can aid orientation of the quantitative data with the occurrence of these incidents, and may be clearer than presentation of separate time series plots and time lines. However, care must be taken to ensure that the key information is not lost due to graphic clutter.

Grey-scale figures

Shading in grey (or in colour) can be used to highlight key features. Thus, *Figure 12.13* uses a grey scale to illustrate data presented in *Figure 12.11* and *Table 8.3*. This aids comparison of monthly data across the years, revealing, for example, that there is an increase in the percentage of lungs condemned in the later years, particularly in September and November.

Grey-scale shading can be complemented by texturing (e.g., *Figures 4.5* and *4.8*).

Monitoring performance: control charts

It is sometimes desirable to monitor aspects of live-stock production, such as mean litter size in a herd of pigs, so that any significant negative or undesirable

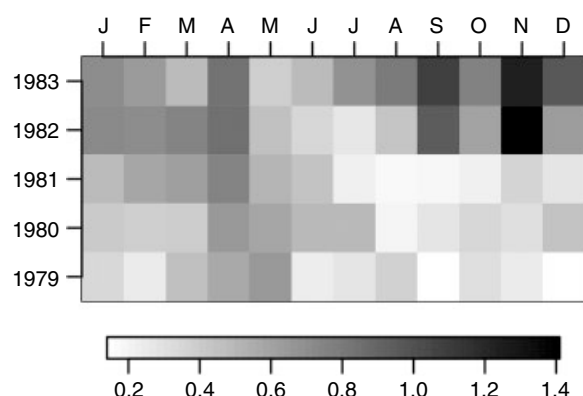


Fig. 12.13 Grey-scale image depicting the percentage of sheep lungs condemned monthly because of pneumonia and/or pleurisy at a Scottish abattoir (1979–1983). (Data from Table 8.3.)

deviations in the values from a **predefined standard** or **acceptable range** can be detected. It is then possible to undertake appropriate remedial action. When data are recorded in tables, it is often difficult to appreciate the full significance of values or of their differences from one another. A suitable technique for monitoring data when they accrue sequentially is the use of **control charts**, which are graphs on which successive results are plotted in sequence while production is proceeding. These charts are important in modern statistical process control methods (Naikan, 2008). The significance of changes then can be evaluated, and corrective action taken at the earliest possible moment, thereby ensuring a smooth level of production.

Shewhart charts

The change in a variable's value can be monitored easily over a period of time by a simple extension of the time series plot. This technique was developed by Shewhart (1931) to control the quality of manufactured articles, and an example is given in Figure 12.14, which charts the mean of the number of piglets born alive/litter in a herd of pigs over a two-year period (Wrathall and Hebert, 1982).

In addition to plotting the results, a **standard** or **reference value** is selected and plotted as a horizontal line on the chart. This value is derived from the average of (ideally, at least 100) previous observations of the plotted variable in the herd. Alternatively, a composite value from other herds of a similar type in the national population may be used.

Decision boundaries (also termed **action levels** or **interference levels**; see Chapter 26) also are included on the chart, and remedial action is taken if a value of the variable crosses these boundaries.

A mathematically-derived decision boundary is based on the e.s.e.m. of a typical sample. It requires knowledge not only of the standard deviation of the reference figure but also of the average batch size. In some applications, two decision boundaries above and two below the target level may be used. The inner pair give 'warning' and the outer pair indicate 'action'. Selection of boundaries is discussed by Wheeler and Chambers (2010).

In Figure 12.14 (with only one pair of decision boundaries) the decision boundary is set at two e.s.e.m. (two here used as an approximation to 1.96; visually the difference should be of little consequence). A problem was detected in September 1977, and another, more severe problem was detected six months later. Such a problem may have been caused by reproductive disease in the sow, and subsequent microbiological investigations identified parvovirus in mummified fetuses.

If two standard errors are selected, it is expected that 2.5% of observations from the population will lie outside each boundary by chance alone. Therefore, remedial action will be taken on 2.5% of occasions when it is not justified. Similarly, if 2.33 standard errors are chosen as the boundary, then action will be unjustifiably taken on approximately 2% of occasions; if three standard errors are chosen, then unjustifiable action will be taken on approximately 0.28% of occasions. While an interval of three standard errors may seem attractive, this is accompanied by a greater probability of failing to take action, when desirable, than if a smaller number of standard errors had been chosen.

Although a decision boundary based on the e.s.e.m. is valuable, rigid adherence to such boundaries is not always valid because batch sizes tend to vary over time and so the e.s.e.m. will vary. However, average batch sizes can be used in calculating the e.s.e.m.; and this approach is more valuable than the arbitrary selection of a boundary level where individual values may cross the boundary level purely at random.

Note that the use of Shewhart charts for monitoring performance is only appropriate for stable processes or processes in control.

Cusum charts

The Shewhart chart is effective for detecting large, abrupt changes in the value of a monitored variable. However, it is less effective when the change is small and results from a slight shift or 'drift' away from the reference or standard value over a period of time. The 'cumulative sum' technique is a more sensitive method for detecting such trends, and control charts incorporating this technique are known as **cusum charts** (Woodward and Goldsmith, 1964). The

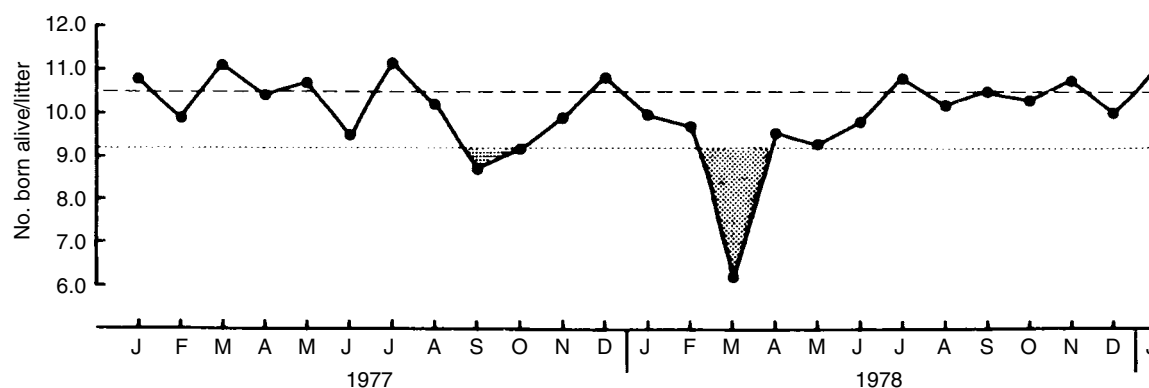


Fig. 12.14 Shewhart chart: number of piglets born alive/litter in a herd of pigs, 1977 and 1978. — — — Reference value; decision boundary. (Data from Table 12.6.) (Source: Wrathall and Hebert, 1982.)

technique is useful for detecting changes in average levels, determining the point of onset of these changes, obtaining reliable estimates of current average values, and making short-term predictions of future average levels.

A cusum chart consists of a continuous plot (usually against time) of deviations of the cumulative value of the monitored variable from its reference value, k . Table 12.6 lists the mean monthly number of piglets born alive in a pig herd over a two-year period. The selected reference value (obtained, for example, from previous production data on the farm) is 10.5.

In each successive time period, as values $x_1, x_2, \dots$ for the batches become known, the cumulative sums, $C_1, C_2, \dots$ are calculated thus:

$$\begin{aligned} \text{period 1: } C_1 &= x_1 - k \\ &= 10.9 - 10.5 \\ &= 0.4; \end{aligned}$$

$$\begin{aligned} \text{period 2: } C_2 &= (x_1 - k) + (x_2 - k) \\ &= C_1 + (x_2 - k) \\ &= 0.4 - 0.6 \\ &= -0.2; \end{aligned}$$

$$\begin{aligned} \text{period 3: } C_3 &= C_2 + (x_3 - k) \\ &= -0.2 + 0.6 \\ &= 0.4; \end{aligned}$$

and so on (see the last column in Table 12.6).

The cusum is plotted in Figure 12.15. So long as the mean for a series of batch values remains close to the reference value, k , some of the individual cusum values will be positive, and some negative, and so they will tend to cancel each other out. The cusum plot will

Table 12.6 Mean monthly values for piglets born alive per litter in a 125-sow herd, January 1977–December 1978. The reference value, $k = 10.5$; the cumulative sum, $C_j = C_{j-1} + (x_j - k)$. (See text for explanation.) (From Wrathall and Hebert, 1982; raw data supplied by the authors.)

j	Month	Mean number of piglets born alive/litter (x_j)	$x_j - k$	C_j
1	1977 Jan	10.9	0.4	0.4
2	Feb	9.9	-0.6	-0.2
3	Mar	11.1	0.6	0.4
4	Apr	10.5	0.0	0.4
5	May	10.7	0.2	0.6
6	June	9.4	-1.1	-0.5
7	July	11.0	0.5	0.0
8	Aug	10.3	-0.2	-0.2
9	Sept	8.7	-1.8	-2.0
10	Oct	9.2	-1.3	-3.3
11	Nov	9.9	-0.6	-3.9
12	Dec	10.7	0.2	-3.7
13	1978 Jan	9.8	-0.7	-4.4
14	Feb	9.6	-0.9	-5.3
15	Mar	6.5	-4.0	-9.3
16	Apr	9.4	-1.1	-10.4
17	May	9.3	-1.2	-11.6
18	June	9.6	-0.9	-12.5
19	July	10.7	0.2	-12.3
20	Aug	10.1	-0.4	-12.7
21	Sept	10.5	0.0	-12.7
22	Oct	10.3	-0.2	-12.9
23	Nov	10.7	0.2	-12.7
24	Dec	10.1	-0.4	-13.1

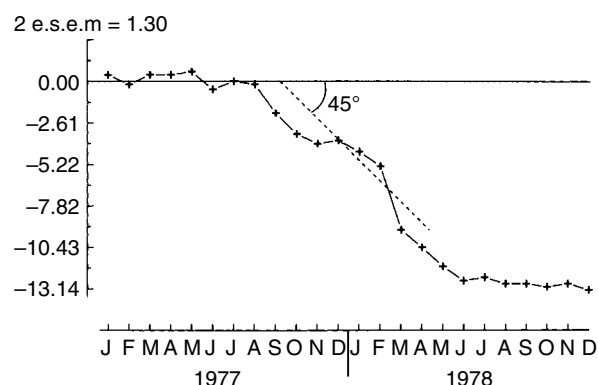


Fig. 12.15 Plot of the cumulative sum values (cusum) for the mean monthly number of piglets born alive per litter in a herd of pigs in a 125-sow herd, January 1977–December 1978. (Data from Table 12.6.)

then be essentially horizontal, indicating that the monitored variable is in a state of control.

Important indicators of abnormality on the cusum chart are changes in direction and slope of the plotted line. The scales of the horizontal and vertical axes must therefore be chosen carefully. If one time period equals

one unit on the horizontal axis, and two e.s.e.m. of cusum are equal to one unit on the vertical axis (the e.s.e.m. being obtained from previous production data), then a plot's slope of 45° or more will indicate a change that is significant at the 2.5% level (see Chapter 14). Often, the slope is examined over a period of 10 time points (Owen, 1989). Thus, in Figure 12.15, it can be seen that in the 10 months preceding May 1978, the slope is approximately 45° . Consequently, this cusum analysis suggests a drift from the reference value. If smaller changes are of interest – and this is quite likely – then techniques such as the use of a 'V-mask' (Barnard, 1959) and a decision interval scheme (Wetherill, 1969) are available. Page (1961), Davies and Goldsmith (1972) and Wrathall and Hebert (1982) discuss this topic in detail, and Tam (2009) reviews cusum techniques generally. They have been extended into clinical areas such as healthcare improvement (Thor *et al.*, 2007), surgical results and post-operative mortality (Grunkemeier *et al.* 2003), and infectious-disease surveillance (Carpenter, 2002).

Cusum charts do not detect steady trends and regular cyclical variations. These can be detected by other techniques such as time series analysis (see Chapter 8).

Further reading

- Altman, D.G. and Bland, J.M. (1996) Presentation of numerical data. *British Medical Journal*, **312**, 572
- Altman, D.G., Machin, D., Bryant, T.N. and Gardner, M.J. (Eds) (2000) *Statistics with Confidence*, 2nd edn. BMJ Books, London. (*Estimation of confidence intervals and statistical guidelines*)
- Bland, M. (2015) *An Introduction to Medical Statistics*, 4th edn. Oxford University Press, Oxford
- Cleveland, W.S. (1985) *The Elements of Graphing Data*. Hobart Press, Summit
- Daly, L.E. and Bourke, G.J. (2000) *Interpretation and Uses of Medical Statistics*, 5th edn. Blackwell Science, Oxford
- Essex-Sorlie, D. (1995) *Medical Biostatistics and Epidemiology*. Appleton and Lange, East Norwalk
- Katz, D.L., Wild, D., Elmore, J.G. and Lucan, S.C. (2014) *Jekel's Epidemiology, Biostatistics, Preventive Medicine, and Public Health*, 4th edn. Saunders, Philadelphia
- Kaye, D.H. and Freedman, D.A. (2011) Reference guide on statistics. In: *Reference Manual on Scientific Evidence*, 3rd edn, pp. 211–304. Federal Judicial Center, Washington DC. (*A general guide to statistical methods, with emphasis on interpretation*)
- Mead, R., Curnow, R.N. and Hasted, A.M. (2003) *Statistical Methods in Agriculture and Experimental Biology*, 3rd edn. Chapman and Hall, Boca Raton and London
- Newcombe, R.G. (2013) *Confidence Intervals for Proportions and Related Measures of Effect Size*. CRC Press, Boca Raton
- Petrie, A. and Watson, P. (2013) *Statistics for Veterinary and Animal Science*, 3rd edn. John Wiley and Sons, Chichester
- Stewart, A. (2016) *Basic Statistics and Epidemiology: A Practical Guide*, 4th edn. CRC Press, Boca Raton
- Tufte, E.R. (2001) *The Visual Display of Quantitative Information*, 2nd edn. Graphics Press, Cheshire

13

Surveys

Information on disease and associated events, such as productivity, can be obtained from **surveys** (introduced in Chapter 2). These involve **counting** members of an aggregate of units and measuring their characteristics. They may be conducted to estimate either a continuous variable such as weight and milk yield, or discrete events such as diseased animals. An important application of surveys in epidemiology is estimation of the **prevalence** of clinical disease, infection, or seropositive animals from samples of an animal population.

A survey of prevalence can involve:

- a single sample, either to determine prevalence or to determine whether or not disease is present in a group of animals;
- two samples, to compare prevalence;
- three or more samples.

The first situation commonly is encountered in epidemiological investigations and is the main subject of this chapter; the statistical methods are those used in estimating proportions¹. Comparisons between two samples are described in the next chapter.

Censuses and sample surveys

If all animals in a population are investigated then the survey is a **census**. Demographic censuses of human and animal populations are conducted regularly in many countries to determine their size and structure. A census is the only means of measuring *exactly* the distribution of a variable in a population. Some prevalence surveys that are 'almost' censuses have been conducted. For example, Jasper and others (1979) surveyed 2400 out of 2800 farms in California to determine the prevalence of mycoplasmal mastitis in

dairy cows. Censuses can be expensive and may be difficult or impossible to conduct. However, if a survey is designed well, then a reasonably accurate and acceptable estimate of a variable can be made by examining some of the animals in the relevant population; that is, a **sample**. This involves analysis of the inherent variation in values of a variable that occurs in a population, which is reflected in samples, leading to differences between sample results and the value of the population characteristic that is being estimated (the **sampling error**).

Sampling: some basic concepts

The validity of sampling theory is based on the assumption that an aggregate of units can be divided into representative sub-units, and that characteristics of the aggregate can be estimated from the sub-units.

Some definitions

The **target population** is the total population about which information is required. Ideally, this should be the population at risk. The **study population** is the population from which a sample is drawn. These two populations should be the same. However, for reasons of practicality, this may not be possible. For instance, if an investigation of periodontitis in Maltese terriers were to be undertaken, then, ideally, all Maltese terriers (the target population) should be sampled, although it may only be possible to investigate animals at dog shows or attending veterinary practices (the study population). If the study population is not 'representative' of the target population, then results should not be generalized beyond the study population.

The study population consists of **elementary units**, which cannot be divided further. In veterinary surveys, these are usually individual animals.

A **stratum** is one of several groups into which a population can be divided according to common

¹ Surveys of continuous variables are not discussed here; suitable methods are described in the general texts on survey methods listed at the end of this chapter. Similarly, surveys to determine the size of animal populations are not discussed; Levy and Lemeshow (2008) give appropriate formulae.

characteristics. For example, dairy cows are a stratum of the livestock population.

Before a sample is taken, members of the study population must be identified by constructing a list; this is the **sampling frame**. Each member of the sampling frame is a **sampling unit**. Thus, a list of hunting licence receipts was used as the sampling frame to identify hunters in an investigation of the recreational importance of feral pigs in relation to the possible control of African swine fever in the US (Degner *et al.*, 1983). A register of veterinarians can provide a sampling frame for sampling veterinary practices.

In areas in which telephone ownership is widespread, a sampling frame can be constructed from the list of names and numbers in telephone directories. Alternatively, all possible numbers can be used as the basis of sample selection. This second approach uses **random-digit dialling** (Waksberg, 1978) and is preferred to the first because it can include unlisted numbers and therefore reduces the likelihood of selection bias (Roslow and Roslow, 1972). It is a useful sampling method for surveys of pet populations (e.g., Lengerich *et al.*, 1992; Teclaw *et al.*, 1992; Slater, 1996a; Patronek *et al.*, 1997; Ortega-Pacheco *et al.*, 2007). However, the newer telephone technologies, such as mobile phones, pose new challenges to identification of sampling frames and other telephone-survey issues (Kempf and Remington, 2007).

The Internet also is a potential source of sampling frames. However, users of the Internet may not be representative of the target population (Best and Harrison, 2009), thus posing challenges to probability sampling, although this approach may be more applicable to non-probability sampling (see 'Types of sampling' following).

The **sampling fraction** is the ratio of sample size to study population size. Thus, if 10 animals were chosen from 1000, the sampling fraction would be 1%.

Veterinary sampling frames include lists of abattoirs, farms and veterinary practices, and can be constructed from some of the other sources of data described in Chapter 21. The objective of sampling is to provide an unbiased estimate of the variable that is being measured in the population. Many veterinary sampling frames (e.g., abattoirs) themselves relate to biased sectors of the total animal population and so caution must be exercised when extrapolating results to other sectors (e.g., farms). However, biased estimates also can be produced *within* a sampling frame when:

- lists of members of the frame are incomplete;
- information is obsolescent;
- segments of the frame are untraceable;

- there is lack of cooperation by some members of the frame;
- sampling procedures are not random (see 'Types of sampling' following).

These sources of bias are **non-compensating errors** because they cannot be reduced by increasing the size of the sample. They are part of a larger group of **non-sampling errors** (Groves, 2004), such as interviewer errors and poor response to questionnaires (see Chapter 11), which, although difficult to quantify, can have a large impact on the overall error (Zarkovich, 1966).

The nature of sampling units

Sampling units may be individual animals (i.e., elementary units) or they may be aggregates such as herds, farms or administrative regions, and prevalence may be calculated in relation to these different units. Thus, individual-animal prevalence (the proportion of affected animals) may be quoted, or herd prevalence (the proportion of affected herds) may be cited². Occasionally, the sampling units may be parts of individuals; for example, teats, limbs or eyes³.

It is important to distinguish between the 'epidemiological unit' and the 'sampling unit' when dealing with infectious diseases (Tyler, 1991). The former unit is the group of animals that is of epidemiological significance in terms of the transmission and maintenance of infection, and therefore of disease control. It is convenient if the two units are identical. Thus, sheep 'hefts'⁴, and large herds that are managed independently, constitute different epidemiological units that may be considered as separate sampling units. In contrast, in less-economically-developed countries, several small village herds that are put together and are

² Exceptionally, the herd may be the sampling unit, but a prediction can be made about individual-animal prevalence: Hartman and others (1997), for example, discuss the use of ELISA testing of bulk milk samples to predict within-herd prevalence of bovine herpesvirus 1. Cowling and others (1999) and Evers and Nauta (2001) describe methods for estimation of individual-animal prevalence based on pooled samples, which are eased by the use of appropriate software (Sergeant, 2015). Græsbøll and others (2016) discuss pooling of samples to detect disease.

³ This may complicate analysis because basic sampling theory assumes that each sampling unit is *independent*; that is, that one unit does not exert an effect on another. However, structures in the same animal may not be independent (e.g., the mammary quarters of a cow in relation to mastitis). The analytical implications in such circumstances are discussed by Barkema and others (1997) and Schukken and his colleagues (2003).

⁴ Populations of sheep, resident on extensive hills or mountains, the populations often retaining themselves within a localized area without fences.

herded on common grazing constitute a single epidemiological unit, and the village could be considered as the sampling unit.

Types of sampling

There are two main types of sampling:

1. **non-probability sampling** in which the choice of the sample is left to the investigator;
2. **probability sampling** in which the selection of the sample is made using a *deliberate, unbiased* process, so that *each sampling unit in a group has an equal probability of being selected*; this is the basis of **random sampling**.

Non-probability sampling methods

Convenience sampling

Convenience (availability/accidental/opportunity) sampling is the collection of easily-accessible sampling units. When convenience is the main criterion for selecting a sample, it is very unlikely that the sample will be truly representative of the study population, resulting in biased estimates. For instance, if a survey were undertaken to estimate the prevalence of lameness in cattle, and if a sample were selected by choosing the first 10 out of 100 animals that entered a milking parlour, then the sample probably would underestimate the prevalence because the leading animals would be less likely to be lame than those who entered the parlour last. In other cases, the selection procedure may not induce bias as obviously as in this example, but bias may nevertheless occur.

Purposive sampling

Purposive (judgemental) sampling is the selection of a sample, the averages of whose quantitative characteristics (e.g., weight) or distribution of whose qualitative characteristics (e.g., sex and breed) are similar to those that are to be studied in the target population. The object is to select a sample where characteristics are *balanced* with those of the target population. For example, a veterinarian who is undertaking a tuberculosis test on several herds may be asked to take blood samples from a 'representative' (i.e., balanced) sample for titration of antibodies against various bacteria and viruses. Purposive selection of so-called 'average' samples can produce a sample, none of whose members is far from the population mean. This sample therefore will only be representative of those collections of sampling units for which none of the members is

far from the population mean. The sample will not be representative of all the possible samples that may be taken from the population, some of which will have means far from the population mean. Therefore, the variability of the population that is being sampled may be underestimated. Additionally, experience and experimental evidence have demonstrated that consciously-selected 'representative' samples are always biased. Yates (1981) and Brink (1996) discuss the disadvantages of purposive sampling in detail.

Probability sampling methods

Simple random sampling

A simple random sample is selected by drawing up a list of all animals or other relevant sampling units (e.g., herds) in the study population, and then selecting the sampling units randomly.

Selecting a simple random sample There are several ways of selecting a simple random sample. For instance, each animal in the study population could be represented by a numbered piece of paper in a hat. The desired number of pieces of paper, corresponding to the sample size, could then be drawn from the hat to identify the sample's members. This method assumes that selection is random, and could be laborious for large study populations. A more convenient and less haphazard way of random selection uses random numbers. These are listed in published tables, one of which is given in Appendix X, which also includes an example of random number selection.

Random numbers also can be generated by pocket calculators and using appropriate software (see Appendix III). The former generate random numbers between zero and one. These values can be multiplied by the study population size to produce the required numbers. For example, if a random sample of 50 animals were required from a population of 2000 animals, then the calculator's random number generator might produce the values:

0.969, 0.519, 0.670 and 0.164 . . . ,

which, when multiplied by 2000, produce the sample numbers:

1938, 1038, 1340 and 328 . . . ,

respectively; and so on, until 50 animals are selected.

Systematic sampling

Systematic sampling involves selection of sampling units at equal intervals, the first animal being selected

randomly. For example, if one animal in every 100 were required, then the first animal would be selected randomly from the first 100. If this were animal 63, then the sample would comprise animals, 63, 163, 263, 363 and so on. Systematic sampling frequently is used in industrial quality control, such as selecting samples of goods on a conveyor belt.

Simple random versus systematic sampling

A systematic sample does not require knowledge of the total size of the study population. A simple random sample, however, can only be selected when all of the animals in the study population are identified. If lists are available with which to compile the sampling frame (e.g., lists of farms), then the random sample can be selected relatively easily. However, if lists are not available, it may be difficult – even impossible – to draw up the sampling frame and therefore to select the random sample.

Systematic samples tend to be more evenly spread in the population than random samples, but in practice give results similar to simple random samples (Armitage *et al.*, 2002). However, the technique, although having the advantage of simplicity, can be dangerous if there is periodicity in the sampling frame. For example, if a farmer sends his animals to slaughter on only Tuesdays, and abattoir samples are selected on

only Wednesdays, then that farmer's animals cannot be represented in the samples.

Stratified sampling

A stratified random sample is obtained by dividing the study population into exclusive groups (**strata**), then randomly sampling units from all of the individual strata. For example, the strata may be different ranges of herd or flock size, or different geographical regions. The latter may need to be identified after a detailed scrutiny of topography and livestock distribution. Thus, the Kingdom of Bhutan, lying in the Himalayas, has three main routes of livestock movement, separated by mountainous spurs (Figure 13.1). This suggests that the three regions, A, B and C, should be treated as separate strata.

Stratification can improve the accuracy of a sample because it overcomes the tendency of a simple random sample to either over-represent or under-represent some sections of the sampling frame. Thus, if a simple random sample of animals in all herds in a country were selected, it is possible that no animals in very small herds would be chosen. Stratification, which ensures that each group in the population is represented, overcomes this problem.

The number of sampling units selected from each stratum can be determined by several methods. A common method is **proportional allocation**, where

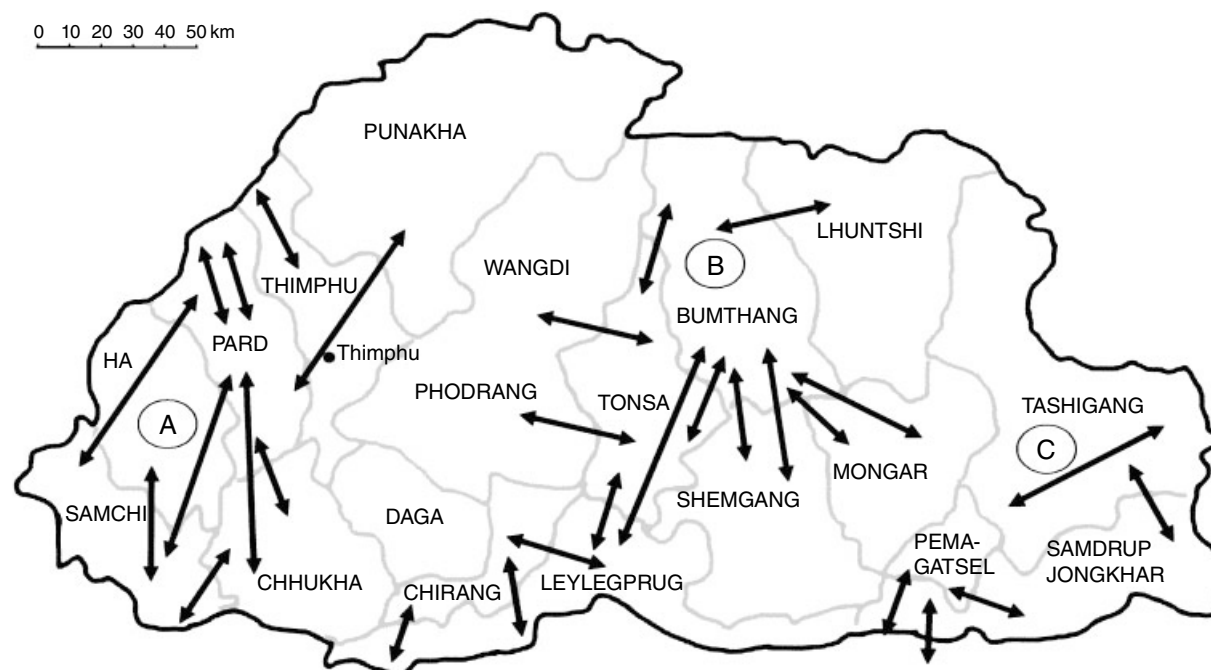


Fig. 13.1 The Kingdom of Bhutan, showing the main routes of livestock movement. $\longleftrightarrow$ Routes of animal movement. Administrative regions (Dzongkhag) are outlined in grey.

Table 13.1 An example of stratification: selection of a stratified random sample of cows from different regions of Great Britain based on a 5% sample of all cows (147 000). (Data extracted from Wilson *et al.*, 1983.)

Region	Number of cows	Number sampled
Devon and Cornwall	302 647	$302\,647 \times 0.05 = 15\,132$
SW England other than Devon and Cornwall	469 486	$469\,486 \times 0.05 = 23\,474$
S England	271 225	$271\,225 \times 0.05 = 13\,561$
E England	119 835	$119\,835 \times 0.05 = 5\,992$
East Midlands	189 817	$189\,817 \times 0.05 = 9\,491$
West Midlands	462 826	$462\,826 \times 0.05 = 23\,141$
Wales	342 346	$342\,346 \times 0.05 = 17\,117$
Yorkshire/Lancashire	255 626	$255\,626 \times 0.05 = 12\,781$
N England	273 838	$273\,838 \times 0.05 = 13\,692$
Scotland	260 366	$260\,366 \times 0.05 = 13\,018$
Totals	2 948 012	147 399

the number of sampling units selected is proportional to the number in each stratum. For instance, if a sample of cows were required from the British dairy herd by region, the number of cows selected from each region would be proportional to the number of cows in each region, to ensure that cows in regions with large numbers of dairy cattle are not under-represented. *Table 13.1* illustrates this method of selection based on a 5% sampling fraction. Proportional allocation is the most efficient method of selection if there is equal cost in sampling each stratum, and is generally recommended (Sadooghi-Alvandi, 1988). If this assumption is not true, then other, more complex, allocation methods should be used (Levy and Lemeshow, 2008).

Cluster sampling

Sometimes, strata are defined as relatively small geographical groups, such as different parishes, villages and herds, or by other categories such as veterinary practices or periods of time during which samples are selected. The strata are then termed **clusters**. Sampling randomly from all of these clusters can be time-consuming and costly and so some clusters are selected, and animals are sampled only in these clusters; for example, animals in a few villages or herds could be sampled. This is **cluster sampling**. Commonly, *all* animals in each selected cluster are sampled; this is **one-stage cluster sampling**.

A sample also may be selected in more than one stage. Thus, a sample of clusters can be selected, followed by sub-sampling of *some* animals in the clusters (in contrast to *all* animals in one-stage cluster sampling). This procedure therefore is called **two-stage cluster sampling**; the clusters are the **primary units**, and the selected members of the sub-samples are the **secondary units**.

If the secondary units are the individual members of the study population, there is no point in going further. However, if they consist of **groups** of population members, either all of their constituent members could be sampled or further stages of sampling could be undertaken, corresponding to progressively higher levels of sub-sampling; for example, sampling regions, then dairy farms in each selected region, then cows on each selected farm. This is **multistage cluster sampling**. The sampling technique at each stage is usually simple random sampling.

Cluster sampling is sometimes used when there is an incomplete list of all members of a population: a list of primary units is required, but secondary units need only be listed within the selected primary units. The technique is therefore convenient and relatively cheap because resources can be concentrated on limited parts of the full sampling frame. However, information is less precise than if either a systematic or random sample comprising the same number of animals were selected, because disease prevalence tends to be more variable *between* groups than *within* them – particularly with contagious diseases where herds are likely to have either high or low levels of infection.

Sometimes it is impossible to construct a sampling frame because reliable demographic data are not available. In such circumstances clusters can be defined using coordinates of a map grid. Grids may need to be constructed on maps that lack them, and grid lines identified by letters should be relabelled with numbers. *Figure 13.2*

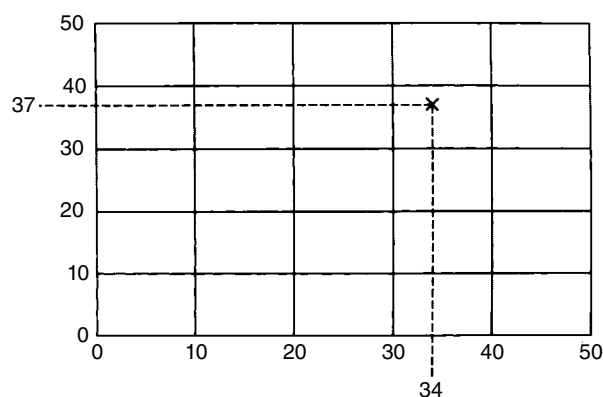


Fig. 13.2 An example of sampling using map grids.

is an example. The coordinates 37 ('latitude') and 34 ('longitude') are chosen randomly (e.g., using random number tables). If herds are being selected, all herds within a defined radius of the selected coordinates should be sampled, the radius depending on the density of herds. If no herds are present within the radius, then no animals are sampled. The nearest herd to selected coordinates should *not* be the only herd sampled because this would bias the result by including too many herds from sparsely-populated areas, and too few from densely-populated ones.

What sample size should be selected?

The question that should be answered in all sample surveys is 'How many animals should be chosen for the survey?' An answer cannot be given without considering the objectives and circumstances of the investigation.

The choosing of sample size depends on non-statistical and statistical considerations. The former include the availability of manpower and sampling frames. The latter are the desired **precision** of the estimate of prevalence and the **expected prevalence** of the disease.

Precision of the estimate of prevalence

The ability of an estimator to determine the true population value of a variable (i.e., the estimator's precision) can be expressed in terms of the bound on the

error of estimation that can be tolerated. The error can be defined either absolutely or relatively. For example, an acceptable absolute error of $\pm 2\%$ of a prevalence of 40% represents an acceptable range of 38–42%. A relative error of $\pm 2\%$ of the same prevalence corresponds to 2% of 40%; that is, $40\% \pm 0.8\%$, representing an acceptable range of 39.2–40.8%.

Expected prevalence of the disease

It may appear paradoxical to suggest that some idea of disease prevalence is necessary before a survey is undertaken, because the objective of the survey is to determine the prevalence. However, a general notion is required; if the prevalence is thought to be close to either 0% or 100%, then the confidence interval for a given sample size will be narrower than if the prevalence were close to 50%; that is, fewer animals will be required in the sample to achieve a stipulated width of confidence interval in the former case (*Table 13.2*). Information on prevalence might be obtained from other related surveys. However, frequently this information is not available and so estimates have to be made that may be little more than informed guesses ('guesstimates').

Estimation of disease prevalence

Simple random sampling

The approximate sample size required to estimate prevalence in a large (theoretically 'infinite') population can be determined for a defined precision and level of confidence. The limits of the associated interval indicate the specified bounds within which the

Table 13.2 The approximate sample size required to estimate prevalence in a large population with the desired fixed width confidence limits. (Adapted from Cannon and Roe, 1982.)

Expected prevalence	Level of confidence								
	90%			95%			99%		
	Desired absolute precision			Desired absolute precision			Desired absolute precision		
	10%	5%	1%	10%	5%	1%	10%	5%	1%
10%	24	97	2 435	35	138	3 457	60	239	5 971
20%	43	173	4 329	61	246	6 147	106	425	10 616
30%	57	227	5 682	81	323	8 067	139	557	13 933
40%	65	260	6 494	92	369	9 220	159	637	15 923
50%	68	271	6 764	96	384	9 604	166	663	16 587
60%	65	260	6 494	92	369	9 220	159	637	15 923
70%	57	227	5 682	81	323	8 067	139	557	13 933
80%	43	173	4 329	61	246	6 147	106	425	10 616
90%	24	97	2 435	35	138	3 457	60	239	5 971

estimate will lie with the defined level of confidence. The relevant formula for a 95% confidence interval is:

$$n = \frac{1.96^2 P_{\text{exp}} (1 - P_{\text{exp}})}{d^2},$$

where:

n = required sample size;

P_{exp} = expected prevalence;

d = desired absolute precision.

For example, if an expected prevalence of 30% is to be estimated with a desired absolute precision of $\pm 5\%$ (i.e., the limits of the associated 95% interval are 25% and 35%), then:

$$P_{\text{exp}} = 0.30,$$

and

$$d = 0.05.$$

Substituting these values in the formula:

$$\begin{aligned} n &= \frac{1.96^2 \cdot 0.30(1 - 0.30)}{0.05^2} \\ &= \frac{3.84 \times 0.21}{0.0025} \\ &= 323. \end{aligned}$$

If other confidence intervals are required, then 1.96 is replaced by the appropriate multiplier (see Appendix VI).

Table 13.2 also can be used. This table lists expected prevalence values and the desired absolute precision of $\pm 10\%$, 5% and 1% for three levels of confidence (90%, 95% and 99%). Thus, if the expected prevalence is again thought to be about 30%, and it is desired to estimate this with an absolute precision of $\pm 5\%$ for a 95% confidence interval, then 323 animals are required – the same value as that given by the formula.

In this example, the use of 30% might be based on prior evidence. In other circumstances the approximate prevalence might not be known. A suitable procedure would be either to choose the 50% figure to give the maximum sample size, or to select the 20% figure and take more samples, if necessary.

Appendix XI also can be used. The figures in this appendix give the sample sizes required to attain either a 95% or 99% confidence interval for various prevalence values at various values of absolute precision. Using the same example of a 30% expected prevalence and absolute precision of $\pm 5\%$, the sample would be selected using Figure 4 of the appendix, giving a sample size of approximately 400 (judged by eye from the curve) at the 95% level of confidence.

The formula and Table 13.2 are based on the Normal approximation to the binomial distribution (see Chapter 12). This is acceptable if the size of the study population is large in relation to the sample. However, as the size of the sample relative to the study population increases, the variance of the estimator of the mean of the study population is decreased and the width of the confidence interval is reduced accordingly. Therefore, in relatively small populations, it is possible to select a smaller sample than one from a theoretically infinite population to achieve the same degree of precision.

The required sample size, n_{adj} , is given by the following formula:

$$n_{\text{adj}} = \frac{N \times n}{N + n},$$

where n is the sample size, based on an infinite population (obtained from the formula above or Table 13.2) and N is the size of the study population.

For example, if prevalence were to be estimated using values similar to those in the example above, but in a small study population, say 900 animals, then:

$$n = 323,$$

$$N = 900.$$

Therefore:

$$\begin{aligned} n_{\text{adj}} &= \frac{900 \times 323}{900 + 323} \\ &= \frac{290\,700}{1223} \\ &= 238. \end{aligned}$$

Thus, the number of animals required to estimate the prevalence to the same absolute precision as the example above (which was based on an infinite population) is 238.

It is difficult to give a strict rule regarding application of this formula. It will always give the correct sample size, but n_{adj} will be very close to n if adjustment is unnecessary. A rough guideline is to calculate n_{adj} if n is 5% or more of N .

These approximate methods are usually sufficient, but, if the more complex exact methods are required, Levy and Lemeshow (2008) should be consulted.

Imperfect tests Frequently, surveys utilize diagnostic tests to identify disease, and such tests are not 'perfect'; that is, they have a diagnostic sensitivity and diagnostic specificity of less than 100% (see Chapters 10 and 20), thereby generating false-negative and false-positive

results, respectively, and therefore producing estimates of *test* prevalence, rather than *true* prevalence.

The relevant formula for determining sample size in a large population, for a 95% confidence interval, accommodating test imperfections (derived from Humphry *et al.*, 2004), is:

$$n = \left(\frac{1.96}{d} \right)^2 \times \frac{[(Se \times P_{\text{exp}}) + (1 - Sp)(1 - P_{\text{exp}})][(1 - Se \times P_{\text{exp}}) - (1 - Sp)(1 - P_{\text{exp}})]}{(Se + Sp - 1)^2},$$

where n , P_{exp} and d follow the same notation as before, Se = sensitivity, and Sp = specificity.

For example, if an expected prevalence of 30% again is to be estimated with a desired absolute precision of $\pm 5\%$, with 95% confidence, and the test used has a sensitivity and specificity of 90% and 80%, respectively, then:

$$P_{\text{exp}} = 0.30,$$

$$d = 0.05,$$

$$Se = 0.90,$$

$$Sp = 0.80;$$

and:

$$\begin{aligned} n &= \left(\frac{1.96}{0.05} \right)^2 \times \frac{[(0.90 \times 0.30) + (1 - 0.80)(1 - 0.30)][(1 - 0.90 \times 0.30) - (1 - 0.80)(1 - 0.30)]}{(0.90 + 0.80 - 1)^2} \\ &= 1536.64 \times \frac{(0.41)(0.59)}{0.49} \\ &= 1536.64 \times \frac{0.2419}{0.49} \\ &= 758.6; \end{aligned}$$

that is, 759 animals are required.

Note that more animals are required than if the test were assumed to be perfect (which only requires 323 animals), and the number will increase as values of sensitivity and specificity decrease.

Again, in relatively small populations, a smaller sample, n_{adj} , can be selected, using the previous formula.

The formula for determining sample size using an imperfect test also can be used when aggregates of animals (e.g., herds or flocks) are the sampling units. This issue is addressed later in this chapter, at the end of the section 'Detecting the presence of disease', where the relevant statistical theory is presented.

Systematic sampling

If it is reasonable to assume that a systematic sample is as representative as a simple random sample, then sample size can be calculated using the same formulae

as those used previously for calculating sizes of simple random samples. However, if periodicity is likely to be present in the sampling frame, more sophisticated formulae should be applied (Levy and Lemeshow, 2008).

Stratified sampling

Sample sizes for proportionally-allocated stratified samples again can be calculated using the methods appropriate to simple random samples. However, if more complex methods of allocation are adopted, then other formulae should be used (Levy and Lemeshow, 2008).

Cluster sampling

The formulae for sample size determination for simple random samples cannot be applied to cluster samples because they do not take account of the potentially large variation that can occur *between* clusters, and so different methods therefore need to be applied.

Two approaches can be used to address the variability between clusters. The first determines the between-cluster variance component, V_c . This is the variation expected between clusters if all animals in the clusters were sampled (i.e., there is no sampling variation within the clusters). If previous cluster sample data are available, then the between-cluster variance can be calculated approximately:

$$V_c = c \left\{ \frac{K_1 c V}{T^2 (c - 1)} - \frac{K_2 \hat{P}(1 - \hat{P})}{T} \right\},$$

where:

c = number of clusters in the sample;

T = total number of animals sampled;

$$K_1 = (C - c)/C;$$

where:

C = number of clusters in the population;

$$K_2 = (N - T)/N;$$

where:

N = total number of animals in the population;

$$V = \hat{P}^2 (\Sigma n^2) - 2 \hat{P} (\Sigma nm) + (\Sigma m^2);$$

where:

$\hat{P}$ = sample estimate of overall prevalence;

n = number of animals sampled in each cluster;

m = number of diseased animals sampled in each cluster.

Table 13.3 presents information from 14 farms selected as clusters from a total of 865 farms, all

Table 13.3 An example of cluster sampling: a survey of 14 farms selected randomly from 865 farms; all animals in the selected farms investigated.

Farm	Total number of animals	Number of diseased animals	Farm prevalence
1	272	17	0.063
2	87	15	0.172
3	322	71	0.221
4	176	17	0.097
5	94	9	0.096
6	387	23	0.059
7	279	78	0.280
8	194	59	0.304
9	65	37	0.569
10	110	34	0.309
11	266	23	0.087
12	397	57	0.144
13	152	19	0.125
14	231	17	0.074
Totals	3032	476	

animals in each cluster being sampled (i.e., one-stage cluster sampling). If such data are available, they can be used to determine between-cluster variance for determination of sample size for a subsequent cluster sample.

The sample estimate, $\hat{P}$, of overall prevalence = $476/3032 = 0.157$. (It would be misleading to calculate the overall prevalence by taking the mean of the individual cluster prevalences. In this example, such a calculation would give a value of 0.186, i.e., $[0.063 + 0.172 + \dots + 0.074]/14$ —somewhat higher than the actual overall prevalence.)

In this example, $C = 865$ and $c = 14$. Thus, K_1 is approximately equal to one and so K_1 can be omitted from subsequent calculations. Similarly, N is assumed to be large, compared with T , and so K_2 is approximately equal to one and so also can be omitted from the calculations.

Calculate V thus:

$$\begin{aligned}\Sigma n^2 &= 272^2 + 87^2 + \dots + 231^2 \\ &= 811\,450. \\ \Sigma nm &= (272 \times 17) + (87 \times 15) + \dots + (231 \times 17) \\ &= 116\,445. \\ \Sigma m^2 &= 17^2 + 15^2 + \dots + 17^2 \\ &= 22\,972.\end{aligned}$$

Thus:

$$\begin{aligned}V &= (0.157^2 \times 811\,450) - \{(2 \times 0.157) \times 116\,445\} + 22\,972 \\ &= 20\,001 - 36\,564 + 22\,972 \\ &= 6409.\end{aligned}$$

Now substitute the values $c = 14$, $T = 3032$ and $V = 6409$ into the formula:

$$\begin{aligned}V_c &= 14 \left\{ \frac{14 \times 6409}{3032^2 \times 13} - \frac{0.157 \times (1 - 0.157)}{3032} \right\} \\ &= 14 \times 0.000\,707 \\ &= 0.009\,90.\end{aligned}$$

This value of V_c can be used to determine sample size for a subsequent cluster sample if the expected prevalence, P_{exp} , in the latter is assumed to be the same as that in the previous sample, $\hat{P}(15.7\%)^5$. If the expected prevalence is different, then an adjustment must be made:

$$V_{c,\text{adj}} = \frac{V_c P_{\text{exp}}(1 - P_{\text{exp}})}{\hat{P}(1 - \hat{P})}.$$

For example, if the expected prevalence in the new survey is 30%, then $P_{\text{exp}} = 0.30$, $\hat{P} = 0.157$, and:

$$\begin{aligned}V_{c,\text{adj}} &= \frac{0.009\,90 \times 0.30(1 - 0.30)}{0.157(1 - 0.157)} \\ &= 0.0157.\end{aligned}$$

(Note that this value is only *coincidentally* one-tenth the value of the prevalence in the previous cluster sample.)

This value is then used for V_c .

One-stage cluster sampling In one-stage cluster sampling, some clusters are selected, then all animals within each selected cluster are sampled. The first step in determining sample size is prediction of the average number of animals per cluster. The appropriate formula for a 95% confidence interval is then:

$$g = \frac{1.96^2 \{ (n-1) V_c + P_{\text{exp}}(1 - P_{\text{exp}}) \}}{nd^2},$$

⁵ Occasionally, a negative estimate of V_c will be obtained. This is usually an indication that there is no additional variation between the clusters, and V_c may be set to zero.

where:

g = number of clusters to be sampled;
 n = predicted average number of animals per cluster;
 P_{exp} = expected prevalence;
 d = desired absolute precision;
 V_c = between-cluster variance.

For example, if an expected prevalence of 30% is to be estimated with a desired absolute precision of $\pm 5\%$, and the predicted average number of animals per cluster is 20:

$$\begin{aligned}n &= 20, \\P_{\text{exp}} &= 0.30, \\d &= 0.05, \\V_c &= 0.0157.\end{aligned}$$

Thus:

$$g = \frac{1.96^2 \{19 \times 0.0157 + 0.30(1 - 0.30)\}}{20 \times 0.05^2} = 39.1.$$

Therefore, rounding up, 40 clusters should be sampled.

If the population of clusters from which the sample is to be drawn is small, the estimated number of clusters should be adjusted by:

$$g_{\text{adj}} = \frac{G \times g}{G + g},$$

where G is the total number of clusters in the population.

For instance, if the sample was to be selected from a population containing only 150 clusters:

$$\begin{aligned}g &= 39.1, \\G &= 150,\end{aligned}$$

and:

$$g_{\text{adj}} = \frac{150 \times 39.1}{150 + 39.1} = 31.0;$$

thus, 31 clusters are required.

If the subsequent sampling results in a value for the average number of animals in each cluster that is less than the predicted value, n , then more clusters should be sampled until the value gn is attained.

If other confidence intervals are required, the appropriate multipliers should be used in place of 1.96 (see Appendix VI).

The second approach to address the variability between clusters involves the use of either the **design effect**, D , or the **rate of homogeneity (intra-class correlation coefficient)**, ρ ('rho'), in sample-size

calculation (Bennett *et al.*, 1991). The design effect is an adjustment factor used to allow for clustering, and, in a specific cluster sample, is the ratio of the cluster sample variance to the variance computed as a simple random sample. It is related to ρ , which is the ratio of the variability between clusters compared with the variability within clusters, and usually takes values between zero and one⁶: the larger the value, the higher the rate of homogeneity. A large value of ρ indicates much homogeneity *within* clusters and large variability *between* clusters; that is, substantial clustering.

The relationship between D and ρ is:

$$D = 1 + (n - 1)\rho;$$

alternatively:

$$\rho = (D - 1)/(n - 1),$$

where n = average number of animals per cluster.

Note that D varies depending on the number of animals per cluster, n . When n is higher, the relative effect of clustering on the overall sample variance will be greater. The parameters, D and ρ , may be considered as alternatives to V_c for incorporating the additional variance in the sample due to clustering.

When past cluster sample data are available, if V_c has already been adjusted for any difference between the expected prevalence and that in the original study (see $V_{c,\text{adj}}$ earlier) then:

$$\rho = \frac{V_{c,\text{adj}}}{P_{\text{exp}}(1 - P_{\text{exp}})},$$

or alternatively it may be obtained from the unadjusted value of V_c as:

$$\rho = \frac{V_c}{\hat{P}(1 - \hat{P})}.$$

$V_{c,\text{adj}}$ may be written in terms of ρ by:

$$V_{c,\text{adj}} = P_{\text{exp}}(1 - P_{\text{exp}})\rho,$$

or in terms of D by:

$$V_{c,\text{adj}} = \frac{(D - 1) \times P_{\text{exp}}(1 - P_{\text{exp}})}{(n - 1)}.$$

D may be written in terms of $V_{c,\text{adj}}$ by:

$$D = 1 + (n - 1) \frac{V_{c,\text{adj}}}{P_{\text{exp}}(1 - P_{\text{exp}})}.$$

⁶ Very occasionally an estimate of ρ may be negative when there is less variability observed between clusters than would be expected by chance. Usually in this situation it will be appropriate for ρ to take a value of 0.

For the example above, taking $V_{c,adj} = 0.0157$ and $P_{exp} = 0.30$:

$$\rho = \frac{0.0157}{0.30 \times (1 - 0.30)} = 0.0748.$$

The number of clusters required, g , may be equivalently expressed in terms of ρ by:

$$g = \frac{1.96^2 \{1 + (n-1)\rho\} \times P_{exp}(1 - P_{exp})}{nd^2},$$

or in terms of D by:

$$g = \frac{1.96^2 D \times P_{exp}(1 - P_{exp})}{nd^2}.$$

Taking $\rho = 0.0748$ and calculating for a new cluster sample with $P_{exp} = 0.30$, then:

$$g = \frac{1.96^2 \times \{1 + (19 \times 0.0748)\} \times 0.30 \times (1 - 0.30)}{200.05^2} = 39.1,$$

giving 40 clusters to be sampled, as before. These calculations may be eased by appropriate software (e.g., *CSurvey* and *WinPepi*: see Appendix III).

Two-stage cluster sampling In two-stage cluster sampling, only a sample of animals within the selected clusters are sampled. Determination of sample size for estimation of prevalence using two-stage cluster sampling depends on whether:

- the total sample size is fixed, and the number of clusters to be sampled is required;
- the number of clusters is fixed, and the number of animals to be sampled is required; or
- the sample size per cluster is fixed and the number of clusters (and hence the total sample size) is required.

(1) *Number of clusters to be sampled when the total sample size is fixed* If the population of clusters is large compared with the number to be sampled, and the total number of animals is also large (which is the usual circumstance), the relevant formula for a 95% confidence interval is:

$$g = \frac{1.96^2 TV_c}{d^2 T - 1.96^2 \{P_{exp}(1 - P_{exp}) - V_c\}},$$

where:

g = number of clusters to be sampled;
 P_{exp} = expected prevalence;
 d = desired absolute precision;
 T = total number of animals to be sampled.

If an expected prevalence of 30% is to be estimated with a desired absolute precision of $\pm 5\%$, 1000

animals are to be sampled, and the between-cluster variance is assumed to be 0.0157 (estimated from the previous cluster sample data in *Table 13.3* with an adjustment for the difference between the prevalence in the previous cluster sample and the expected prevalence in the proposed sample):

$$\begin{aligned} P_{exp} &= 0.30, \\ d &= 0.05, \\ T &= 1000, \\ V_c &= 0.0157. \end{aligned}$$

Thus:

$$g = \frac{1.96^2 \times 1000 \times 0.0157}{0.05^2 \times 1000 - 1.96^2 \{0.30(1 - 0.30) - 0.0157\}} = 34.4.$$

Thus, 35 clusters need to be sampled, each sample ideally containing 29 animals ($35 \times 29 \approx 1000 = T$). The selection of unequal numbers of animals from each cluster should not affect the result materially, unless there are major disparities. However, if fewer than 29 animals, on average, are sampled (i.e., $T < 1000$), then the desired level of precision may not be attained.

If the number of clusters to be sampled is negative, then it is not possible to obtain the desired absolute precision, even with a very large number of clusters. It therefore would be necessary to increase the total sample size, T , or decrease precision (i.e., increase the value of d). If g is unrealistically high, it can be reduced by increasing d and/or increasing T , and then recalculating g .

If the population of clusters from which the sample is to be drawn is small, the adjustment formula, $g_{adj} = Gg/(G + g)$, again should be applied.

Thus, if the cluster sample designed above involved selection from a population of only 100 clusters, then $G = 100$, $g = 34.4$, and:

$$g_{adj} = \frac{100 \times 34.4}{100 + 34.4} = 25.6.$$

Therefore, rounding up, 26 clusters are required, each sample containing approximately 39 animals.

If the total number of animals in the population, N , is not large compared with T , then, for a 95% confidence interval, the number of clusters is approximately:

$$g = \frac{1.96^2 NTV_c}{d^2 NT - 1.96^2 (N - T) \{P_{exp}(1 - P_{exp}) - V_c\}},$$

where N = total number of animals in the population, and P_{exp} , g , d , T and V_c follow the same notation as before.

For example, if 1000 animals are to be sampled from a total population of 10 000 animals to estimate a prevalence of 30% with an absolute precision of $\pm 5\%$, then $N = 10\,000$, $T = 1000$, $d = 0.05$ and, for a 95% confidence interval:

$$g = \frac{1.96^2 \times 10000 \times 1000 \times 0.0157}{(0.05^2 \times 10000 \times 1000) - 1.96^2 \times (10000 - 1000) \times \{0.30 \times (1 - 0.30) - 0.0157\}}$$

$$= \frac{603131}{25000 - 6717}$$

$$= 33.0.$$

Therefore, 33 clusters are required, each containing, on average, 31 animals.

If the total number of animals and clusters in the population are both small, then the value of g obtained should be adjusted using the formula for g_{adj} , given previously. Thus, if the total population of 10 000 animals were contained in 100 herds:

$$G = 100,$$

$$g = 33,$$

and:

$$g_{\text{adj}} = \frac{100 \times 33}{100 + 33} = 24.8.$$

Therefore, 25 clusters are required, each sample containing approximately 40 animals.

Again, the appropriate multipliers (see Appendix VI) should be used if other confidence intervals are required.

Using the alternative approach, g may be obtained in terms of ρ as:

$$g = \frac{1.96^2 T \rho P_{\text{exp}} (1 - P_{\text{exp}})}{\{d^2 T - 1.96^2 (1 - \rho) P_{\text{exp}} (1 - P_{\text{exp}})\}}.$$

Thus, if $\rho = 0.0748$, g may again be calculated for the above example as:

$$g = \frac{1.96^2 \times 1000 \times 0.0748 \times 0.30(1 - 0.30)}{(0.05^2 \times 1000) - 1.96^2 \times (1 - 0.0748) \times 0.30 \times (1 - 0.30)} = 34.4,$$

giving 35 clusters as before.

Again if the population of clusters from which the sample is to be drawn is small, the adjustment formula, $g_{\text{adj}} = Gg/(G + g)$, should be applied.

If the total number of animals in the population, N , is not large compared with T , then, for a 95% confidence interval, the number of clusters in terms of ρ is:

$$g = \frac{1.96^2 NT \rho P_{\text{exp}} (1 - P_{\text{exp}})}{d^2 NT - 1.96^2 (N - T)(1 - \rho) P_{\text{exp}} (1 - P_{\text{exp}})}.$$

(2) *Number of animals to be sampled when the number of clusters is fixed* To determine the number of animals

to be sampled when the number of clusters is fixed, the appropriate formula for a 95% confidence interval is:

$$T = \frac{1.96^2 g \{P_{\text{exp}} (1 - P_{\text{exp}}) - V_c\}}{gd^2 - 1.96^2 V_c}.$$

If the total number of clusters in the population, G , is not large compared with g , then:

$$T = \frac{1.96^2 Gg \{P_{\text{exp}} (1 - P_{\text{exp}}) - V_c\}}{Ggd^2 - 1.96^2 V_c (G - g)}.$$

If the total number of animals in the population, N , is not large compared with T , then adjust T thus:

$$T_{\text{adj}} = \frac{N \times T}{N + T}.$$

For example, if $V_c = 0.0157$, $P_{\text{exp}} = 0.30$, $g = 30$, $d = 0.05$, and the total population of clusters is large compared with g , then:

$$T = \frac{1.96^2 \times 30 \times \{0.30 \times 0.70 - 0.0157\}}{30 \times 0.05^2 - 1.96^2 \times 0.0157} = 1525,$$

that is, $1525/30 = 51$ animals per cluster.

If there are only 50 clusters in the total population, then:

$$T = \frac{1.96^2 \times 50 \times 30 \times \{0.30(1 - 0.30) - 0.0157\}}{50 \times 30 \times 0.05^2 - 1.96^2 \times 0.0157 \times (50 - 30)}$$

$$= 440.2,$$

that is, $441/30 = 15$ animals per cluster.

If there are only 2000 animals in the total population, but a large number of clusters, then:

$$T_{\text{adj}} = \frac{2000 \times 1525}{2000 + 1525} = 865.3,$$

that is, $866/30 = 29$ animals per cluster.

If there are only 2000 animals and 50 clusters in the total population, then:

$$T_{\text{adj}} = \frac{2000 \times 441}{2000 + 441}$$

$$= 362,$$

that is, $362/30 = 12$ animals per cluster.

Using the alternative approach, T may be estimated from ρ :

$$T = \frac{1.96^2 g (1 - \rho) P_{\text{exp}} (1 - P_{\text{exp}})}{gd^2 - 1.96^2 \rho P_{\text{exp}} (1 - P_{\text{exp}})},$$

using the same notation as before.

Taking $\rho = 0.0748$, T may be calculated as:

$$T = \frac{1.96^2 \times 30 \times (1 - 0.0748) \times 0.30 \times 0.70}{30 \times 0.05^2 - 1.96^2 \times 0.0748 \times 0.30 \times 0.70} = 1528;$$

that is, $1528/30 = 51$ animals per cluster. Note this value of T differs slightly from the value obtained using V_c , due to the value of ρ being rounded to three significant numbers.

If the total number of clusters in the population, G , is not large compared with g , then:

$$T = \frac{1.96^2 G g (1 - \rho) P_{\text{exp}} (1 - P_{\text{exp}})}{G g d^2 - 1.96^2 (G - g) \rho P_{\text{exp}} (1 - P_{\text{exp}})}.$$

(3) Number of clusters to be sampled when the number of animals per cluster is fixed. The number of clusters to be sampled when the average sample size per cluster, n , is fixed is given by:

$$g = \frac{1.96^2 \{ (n - 1) V_c + P_{\text{exp}} (1 - P_{\text{exp}}) \}}{n d^2}.$$

For example, assuming the number of animals per cluster (n) is 200, and the other parameters are as in scenario (1) ($V_c = 0.0157$, $P_{\text{exp}} = 0.30$ and $d = 0.05$), then:

$$g = \frac{1.96^2 \{ 199 \times 0.0157 + 0.30 \times (1 - 0.30) \}}{200 \times 0.05^2} = 25.6,$$

which rounds to 26 clusters, giving a total of $26 \times 200 = 5200$ animals.

Using the alternative approach, the number of clusters required may be written in terms of ρ as:

$$g = \frac{1.96^2 \{ 1 + (n - 1) \rho \} P_{\text{exp}} (1 - P_{\text{exp}})}{n \times d^2};$$

or in terms of D as:

$$g = \frac{1.96^2 \times D \times P_{\text{exp}} (1 - P_{\text{exp}})}{n \times d^2}.$$

Using $\rho = 0.0748$, g may be calculated:

$$g = \frac{1.96^2 \{ 1 + (200 - 1) 0.0748 \} \times 0.30 \times 0.70}{200 \times 0.05^2} = 25.6,$$

which again rounds to 26 clusters, giving a total of $26 \times 200 = 5200$ animals.

Ideally, V_c or ρ should be based on past data. If such data are not available, there are two options. First, a small random sample of clusters can be selected to provide a rough idea of the between-cluster variance before a full study is undertaken. Secondly, the between-cluster variance component can be guessed. This is most easily done by guessing the standard deviation (i.e., the average difference expected between an

individual cluster prevalence and the overall mean cluster prevalence), and then squaring it to give the between-cluster variance component. Thus, if an overall mean cluster prevalence of 0.30 (30%) is anticipated, and the average difference between this and the individual-cluster prevalence is guessed to be 0.09 (9%), then the between-cluster variance component would be $0.09^2 = 0.0081$. Thus, $\rho = 0.0081 / \{0.30 \times (1 - 0.30)\} = 0.039$. There is potential for error in determining the most appropriate sample size when there is greater variability between clusters than is guessed. It therefore may be prudent to assume a larger standard deviation than is guessed; for example 20%, giving a between-cluster variance component, V_c , of 0.04 (0.2²) and ρ of 0.19.

Table 13.4 presents some estimates of ρ for various infectious diseases. These suggest that ρ for highly infectious diseases (e.g., bovine virus diarrhoea, infectious bovine rhinotracheitis, infectious bursal disease and Newcastle disease) may range from 0.2 to 0.4, although some estimates were below this range. Low values may occur if there is substantial inter-herd spread (e.g., by frequent mixing or contact of animals at communal water sources). Values for some moderately-infectious diseases (e.g., enzootic bovine leucosis and bovine leptospirosis) range from 0.08 to 0.12. Values of ρ for vector-borne infections are likely to be very variable, depending on the distribution and abundance of vectors, and efficiency of transmission (e.g., Di Nardo *et al.*, 2014).

Since a value of ρ should be obtained from previous cluster sample data with characteristics similar to the sample that is being planned, estimates or guesses should only be used as rough guides to the selection of suitable values of ρ , and the impact of other values should be evaluated as part of sample-size calculation. Several authors contend that ρ rarely exceeds 0.2 (Deem *et al.*, 1993; McDermott and Schukken, 1994; Uilenberg, 1998), in which circumstance, therefore, sampling approximately 30 clusters with approximately 10 animals sampled in each cluster is adequate to estimate a conservatively-assumed prevalence of 50% with absolute precision of $\pm 10\%$ at the 95% level of confidence.

There are no simple formulae for sample-size estimation involving three or more stages. If the simple random sample formula is applied, it is likely to produce conservative estimates. (Some authorities recommend applying the simple random sampling formula and then, for all types of cluster sampling, multiplying the estimated sample size by between two and four. This approach is empirical and can be inaccurate.)

Bennett and others (1991), Otte and Gumm (1997) and Fosgate (2009) discuss cluster sampling further.

Table 13.4 Summary of survey data used for the calculation of the design effect, D , and rate of homogeneity, ρ , for selected infections of domestic animals. (Reduced from Otte and Gumm, 1997.)

Infection	Species	Prevalence		Clusters		D	ρ
		%	Number tested	Number	Average size*		
<i>Anaplasma marginale</i>	Ox	3.78	2909	104	28.0	2.19	0.04
		4.32	1111	91	12.2	2.11	0.10
Bovine virus diarrhoea	Ox	6.30	2799	102	27.4	6.95	0.23
		19.07	970	82	11.8	5.74	0.42
		69.74	466	90	5.2	2.76	0.42
<i>Brucella abortus</i>	Ox	7.74	1512	104	14.5	2.18	0.09
<i>Brucella ovis</i>	Sheep	11.71 [†]	1529	40	38.2	6.94	0.16
		10.99 [‡]	1529	40	38.2	9.20	0.22
<i>Eimeria</i> spp.	Ox	27.13	1010	104	9.7	2.53	0.18
		15.63	1113	91	12.2	4.32	0.30
Enzootic bovine leucosis	Ox	1.51	2907	104	28.0	3.52	0.09
		11.75	945	81	11.7	2.11	0.10
		1.93	466	90	5.2	1.34	0.08
<i>Fasciola</i> spp.	Ox	6.92	1113	91	12.2	4.09	0.27
Infectious bovine rhinotracheitis	Ox	31.97	2852	104	27.4	2.76	0.07
		47.88	969	82	11.8	1.71	0.07
		28.11	466	90	5.2	2.62	0.39
Infectious bursal disease	Chicken	41.56	1470	253	5.8	2.56	0.37
<i>Leptospira canicola</i>	Ox	5.38	2861	104	27.5	3.04	0.08
<i>Leptospira grippityphosa</i>	Ox	16.57	2861	104	27.5	3.91	0.11
<i>Leptospira hardjo</i>	Ox	38.55	2861	104	27.5	2.54	0.06
<i>Leptospira icterohaemorrhagiae</i>	Ox	13.60	2861	104	27.5	4.24	0.12
<i>Moniezia</i> spp.	Ox	3.07	1010	104	9.7	1.46	0.05
		15.90	1113	91	12.2	3.21	0.20
Newcastle disease	Chicken	37.89	1470	253	5.8	1.89	0.18
<i>Strongyloides</i> spp.	Ox	12.08	1010	104	9.7	2.35	0.16
		4.04	1113	91	12.2	2.27	0.11
Trichostrongyles	Ox	69.01	1010	104	9.7	1.70	0.08
		48.43	1113	91	12.2	2.22	0.10
<i>Trypanosoma brucei</i>	Ox	24.39	1111	91	12.2	2.39	0.12
<i>Trypanosoma congolense</i>	Ox	23.94	1111	91	12.2	2.51	0.13
<i>Trypanosoma vivax</i>	Ox	2.75	2909	104	28.0	2.56	0.06
		30.87	1111	91	12.2	2.68	0.15

* Disparities in average cluster size for the same survey result from not all samples being subjected to all laboratory examinations.

[†] Diagnosed by ELISA.[‡] Diagnosed by immunodiffusion.

Sampling using probability proportional to size Cluster sampling may be applied when the **primary sampling frame** is a list of places where herds or flocks are kept (e.g., villages), and the lists of herds or flocks in each place constitute **secondary sampling frames**.

However, it is often impossible to create, from the very first, a complete list of all herd or flocks. Thus, it would be more likely first to draw up a list of places where herds or flocks are known to be kept and then randomly select some of the places. Lists of herds or flocks

in each selected place would only then be compiled, and some herds then chosen at random.

A problem arises if some of the members of the primary sampling frame (e.g., villages) contain substantially more members (e.g., herds) than others because, if a fixed number of herds is selected from each village (a common sampling practice), then the probability of selection of a herd will be smaller in villages with many herds than in villages with few herds. This can be overcome by sampling with **probability proportional to size (PPS)**, in which sampling elements are aggregated or arranged into groups or units of different sizes (Czaja, 2005). For example (Table 13.5), if five herds were to be selected from each of three villages, first compile a primary sampling frame of all villages, labelled A–F. Then list the cumulative number of herds in all villages: 56 in this example. Then randomly select three numbers between one and 56 – say, 13, 49 and 51. Five herds are then randomly selected from the villages in which these three numbers fall: villages B, E and F.

It is important that the villages are not listed according to either increasing or decreasing herd numbers, but in a random fashion (listing by village name would often suffice).

The PPS method assumes that data are of high quality; for instance, that all villages in a region are known, and that the number of herds (or other appropriate epidemiological unit) in each village also is known.

Detecting the presence of disease

If an investigator only wishes to know whether or not a disease is present in a group of animals (rather than determining the prevalence), a suitable sample size can be selected using the formula:

$$n = \left\{ 1 - (1 - p_1)^{1/d} \right\} \{N - d/2\} + 1,$$

Table 13.5 Example of sampling using probability proportional to size.

Village	Number of herds	Cumulative number of herds	Random number
A	7	7	
B	8	15	13
C	10	25	
D	9	34	
E	15	49	49
F	7	56	51

where:

N = population size;

d = minimum number of affected animals expected in the population;

n = required sample size;

p_1 = probability of finding at least one case in the sample.

For example, during the *Pan-African Rinderpest Campaign*, at the stage of serological surveillance, it was necessary to sample herds to ascertain if unvaccinated animals had seroconverted (i.e., had been exposed to natural infection)⁷. It is unlikely that only a small number of animals would have seroconverted in infected herds, and it was considered reasonable to assume that at least 5% of animals in such herds would be seropositive; that is, a *minimum* seroprevalence could be postulated in affected herds⁸. Therefore, the sampling protocol was designed to detect a seroprevalence of at least 5%. Thus, if p_1 is set at 0.95, and if a herd containing 200 animals is to be sampled, then, substituting these values into the formula:

$$N = 200,$$

$$d = 10 \text{ (5\% of 200)},$$

$$p_1 = 0.95.$$

Therefore:

$$\begin{aligned} n &= \left\{ 1 - (1 - 0.95)^{1/10} \right\} \{200 - 10/2\} + 1 \\ &= (1 - 0.05^{1/10}) \times 195 + 1 \\ &= \{1 - 0.74113\} \times 195 + 1 \\ &= 0.2589 \times 195 + 1 \\ &= 50 + 1 \\ &= 51. \end{aligned}$$

Thus, if the seroprevalence is 5%, 51 animals need to be sampled to detect at least one seropositive animal with probability 0.95⁴. The probability of missing

⁷ Serological tests often are used to certify freedom from infection. This is because antibodies generally persist longer than clinical disease, and so are present at a higher prevalence than clinical cases, thereby necessitating a smaller sample size for detection.

⁸ Surveys of diseases that are not highly contagious are generally concerned with the maximum amount of disease that can be tolerated (lower levels being considered acceptable). Accordingly, the same formula can be used, but a *maximum* prevalence is specified.

⁴ If the population size is large, the calculation also can be performed using natural logarithms: $n = \left\{ \log_e(1 - p_1) \right\} / \log_e(1 - P)$, where P = expected minimum seroprevalence. Thus, if $p_1 = 0.95$, and $P = 0.05$ then $n = \log_e 0.05 / \log_e 0.95 = -2.9957 / -0.0513 = 59$ (Table 13.6).

infection is therefore $1 - 0.95 = 0.05$, and, colloquially, one is '95% confident' that the disease is absent if 51 animals are sampled and no seropositive animals are detected⁹. Note that p_1 is also the **aggregate sensitivity** (see Chapter 20), for a test with sensitivity and specificity = 100%, because it is also the proportion of affected herds that will be detected. Therefore, the formula above also can be used to calculate the sample size needed to attain a desired aggregate sensitivity. More complicated formulae are required when sensitivity and specificity $\neq 100\%$ (see 'Imperfect tests' later in this chapter).

Table 13.6 also can be used. This lists the sample sizes, with p_1 set at 0.95, for detecting at least one case of disease for various prevalence values and population sizes, offering a rapid means of determining sample size, rather than working the formula given above. For example, if the anticipated prevalence is 25%, and the population size is 120, then 10 animals need to be sampled to detect at least one case with probability 0.95.

Some protocols involve sampling in more than one stage. For example, following the foot-and-mouth disease epidemic in the UK in 2001, demonstration of freedom from the infection required that samples were taken from as many small-ruminant holdings within the perimeters of the 10 km surveillance zones surrounding previously infected premises to detect, serologically, with probability 0.95, at least one infected holding if the estimated **flock seroprevalence** of disease was 2% (European Commission decision 2001/295/EC). The holdings thus identified were then subjected to second-stage sampling to detect a minimum within-flock/herd prevalence (i.e., **animal seroprevalence**) of 5%, again with probability 0.95[†].

If a sample of size n has been selected from a population of size N and no cases have been detected, then the upper 100 ξ % confidence limit, u , to the number of cases that may be present can be estimated from:

$$u = \left\{ 1 - (1 - \xi)^{1/n} \right\} \{ N - n/2 \} + 1.$$

(ξ is the Greek letter 'xi'.)

For example, if $N = 400$, $n = 50$ and $\xi = 0.95$, then the upper 95% confidence limit, $u = 23$.

Again, Table 13.6 can be used for this estimation. For instance, if 25% of 1400 animals were sampled

and found to be disease-free, the upper 95% confidence limit of diseased animals is 11. Similar tables, constructed for 90% and 99% confidence limits, are listed in Cannon and Roe (1982).

The probability of detecting at least one positive animal, with various sampling fractions, is given in Appendix XII. The probability of *failing* to detect positive animals for various sample sizes and prevalence values is given in Appendix XIII.

This general approach to demonstrating freedom from infection is based on an assumed minimum prevalence of infection, if disease were present. Mintiens and others (2007) offer an approach allowing for a zero prevalence.

Sample optimization

Optimizing the number of herds and the number of animals selected in two-stage sampling to demonstrate freedom from disease is an important consideration. For example, in serosurveillance of sheep following outbreaks of foot-and-mouth disease, sampling aims to detect a flock prevalence of 2%, and a within-flock prevalence of 5%, and application of the previous formula would indicate that, in a 10 km zone containing 200 flocks, 105 flocks are required; and if a selected flock is of size 200 then 51 animals are needed. Note, however, that this approach will only detect evidence of infection in 95% of affected flocks. Thus, to guarantee detection of evidence of infection with probability 0.95 if the flock prevalence was 2%, *all* animals in the selected flocks essentially would need to be sampled (200 in this example). This clearly has resource implications.

An alternative approach involves balancing the number of herds or flocks to be sampled with the number of sampled animals in selected herds or flocks, to optimize resources, whilst also maintaining the required probability of detection of infection at a specified herd or flock prevalence. This requires an understanding of the concept of **detectable prevalence**. Thus, if the true prevalence of affected flocks was 2% and the probability of detecting them was set at 0.95, only 95% of seropositive flocks would be detected. In this example, the detectable prevalence would be $2\% \times 0.95$ (the product of the true prevalence and the probability of detection) = 1.9%.

In order to guarantee detection of the minimum flock prevalence, this loss of efficiency in detecting affected flocks needs to be addressed by increasing the number of flocks that are sampled. This is calculated by multiplying the number of flocks required (assuming 100% detection) – 105 in the example – by the true prevalence divided by the detectable prevalence. That is, 111 flocks (i.e., $105 \times [2/1.9]$ flocks) are

⁹ This expression may aid understanding, but should not be confused with confidence intervals, which indicate the range of values within which a population parameter lies (see Chapter 12).

[†] Sheep flocks that existed as hefts had each heft treated as a separate epidemiological unit, with the sampling procedure applied individually to each unit.

Table 13.6 (i) Sample size required for detecting disease where the probability of finding at least one case in the sample is 0.95; (ii) upper 95% confidence limits for number of cases. (From Cannon and Roe, 1982.)

Population size (N)	(i) Percentage of diseased animals in population (d/N)											
	OR											
	(ii) Percentage sampled and found clean (n/N)											
	50%	40%	30%	25%	20%	15%	10%	5%	2%	1%	0.5%	0.1%
10	4	5	6	7	8	10	10	10	10	10	10	10
20	4	6	7	9	10	12	16	19	20	20	20	20
30	4	6	8	9	11	14	19	26	30	30	30	30
40	5	6	8	10	12	15	21	31	40	40	40	40
50	5	6	8	10	12	16	22	35	48	50	50	50
60	5	6	8	10	12	16	23	38	55	60	60	60
70	5	6	8	10	13	17	24	40	62	70	70	70
80	5	6	8	10	13	17	24	42	68	79	80	80
90	5	6	8	10	13	17	25	43	73	87	90	90
100	5	6	9	10	13	17	25	45	78	96	100	100
120	5	6	9	10	13	18	26	47	86	111	120	120
140	5	6	9	11	13	18	26	48	92	124	139	140
160	5	6	9	11	13	18	27	49	97	136	157	160
180	5	6	9	11	13	18	27	50	101	146	174	180
200	5	6	9	11	13	18	27	51	105	155	190	200
250	5	6	9	11	14	18	27	53	112	175	228	250
300	5	6	9	11	14	18	28	54	117	189	260	300
350	5	6	9	11	14	18	28	54	121	201	287	350
400	5	6	9	11	14	19	28	55	124	211	311	400
450	5	6	9	11	14	19	28	55	127	218	331	450
500	5	6	9	11	14	19	28	56	129	225	349	500
600	5	6	9	11	14	19	28	56	132	235	379	597
700	5	6	9	11	14	19	28	57	134	243	402	691
800	5	6	9	11	14	19	28	57	136	249	421	782
900	5	6	9	11	14	19	28	57	137	254	437	868
1000	5	6	9	11	14	19	29	57	138	258	450	950
1200	5	6	9	11	14	19	29	57	140	264	471	1102
1400	5	6	9	11	14	19	29	58	141	269	487	1236
1600	5	6	9	11	14	19	29	58	142	272	499	1354
1800	5	6	9	11	14	19	29	58	143	275	509	1459
2000	5	6	9	11	14	19	29	58	143	277	517	1553
3000	5	6	9	11	14	19	29	58	145	284	542	1895
4000	5	6	9	11	14	19	29	58	146	268	556	2108
5000	5	6	9	11	14	19	29	59	147	290	564	2253
6000	5	6	9	11	14	19	29	59	147	291	569	2358
7000	5	6	9	11	14	19	29	59	147	292	573	2437
8000	5	6	9	11	14	19	29	59	147	293	576	2498
9000	5	6	9	11	14	19	29	59	148	294	579	2548
10 000	5	6	9	11	14	19	29	59	148	294	581	2588
∞	5	6	9	11	14	19	29	59	149	299	598	2995

The table gives:

- (i) the sample size (n) such that the probability (p_1) of including at least one positive if the disease is present at the specified level is 0.95;
- (ii) the upper 95% confidence limit (u) to the number of diseased animals in a population, given that the specified proportion was tested and found to be negative.

Examples:

- (i) expected proportion of positives is 2%; the population size is 480 – use 500; from the table, a sample of 129 is required to detect at least one positive with probability 0.95;
- (ii) for a population of 1000, a sample of 10% were all found to be negative; from the table, the upper 95% confidence limit for the number of positives is 29.

Table 13.7 Numbers of herds and animals to be tested for 0.95 probability of detecting 1% prevalence of positive herds with a within-herd prevalence of 5%. (Adapted from James, 1998.)

		Number of animals in herd				
Herd size	Infected*	Sampled				
10	1	10	10	10	8	6
20	1	20	19	19	16	11
30	1	30	29	28	23	16
40	2	40	31	28	20	12
50	2	50	39	34	25	15
75	3	75	47	40	28	16
100	5	100	45	37	24	13
200	10	200	51	41	26	14
500	20	500	56	43	27	14
Infinite	Infinite	–	59	45	28	14
Probability of detecting an infected herd if the specified number of animals are sampled		1.00	0.95	0.90	0.75	0.50
Detectable prevalence		1.00%	0.95%	0.90%	0.75%	0.50%
Number of herds to be sampled		299	315	332	398	598
Probability of detecting infection		0.95	0.95	0.95	0.95	0.95

* Prevalence is conservatively rounded down to the lowest integer if a non-integer value is generated for the number of affected animals at a prevalence of 5%. Thus, in a herd of size 30, 5% yields 1.5 affected animals, which is rounded down to 1.

required (with 51 animals selected from a flock of size 200, to detect a within-flock prevalence of 5% with the probability 0.95).

Consider the detection of rinderpest infection, where, during the eradication campaign, the *Office International des Epizooties* guidelines prescribed that samples of herds, and of animals within selected herds, should be taken such that the probability of detection of a herd prevalence of 1%, and within-herd seroprevalence of 5%, was set at 0.95. *Table 13.7* presents various values for the number of herds selected, the number of animals selected, the probability of detecting an affected herd if the specified number of animals is sampled, and the detectable prevalence.

To have certainty (probability = 1) of detecting infection in a herd of size 100, essentially all animals need to be tested; and 299 herds would need to be sampled from a large population to detect a herd prevalence of 1% with probability 0.95. This totals 29 900 samples.

To have a probability of detecting an infected herd of size 100 (within-herd prevalence of 5%) with probability of 0.95, 45 animals are required. The detectable prevalence is $1\% \times 0.95 = 0.95\%$. Thus, to maintain the probability of detecting infection in 1% of herds,

the number of herds to be sampled rises to 315 (i.e., $299 \times [1/0.95]$). This totals 14 175 samples.

Similarly, to have a probability of detecting an infected herd (within-herd prevalence of 5%) with probability of 0.90, 37 animals are required. The detectable prevalence is $1\% \times 0.90 = 0.90\%$. Thus, to maintain a probability of 0.95 of detecting infection in 1% of herds, the number of herds to be sampled rises to 332 (i.e., $299 \times [1/0.90]$).

Balancing the probability of detecting infection in a herd with the number of herds sampled can achieve a substantial reduction in the total number of sera sampled. Thus, to detect infection with probability 0.95 in a herd typically of size 500, 56 samples would be required. If the number of herds is large, 315 would be required to detect infection in 1% of herds with probability 0.95, and 17 640 samples (315×56) samples would be required. In contrast, if the probability of detection within a herd were reduced to 0.75, the total number of sera would only be 10 746 ($398 \text{ herds} \times 27 \text{ samples per herd}$).

In practice, a decision needs to be made concerning the cost of samples versus the cost of travelling to herds or flocks, and therefore the best balance between the number of herds or flocks sampled and the number of animals sampled per herd or flock.

Table 13.8 Sample size required for detecting disease, with probability, $p_1 = 0.95$, and threshold number of positives (in brackets), according to prevalence, and probability of incorrectly concluding that a healthy population is diseased [in square brackets]. Sensitivity = 98%; specificity = 98%.

Population size	Prevalence											
	10%			5%			2%			1%		
	[0.10]	[0.05]	[0.01]	[0.10]	[0.05]	[0.01]	[0.10]	[0.05]	[0.01]	[0.10]	[0.05]	[0.01]
30	26 (1)	30 (2)	*	*	*	*	*	*	*	*	*	*
50	39 (2)	39 (2)	45 (3)	48 (1)	*	*	*	*	*	*	*	*
100	45 (2)	55 (3)	64 (4)	85 (3)	*	*	*	*	*	*	*	*
150	48 (2)	58 (3)	78 (5)	105 (4)	119 (5)	*	*	*	*	*	*	*
200	49 (2)	60 (3)	80 (5)	115 (4)	131 (5)	173 (8)	*	*	*	*	*	*
300	50 (2)	62 (3)	83 (5)	121 (4)	155 (6)	202 (9)	*	*	*	*	*	*
500	51 (2)	63 (3)	85 (5)	144 (5)	162 (6)	229 (10)	469 (13)	*	*	*	*	*
1000	52 (2)	64 (3)	87 (5)	148 (5)	185 (7)	238 (10)	555 (15)	693 (20)	931 (29)	*	*	*
5000	52 (2)	65 (3)	88 (5)	151 (5)	190 (7)	263 (11)	640 (17)	788 (22)	1133 (34)	2100 (50)	2575 (63)	–
10 000	53 (2)	65 (3)	88 (5)	152 (5)	191 (7)	265 (11)	677 (18)	827 (23)	1178 (35)	2244 (53)	2841 (69)	–

*: Required accuracy cannot be achieved, even by sampling all in the population; –: too large a number to compute.

Imperfect tests

The formulae given assume that the diagnostic tests applied to samples have a sensitivity and specificity of 100%*. Thus, consideration is not given to the possibility that a seropositive animal detected in a sample is a false-positive (arising from a specificity less than 100%), in which circumstance the population actually may be disease-free. Similarly, a test-negative sampled animal may be a false-negative (arising from a sensitivity of less than 100%), in which circumstance disease may be missed. The consequences of such errors could be serious: in the first instance, a healthy herd or flock may be slaughtered, whereas, in the second situation, an infected population may remain free to infect others.

Sensitivity and specificity can be included in sample size calculations for detecting disease, facilitating a more informed interpretation of any positive results that occur in samples (Cameron and Baldock, 1998a), and can be applied to two-stage sampling protocols (Cameron and Baldock, 1998b). The formulae (which include sampling without replacement) are complex, but are incorporated in software (*Freecalc* in *Epi Tools*: see Appendix III).

* A further assumption is that animals are sampled 'with replacement', that is, are placed back into the herd or flock immediately after sampling, and may therefore be selected again during sampling. In practice, this is usually not the case: sampling 'without replacement' (also see Appendix X). However, the practical violation of this assumption is usually of little consequence (Hsia, 2005).

The input parameters for the calculation are:

- the minimum expected prevalence, if disease is present;
- the probability of detecting disease at the minimum prevalence, p_1 ;
- the probability of incorrectly concluding that disease is present, when it is absent, p_0 ;
- the population size.

The output includes two numbers:

- the number of animals to be sampled;
- an upper 'threshold number' of test-positive animals that can occur in the sample, whilst still inferring that disease is absent at the minimum specified prevalence (the assumption being that this number of test-positives comprises false-positives).

Table 13.8 lists the sample sizes and threshold numbers, with p_1 again set at 0.95, for various prevalence values, and values of p_0 , using a test with a sensitivity and specificity both equal to 98%. For instance, if the minimum prevalence is assumed to be 5%, then 121 animals need to be sampled from a population of 300, if p_0 is set at 0.10. Moreover, up to four of these animals can test positive, while still being able to conclude, with '95% confidence', that disease is absent from the population at the specified minimum prevalence. Again, there is a probability, $1 - p_1$ (i.e., 0.05) of missing disease. This is the probability of observing four or fewer test-positive animals in a sample of

121 animals drawn from a population of 300 animals in which the prevalence is 5%, and, again, p_1 is also the aggregate sensitivity.

The interpretation of p_0 is most easily understood in the context of the number of test positives that are detected in the sample, and the consequent value of p_1 . First, the threshold number may be exceeded. For example, five test positives may be identified in the sample of 121 animals. Using the appropriate formula (Cameron and Baldock, 1998a), the value of p_1 is calculated to be 0.88, in which circumstance the probability of concluding that the population is not diseased when it actually is $(1-p_1)$ rises to 0.12: one is only '88% confident' that disease is absent at a minimum prevalence of 5%. The value of p_0 is calculated to be 0.10; this is the probability of observing five reactors or more in a sample of 121 animals drawn from a *disease-free* population. (In setting the value of p_0 at 0.10 in the sample size calculation, it is this value that is achieved if the threshold number is exceeded by one.) Thus, one can be '90% confident' that the population is diseased. Moreover, because p_0 is the probability of incorrectly classifying a healthy population as being diseased, $1-p_0$ (0.90) is the probability of correctly classifying a healthy population being healthy; that is, the **aggregate specificity** (see Chapter 20).

Secondly, fewer reactors than the threshold number may be identified. Thus, if only three reactors were detected in the sample of 121 animals, p_1 and p_0 are computed to be 0.98 and 0.44, respectively. Therefore, it is now possible to conclude that disease is absent, at a prevalence of 5%, with an increased probability of 0.98 ('98% confidence'), and there is now only '56% confidence' that disease is present. (A high value of p_1 and a low value of p_0 would indicate that the population was not free from disease, but that the prevalence was less than the expected level.)

The value of p_1 that is used in sample size determination dictates the aggregate sensitivity and level of confidence attached to declaring freedom from disease (a high value of p_1 specifies a high degree of confidence), whereas the value of p_0 decides aggregate specificity and level of confidence attached to identifying a diseased population (a low value of p_0 specifies a high degree of confidence). The selected values are governed by the goal of the survey. If a herd or flock is to be certified free from disease as part of an eradication campaign, then p_1 should be set quite high (conventionally at 0.95). However, the value of p_0 could be lower, because the consequences of, say, quarantining a false-positive herd or flock are not severe. High values of p_1 linked to low values of p_0 demand large sample sizes. This is indicated in *Table 13.8* and Appendix XIV, which tabulates sample sizes and

threshold numbers for various values of p_1 , p_0 , population size and prevalence. Accordingly, a compromise between p_1 and p_0 is usually sought.

If the population is large, and specificity is assumed to be 100% (i.e., there are no false-positive results), the sample size, n , can be determined thus:

$$n = \frac{\log_e(1-p_1)}{\log_e(1-P \times Se)},$$

where

P = the minimum expected prevalence,

Se = sensitivity,

p_1 = probability of detecting disease at the minimum expected prevalence.

Thus, if $P = 0.05$,

$p_1 = 0.95$,

$Se = 0.95$,

$$\begin{aligned} n &= \frac{\log_e 0.05}{\log_e(1-0.05 \times 0.95)} \\ &= \frac{\log_e 0.05}{\log_e 0.9525} \\ &= -2.9957 / -0.0487 \\ &= 62. \end{aligned}$$

Thus, 62 animals need to be sampled to detect at least one positive animal with probability 0.95. Note that if the test was perfect (i.e., sensitivity also was 100%) then only 59 animals would be required (see *Table 13.6*).

Sample-size calculation for aggregate-level prevalence surveys

The method for determining sample size for detecting the presence of disease using imperfect tests that has just been described is also of relevance to sample-size calculations for prevalence surveys when herds, flocks or other aggregates of animals are the sampling units.

The sample-size calculation, accommodating imperfect tests, is a two-stage procedure, involving determination of the number of aggregates to be sampled, and the number of animals to be sampled in each aggregate.

First, the number of herds or flocks to be sampled is determined using the formula for determining sample size for estimation of prevalence, using an imperfect test, but the sensitivity and specificity are now the aggregate sensitivity (Se_{agg}) and aggregate specificity (Sp_{agg}), which must be *pre-specified* by the investigator:

$$n = \left(\frac{1.96}{d}\right)^2 \times \frac{[(Se_{agg} \times P_{exp}) + (1 - Sp_{agg})(1 - P_{exp})][(1 - Se_{agg} \times P_{exp}) - (1 - Sp_{agg})(1 - P_{exp})]}{(Se_{agg} + Sp_{agg} - 1)^2},$$

where:

n = required number of herds or flocks to be sampled,
 P_{exp} = expected herd or flock prevalence,
 d = desired absolute precision.

For example, if an expected herd or flock prevalence of 20% is to be estimated with a desired absolute precision of $\pm 5\%$, and aggregate sensitivity and aggregate specificity have the pre-specified values, 95% and 90%, respectively, then:

$$P_{\text{exp}} = 0.20,$$

$$d = 0.05,$$

$$Se_{\text{agg}} = 0.95,$$

$$Sp_{\text{agg}} = 0.90,$$

and:

$$n = \left(\frac{1.96}{0.05} \right)^2 \times \frac{[(0.95 \times 0.20) + (1 - 0.90)(1 - 0.20)][(1 - 0.95 \times 0.20) - (1 - 0.90)(1 - 0.20)]}{(0.95 + 0.90 - 1)^2}$$

$$= 419 \text{ herds}^{10}.$$

Secondly, the number of animals in each herd or flock that is required to achieve the pre-specified values of aggregate sensitivity and aggregate specificity needs to be calculated. The value of Se_{agg} is determined by the individual-animal sensitivity, the number of animals sampled in the aggregate, and disease prevalence within the aggregate; whereas the value of Sp_{agg} is dictated by the individual-animal specificity and the number of animals sampled in the aggregate (see Chapter 20). It is therefore necessary to postulate the minimum prevalence of disease likely to obtain in an aggregate, as well as having estimates of individual-animal sensitivity and specificity.

For example, if individual-animal sensitivity and specificity are each estimated as being 98%, the minimum within-herd prevalence is 10%, and Se_{agg} and Sp_{agg} are pre-specified as 95% and 90%, respectively, then Table 13.8 can be used to determine the number of animals that need to be sampled. The table lists sample sizes for $Se_{\text{agg}} = 0.95$ (p_1 set at 0.95), and various values of Sp_{agg} ($1 - p_0$, where values of p_0 are given in square brackets). Thus, to determine how many animals need to be sampled from a herd of 200 animals, the column headed [0.10], representing $Sp_{\text{agg}}(1 - p_0) = 0.90$ (i.e., $p_0 = 0.10$) is scrutinized, and 49 animals are required; if the threshold number (2) is not exceeded, the herd

is interpreted as diseased, and if the threshold number is not exceeded, the herd is interpreted as unaffected.

Appendix XIV (or the *Freecalc* software) is used for other values of sensitivity, specificity, p_1 and p_0 .

In pre-specifying Se_{agg} and Sp_{agg} it should be noted that the larger the values, the smaller the number of herds or flocks that are required. However, scrutiny of Table 13.8 and Appendix XIV reveals that large values of Se_{agg} and Sp_{agg} require more animals to be sampled from each aggregate, and there may be insufficient animals in small herds to achieve such values. Thus, a trade-off may be required between the number of animals within each aggregate (i.e., Se_{agg} and Sp_{agg}) and the number of aggregates that need to be selected. However, high levels of precision and confidence for aggregate-level prevalence require large numbers of herds, particularly when individual-animal sensitivity and specificity are poor (Table 13.9)¹¹.

The design of surveys based on imperfect tests is discussed further by Audigé and others (2001), Cannon (2001), Humphry and others (2004), Huzurbazar and others (2004) and Wagner and Salman (2004).

The cost of surveys

Sampling the study population incurs a cost. For example, sampling of cows to determine the prevalence of mastitis by bacteriological examination of milk specimens involves a laboratory cost. The most economic sample size can be determined with defined precision. Alternatively, if a fixed amount of money is available, then the sample size can be determined to maximize precision. Levy and Lemeshow (2008) and Scheaffer and others (2012) describe techniques for estimating sample size for simple random, stratified, systematic and cluster sampling that include cost functions in the estimations. An example of estimation of optimum sample size for a fixed cost, relating to a national British bovine mastitis survey, is given by Wilson and others (1983).

Calculation of confidence intervals

There may be occasions when sample size is predetermined (e.g., by the availability of animals). Moreover, even when an appropriate sample size has been selected before a survey is undertaken, it is unlikely that the prevalence will be exactly what is anticipated, and that the specified number of animals will be sampled. Thus, in all circumstances, a confidence interval should be calculated from the sample that is available.

¹⁰ Again, this should be adjusted (n_{adj}) if the population of herds or flocks from which the sample is drawn is not large.

¹¹ For example, tests for Johne's disease (paratuberculosis) have been reported to have an individual-animal sensitivity as low as 35% (Whitlock *et al.*, 2000).

Table 13.9 The approximate number of herds required to estimate an aggregate-level (herd) prevalence of 10% in a large population with the desired fixed-width confidence limits for various values of aggregate sensitivity and specificity. (Adapted from Humphry, *et al.*, 2004. Reproduced with permission from Elsevier.)

Desired absolute precision (%)	Level of confidence (%)	Aggregate specificity (%)	Aggregate sensitivity (%)			
			55	70	85	90
5	95	55	38 169	6 131	2 400	1 897
5	95	70	5 394	2 156	1 164	984
5	95	85	1 479	828	539	477
5	95	90	941	574	395	355
5	90	55	26 883	4 319	1 691	1 336
5	90	70	3 799	1 518	820	693
5	90	85	1 041	584	379	336
5	90	90	663	405	278	250
7.5	95	55	16 964	2 725	1 067	844
7.5	95	70	2 398	958	517	438
7.5	95	85	657	368	240	212
7.5	95	90	419	255	176	158
7.5	90	55	11 948	1 920	752	594
7.5	90	70	1 689	675	365	308
7.5	90	85	463	260	169	150
7.5	90	90	295	180	124	111

Simple random sampling

A 95% confidence interval can be calculated from a simple random sample for a given sample size, n , and estimated prevalence, $\hat{P}$, using Wald's method for calculation of a confidence interval for a proportion, based on the Normal approximation to the binomial distribution, introduced in Chapter 12, and expressed in this chapter in terms of $\hat{P}$:

$$\hat{P} - 1.96\sqrt{\frac{\hat{P}(1-\hat{P})}{n}}, \quad \hat{P} + 1.96\sqrt{\frac{\hat{P}(1-\hat{P})}{n}}.$$

For example, if 200 animals were sampled, and 80 were found to be diseased, then the estimated prevalence would be 40%, and:

$$\begin{aligned}\hat{P} &= 0.4, \\ n &= 200.\end{aligned}$$

Thus, the 95% confidence interval is:

$$\begin{aligned}&0.40 - 1.96\sqrt{\frac{0.40(1-0.40)}{200}}, \\ &0.40 + 1.96\sqrt{\frac{0.40(1-0.40)}{200}} \\ &= 0.40 - 0.068, 0.40 + 0.068 \\ &= 0.332, 0.468;\end{aligned}$$

that is, 33.2%, 46.8%.

If other confidence intervals are required, then 1.96 is replaced by the appropriate multiplier (see Appendix VI).

This formula assumes that the population from which the sample is drawn is large, and therefore that the sampling fraction, f , is small, which is usually the case. If, however, the sampling fraction is large (greater than 10%), then the numerator, $\hat{P}(1-\hat{P})$ needs to be multiplied by $(1-f)$. Thus, if the sample of 200 animals were drawn from a population of 1000 animals, $f = 0.2$, $(1-f) = 0.8$, and the confidence interval is:

$$\begin{aligned}&0.40 - 1.96\sqrt{\frac{0.80 \times 0.40(1-0.40)}{200}}, \\ &0.40 + 1.96\sqrt{\frac{0.80 \times 0.40(1-0.40)}{200}} \\ &= 0.40 - 0.061, 0.40 + 0.061 \\ &= 0.339, 0.461;\end{aligned}$$

that is, 33.9%, 46.1% (slightly narrower – i.e., more precise – than if the large-population formula had been applied).

The formula also assumes that:

$$\begin{aligned}P &\geq 0.05, \text{ and } \leq 0.95, \\ nP \text{ and } n(1-P) &\geq 5,\end{aligned}$$

where n = sample size, and P = prevalence in the study population.

In the example just given, P is replaced by its estimate from the sample, $\hat{P}$:

$$\begin{aligned}\hat{P} &= 0.40, \\ n\hat{P} &= 200 \times 0.4 \\ &= 80; \\ n(1 - \hat{P}) &= 200(1 - 0.4) \\ &= 120;\end{aligned}$$

and so the formula may be applied.

If $P < 0.05$ or (most unlikely) > 0.95 , appropriate methods are outlined in Chapter 12.

Small sample size If only a small-sized sample is available, then nP and $n(1 - P)$ may be less than five. It is then necessary to use alternative methods (see Chapter 12). The intervals also can be obtained conveniently by consulting Appendix VII.

Diseases of low prevalence Some diseases (e.g., tumours) may be rare. If the Normal approximation to the binomial distribution is applied, then a very large sample would be required to estimate a confidence interval accurately. Moreover, Appendix VII can only be used for prevalence values greater than 0.2%. Therefore, if the estimated prevalence is lower than this, an alternative method, utilizing the Poisson distribution (see Chapter 12) should be used. For example, a random sample of 2000 dogs may yield two cases of osteosarcoma. The point estimate of the prevalence per 100 000 animals is therefore $2/2000 \times 100\,000 = 100$ cases per 100 000 dogs. To construct a 95% confidence interval, consult columns 4 and 5 of Appendix VIII. The lower limit for two cases ($x = 2$) is derived from the value of $x_L = 0.242$, thus: $0.242/2000 \times 100\,000 = 12$ cases per 100 000 animals. The upper limit is derived from the value of $x_U = 7.225$, thus: $7.225/2000 \times 100\,000 = 361$ cases per 100 000 animals.

Imperfect tests If prevalence is being estimated with a diagnostic test whose sensitivity and specificity are known, then a corrected estimate of the true prevalence, P , can be made by:

$$P = \frac{P^T + \text{specificity} - 1}{\text{sensitivity} + \text{specificity} - 1},$$

where P^T is the test prevalence (Rogan and Gladen, 1978). (The value of 1 is replaced by 100 if the parameters are quoted as percentages.)

For example, if $P^T = 0.20$, sensitivity (Se) = 0.90 (90%), and specificity (Sp) = 0.95 (95%), then:

$$\begin{aligned}P &= \frac{0.20 + 0.95 - 1}{0.90 + 0.95 - 1} \\ &= 0.1765.\end{aligned}$$

An approximate 95% confidence interval for the true prevalence, based on Wald's method, can be calculated thus (Rogan and Gladen, 1978):

$$\hat{P} - 1.96\sqrt{\text{var } \hat{P}}, \hat{P} + 1.96\sqrt{\text{var } \hat{P}}$$

where:

$\text{var } \hat{P}$ = the variance of the true prevalence

$$= \frac{\hat{P}^T (1 - \hat{P}^T)}{n(Se + Sp - 1)^2},$$

where n = sample size.

Thus, if the test prevalence, above, was estimated from a sample of 400 animals, $n = 400$, and:

$$\begin{aligned}\text{var } \hat{P} &= \frac{0.20(1 - 0.20)}{400 \times (0.90 + 0.95 - 1)^2} \\ &= \frac{0.20 \times 0.80}{400 \times 0.85^2} \\ &= 0.000\,553\,63.\end{aligned}$$

Thus, the approximate 95% confidence interval for the true prevalence is:

$$\begin{aligned}&0.1765 - 1.96\sqrt{0.000\,553\,63}, 0.1765 + 1.96\sqrt{0.000\,553\,63} \\ &= 0.1765 - 0.0461, 0.1765 + 0.0461 \\ &= 0.1304, 0.2226;\end{aligned}$$

that is, 13.0%, 22.3%.

If other confidence intervals are required, then 1.96 is replaced by the appropriate multiplier (see Appendix VI).

If the sampling fraction, f , is large (greater than 10%), then the numerator of the variance formula, $\hat{P}^T (1 - \hat{P}^T)$ again should be multiplied by $(1 - f)$.

This formula also can be applied when herds or flocks (rather than individual animals) are the sampling units, in which circumstance sensitivity and specificity are replaced by aggregate sensitivity and aggregate specificity (see Chapter 20 and 'Detecting the presence of disease: Imperfect tests' earlier in this chapter).

Alternative methods for constructing approximate and exact confidence intervals are described by Lang and Reiczig (2014) and Reiczig and others (2010), respectively.

Systematic sampling

Confidence intervals for prevalence can be calculated for systematic samples using the formulae applied to simple random samples, assuming that there is no periodicity in the samples. More complex formulae should be applied if the latter may be present (Levy and Lemeshow, 2008).

Stratified sampling

The simple random sample formulae also are satisfactory for proportionally-allocated stratified samples. However, if other methods of allocation are undertaken, more complex formulae are required (Levy and Lemeshow, 2008).

Cluster sampling

Confidence intervals for cluster samples need to be calculated differently from those for simple random samples, to take account of the variability that is likely to exist between the groups that constitute the clusters.

The data in *Table 13.3* will again be used to illustrate how confidence intervals are calculated. The sample estimate, $\hat{P}$, has already been calculated in the preceding section on sample-size determination for cluster samples, and was found to be 0.157.

An approximate 95% confidence interval may be calculated using the formula¹²:

$$\hat{P} - 1.96 \left\{ \frac{c}{T} \sqrt{\frac{V}{c(c-1)}} \right\}, \hat{P} + 1.96 \left\{ \frac{c}{T} \sqrt{\frac{V}{c(c-1)}} \right\},$$

where:

c = number of clusters in the sample;

T = total number of animals in the sample;

and:

$$V = \hat{P}^2 (\Sigma n^2) - 2\hat{P}(\Sigma nm) + (\Sigma m^2),$$

where:

n = number of animals sampled in each cluster;

m = number of diseased animals sampled in each cluster.

Now, substitute the values $c = 14$, $T = 3032$ and $V = 6409$ (derived in the preceding section on

sample-size determination for cluster samples) into the formula:

$$\begin{aligned} & 0.157 - 1.96 \left\{ \frac{14}{3032} \sqrt{\frac{6409}{14(14-1)}} \right\}, \\ & 0.157 + 1.96 \left\{ \frac{14}{3032} \sqrt{\frac{6409}{14(14-1)}} \right\} \\ & = 0.157 - (1.96 \times 0.0046 \times \sqrt{35.214}), \\ & 0.157 + (1.96 \times 0.0046 \times \sqrt{35.214}) \\ & = (0.157 - 0.0535), (0.157 + 0.0535) \\ & = 0.1035, 0.2105; \end{aligned}$$

that is, 10.35%, 21.05%.

Again, if other confidence intervals are required, the appropriate multipliers should be used (see Appendix VI).

Note that the value for the confidence interval is much wider than that which would have been obtained if the formula for simple random sampling had been inappropriately used (14.4%, 17.0% for a 95% confidence interval), illustrating that, for a given sample size, cluster samples usually produce less precise estimates than simple random samples¹³.

This formula can be used for both one-stage and two-stage cluster samples. In the former, n is all animals in each cluster, whereas, in the latter, n is a sample of animals in each cluster.

Calculations are facilitated by the use of appropriate software (e.g., *EPI INFO*, *WinPepi* and *R*: see Appendix III).

A simple rule stating when this Normal approximation method for calculating confidence intervals may be applied does not exist. This is because the distribution of a cluster sample proportion is complex due to the presence of the two variance components (between clusters and between animals within clusters)¹⁴. An indication of the validity of the method can be obtained by plotting the frequency distribution

¹³ This is because the variance of the prevalence (i.e., the square of the standard error of a proportion: see Chapter 12) derived by cluster sampling, when there is variability between clusters, is larger than the variance derived when the data are treated as a simple random sample (see the earlier discussion of the design effect).

¹⁴ This means that it is unclear when the **central limit theorem** holds. This theorem states that, as more observations are included, eventually the mean of any distribution will tend towards a Normal distribution, providing its variance is finite. (See also Chapter 12: 'Normal approximations to the binomial and Poisson distributions'.) The central limit theorem should hold before Normal approximation methods are used with complete trust. In cluster sampling, the larger the number of clusters and the number of animals, the more likely that the theorem will hold.

¹² Again, this formula assumes that the fraction of clusters sampled (f) is small (less than 10%). If the fraction is not small, the value $V/\{c(c-1)\}$ needs to be multiplied by $(1-f)$. In this example, $f = 14/865 = 0.016$; thus $(1-f)$ is small and so can be omitted from the formula without affecting the result materially.

of the individual cluster prevalence values. If this has a smooth, symmetrical distribution, the Normal-approximation method is likely to be valid. Otherwise, the calculated confidence intervals may only be approximate, particularly if a small number of clusters has been sampled. Thus, a plot of the individual cluster prevalence values in *Table 13.3* does not show a smooth distribution, and so the confidence intervals should be

regarded as approximate. In such circumstances, an alternative approach to presenting precision would be to simply state the standard error of the cluster sample's overall prevalence (i.e., the value derived in the worked example before being multiplied by the multiplier 1.96: 0.0273).

Samples with three or more stages require more complicated methods (Levy and Lemeshow, 2008).

Further reading

- Abramson, J.H. and Abramson, Z.H. (2008) *Research Methods in Community Medicine*, 6th edn. John Wiley and Sons, Chichester
- Audigé, L., Doherr, M.G. and Salman, M. (1999) A quantitative approach in declaring a country free of a disease. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Bristol, 24–26 March 1999. Eds Goodall, E.A. and Thrusfield, M.V., pp. 78–87.
- Barnett, V. (2002) *Sample Survey Principles and Methods*, 3rd edn. Arnold, London
- Bennett, S., Woods, T., Liyanage, W.M. and Smith, D.L. (1991) A simplified general method for cluster sampling surveys of health in developing countries. *World Health Statistics Quarterly*, **44**, 98–106
- Cameron, A. (2004) *Principles for the Design and Conduct of Surveys to Show Presence or Absence of Infectious Disease in Aquatic Animals*. National Aquatic Animal Health Technical Working Group – Policy Document. AusVet Animal Health Services, Wentworth Falls
- Casley, J.D. and Lury, D.A. (1981) *Data Collection in Developing Countries*. Clarendon Press, Oxford
- Cochran, W.R. (1977) *Sampling Techniques*, 3rd edn. John Wiley, New York
- Condon, J., Kelly, G., Bradshaw, B. and Leonard, N. (2004) Estimation of infection prevalence from correlated binomial samples. *Preventive Veterinary Medicine*, **64**, 1–14
- Donner, A. and Klar, N. (2000) *Design and Analysis of Cluster Randomization Trials in Health Research*. Arnold, London
- Dufour, B., Pouillot, R. and Toma, B. (2001) Proposed criteria to determine whether a territory is free of a given animal disease. *Veterinary Research*, **32**, 545–563
- Farver, T.B. (1987) Disease prevalence estimation in animal populations using two-stage sample designs. *Preventive Veterinary Medicine*, **5**, 1–20
- Farver, T.B., Holt, D., Lehenbauer, T. and Greenley, W.M. (1997) Application of composite estimation in studies of animal population production with two-stage repeated sample designs. *Preventive Veterinary Medicine*, **30**, 109–119
- Greiner, M. and Dekker, A. (2005) On the surveillance for animal diseases in small herds. *Preventive Veterinary Medicine*, **70**, 223–234. (*A discussion of disease detection in small herds*)
- Hadorn, D.C., Rüfenacht, J., Hauser, R. and Stärk, K. (2002) Risk-based design of repeated surveys for the documentation of freedom from non-highly contagious diseases. *Preventive Veterinary Medicine*, **56**, 179–192
- Humphry, R.W., Cameron, A. and Gunn, G.J. (2004) A practical approach to calculate sample size for herd prevalence surveys. *Preventive Veterinary Medicine*, **65**, 173–188
- Kish, L. (1995) *Survey Sampling*. John Wiley, New York
- Krótki, K.P. (2005) Sampling in developing countries. In: *Encyclopedia of Biostatistics*, Vol. 7, 2nd edn. Eds Armitage, P. and Colton, T., pp. 4758–4763. John Wiley and Sons, Chichester
- Lavrakas, P.J. (2009) Methods for sampling and interviewing in telephone surveys. In: *The SAGE Handbook of Applied Social Research Methods*, 2nd edn. Eds Bickman, L. and Rog, D.J., pp. 509–542. Sage Publications, Thousand Oaks
- Leech, F.B. and Sellers, K.C. (1979) *Statistical Epidemiology in Veterinary Science*. Charles Griffin, London and High Wycombe
- Levy, P.S. and Lemeshow, S. (2008) *Sampling of Populations: Methods and Applications*, 4th edn. John Wiley, New York
- Lohr, S.L. (2010) *Sampling: Design and Analysis*. Brooks/Cole, Boston
- Lwanga, S.K. and Lemeshow, S. (1991) *Sample Size Determination in Health Studies: A Practical Manual*. World Health Organization, Geneva
- Martin, P.J., Cameron, A.R. and Greiner, M. (2007) Demonstrating freedom from disease using multiple complex data sources: 1. A new methodology based on scenario trees. *Preventive Veterinary Medicine*, **79**, 71–97
- Miller, E., Huppert, A., Novikov, I., Warburg, A., Hailu, A. *et al.* (2015) Estimation of infection prevalence and

- sensitivity in a stratified two-stage sampling design employing highly specific diagnostic tests when there is no gold standard. *Statistics in Medicine*, **34**, 3349–3361
- Moser, C.A. and Kalton, G. (1971) *Survey Methods in Social Investigation*, 2nd edn. Heinemann Educational Books, London
- Naing, L., Winn, T. and Rusli, B.N. (2006) Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Sciences*, **1**, 9–14
- Salman, M.D. (Ed.) (2003) *Animal Disease Surveillance and Survey Systems: Methods and Applications*. Iowa State Press, Ames
- Sergeant, E. and Toribio, J.-A. (2004) *Estimation of Animal-Level Prevalence from Testing of Pooled Samples*. AusVet Animal Health Services, South Brisbane
- Slater, M.R. (1996) Methods and issues in conducting breed-specific canine health surveys. *Preventive Veterinary Medicine*, **28**, 69–79
- Thompson, S.K. (2012) *Sampling*, 3rd edn. John Wiley, Hoboken
- Tryfos, P. (1996) *Sampling Methods for Applied Research*. John Wiley, New York
- Wagner, B. and Salman, M.D. (2004) Strategies for two-stage sampling designs for estimating herd-level prevalence. *Preventive Veterinary Medicine*, **66**, 1–17
- Woods, A.J. (1985) Sampling in animal health surveys. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Reading, 27–29 March 1985. Ed. Thrusfield, M.V., pp. 36–54
- Yates, F. (1981) *Sampling Methods for Censuses and Surveys*, 4th edn. Charles Griffin, London and High Wycombe
- Ziller, M., Selhorst, T., Teuffert, J., Kramer, M. and Schlüter, H. (2001) Analysis of sampling strategies to substantiate freedom from disease in large areas. *Preventive Veterinary Medicine*, **52**, 333–343

14

Demonstrating association

A valuable step in the identification of the cause of a disease is the detection of a **statistically significant** association between the disease and hypothesized causal factors; this is the basis of the first three of Evans' rules (see Chapter 3).

Some basic principles

Demonstration of association can be approached in three ways.

1. The difference, under two different circumstances, between the mean of the probability distribution of a set of values of a variable can be measured. If there is a significant difference between the means in the two circumstances, the different circumstances may lead to an explanation that reflects a causal association. For example, the weights of two groups of piglets, one group of which has developed neonatal diarrhoea and one group of which has not, can be measured. The effect of diarrhoea on weight can then be assessed by analysing the difference between the mean weights of the two groups. A similar approach can be adopted when comparing medians.
2. Variables can be categorized, and a significant association sought between various categories. Thus, bitches can be categorized according to whether or not they have physiological urinary incontinence and whether or not they are neutered. Evidence of an association between the syndrome and neutering can then be sought.
3. A correlation between variables can be sought. For instance, the incidence of lameness in cattle and the amount of rainfall can be recorded to investigate whether increased rainfall is significantly associated with an increased incidence of lameness.

Although approaches 1 and 2 are introduced in this chapter in the context of causal studies, they can be applied in any circumstance in which two groups are being **compared**.

Many of the statistical techniques that adopt these three approaches were developed for use in agricultural science. Their use is confined to epidemiological investigations in this book, but they also have a wide application to experimental and observational biological and social sciences, and therefore are described in most general statistics textbooks.

The principle of a significance test

The bell shape of the Normal distribution (see *Figure 12.1*) reveals that there is a probability, albeit a small one, of an observation occurring at the extreme tails of the distribution. This distribution may be used to describe the frequency distribution not only of the values of a continuous variable that has a Normal distribution but also of the means of repeated samples taken from that population (here termed the 'reference population'). This also includes means of parameters of other distributions, such as the binomial, when Normal approximations are applied. There is, therefore, a high probability of the mean of a sample being under the peak, and a much lower probability of this mean being close to either of the two tails. If the mean is close to a tail, this indicates either that the sample is one of those improbable samples taken from the reference population or that it has been drawn from a population with a different population mean.

It is necessary to decide when it is improbable that a sample mean has come from the reference population. This decision is taken when the probability, P , of obtaining a value for the sample mean at least as extreme as the one observed, *assuming that the sample is drawn from the reference population*, is less than

a value corresponding to the **level of significance**. This level is represented by a probability called α ('alpha'). Conventionally, in biological sciences, α is taken to be 0.05, representing the 5% significance level. In the event of $P < 0.05$, the result is reported as 'significant $P < 0.05$ ' and supports the claim that the sample was not drawn from the reference population. The 5% significance level is purely conventional. If more caution in inferring a difference were necessary, the 1% level ($P < 0.01$) or 0.1% level ($P < 0.001$) could be chosen. Some reporting procedures use *, **, and *** to record significance at these 'critical' levels of 5%, 1% and 0.1%, respectively. This decision-making procedure is the principle of a significance test¹.

Significance tests originally were conducted in conjunction with tables that presented only a limited number of significance levels, including the critical ones. However, most statistical software packages (see Appendix III) calculate exact P values, and these should always be quoted in preference to the relevant critical levels.

The null hypothesis

In the previous discussion, a statistical test was undertaken on the basis that the sample came from a population with a mean no different from that of the reference population. This constitutes the **null hypothesis**; the null hypothesis thus is one of *no difference*. A significant result indicates that the null hypothesis is rejected in favour of an alternative one, which states that the sample has been drawn from a population with characteristics that are different from the reference population. Demonstration of a significant difference implies rejection of the null hypothesis.

Notice that **confidence intervals** (see Chapter 12) and the outcomes of significance tests are closely related. For example, suppose that the null hypothesis states a particular value for the mean of a Normal distribution. A sample is taken and the significance test rejects the null hypothesis at the 5% level. The corresponding 95% confidence interval (sample mean $\pm$ two e.s.e.m.) then will *not* contain the value of the mean specified by the null hypothesis. Conversely, if

the significance test does not reject the null hypothesis at the 5% level, the corresponding 95% confidence interval will contain the value of the mean specified by the null hypothesis.

Errors of inference

Five per cent of samples from the reference population lie within the region that would lead to rejection of the null hypothesis at the 5% level. If this happens, it constitutes a rejection of the null hypothesis when the hypothesis is true. This error is an example of a **Type I error**: false rejection of a true null hypothesis. The probability of a Type I error is similar to, but distinct from, the level of significance discussed above².

A **Type II error** is a failure to reject the null hypothesis when it is untrue. The probability of committing this error is called β ('beta'). Ideally, both α and β should be known by the investigator before the study begins.

Depending on the alternative hypothesis, β may or may not be determinable. In the previous discussion, if the alternative hypothesis specified the mean of the population from which the sample was drawn, then β could be determined for a stated value of α and sample size. However, if the alternative hypothesis only hypothesized that the mean of the population from which the sample was drawn took one of several values, then β cannot be calculated. It is known that β can be kept small by increasing either α or the sample size. The probabilities of Type I and II errors decrease as sample size increases. For a fixed sample size, the larger the probability of a Type I error is chosen to

¹ Note, however, that scientific conclusions and policy decisions should not be based only on whether a P value passes a specific threshold: many other contextual factors, such as study design, validity of assumptions underlying data analysis, quality of measurements, and external evidence for the phenomenon under study, also should be considered (Wasserstein and Lazar, 2016).

² The difference between the probability of a Type I error and a P value (Goodman, 1993) is not usually addressed in introductory texts. Moreover, the statistical approach described here, which focusses on error rates and is termed 'frequentist statistics', attempts to draw a conclusion about the truth of a hypothesis based on the results of a single study. Another school of statistical thinking, 'Bayesian statistics', uses probability distributions as a mechanism to describe uncertainty about the real world. The approach takes a prior belief about parameters of interest, based on previous studies, and updates it with evidence from a subsequent study (Louis, 1991). Bayesian methods are introduced in Chapter 20 in the context of predictive values and likelihood ratios. For a detailed discussion of these issues, including statistical inference in the context of inductive and deductive reasoning, see Hacking (1975), Holland (1986), Casella and Berger (1987), Louis (1991), Goodman (1999a,b) and Pearl (2009b). Huberty and Pike (1999), Mulaik and others (1997) and Hubbard and Ryan (2000) trace the evolution of significance testing and the theory that underpins it.

be, the smaller the probability of a Type II error will be, and vice versa.

Two remaining alternative decisions are possible. These represent correct inferences, rather than errors. The first inference is not rejecting the null hypothesis when it is true. The second is rejecting the null hypothesis when it is false (i.e., demonstrating a significant difference). The probability of the latter is called the 'power' of a test; it is denoted by $1 - \beta$.

Most tests are calibrated by prescribing the significance level, α , and the power, $1 - \beta$.

Multiple significance testing

Veterinary research commonly involves the running of several significance tests. Scenarios include:

- the testing of multiple hypotheses (e.g., the relationship between breed, sex, age, herd size, vaccination status, farm density, insemination methods and brucellosis seropositivity);
- the analysis of sub-groups (e.g., dogs of different breeds or age categories);
- the undertaking of a series of interim analyses (e.g., during sequential clinical trials: see Chapter 17);
- assessment of several response variables in relation to one explanatory variable (e.g., a primary and several secondary endpoints in clinical trials: see Chapter 17);
- 'data dredging' (i.e., when several comparisons are made between variables in a data set with no particular comparison being of primary interest).

In such circumstances, it is clear that, if sufficient testing occurs, something 'significant' is likely to crop up, and therefore that there is a danger of attaching too much importance to an isolated significant result among the many non-significant ones.

As noted above, the probability of falsely rejecting a true null hypothesis is given by the probability of a Type I error, which is usually set at 0.05. Importantly, this is the probability *per* hypothesis test. When multiple independent hypothesis tests are performed, the probability of obtaining at least one significant result when all the null hypotheses are true is $1 - (1 - \alpha)^n$, where n is the number of tests performed³. Hence, if a study included 10 hypothesis tests, conducted with a critical significance value, α , of 0.05, the probability of at least one significant result (when all the null

hypotheses are, in fact, true) is $1 - (1 - 0.05)^{10} = 0.40$ (i.e., there is a 40% chance of obtaining at least one spurious significant result – considerably higher than the 0.05 critical value). With 20 hypothesis tests, this increases to 64% and with 50 it increases to 92%. These numbers of hypothesis tests are not uncommon in veterinary research and so such studies are at great risk of drawing false conclusions. Ideally, the number of hypothesis tests performed should be kept to a minimum; hypotheses to be tested should be determined *a priori* where possible; and interpretation of test results should account for any multiple testing⁴.

Several methods have been described to reduce the impact of multiple testing on the probability of Type I errors. The most common method is the **Bonferroni approach** (Bland and Altman, 1995), in which the critical value of significance for each test, α_i , is given by:

$$\alpha_i = \frac{\alpha}{n},$$

where n is the number of tests performed.

Thus, if 10 hypothesis tests are performed, the critical value for each test, α_i is $0.05/10 = 0.005$. The Bonferroni approach ensures that the sum of all α_i is equal to α . This method is rather conservative; that is, the reduction in the probability of a Type I error in each test occurs at the expense of an increased probability of a Type II error in each test.

For further discussion of this method and other methods for counteracting the problem of multiple comparisons, see Shaffer (1995) and Bender and Lange (2001).

One- and two-tailed tests

The previous discussion has been concerned with demonstrating any differences between a sample and a reference population, irrespective of the 'direction' of the difference; that is, whether the sample differs because it comes from a distribution with a mean to the left or right of the mean of the reference population. In this case, the investigator is concerned with significant departures towards either of the two tails of the distribution. A test that considers these departures therefore is called a **two-tailed** test.

Sometimes, an investigator may be reasonably certain that significant departures only occur in one direction. An example would be investigating whether

³ In veterinary and medical research, tests often are not independent because they are carried out on the same individuals, and so this formula does not apply exactly.

⁴ Noting that opinions on the use of multiple significance tests vary (Wright, 1992; Perneger, 1998), their use implies that the results of a test are interpreted differently, depending on how many other tests are performed, and that Type II errors may occur.

Table 14.1 Summary of some parametric statistical techniques for testing hypotheses relating to means of Normally-distributed data.

Level of measurement	Variance	One-sample case	Parametric statistical test				Parametric measure of correlation
			Two-sample case		Case with three or more samples		
			Related samples	Independent samples	Related samples	Independent samples	
Interval and ratio	Known	Normal test	Normal test	Normal test			Pearson correlation coefficient, ρ^*
Interval and ratio	Unknown	t -test	t -test	t -test* (equal variance) Welch t -test* (unequal variances)	F -test (equal variance) Welch F -test (unequal variances)	F -test (equal variance) Welch F -test (unequal variances)	Pearson correlation coefficient, ρ^*

* Indicates a technique that is exemplified in this book.

diarrhoea depressed weight gain in piglets, rather than either depressed or increased it (the latter is very unlikely). This investigation requires a **one-tailed** test. The 5% significance level in a two-tailed test represents approximately two standard errors to either side of the mean. If the same criterion of approximately two standard errors were used in a one-tailed test, then the actual significance level would only be 2.5% (corresponding to the tail with which the investigator is concerned). Therefore, rejection of the null hypothesis at the 5% level in a one-tailed test requires a deviation corresponding to that required for rejection at the 10% level in a two-tailed test.

Significance tests involving other distributions, and conducted on large samples for which Normal approximations to these distributions are valid, often compute values of the standardized Normal deviate, *z* (see Chapter 12). These are then compared with tabulated values using values of mean and variance specified by the null hypothesis (see Appendix XV). Some examples are given later.

Independent and related samples

Groups (samples) that are compared may be **independent** or **related**, and different statistical techniques are required for these two circumstances. Independent samples require **unpaired** tests, whereas related samples require **paired** tests.

Samples are related when:

- comparisons are made between repeated measurements on the same individuals;
- they are matched for other variables.

An example of the first situation is the measuring of the weights of individual calves one week, and then a

week later. Similarly, the completion of two similar questionnaires by the same respondents constitutes related samples.

Matching is a feature of some observational studies (see Chapter 15) and clinical trials (see Chapter 17).

Pairing can have the benefit of removing a source of variation, therefore providing a more sensitive test than its unpaired counterpart.

Parametric and non-parametric techniques

Parametric tests

Some tests are **parametric** because they are concerned with the mean, which is one of the parameters of the Normal distribution. Parametric techniques make the following assumptions of the data that are to be analysed:

- the distribution is Normal;
- the variables are measured on the interval or ratio scale; that is, they are continuous (see Chapter 10)⁵.

Additionally:

- some tests require that the two populations being compared have equal variances; if this is not the case, then these tests must be modified.

Table 14.1 lists some commonly used parametric techniques that test hypotheses relating to the mean. This chapter focusses on comparing two populations (i.e., two-sample methods) and correlation.

⁵ Although visual analogue measurements are subjective, they can be treated as continuous data for the purpose of analysis.

Non-parametric tests

If the assumptions of a parametric test cannot be met, then **non-parametric** techniques should be used. These can be applied to nominal and ordinal data, as well as interval and ratio data. Most of these tests are distribution-free; that is, they do not require assumptions such as the underlying distribution being Normal, but they do assume symmetry.

Table 14.2 lists some commonly used non-parametric techniques. These are described fully by Siegel and Castellan (1988).

Parametric tests are more powerful than non-parametric tests if the distributional assumptions hold; that is, the former require a smaller sample size than the latter to detect a significant difference of a given size. Moreover, parametric tests are more robust to deviations from Normality than are non-parametric tests to deviations from symmetry. Hence, in some circumstances, it is possible to use parametric tests where the assumptions stated above are not met. This remains an area of controversy: Boneau (1960), Chen and Popovich (2002), Choi and others (2010) and Norman (2010) discuss the impact of these assumptions on a number of parametric tests.

Hypothesis testing versus estimation

Comparisons have traditionally been made by **testing** the null hypothesis using an appropriate significance test. However, such tests do not indicate the *magnitude* of the difference between groups that are being compared, with defined *precision*, and it is the magnitude that may be of interest (e.g., when assessing the prophylactic or therapeutic impact of a compound in a clinical trial). An alternative approach, which **estimates** the difference with defined precision, therefore conveys more information, and is frequently preferred (Cohen, 1994; Brandstätter, 1999; Altman *et al.*, 2000). This involves calculating confidence intervals for the parameters that are being considered. Both approaches are outlined in this chapter.

Sample-size determination

The importance of determining appropriate sample sizes before undertaking an investigation was introduced in relation to surveys in Chapter 13. It is also desirable to determine sample sizes before two groups are compared. This ensures that results will be obtained according to specified parameters. These are:

- the acceptable level of Type I error, α (the probability of inferring a difference between the groups when one does not exist);
- test power, $1 - \beta$ (the probability of correctly inferring a difference);
- the magnitude of the difference to be detected (i.e., the difference between means, medians or proportions);
- the choice of alternative hypothesis: one-tailed or two-tailed;

and, additionally, for ordinal, interval and ratio response variables:

- the variability of the response variable between the two groups.

Conventionally, α is set at 0.05 and β at 0.20 (power = 0.80), but these values may be different, depending on inferential requirements. Unless there is strong evidence that a one-tailed test is justifiable, it is prudent to estimate sample sizes for two-tailed conditions because the latter require a larger sample size than the former for a specified value of α , power and magnitude of the difference to be detected.

Statistical versus clinical (biological) significance

Statistical significance tests give an indication of the probability that observed differences between groups are due to chance. **Clinical (biological) significance**, however, concerns the relevance of findings to clinical veterinary practice. Because statistical significance is partly dependent on sample size, it is possible that small and clinically unimportant differences may become statistically significant. It also is possible that clinically important results may be overlooked because a study's sample size is too small to allow sound conclusions to be drawn. For example, in a clinical study to compare the effect of diet on bilirubin levels in two groups of dogs, the sample sizes may be large enough to indicate statistical significance when the difference in means between the two groups is two mmol/l. However, an investigator may judge a two mmol/l change in means to be of no clinical relevance. In contrast, if the investigator considers a one mmol/l change in means to be important to health, the sample sizes may not be large enough to conclude that the difference is significant. In this case, there is a serious risk of failing to recognize a genuine dietary effect. This is why the sample sizes for prospective studies should be justified (Lipsey and Hurley, 2009).

Table 14.2 Summary of some non-parametric statistical techniques. (Reprinted, with modifications, from Siegel, S. and Castellan, N.J., *Nonparametric Statistics for the Behavioral Sciences*, 2nd edn. Copyright © 1988 McGraw-Hill Inc., New York.)

Level of measurement	Non-parametric statistical test					Non-parametric measure of correlation
	Two-sample case		Case with three or more samples			
	One-sample case	Related/matched samples	Independent samples	Related samples	Independent samples	
Nominal	Binomial test	McNemar's change test	Fisher exact test for 2×2 contingency tables*	Cochran Q test	χ^2 test for $r \times k$ tables	Cramer coefficient, C Phi coefficient, r_ϕ
	χ^2 goodness-of-fit test		χ^2 test for: 2×2 contingency tables* $r \times 2$ contingency tables			Kappa coefficient of agreement, K^*
Ordinal	Kolmogorov–Smirnov one-sample test, $D_{m,n}$	Sign test	Median test		Extension of the median test	Asymmetrical association, the lambda statistic, L_B Spearman rank-order correlation coefficient, r_s
	One-sample runs test	Wilcoxon signed ranks test, T^{++}	Wilcoxon–Mann–Whitney test, W_x^*	Friedman two-way analysis of variance by ranks, F_r	Kruskal–Wallis one-way analysis of variance, KW	Kendall rank-order correlation coefficient, T
	Change-point test		Robust rank-order test, U	Page test for ordered alternatives, L	Jonckheere test for ordered alternatives, J	Kendall partial rank-order correlation coefficient, T_{xyz} Kendall coefficient of concordance, W Kendall coefficient of agreement, U
Interval and ratio			Kolmogorov–Smirnov two-sample test, $D_{m,n}$			Correlation between k judges and a criterion, T_c Gamma statistic, G
	Test for distributional symmetry	Permutation test for paired replicates	Siegel–Tukey test for scale differences	Permutation test for two independent samples		
			Moses rank-like test for scale differences			Somers' index of asymmetric association, d_{BA}

Each column lists, cumulatively downwards, the tests applicable for the given level of measurement. For example, in the case of three or more related samples, when the variables are measured on the ordinal scale, both the Friedman two-way analysis of variance and the Cochran Q test are applicable.

* Indicates a technique that is exemplified in this book.

Interval and ratio data: comparing means

Hypothesis testing

Independent samples: Student's *t*-test

Student's *t*-test (also called an **unpaired, independent** or **two-sample *t*-test**) is a parametric significance test, commonly used for small samples. This involves calculation of a test statistic called *t*, which measures departures from the mean that is specified by the null hypothesis. The distribution of this test statistic follows a *t*-distribution (illustrated in Figure 12.4) – hence the name of the test.

Student's *t*-test can be applied to samples from different populations and a hypothesis that the data come from Normal distributions with a known difference, $\mu_1 - \mu_2 = \delta$ ('delta'), between the two population means, μ_1 and μ_2 , and common unknown variance, σ^2 . If $n_1, \bar{x}_1, s_1^2$ are the sample size, sample mean and sample variance from the first population, and $n_2, \bar{x}_2, s_2^2$ are similar statistics from the second population, then the estimate of the common unknown variance, σ^2 , is s^2 :

$$s^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 + n_2 - 2)}.$$

The test statistic, *t*, is:

$$t = \frac{(\bar{x}_1 - \bar{x}_2 - \delta)}{\sqrt{s^2 \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}},$$

and there are $n_1 + n_2 - 2$ degrees of freedom (see Chapter 12). (In this two-sample case, with sample sizes of n_1 and n_2 , two means are evaluated. There are thus $n_1 + n_2 - 2$ 'pieces' of information for calculation of the variance.)

The standard error of the difference between the two sample means, SE_{diff} , is:

$$SE_{\text{diff}} = s \sqrt{1/n_1 + 1/n_2}.$$

Hence, the test statistic, *t*, also can be written as:

$$t = \frac{(\bar{x}_1 - \bar{x}_2 - \delta)}{SE_{\text{diff}}}.$$

This test is exemplified using the data in Table 12.1:

$$n_1 = 49; \bar{x}_1 = 5.76 \text{ kg}; s_1 = 0.60 \text{ kg};$$

$$n_2 = 49; \bar{x}_2 = 4.69 \text{ kg}; s_2 = 0.67 \text{ kg}.$$

The hypothesis to be tested is that there is no difference in three-week weaning weights between the two groups of piglets; that is, $\delta = 0$.

$$s^2 = \{ (48 \times 0.60^2) + (48 \times 0.67^2) \} / 96$$

$$= (17.280 + 21.547) / 96$$

$$= 0.404.$$

$$t = (5.76 - 4.69 - 0) / \sqrt{\{0.404(1/49 + 1/49)\}}$$

$$= 1.07 / \sqrt{0.0165}$$

$$= 8.33.$$

There are 96 degrees of freedom ($49 + 49 - 2$). When Appendix V is consulted, it does not have a row corresponding to 96 degrees of freedom, and so the row with the greatest number of degrees of freedom less than 96 is chosen (60 in this instance). This is a conservative procedure. The 0.1% value for 60 degrees of freedom is 3.460. This is considerably less than 8.33. Therefore, in this example, the two groups (A and B) of piglets have mean values that differ significantly at the 0.1% level (that is $P < 0.001$). This is a two-tailed test for which Appendix V is directly applicable. However, if a one-tailed test is being conducted, the probability should be halved (the probability of a figure falling outside a certain value of *t* at one end, only, of a distribution is only half as great as the probability of it falling at either end).

Often, when sample sizes are large, the e.s.e.m.s are regarded as the true s.e.m.s and the *t*-test is conducted with degrees of freedom corresponding to infinity (∞). The test then becomes a **two-sample *z*-test**.

If the variances of the two samples are not equal, the test is modified as the **Welch *t*-test**, an example of which is presented in Chapter 20.

Related samples: paired *t*-test

Student's *t*-test can be adapted for comparisons in which there are pairs of observations on individuals (e.g., blood glucose concentrations in diabetic dogs before, and after, treatment with a hypoglycaemic drug)⁶, and is usually called a paired *t*-test. The test assumes that the differences between the observations on each pair are Normally distributed with mean, δ , and an unknown variance, σ^2 . If $\bar{d}$ and s^2 are the sample

⁶ Note, however, that the use of multiple *t*-tests to assess differences arising from *serial* measurements on the same individuals (e.g., blood glucose concentrations recorded several times after feeding) is incorrect, and other methods therefore should be used (Matthews *et al.*, 1990).

mean and variance of the individual **differences**, respectively, then the test statistic is:

$$t = \frac{\bar{d} - \delta}{s \sqrt{(1/n)}},$$

where n is the sample size. There are $(n-1)$ degrees of freedom.

As the standard error of the mean of the individual differences, $SE(\bar{d})$, is:

$$SE(\bar{d}) = s \sqrt{(1/n)},$$

the equation for the paired t -test statistic also can be written as:

$$t = \frac{\bar{d} - \delta}{SE(\bar{d})}.$$

Calculation of confidence intervals

Independent samples

Confidence intervals can be calculated for the difference $\mu_1 - \mu_2$ between the true population means for independent samples. The t -distribution again is used if the data have an approximately Normal distribution.

First, an estimate of the common standard deviation, s , is calculated:

$$s = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}},$$

using the notation of Student's t -test.

The standard error of the difference between the two sample means, SE_{diff} , is:

$$SE_{\text{diff}} = s \sqrt{1/n_1 + 1/n_2}.$$

Using the data in Table 12.1:

$$\begin{aligned} s &= \sqrt{\frac{(49-1)0.60^2 + (49-1)0.67^2}{49+49-2}} \\ &= \sqrt{0.404} \\ &= 0.636. \end{aligned}$$

Therefore:

$$\begin{aligned} SE_{\text{diff}} &= 0.636 \sqrt{1/49 + 1/49} \\ &= 0.128. \end{aligned}$$

A 95% confidence interval is calculated thus:

$$\bar{x}_1 - \bar{x}_2 \pm (t_{\nu(0.95)} \times SE_{\text{diff}}),$$

where $t_{\nu(0.95)}$ is the 95% point of the t -distribution with ν ('nu') degrees of freedom. This may be expressed alternatively as $t_{\nu(1-\alpha)}$, where α is the level of significance. Consulting Appendix V, there are 96 degrees of freedom, and 60 is again chosen conservatively; $t_{\nu(0.95)}$ is located in the column headed '0.05' ($\alpha = 0.05$); this is 2.000 for 60 degrees of freedom.

$$\bar{x}_1 = 5.76,$$

$$\bar{x}_2 = 4.69.$$

The 95% confidence interval is therefore:

$$\begin{aligned} &(5.76 - 4.69) \pm (2.000 \times 0.128) \\ &= 1.07 \pm 0.256 \\ &= 0.81, 1.33. \end{aligned}$$

If the 95% confidence interval *includes* zero, the two means are not significantly different at the 5% level. In this example, the confidence interval *excludes* zero, and so a significant difference can be inferred at the 5% level. This result is consistent with the results of Student's t -test, which indicated a significant difference at the 1% (and therefore 5%) level⁷.

Confidence intervals of 90%, 99% and 99.9% (corresponding to respective values of α of 0.10, 0.01 and 0.001) can be calculated utilizing values of t_{ν} of 1.671, 2.660 and 3.460.

Note that the confidence interval conveys more information than the result of the t -test. It gives an indication of the **precision** of the **magnitude** of the difference between the two means of the populations from which the samples were selected.

This method is not appropriate when the standard deviations of the two samples differ considerably; more complex methods must then be used (Armitage *et al.*, 2002).

⁷ Some data analysts also explore differences by comparing the overlap between confidence intervals constructed for each sample. If two statistics (the mean or other parameters) have non-overlapping confidence intervals, they are necessarily significantly different. However, the converse is not the case: it is not necessarily true that overlapping confidence intervals indicate a lack of a significant difference (Schenker and Gentleman, 2001; Payton *et al.*, 2003). Therefore – although this approach is relatively simple (e.g., if confidence intervals are displayed graphically) – it should not be used for formal significance testing, unless information required to undertake a more appropriate procedure is unavailable, and its deficiencies are noted.

Related samples

Confidence intervals for related samples are calculated in the same way as those for a single sample (see Chapter 12), but $\bar{x}$ and s are now the mean and standard deviation of the individual *differences* between the first and second samples (Altman *et al.*, 2000).

What sample size should be selected?

The approximate sample size required to detect the difference between the true means of two populations, using a two-tailed test at significance level α , can be determined thus:

$$n = 2 \left\{ \frac{\sigma(M_{\alpha/2} + M_{\beta})}{\mu_1 - \mu_2} \right\}^2,$$

where:

n = sample size for each population;

μ_1 = true mean in population 1;

μ_2 = true mean in population 2;

σ = common standard deviation of the two populations;

$M_{\alpha/2}$ = multiplier associated with the required significance level, α ;

M_{β} = multiplier associated with β , the probability of a Type II error.

For example, suppose that an investigator wishes to identify a difference in the mean day at which bacon pigs reach slaughter weight under two different systems of management, and that this difference is specified as a change of five days (i.e., the alternative hypothesis is two-tailed). An estimate of the mean day under the first system of management is required: say, day 160. Next, an estimate of the standard deviation is required. Previous production data indicate that this is approximately seven days, and so this value is used as an estimate of the common standard deviation, σ . These estimates are then applied to the formula.

The two multipliers, $M_{\alpha/2}$ and M_{β} , are obtained from Appendix XV. If the level of significance is set at 5%, $\alpha = 0.05$. The value of z corresponding to $P = 0.025$ is 1.96; this is therefore chosen as the multiplier, $M_{\alpha/2}$. If the investigator wishes to be 80% confident of detecting this difference, test power $(1 - \beta) = 0.80$, and $\beta = 0.20$ [†]. Consulting Appendix XV again, the value of z corresponding to a probability of 0.20 is 0.84, and this is used as the multiplier M_{β} .

Therefore:

$$\begin{aligned} n &= 2\{7(1.96 + 0.84)/(160 - 165)\}^2 \\ &= 2\{(7 \times 2.80/-5)\}^2 \\ &= 2 \times 15.37 \\ &= 30.7. \end{aligned}$$

Under the given error criteria, 62 pigs are therefore required: 31 under each system of management.

If one specific group were expected to have an *increase* over the other of five days in the mean day to slaughter, the alternative hypothesis would be one-tailed. The multiplier $M_{\alpha/2}$ should then be replaced by M_{α} in the appropriate formula. Thus, from Appendix XV, $z = 1.64$, and $M_{\alpha} = 1.64$. This would require 50 pigs: 25 under each system of management. This exemplifies that two-tailed tests require a *larger* sample size than their one-tailed counterparts.

Note that, in addition to the nature of the alternative hypothesis, the sample size is determined by the *difference* between the two means, *not* their *absolute* values: the smaller the difference to be detected, the larger the required sample size. Additionally, a large common standard deviation (indicating considerable variability within the two groups) results in a large sample size.

This formula will slightly underestimate sample sizes for independent samples, and overestimate sample sizes for related samples.

Ordinal data: comparing medians

Hypothesis testing

Independent samples: the Wilcoxon–Mann–Whitney test

The **Wilcoxon–Mann–Whitney (Mann–Whitney U/Mann–Whitney–Wilcoxon/Wilcoxon rank-sum) test** is used to compare two independent groups when at least ordinal measurement has been achieved for the study variables. It also is an appropriate test for interval and ratio data that are not Normally distributed.

Table 14.3a lists the summer body-condition scores from samples of two groups of ponies on Assateague Island off the coast of Maryland, US. One group lives on the north of the island, and the other group lives on the south. The medians of the two samples are 2.5 and 3.5, respectively. The question posed is: ‘Do the body condition scores of north-end and south-end ponies differ significantly?’ (i.e., the test is two-tailed). Body-condition score is an ordinal measurement, and the

[†]Recall that β is conventionally set at four times the value of α .

Table 14.3 Summer body-condition scores of feral ponies on the northern and southern parts of Assateague Island, 1988. (From Rudman and Keiper, 1991; raw data supplied by the authors.)

<i>(a) Scores for 10 ponies</i>			
<i>North-end ponies</i>		<i>South-end ponies</i>	
<i>Score</i>		<i>Score</i>	
1		2	
2.5		3.5	
3		4	
2.5		3.5	
4		3	
<i>(b) Scores and ranks for 44 ponies</i>			
<i>North-end ponies</i>		<i>South-end ponies</i>	
<i>Score</i>	<i>Rank</i>	<i>Score</i>	<i>Rank</i>
3	35	4	44
3	35	3	35
2.5	22	3	35
2.5	22	3.5	43
1	2.5	1	2.5
2.5	22	3	35
2	11	1	2.5
2	11	3	35
2	11	3	35
2.5	22	3	35
2.5	22	2.5	22
2	11	2	11
2	11	3	35
2	11	2	11
2.5	22	3	35
2	11	3	35
1.5	5	1	2.5
3	35	3	35
2	11	3	35
3	35	2.5	22
		2.5	22
		2	11
		2.5	22
		2.5	22
<i>Totals $W_x = 367.5$</i>		<i>$W_y = 622.5$</i>	

two groups of ponies are independent; therefore the Wilcoxon–Mann–Whitney test is appropriate.

The test is based on **ranking** of the scores, and tests the null hypothesis that the median values of each group are the same; the alternative hypothesis being that either: (1) the median of one group is greater than the other, or vice versa (a two-tailed test); or (2) the median of *one* specified group is greater than the other (a one-tailed test).

First, the smallest group, X , of size m , is selected. If both groups are of equal size, as in this example, then either can be chosen (say, the north-end ponies). The value of the statistic used in the test, W_x , is the sum of the ranks in this group.

The scores in group X and the other group, Y , of size n (south-end ponies), are then ranked in order of increasing size, retaining their identity. Scores are relatively crude measures, usually with a limited range, and so samples may include two or more individuals with the same scores. Identical scores constitute **ties**. The rank given to such ties is the average of the tied ranks that would have been assigned if the scores had differed slightly. For example, if three individuals are given the lowest score, then they are each assigned the rank of 2, that is, $(1 + 2 + 3)/3$. The next score would then be ranked 4. If the lowest score was shared by two individuals, they would each be ranked 1.5 $([1 + 2]/2)$ and the next score would be ranked 3.

The scores and ranks of the ponies are therefore:

Score:	1	2	2.5	2.5	3	3	3.5	3.5	4	4
Group:	X	Y	X	X	X	Y	Y	Y	X	Y
Rank:	1	2	3.5	3.5	5.5	5.5	7.5	7.5	9.5	9.5

The sum of the ranks is then calculated for the first group:

$$W_x = 1 + 3.5 + 3.5 + 5.5 + 9.5 = 23;$$

and similarly for the second group:

$$W_y = 2 + 5.5 + 7.5 + 7.5 + 9.5 = 32.$$

The total sample size, $N = m + n = 10$.

If there were no difference between the two groups, then the average ranks of each group would be about the same. However, if there were a difference, then the average ranks would not be the same. In this example, the average rank of group X is $23/5 = 4.6$, and the average rank of group Y is $32/5 = 6.4$, and so there is reason to suspect a difference.

The sampling distribution of W_x under the null hypothesis is given in Appendix XVI. This is used to determine the significance of the result, using the values W_x , m and n . The lower limit is used for observations at the lower end of the distribution. The table appropriate to the values $m=5$ and $n=5$ is selected, and the probability of observing a value of $W_x < 23$ when the null hypothesis is true is located by finding the entry to the right of the lower critical limit, c_L , with a value equal to W_x (i.e., 23). This (one-tailed) probability is 0.2103. Thus, in a two-tailed test (as in this example), the probability is doubled (0.420); this is considerably greater than 0.05 and so the result is not significant at the 5% level.

If W_y had been chosen to determine the significance of the results, the upper limit is used for observations at the upper end of the distribution. Thus, the probability of observing a value of $W_y > 32$ is similarly 0.2103, and the same conclusion is reached.

Large samples Appendix XVI can only be used when m and n are less than 11 ($n < 13$ if $m = 3$ or 4). For larger values of m and n , however, the sampling distribution of W_x is approximately Normal. It is therefore possible to use Appendix XV to assess the significance of any observed differences between large samples.

A value of the standardized Normal deviate, z , can be calculated using the formula:

$$z = \frac{W_x + 0.5 - m(N + 1)/2}{\sqrt{mn(N + 1)/12}},$$

using the same notation as above.

The value 0.5 is added when looking at the lower end of the distribution, and subtracted when considering the upper end.

Table 14.3b lists a larger number of records of scores of ponies on Assateague Island than Table 14.3a. The median score values for north-end and south-end ponies are 2.5 and 3.0, respectively, and the significance of this sample difference can again be assessed. The total number is too great to be accommodated by Appendix XVI and so Appendix XV should be used. There are also ties, which are accommodated by the ranking given in the table.

The average of the ranks for the north-end ponies is $367.5/20 = 18.4$, whereas the average for the south-end ponies is 25.9, also suggesting that the latter are in better condition in the summer than the former.

$$W_x = 367.5,$$

$$m \text{ (north-end ponies)} = 20,$$

$$n \text{ (south-end ponies)} = 24,$$

$$N = m + n = 44.$$

These values are substituted into the formula, adding the value 0.5 (because W_x is associated with the average rank of the smaller group, hence the lower end of the distribution is being considered), and the alternative hypothesis again is two-tailed:

$$\begin{aligned} z &= \frac{367.5 + 0.5 - 20(44 + 1)/2}{\sqrt{20 \times 24(44 + 1)/12}} \\ &= \frac{367.5 + 0.5 - 450}{\sqrt{1800}} \\ &= -1.93. \end{aligned}$$

The calculated value of z is negative, corresponding to the lower tail of the Normal distribution. Appendix XV tabulates only the upper tail, and so the sign is ignored. The value 1.93 corresponds to a one-tailed P value of 0.0268 (a two-tailed P value of 0.0536), and indicates that the observed condition scores between the two groups are not significantly different at the 5% level. Note, however, the prudence of quoting the P value in view of it being so close to the 5% significance level.

Related samples: the Wilcoxon signed ranks test

Related samples can be compared using the **Wilcoxon signed ranks test**. This tests a similar hypothesis to its unpaired analogue, described above.

Table 14.4a lists the body-condition scores of a group of seven sheep recorded in the summer and then again in the winter. These therefore constitute related samples. The median summer and winter scores are 4.0 and 3.5, respectively. It is reasonable to assume that scores are likely to be higher in the summer than the winter – not vice versa – and so the alternative hypothesis is one-tailed. To perform a Wilcoxon signed ranks test to assess the significance of these sample differences, the *differences* between the paired observations are determined; then the differences are ranked, without reference to sign. If there is no difference between a pair, that is, there is a tie with the value zero (e.g., sheep number 5 in Table 14.4a), the pair is excluded from further analysis. Ties not taking the value zero are ranked in the same way as in the Wilcoxon–Mann–Whitney test. The sign (+ or –) that the

Table 14.4 Winter and summer body-condition scores of sheep. (Hypothetical data.)

<i>(a) Scores and ranks for seven sheep</i>				
<i>Sheep no.</i>	<i>Winter score</i>	<i>Summer score</i>	<i>Difference in scores (summer–winter)</i>	<i>Rank of difference</i>
1	3.5	5	1.5	5.5
2	3.5	4	0.5	2
3	3	4	1	4
4	3.5	4	0.5	2
5	3.5	3.5	0	–
6	3.5	3	–0.5	–2
7	3.5	5	1.5	5.5
<i>(b) Scores and ranks for 25 sheep</i>				
<i>Sheep no.</i>	<i>Winter score</i>	<i>Summer score</i>	<i>Difference in scores (summer–winter)</i>	<i>Rank of difference</i>
1	4.5	3	–1.5	–19
2	4	3.5	–0.5	–6.5
3	2.5	3.5	1	15
4	3	3.5	0.5	6.5
5	3	3	0	–
6	3	3.5	–0.5	–6.5
7	5	4	–1	–15
8	4.5	5	0.5	6.5
9	2	2.5	0.5	6.5
10	4.5	5	0.5	6.5
11	2	3.5	1.5	19
12	2.5	3	0.5	6.5
13	4	4.5	0.5	6.5
14	4	4	0	–
15	2.5	3.5	1	15
16	3	3.5	0.5	6.5
17	2	4.5	2.5	22
18	2.5	2.5	0	–
19	3.5	4.5	2	21
20	4	4.5	0.5	6.5
21	3.5	4.5	1	15
22	2.5	4.5	2	21
23	4	4.5	0.5	6.5
24	3	4.5	1.5	19
25	3.5	4	0.5	6.5
			$T^+ = 212.0$	

difference represents is then attached to each rank. Next, the sum of the positive ranks, T^+ , is calculated. The ranking of the differences in the sheep body-condition scores is listed in *Table 14.4a*, and:

$$\begin{aligned} T^+ &= 5.5 + 2 + 4 + 2 + 5.5 \\ &= 19. \end{aligned}$$

Appendix XVII is then consulted. This gives the corresponding one-tailed significance levels for T^+ and the sample size, N , for pairs with non-zero differences. In this example, $N = 6$ and $T^+ = 19$. The table entry for this combination is 0.0469. This is less than 0.05, indicating that there is a significant difference between median summer and winter condition scores. (If the alternative hypothesis were two-tailed, the significance level would be 0.094, and the difference would not be significant at the 5% level.)

Large samples Appendix XVII cannot be used when N is larger than 15, but the sampling distribution of T^+ is then approximately Normally distributed, and:

$$z = \frac{T^+ - N(N+1)/4}{\sqrt{N(N+1)(2N+1)/24}}.$$

Table 14.4b lists the winter and summer condition scores for 25 sheep, with medians of 3.0 and 4.0, respectively. Sheep 5, 14 and 18 have no difference in winter and summer scores and so are not considered further; thus $N = 22$, and $T^+ = 212.0$.

Thus:

$$\begin{aligned} z &= \frac{212.0 - 22(22+1)/4}{\sqrt{22(22+1)(2 \times 22 + 1)/24}} \\ &= \frac{85.5}{\sqrt{948.75}} = 2.78. \end{aligned}$$

Appendix XV is consulted, and the alternative hypothesis again is one-tailed. The probability of obtaining a value of 2.78 if the null hypothesis were true is 0.0027. Thus, a significant difference between the summer and winter median condition scores can be inferred.

Calculation of confidence intervals

Confidence intervals for the difference between two medians can be calculated on the assumption that

Table 14.5 Difference in condition scores of two groups of ponies. (Data from *Table 14.3a*.)

South-end ponies	North-end ponies				
	1	2.5	2.5	3	4
2	-1	0.5	0.5	1	2
3	-2	-0.5	-0.5	0	1
3.5	-2.5	-1	-1	-0.5	0.5
4	-3	-1.5	-1.5	-1	0

the two samples have frequency distributions that are identical in shape, but differ in location.

Independent samples

If there are n_1 and n_2 observations in the two respective samples, the difference between the two medians is first estimated as the median of all possible $n_1 \times n_2$ differences. The differences for the data in *Table 14.3a* are given in *Table 14.5*.

The differences are then sorted:

-3 -2.5 -2.5 -2 -1.5 -1.5 -1 -1 -1 -1
-1 -1 -0.5 -0.5 -0.5 -0.5 0 0 0.5 0.5 0.5 0.5 1 1 2.

The point estimate of the median is thus -0.5.

A parameter, K , based on the Wilcoxon–Mann–Whitney test statistic, is then calculated (Altman *et al.*, 2000). This is tabulated for 95% confidence limits in Appendix XVIII for sample sizes between 5 and 25. The value of K for appropriate values of $n_1 = 5$ (north-end ponies) and $n_2 = 5$ (south-end ponies) is identified; this is 3. The approximate 95% limits are then the *third* smallest and *third* largest difference: -2.5 and 1, respectively. The 95% confidence interval therefore contains the value zero, and so a significant difference between condition scores cannot be inferred at the 5% level. This is in accord with the result of the Wilcoxon–Mann–Whitney test.

Altman and others (2000) give appropriate values of K for determining 90% and 99% confidence intervals.

Large samples When each sample size is greater than 25, an approximate value of K for a 95% confidence interval, based on the Normal-approximation multiplier, is:

$$K = \frac{n_1 n_2}{2} - \left\{ 1.96 \sqrt{\frac{n_1 n_2 (n_1 + n_2 + 1)}{12}} \right\},$$

rounding K up to the nearest integer.

Related samples

Confidence intervals for related samples are calculated in a similar way to those for unrelated samples. If there are n differences for all matched pairs, the averages of all possible differences are calculated, including each difference with itself. For example, in *Table 14.4b*, the average of the difference of sheep number 1 with itself is $(-1.5 - 1.5)/2 = -1.5$; the average of the difference of sheep number 1 with sheep number 2 is $(-1.5 - 0.5)/2 = -1$; and so on:

	Change			
Change	-1.5	-0.5	1	0.5
-1.5	-1.5	-1	-0.25	-0.5
-0.5		-0.5	0.25	0
1			1	0.75
0.5				0.5

These differences are again sorted, and the point estimate of the median identified. A parameter, K^* , based on the Wilcoxon signed ranks test statistic, is then calculated (Altman *et al.*, 2000). This is tabulated in Appendix XIX for 95% confidence limits for sample sizes, n , between 6 and 50. The value of K^* is applied to the sorted differences in the same way as K , previously. Altman and others (2000) give appropriate values of K^* for determining 90% and 99% confidence intervals.

Large samples When each sample size is greater than 50, an approximate value of K^* for a 95% confidence interval is:

$$K^* = \frac{n(n+1)}{4} - \left\{ 1.96 \sqrt{\frac{n(n+1)(2n+1)}{24}} \right\},$$

rounding K^* up to the nearest integer.

What sample size should be selected?

The calculation of sample sizes for the comparison of two sets of ordinal data is not simple (Campbell *et al.*, 1995). One method, involving some pragmatic simplification, will be described and exemplified.

First, an indication of the frequency distribution of the scores in each of the two groups is needed. This

requires data from an earlier study (e.g., a 'pilot' study). The values in the two unrelated samples in *Table 14.3b* will be used in the worked example that follows.

A sufficiently large 'pilot' sample and number of score categories may allow the data to be considered as Normally distributed. Therefore, sample size can be computed using the formula for comparing two means, described earlier, but with an increase in sample size to account for the inefficiency of the Wilcoxon–Mann–Whitney test, relative to Student's t -test ($3/\pi$, i.e., about 95%: Lehmann and D'Abrera, 1975). The two histograms of the north-end ponies and south-end ponies in *Table 14.3b* reveal distributions that are approximately Normal. The means and standard deviations of the two groups are 2.275, 0.525 and 2.583, 0.761, respectively. Using the formula for determining sample size for detecting a difference between two means, described earlier in this chapter, with: $\mu_1 = 2.275$, $\mu_2 = 2.583$,

$$\sigma = \sqrt{\frac{0.525^2 + 0.761^2}{2}} \\ = 0.654$$

(the standard deviation based on the average of the two variances), $\alpha = 5\%$, and power = 80%, the required sample size is 73 animals in each group. This is multiplied by $\pi/3$, to compensate for the inefficiency of the Wilcoxon–Mann–Whitney test, giving 77 animals in each group.

A second, more precise, method, considers the difference between the two samples in relative, rather than absolute, terms (Campbell *et al.*, 1995; Julious and Campbell, 1998). However, the calculations are more detailed, and are not suitable for all data sets. (The calculations are eased, however, by the availability of appropriate software such as *nQuery Advisor*: see Appendix III⁸.)

Nominal data: comparing proportions

Counts of nominal data are the basis of comparison of proportions and are commonly encountered in observational studies. Some general methods are outlined

⁸ Indeed, most sample-size calculations can be routinely undertaken using software. Many statistical packages offer limited sample-size options (see Appendix III), and some dedicated software packages, such as *nQuery Advisor* + *nTerim*, offer comprehensive facilities. In general, it is regarded as good practice to consult such software.

in this chapter. Additional techniques, particularly relevant to observational studies, are described in Chapter 15.

Hypothesis testing

Independent samples: the χ^2 test of association

The results of an investigation of physiological urinary incontinence (PUI) in bitches are recorded in *Table 14.6a*. The question being asked was: 'Does an association exist between the development of PUI and spaying?' The investigators categorized dogs into those with PUI that were spayed, those with PUI that were not spayed, those without PUI that were spayed, and those without PUI that were not spayed. These four permutations allow the construction of a two-way table with four 'cells' in it, called a 2×2 contingency table.

The values in *Table 14.6a* need to be assessed. A simple method of assessment would be to express the values in each row as percentages of the total of each row. If each row showed similar percentages, this would imply that the row classification did not affect

the column classification – that there was no association between the two classifications. This reasoning is sound, but requires large numbers, otherwise sampling variation could affect the result. Taking the data in *Table 14.6a*, the percentage of those animals that were spayed and have PUI is 4.3% (34/791); the percentage of those that were not spayed and have PUI is 0.3% (7/2434). This difference could be significant, but it also could merely result from the variation induced by selection of a relatively small sample of the total population at risk.

A common way of conducting a test on these data is to calculate a non-parametric test statistic for independent samples called χ^2 (χ is the Greek letter chi).

The distribution followed by this statistic is known as the χ^2 **distribution**. This statistic indicates the extent to which the observed values in the cells diverge from the values that would be expected if there were no association between row and column categories.

The expected frequencies under the assumption of no association can be calculated from the observed data. For example, from *Table 14.6b*, the

Table 14.6 Cumulative incidence of physiological urinary incontinence (PUI) (excluding congenital incontinence) in spayed and entire bitches six months of age and older (period of observation = seven years). (Hypothetical data.)

<i>(a) Observed numbers, which reflect the proportions of continent and incontinent animals, and spayed and entire animals, in the population from which the sample was drawn</i>			
	Urinary incontinence present	Urinary incontinence absent	Totals
Spayed	34 (<i>a</i>)	757 (<i>b</i>)	791 (<i>a</i> + <i>b</i>)
Entire	7 (<i>c</i>)	2427 (<i>d</i>)	2434 (<i>c</i> + <i>d</i>)
Totals	41 (<i>a</i> + <i>c</i>)	3184 (<i>b</i> + <i>d</i>)	3225 (<i>n</i>)
<i>(b) Calculation of the expected number of animals in each cell, assuming that the occurrence of PUI is not affected by spaying</i>			
	Urinary incontinence present	Urinary incontinence absent	Proportions (<i>P</i>)
Spayed	$3225 \times 0.2453 \times 0.0127 \approx 10$	$3225 \times 0.2453 \times 0.9873 \approx 781$	$P_{(a+b)} = 791/3225 = 0.2453$
Entire	$3225 \times 0.7547 \times 0.0127 \approx 31$	$3225 \times 0.7547 \times 0.9873 \approx 2403$	$P_{(c+d)} = 2434/3225 = 0.7547$
Proportions (<i>P</i>)	$P_{(a+c)} = 41/3225 = 0.0127$	$P_{(b+d)} = 3184/3225 = 0.9873$	
<i>(c) Calculation of the contribution of each cell to the χ^2 statistic</i>			
	Urinary incontinence present	Urinary incontinence absent	
Spayed	$(34 - 10 - 0.5)^2 / 10 = 55.225$	$(757 - 781 - 0.5)^2 / 781 = 0.7071$	
Entire	$(7 - 31 - 0.5)^2 / 31 = 17.815$	$(2427 - 2403 - 0.5)^2 / 2403 = 0.22982$	
	$\chi^2 = 55.225 + 17.815 + 0.7071 + 0.22982 = 73.9769$		

proportion of all animals that were spayed, $P_{(a+b)}$, is $791/3225 = 0.2453$, and the proportion of all animals that had PUI, $P_{(a+c)}$, is $41/3225 = 0.0127$. Therefore, the expected proportion of all animals that were spayed and have PUI is $P_{(a+b)} \times P_{(a+c)} = 0.2453 \times 0.0127 = 0.0031$, and the expected number of animals that were spayed and have PUI is $0.0031 \times 3225 = 10$. The expected number of animals in the other cells is also shown in *Table 14.6(b)*. The χ^2 statistic can be calculated as:

$$\chi^2 = \sum_{i=1}^n \frac{(|O_i - E_i| - \frac{1}{2})^2}{E_i},$$

where O_i is the observed frequency and E is the expected frequency in the i th cell. The vertical bars, $||$, are **moduli**. They indicate the absolute value of $O_i - E_i$; that is, the positive value of the difference is always used. The value $\frac{1}{2}$ is a continuity correction for 2×2 contingency tables, to improve the approximation, because χ^2 is a continuous distribution, yet the data (numbers of animals), and therefore the test statistic, are discrete.

The contribution of each cell to the χ^2 statistic in the example is calculated in *Table 14.6c* and these are summed to give the χ^2 statistic value of 73.98.

A table of the χ^2 distribution is then consulted to decide whether the observed χ^2 value is larger than that which would be expected, based on a null hypothesis postulating no association. Percentage points of the χ^2 distribution are given in Appendix IX for various significance levels and degrees of freedom. As a rule, the degrees of freedom, ν (upsilon), to be selected are given by:

$$\nu = (\text{number of rows} - 1) \times (\text{number of columns} - 1),$$

which, in this example, is:

$$(2 - 1) \times (2 - 1) = 1.$$

In a 2×2 table, where the row and column totals are all known, knowledge of *one* of the values in the four cells in the body of the table immediately implies knowledge of the values in the other three cells. Similarly in a table with r rows and k columns, with the row and column totals all known, the knowledge of $(r-1) \times (k-1)$ of the values in the rk cells in the body of the table implies knowledge of the values in the other cells. This is the idea behind degrees of freedom in contingency tables; namely, the freedom to choose the values in the body of a contingency table when the row and column totals are fixed.

Consulting row 1 (one degree of freedom) of Appendix IX, the observed value, 73.98, is greater than the tabulated statistic at the 5% level of significance (3.841) and so an association can be inferred between spaying and PUI. Note that, in this example, the result is also significant at the 1% and 0.1% levels.

The χ^2 statistic also can be calculated directly from the observed data. This example involves only a 2×2 contingency table, and the χ^2 equation is simplified to refer only to this type of table:

$$\chi^2 = \frac{n(|ad - bc| - n/2)^2}{(a+b)(c+d)(a+c)(b+d)},$$

where $n/2$ is the continuity correction for 2×2 contingency tables, and the moduli again indicate the absolute value of $ad - bc$.

Using the values in *Table 14.6a*:

$$\begin{aligned} \chi^2 &= \frac{3225\{|82\ 518 - 5299| - (3255/2)\}^2}{791 \times 2434 \times 41 \times 3184} \\ &= 73.35. \end{aligned}$$

This value is slightly different to that calculated above due to rounding in the first calculation.

The test also can be performed on contingency tables with several rows and columns; that is, with several categories (Bailey, 1995).

Independent samples: Fisher's exact test

Sometimes sample sizes are limited, resulting in small numbers in some of the cells of a 2×2 contingency table. If, on the assumption that there is no difference between the groups being compared, one *expected* cell value is less than five, the χ^2 test is not reliable, and a different test – **Fisher's exact test** – should be used. (Note that the χ^2 test can be applied when some observed values are less than five, as long as all *expected* values exceed five.)

Table 14.7 presents the same disease/factor relationship as that presented in *Table 14.6a*, but with smaller numbers. The expected values are calculated, on the assumption that there is no difference between the two groups, by considering the marginal totals. The proportion of animals with PUI is then:

$$\begin{aligned} (a+c)/n, &= \frac{9}{772} \\ &= 0.0117. \end{aligned}$$

This proportion can then be applied to each row separately to estimate the expected cell values. For spayed

Table 14.7 Cumulative incidence of physiological urinary incontinence (excluding congenital incontinence) in spayed and entire bitches six months of age and older (period of observation = seven years). (Hypothetical data.)

	Urinary incontinence present	Urinary incontinence absent	Totals
Spayed	7 (<i>a</i>)	121 (<i>b</i>)	128 (<i>a</i> + <i>b</i> = <i>r</i> ₁)
Entire	2 (<i>c</i>)	642 (<i>d</i>)	644 (<i>c</i> + <i>d</i> = <i>r</i> ₂)
Totals	9 (<i>a</i> + <i>c</i> = <i>n</i> ₁)	763 (<i>b</i> + <i>d</i> = <i>n</i> ₂)	772 (<i>n</i>)

animals, the expected number of animals with PUI is $0.0117 \times 128 = 1.5$.

For entire animals, the expected number of animals with PUI is $0.0117 \times 644 = 7.5$.

The first expected value is less than five, indicating that the χ^2 test is inappropriate and that Fisher's exact test therefore should be used.

Fisher's exact test calculates the *P* value associated with the observed contingency table on the hypothesis that there is no difference between the two population proportions. This is performed by summing the probability of occurrence of the observed table and of all tables that have the same marginal totals (*r*₁, *r*₂, *n*₁ and *n*₂), but are as extreme as, or more extreme than, it is. This is a one-tailed formulation.

First, choose the cell entry over which to sum to be the smallest of the four entries, *c**. In this example, *c** = *c* = 2. The formula for *P* is then:

$$\sum_{c=0}^{c^*} \left\{ \frac{r_1! r_2! n_1! n_2!}{n_1! a! b! c! d!} \right\}.$$

Similar expressions can be derived if *a*, *b* or *d* is the smallest entry.

As *c** varies from 0 to 2, the other values (*a*, *b* and *d*) vary also:

<i>c</i> *	<i>a</i>	<i>b</i>	<i>d</i>
2	<i>a</i> + <i>c</i> - 2	<i>b</i> - <i>c</i> + 2	<i>c</i> + <i>d</i> - 2
1	<i>a</i> + <i>c</i> - 1	<i>b</i> - <i>c</i> + 2	<i>c</i> + <i>d</i> - 1
0	<i>a</i> + <i>c</i>	<i>b</i> - <i>c</i>	<i>c</i> + <i>d</i>

Thus, when *c** = 2, the contribution to *P* is:

$$\frac{(7 + 121)!(2 + 642)!(7 + 2)!(121 + 642)!}{772! \times 7! \times 121! \times 2! \times 642!}.$$

Fisher's exact test is best performed using computer software, but the full calculation is shown here for purpose of illustration. Most pocket calculators will compute factorials up to about 70!, but, for larger

values (as in this example), calculations are best performed using logarithms. Logarithms of factorials up to 999! are given in Appendix XX. From this appendix:

$$\begin{aligned} & (\log 128! + \log 644! + \log 9! + \log 763!) \\ & - (\log 772! + \log 7! + \log 121! + \log 2! + \log 642!) \\ & = (215.58616 + 1531.04044 + 5.55976 + 1869.83994) \\ & - (1895.80816 + 3.70243 + 200.90818 + 0.30103 \\ & \quad + 1525.42334) \\ & = 3622.0263 - 3626.14314 \\ & = -4.11684. \end{aligned}$$

Thus, the contribution to *P* = $\text{antilog}^+ - 4.11684$ = 0.000076.

When *c** = 1, the contribution to *P* is:

$$\frac{(7 + 121)!(2 + 642)!(7 + 2)!(121 + 642)!}{772! \times 8! \times 120! \times 1! \times 643!} = 0.0000036.$$

When *c** = 0, the contribution to *P* is:

$$\frac{(7 + 121)!(2 + 642)!(7 + 2)!(121 + 642)!}{772! \times 9! \times 119! \times 0! \times 644!} = 0.000000074.$$

The *P* value is therefore:

$$0.000076 + 0.0000036 + 0.000000074 = 0.0000797.$$

[†]Antilogarithms can be obtained from a pocket calculator. $\text{Antilog}_{10} x = 10^x$. Most calculators have a '10^x' function key, which therefore can be used to compute antilogarithms to the base 10. Thus, $\text{antilog}_{10} -4.11684 = 10^{-4.11684} = 1/10^{4.11684} = 0.00007641$.

The result therefore is significant at the 0.01% level ($P < 0.0001$), and again indicates that an association can be inferred between spaying and PUI.

There is also a two-tailed formulation of Fisher's exact test (Siegel and Castellan, 1988), but many authors advocate simply doubling the one-tailed P value (Armitage *et al.*, 2002). The test also can be generalized to tables with more than two rows and columns (Mehta and Patel, 1983).

Related samples: McNemar's change test

A modification of the χ^2 test – **McNemar's change test** – can be applied to related samples. *Table 14.8* summarizes the possible results of two related samples, '+' denoting the presence of a characteristic, and '-' its absence. This table could be generated, for example, by matching spayed (sample 1) and entire (sample 2) bitches with respect to breed and age, and then recording the cumulative incidence of PUI. The two samples would then comprise matched pairs of dogs.

The modified χ^2 formula only uses the values that are not in agreement between the two tests; that is, values from discordant pairs:

$$\chi^2 = \frac{(|s + t| - 1)^2}{s + t},$$

with one degree of freedom. The value of χ^2 obtained is interpreted by consulting Appendix IX.

Calculation of confidence intervals

Independent samples

Confidence intervals can be calculated for the difference between two unrelated proportions, and the interpretation is similar to that of confidence intervals for the difference between two means and two medians, which have already been described.

First, the standard error of the difference, SE_{diff} , is calculated:

$$SE_{\text{diff}} = \sqrt{\frac{\hat{p}_1(1-\hat{p}_1)}{n_1} + \frac{\hat{p}_2(1-\hat{p}_2)}{n_2}},$$

where:

$\hat{p}_1$ = estimated proportion of individuals with a feature in group 1;

$\hat{p}_2$ = estimated proportion of individuals with a feature in group 2;

n_1 = number of individuals in group 1;

n_2 = number of individuals in group 2.

Applying this formula to the data in *Table 4.10a* relating to the crude leptospiral seroprevalence in samples of dogs in Glasgow and Edinburgh:

$\hat{p}_1$ = estimated proportion of seropositive dogs in Glasgow

$$= 69/251$$

$$= 0.275.$$

$\hat{p}_2$ = estimated proportion of seropositive dogs in Edinburgh

$$= 61/260$$

$$= 0.235.$$

$$n_1 = 251.$$

$$n_2 = 260.$$

Thus:

$$\hat{p}_1 - \hat{p}_2 = 0.275 - 0.235$$

$$= 0.04;$$

and:

$$\begin{aligned} SE_{\text{diff}} &= \sqrt{\frac{0.275(1-0.275)}{251} + \frac{0.235(1-0.235)}{260}} \\ &= \sqrt{0.00079 + 0.00069} \\ &= 0.038. \end{aligned}$$

A 95% confidence interval can then be calculated:

$$\begin{aligned} &(\hat{p}_1 - \hat{p}_2) \pm 1.96 \times SE_{\text{diff}} \\ &= 0.04 \pm 1.96 \times 0.038 \\ &= -0.034, 0.115. \end{aligned}$$

This confidence interval includes zero; thus a significant difference cannot be inferred at the 5% level.

This formula is based on the Normal approximation to the binomial distribution, and only gives approximate values when the sizes of each group are less than 30, and p is greater than 0.9 or less than 0.1. (For this reason, this formula should not, for example, be applied to the data in *Table 14.6a*, where the two values of p are 0.043 and 0.003.) If exact confidence intervals are required, more complex methods should be used (Armitage *et al.*, 2002).

Related samples

Confidence intervals can be calculated for related samples. Using the notation in *Table 14.8*:

proportion positive in the first sample, $\hat{p}_1 = (r + s)/n$,

proportion positive in the second sample, $\hat{p}_2 = (r + t)/n$,

and the difference between $\hat{p}_1$ and $\hat{p}_2 = (s - t)/n$.

Table 14.8 Possible outcomes of two related samples: +, feature present; −, feature absent.

Sample 1	Sample 2	Number of individuals
+	+	<i>r</i>
+	−	<i>s</i>
−	+	<i>t</i>
−	−	<i>u</i>
Total		<i>n</i>

The standard error of this difference is then:

$$SE_{\text{diff}} = \frac{1}{n} \sqrt{s + t - \frac{(s - t)^2}{n}},$$

and the 95% confidence interval is $(\hat{p}_1 - \hat{p}_2) \pm 1.96 \times SE_{\text{diff}}$.

Exact methods again should be employed if sample sizes are small (Armitage *et al.*, 2002).

What sample size should be selected?

The approximate sample size required to detect a difference between two proportions (i.e., a two-tailed test) is obtained from the formula:

$$n = \frac{\{M_{\alpha/2} \sqrt{2p(1-p)} + M_{\beta} \sqrt{\hat{p}_1(1-\hat{p}_1) + \hat{p}_2(1-\hat{p}_2)}\}^2}{(\hat{p}_2 - \hat{p}_1)^2},$$

where:

n = sample size for each population;

*p*₁ = true proportion in population 1;

*p*₂ = true proportion in population 2;

p = (*p*₁ + *p*₂)/2;

*M*_{α/2} = multiplier associated with the required significance level, α;

*M*_β = multiplier associated with β, the probability of a Type II error.

A common one-tailed situation is demonstration that a specified group responds better than another; for example, when a vaccinated group is compared with an unvaccinated one. For instance, suppose that the annual prevalence of footrot in sheep is expected to be approximately 20%, and an investigator wishes to examine the performance of a new vaccine by demonstrating that it can reduce the prevalence by 5%. A flock of sheep is divided into two groups, one of which is vaccinated, and one of which is not vaccinated. Therefore, *p*₁ = 0.20 and *p*₂ = 0.15 (the value for the anticipated prevalence if there is a 5% reduction).

If the level of significance is set at 5%, α = 0.05 and, from Appendix XV, *M*_α = 1.64 (because the hypothesis is one-tailed). If the investigator wishes to be 80% confident of detecting this difference, test power (1 − β) = 0.80, β = 0.20, and *M*_β = 0.84.

Therefore *p* = (0.20 + 0.15)/2 = 0.175; and:

$$n = \frac{\{1.64 \sqrt{2 \times 0.175(1-0.175)} + 0.84 \sqrt{0.20(1-0.20) + 0.15(1-0.15)}\}^2}{(0.15 - 0.20)^2}$$

$$= \frac{(1.64 \sqrt{0.289} + 0.84 \sqrt{0.16 + 0.128})^2}{-0.05^2}$$

$$= 710.8.$$

Thus, a total of 1422 animals is required: 711 in each group.

The sample size depends on the magnitude of the difference to be detected; the greater the magnitude, the smaller the sample size. Thus, the sample of 711 animals per group will detect a difference in annual prevalence of 5% or greater between the two groups.

This formula will slightly underestimate sample sizes for independent samples, and overestimate sample sizes for related samples.

χ² test for trend

Sometimes a hypothesized causal factor may have a *number* of ordered categories. For instance, herd size may be the factor under consideration, and herds may be divided into several categories according to their size. In such circumstances, the ‘method of concomitant variations’ (see Chapter 3) can be used to infer an association, and an appropriate technique for assessing the statistical significance of the association is the **χ² test for trend** (Mantel, 1963). This test yields a χ² statistic on one degree of freedom, which tests for a linear trend over the ordered categories. The categories may be represented by scores, which can be numbers taken as the mid-point of each category (e.g., representing a herd size of 100–200 animals by ‘150’), by a variation of it, such as its logarithm, or by imprecisely defined, but ordered, arbitrary values, such as 0, 1, 2,...

The test statistic is:

$$\chi^2 = \frac{T^2(T-1)\{\sum x(a-E)\}^2}{M_1 M_0 \{T \sum x^2 N - (\sum x N)^2\}},$$

where:

T = total number of individuals in all score categories;

x = score value;

a = observed number of affected individuals in each score category;

Table 14.9 Numbers of dairy herds affected by bovine spongiform encephalopathy according to herd size, Northern Ireland, 1988–1990. (From Denny *et al.*, 1992. Crown Copyright 1992. Produced by the Central Veterinary Laboratory.)

Herd size (number of adult cows)	Number of affected herds	Number of herds at risk	Percentage of affected herds
1–49	47	4802	1.0
50–99	49	1627	3.0
100–199	24	346	6.9
≥ 200	5	28	17.9
Totals	125	6803	

M_1 = total number of affected individuals;

M_0 = total number of unaffected individuals;

N = number of individuals in each score category;

E = expected number of affected individuals in each score category = NM_1/T .

Table 14.9 presents the results of a study of bovine spongiform encephalopathy (BSE) in Northern Ireland. The percentage of affected herds increases with herd size. The χ^2 test for trend can be applied to these results to assess the statistical significance of this apparent trend. (Note that in this example *herds*, not single animals, are the units of concern⁹.)

The herd sizes 1–49, 50–99, 100–199 ($x_1 \dots, x_3$) are scored as their mid-point values, 25, 75 and 150, respectively. The herd size of > 200 (x_4) is arbitrarily scored as 250.

$$T = 6803,$$

$$M_1 = 125,$$

$$M_0 = 6803 - 125$$

$$= 6678.$$

For x_1 , the expected value of the number of affected individuals, $E_1 = 4802 \times 125/6803 = 88.23$; and for $x_2, \dots, x_4$, $E_2, \dots, E_4 = 29.90, 6.36, 0.52$, respectively.

Thus:

$$\begin{aligned} T^2(T-1) &= 6803^2 \times 6802 \\ &= 3.15 \times 10^{11}. \end{aligned}$$

⁹ For a further example of a herd-level analysis, see Martin and others (1997), who assess the relationship between bovine tuberculosis status and distance of herds from badger setts.

$$\begin{aligned} \{\Sigma x(a-E)\}^2 &= \{25(47-88.23) + 75(49-29.90) \\ &\quad + 150(24-6.36) + 250(5-0.52)\}^2 \\ &= (-1030.75 + 1432.50 + 2646.00 \\ &\quad + 1120.00)^2 \\ &= 4167.75^2 \\ &= 1.74 \times 10^7. \\ T\Sigma x^2N &= 6803\{(25^2 \times 4802) + (75^2 \times 1627) \\ &\quad + (150^2 \times 346) + (250^2 \times 28)\} \\ &= 6803(3.00 \times 10^6 + 9.15 \times 10^6 + 7.79 \times 10^6 \\ &\quad + 1.75 \times 10^6) \\ &= 6803 \times 2.17 \times 10^7 \\ &= 1.48 \times 10^{11}. \\ (\Sigma xN)^2 &= \{(25 \times 4802) + (75 \times 1627) \\ &\quad + (150 \times 346) + (250 \times 28)\}^2 \\ &= (1.20 \times 10^5 + 1.22 \times 10^5 + 5.19 \times 10^4 \\ &\quad + 7000)^2 \\ &= (3.01 \times 10^5)^2 \\ &= 9.06 \times 10^{10}. \end{aligned}$$

Thus:

$$\begin{aligned} \chi^2 &= \frac{(3.15 \times 10^{11}) \times (1.74 \times 10^7)}{(125 \times 6678)(1.48 \times 10^{11} - 9.06 \times 10^{10})} \\ &= \frac{5.48 \times 10^{18}}{8.35 \times 10^5 \times 5.74 \times 10^{10}} \\ &= \frac{5.48 \times 10^{18}}{4.79 \times 10^{16}} \\ &= 114.4. \end{aligned}$$

Consulting Appendix IX, row 1 (one degree of freedom), the observed value, 114.4, is greater than the tabulated statistic at the 0.1% level of significance, and so a linear trend can be inferred between the proportion of affected herds and herd size. There is considerable evidence that BSE is transmitted in meat and bone meal (see Chapters 2, 3 and 4) and so the trend can be explained by the larger number of animals at risk in large herds and therefore the greater probability of purchasing a contaminated batch of food.

A continuity correction should be applied to this test if the x scores are one unit apart (e.g., scores of 0, 1, 2, etc.):

$$\{\Sigma x(a-E)\}^2 \text{ should be replaced by } \{\Sigma x(a-E) - 1/2\}^2.$$

This test should be used with caution because a trend may be fitted and may be significant even if inspection of the data does not reveal a regular pattern over the ordered categories. The test, therefore, is appropriate only if there is a reasonable hypothesis of a linear relationship, as in the example above.

Correlation

Table 14.10 records the number of cases of lameness in cattle in relation to rainfall for a one-year period on a farm in south-west England. The question to be answered is: 'Does the amount of rainfall have an effect on the incidence of lameness?' This is a reasonable question; wet feet could be a causal factor. The values in Table 14.10 are plotted graphically in Figure 14.1, each point being a lameness–rainfall pair. Lameness is measured on the horizontal (x) axis and rainfall on the vertical (y) axis.

If there were a positive association between increased rainfall (the explanatory variable) and incidence of lameness (the response variable), most entries

Table 14.10 The joint distribution of fortnightly lameness incidence and rainfall on a farm in south-west England for a one-year period (1977). (Data supplied by the Institute for Research on Animal Diseases, Compton, UK.)

Fortnight	Number of lameness incidents (x)	Fortnightly rainfall totals (mm) (y)
1	40	37.2
2	38	48.8
3	55	72.0
4	38	76.8
5	45	14.8
6	42	53.2
7	51	23.9
8	45	11.0
9	41	79.9
10	23	21.0
11	10	2.3
12	29	81.1
13	19	7.4
14	11	31.5
15	11	33.2
16	19	31.1
17	33	109.2
18	47	25.0
19	42	1.9
20	34	28.8
21	17	24.4
22	30	51.3
23	48	38.2
24	59	18.0
25	41	57.2
26	26	33.2

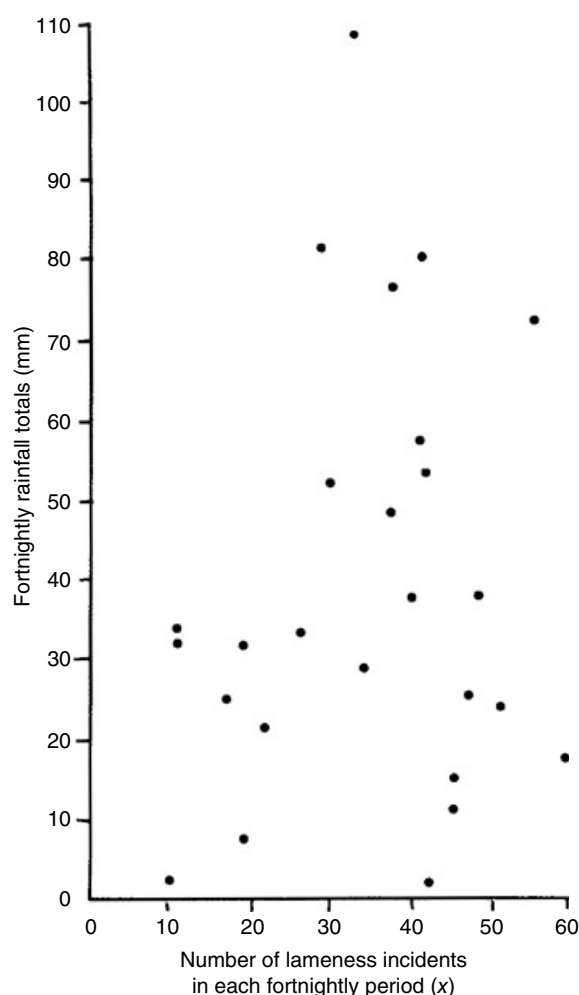


Fig. 14.1 Scatter plot of the joint distribution of fortnightly number of lameness incidents and rainfall on a farm in south-west England for a one-year period (1977). (Data from Table 14.10.)

would be concentrated in a line from the bottom left to the top right of the figure. If there were no association, the pairs would be randomly scattered over the figure. Visually, the results give a slight impression of concentrating along the diagonal, but it is necessary to know whether this clustering is significant.

A useful measure of correlation between x and y for data that fulfil parametric requirements, when successive observations are *independent* of others, is the **Pearson correlation coefficient**, ρ (rho). This ranges from +1, representing a complete positive association, to -1, representing a complete negative association.

In this example, lameness incidence is assumed to be Normally distributed because lameness is fairly common. (If it were rare, then it would more likely have a Poisson distribution.) Similarly, because there are many incidents of lameness, the data are considered to be continuous, although, technically, they are

discrete. It also is assumed that the successive fortnightly observations of rainfall are independent; that is, the rainfall of one fortnight does not influence the rainfall of succeeding fortnights.

The correlation coefficient, ρ , refers to the total population; these measurements come from a sample and so can be used only to estimate the sample's correlation coefficient, r . Estimation involves calculating the means of the two variables and then estimating r , using the equation:

$$r = \frac{\Sigma(x - \bar{x})(y - \bar{y})}{\sqrt{\Sigma(x - \bar{x})^2 \Sigma(y - \bar{y})^2}}.$$

This can be written alternatively in a form that is easier to handle during calculation:

$$r = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\sqrt{\left(\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right) \left(\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right)}}.$$

Using the data from Table 14.10:

$$\Sigma x = 894 \quad \Sigma y = 1012.4$$

$$\Sigma x^2 = 35\,592 \quad \Sigma y^2 = 57\,880.04$$

$$\Sigma xy = 36\,307.5$$

$$n = 26.$$

$$\begin{aligned} \Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n} &= 36\,307.5 - (894)(1012.4)/26 \\ &= 1496.52, \end{aligned}$$

$$\begin{aligned} \left(\Sigma x^2 - \frac{(\Sigma x)^2}{n} \right) &= 35\,592 - 894^2/26 \\ &= 4852.15, \end{aligned}$$

$$\begin{aligned} \left(\Sigma y^2 - \frac{(\Sigma y)^2}{n} \right) &= 57\,880.04 - 1012.4^2/26 \\ &= 18\,458.742. \end{aligned}$$

Thus:

$$\begin{aligned} r &= \frac{1496.52}{\sqrt{4852.15 \times 18\,458.742}} \\ &= \frac{1496.52}{9463.86} \\ &= 0.158. \end{aligned}$$

The significance of this value is obtained by consulting Appendix XXI. If the value is *greater* in absolute terms (i.e., ignoring sign) than the tabulated value, at a defined significance level, then a significant association is demonstrated. In this example, the value (0.158) is less than the

tabulated value at the 5% level, which is 0.381. This value is chosen from the table by selecting the number of degrees of freedom, which is $n - 2 = 24$. This is one degree of freedom less than might be expected because the calculation of the correlation coefficient uses up one further degree of freedom. The most appropriate row therefore is 25 degrees of freedom. In this example, therefore, there is not a significant association between lameness incidence and rainfall, interpreted at the conventional level ($P > 0.05$). Confidence intervals also can be calculated for ρ (Altman *et al.*, 2000).

If parametric assumptions are not met by data, an appropriate non-parametric measure of correlation can be calculated (see Table 14.2). The most commonly used method is the **Spearman rank-order correlation coefficient** (r_s). This involves calculating the ranks of each value *within* each of the variables of interest (i.e., the values for each variable are ranked independently of the other variable), with tied values receiving the ranks that these values would have received if there had been no ties. The Spearman rank-order correlation coefficient is then calculated as the Pearson correlation coefficient of the ranks of each of the variables, and confidence intervals again can be computed (Altman *et al.*, 2000).

Multivariate analysis

The analytical techniques that have been described concern the study of relationships between two variables. In some cases it is necessary to assess the relationship between several variables. This requires statistical techniques that investigate multiple variables; these therefore are called **multivariate techniques** (Porta, 2014)¹⁰ and involve the building of statistical models. Discussion of all these methods is beyond the scope of this book, but those of particular relevance to epidemiology are described in Chapter 22. Comprehensive texts on the subject include Everitt and Dunn (2001) and Hair and others (2014).

¹⁰ The term 'multivariable' also is in use. This, and 'multivariate', have been used interchangeably, but there is now an increasing awareness that they have distinct meanings, particularly in statistical texts (Hidalgo and Goodman, 2013). Multivariate methods generally refer to investigation of several response variables, whereas multivariable methods explore the effects of several explanatory variables. The latter scenario is common in epidemiology; for example, in examining the contribution of breed, parity, pedal lesions, stage of lactation, herd size and herd milk production to the occurrence of bovine lameness (Frankena *et al.*, 1991). Multivariable analysis facilitates control of confounding (see Chapters 3, 15, 18 and 22), evaluation of interaction (see Chapters 5, 15 and 22), and avoids the issue of multiple significance testing when several explanatory variables are assessed.

Statistical packages

There is now a wide range of applications software packages that perform statistical calculations including those of the mean, median, standard deviation and standard error, confidence limits, the tests of association, sample-size calculations (briefly mentioned earlier), time series analysis, multivariate analyses and other statistical procedures relevant to epidemiology. Some of these packages are listed in Appendix III.

Such packages enable data to be managed and analysed efficiently and quickly. However, they can be dangerous. They facilitate easy analysis of data and

so tend to encourage the collection of masses of data without a clear objective. It also is easy to try many of the different tests that are available, without a knowledge of the tests' underlying principles and assumptions.

Expert statistical advice therefore should always be sought if there is any doubt about the analytical technique that should be used in an investigation. This advice should be obtained *before* the investigation begins, not after it is completed; otherwise, time may be spent collecting data, only to discover that they cannot be used to solve the problem that is being posed.

Further reading

- Altman, D.G., Machin, D., Bryant, T.N. and Gardner, M.J. (Eds) (2000) *Statistics with Confidence*, 2nd edn. BMJ Books, London. (*Estimation of confidence intervals and statistical guidelines*)
- Armitage, P., Berry, G. and Matthews, J.N.S. (2002) *Statistical Methods in Medical Research*, 4th edn. Blackwell Science, Malden
- Bailey, N.T.J. (1995) *Statistical Methods in Biology*, 3rd edn. Cambridge University Press, Cambridge. (*An introduction to elementary statistics*)
- Bland, M. (2015) *An Introduction to Medical Statistics*, 4th edn. Oxford University Press, Oxford
- Buskirk, T., Willoughby, L.M. and Tomazik, T.J. (2013) Nonparametric statistical techniques. In: *The Oxford Handbook of Quantitative Methods. Volume 2: Statistical Analysis*. Ed. Little T.D., pp. 106–141. Oxford University Press, New York
- Campbell, M.J. and Swinscow, T.D.V. (2009) *Statistics at Square One*, 11th edn. Wiley-Blackwell/BMJ Books, Chichester. (*An introduction to basic medical statistics*)
- Essex-Sorlie, D. (1995) *Medical Biostatistics and Epidemiology*. Appleton and Lange, East Norwalk
- Fleiss, J.L., Levin, B. and Paik, M.C. (2003) *Statistical Methods for Rates and Proportions*, 3rd edn. John Wiley, Hoboken
- Leech, F.B. and Sellers, K.C. (1979) *Statistical Epidemiology in Veterinary Science*. Charles Griffin, London and High Wycombe
- Mead, R., Curnow, R.N. and Hasted, A.M. (2003) *Statistical Methods in Agriculture and Experimental Biology*, 3rd edn. Chapman and Hall, Boca Raton and London
- Petrie, A. and Watson, P. (2013) *Statistics for Veterinary and Animal Science*, 3rd edn. Wiley Blackwell, Chichester
- Ryan, T.P. (2013) *Sample Size Determination and Power*. John Wiley, Hoboken
- Siegel, S. and Castellan, N.J. (1988) *Nonparametric Statistics for the Behavioral Sciences*, 2nd edn. McGraw-Hill, New York
- Stewart, A. (2016) *Basic Statistics and Epidemiology: A Practical Guide*, 4th edn. CRC Press, Boca Raton
- Whitley, E. and Ball, J. (2002) Statistics review 4: Sample size calculations. *Critical Care*, **6**, 335–341. (*A concise summary of factors governing sample sizes for two-sample comparisons*)
- Woolson, R.F. and Clarke, W.R. (2002) *Statistical Methods for the Analysis of Biomedical Data*, 2nd edn. John Wiley and Sons, New York

15

Observational studies

Observational studies are used to identify risk factors, and to estimate the quantitative effects of the various component causes that contribute to the occurrence of disease. The investigations are based on analysis of natural disease occurrence in populations by **comparing** groups of individuals¹ with respect to disease occurrence and exposure to hypothesized risk factors.

Observational studies differ from experimental studies. In the former, the investigator is not free to randomly allocate factors (disease and hypothesized risk factors) to individuals, whereas in the latter (notably clinical trials: see Chapter 17) the investigator is free to allocate factors to individuals at random.

Risk factors may be categorical (e.g., breed and sex) or quantitative continuous measurements (e.g., weight, age and rainfall). This chapter focusses on categorical data, which are commonly the subject of observational studies. Continuous data also can be analysed using categorical methods by grouping the data into discrete categories (e.g., age intervals).

Types of observational study

Cohort, case-control and cross-sectional studies

There are three main types of observational study: **cohort**, **case-control** and **cross-sectional**. Each classifies animals into those with and without disease, and those exposed and unexposed to hypothesized risk factors. Therefore, they each generate a 2×2 contingency table for each disease/factor relationship (*Table 15.1*). However, the methods of generation differ between the types of study.

¹ Individuals are commonly, but not exclusively, the sampling units in observational studies. Herds, flocks or other aggregates also can be studied.

Table 15.1 The 2×2 contingency table constructed in observational studies.

	<i>Diseased animals</i>	<i>Non-diseased animals</i>	<i>Totals</i>
Hypothesized risk factor present	<i>a</i>	<i>b</i>	<i>a + b</i>
Hypothesized risk factor absent	<i>c</i>	<i>d</i>	<i>c + d</i>
<i>Totals</i>	<i>a + c</i>	<i>b + d</i>	<i>a + b + c + d = n</i>

In **cohort studies** (*a + b*) and (*c + d*) are predetermined.

In **case-control studies** (*a + c*) and (*b + d*) are predetermined.

In **cross-sectional studies** only *n* can be predetermined.

Cohort studies

In a cohort study, a group (cohort) of animals exposed to a hypothesized risk factor, and a group not exposed to the factor, are selected and observed to record development of disease in each group. For example, if spaying were considered to be a risk factor for physiological urinary incontinence (PUI) in bitches, a suitable cohort study would comprise a group of spayed ('exposed') puppies and a group of entire ('unexposed') puppies, each of which would be monitored for the development of PUI. Therefore, incidence is measured, and *a + b* and *c + d* in *Table 15.1* are predetermined.

Case-control studies

In a case-control study, a group of diseased animals (cases) and a group of non-diseased animals (controls) are selected and compared with respect to presence of the hypothesized risk factor. Thus, a case-control study of PUI would involve identification of cases of PUI and comparison of the sexual status (spayed versus entire) of these cases with a control group of bitches that were not incontinent. Therefore *a + c* and *b + d* are predetermined. Case-control studies may be conducted with incident (new) cases or

existing cases and therefore may utilize incidence or prevalence values.

Cross-sectional studies

The cross-sectional study involves the selection of a sample of n individuals from a larger population, and then the determination, for each individual, of the *simultaneous* presence or absence of disease and hypothesized risk factor; prevalence is therefore recorded. For example, in a cross-sectional study of PUI, a sample of bitches would be selected and classified according to sexual status and whether or not the animals were incontinent. At the beginning of a cross-sectional study, only the total number of animals (n in Table 15.1) is predetermined. The numbers of animals with and without disease, and possessing or not possessing the risk factor, are not known initially.

Nomenclature

A variety of alternative names have been applied to case-control and cohort studies. Both of these studies consider two events – exposure to a hypothesized causal factor or factors and development of disease – that are separated by a period of time. Because of this temporal separation of the two events, each of these studies sometimes is termed **longitudinal**.

The case-control study compares diseased animals (cases) with non-diseased animals (controls) and therefore has variously been called a **case-comparison**, **case-referent** or **case-history** study. This study selects groups according to presence or absence of disease and *looks back* to possible causes; it has therefore sometimes been described as a **retrospective study** (looking back from effect to cause).

A cohort study selects groups according to presence or absence of exposure to hypothesized causal factors, and then *looks forward* to the development of disease; it has therefore sometimes been called a **prospective study** (looking forward, from cause to effect). Table 15.2 lists the types of observational study and their synonyms.

The groups may be selected as ‘exposed’ and ‘unexposed’ now, and then observed over a period of time to identify cases; such a cohort study is termed prospective or concurrent (Figure 15.1). Alternatively, if reliable records relating to exposure are available (e.g., from veterinary clinic records and livestock databases, or by tracing animals via adoption records from cat and dog homes/shelters: Beam *et al.*, 1999; Spain *et al.*, 2004a,b; Porphyre *et al.*, 2008; Huynh *et al.*, 2014) then groups may be selected according to presence or absence of previous exposure, and traced to the present to determine disease status; this constitutes a retrospective (non-concurrent) study. The

Table 15.2 Nomenclature of observational studies.

Cross-sectional	Longitudinal	
	Case-control	Cohort
Synonym:	Synonyms:	Synonyms:
Prevalence	Retrospective Case-referent Case-comparison Case-compeer Case-history Trohoc	Prospective Incidence Longitudinal Follow-up

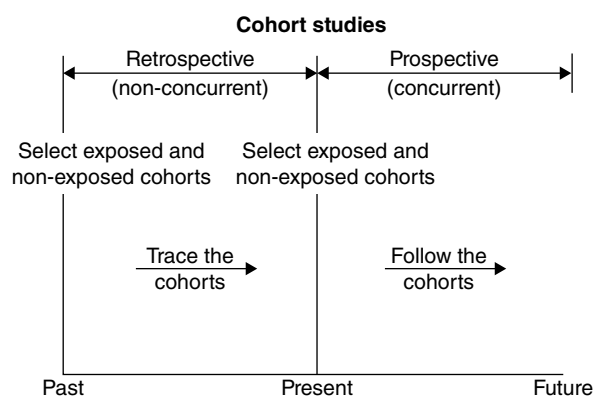


Fig. 15.1 The selection of cohorts in concurrent and non-concurrent cohort studies ('retrospective' and 'prospective' used in the temporal sense.) (Adapted from Lilienfeld and Lilienfeld, 1980.)

merits and limitations of the prospective and retrospective approaches are listed by Pugh and her colleagues (2014).

Some investigators use 'retrospective' to refer to any study that records data from the past, and 'prospective' to refer to any study designed to collect future data. Therefore a non-concurrent cohort (prospective, in the causal sense) study alternatively may be termed a retrospective (in the temporal sense) cohort study. Similarly, a concurrent cohort study also can be called a prospective (in the temporal sense) cohort study (Figure 15.1).

Some studies show characteristics of more than one of the three main types. The range of such 'hybrid' studies, and their nomenclature, are described by Kleinbaum and others (1982); see also Chapter 16.

Causal inference

The three types of study attempt to identify a cause by applying the first three of Evans' rules (see Chapter 3;

rules 1 and 3, rephrased here, using 'prevalence' and 'incidence' in their definitions):

1. the prevalence of a disease should be significantly higher in individuals exposed to the supposed cause than in those who are not (evidence supplied by a cross-sectional study);
2. exposure to the supposed cause should be present more commonly in those with than those without the disease, when all other risk factors are held constant (evidence supplied by a case-control study);
3. the incidence of disease should be significantly higher in those exposed to the supposed cause than in those not so exposed (evidence supplied by a cohort study).

The credibility of cause is strengthened by fulfilling Evans' other rules. Thus, Jarrett's (1980) demonstration of an association between exposure to bracken and the development of intestinal cancer in cattle is more credible because a carcinogen has been isolated from bracken (Wang *et al.*, 1976) (Evans' rule 10).

Causal inference also is strengthened if associations are detected in different circumstances (see Chapter 3) or in several studies, enabling results to be generalized more confidently. Thus, an association between spaying and PUI in bitches has been demonstrated in primary-care cases in Scotland and in referred cases in the south-west of England (Holt and Thrusfield, 1993), and demonstrated again in a subsequent national cohort study in the UK (Thrusfield *et al.*, 1998); this strengthens the inference that the association holds in the general dog population. In contrast, a predisposition to valvular heart disease has been demonstrated in cocker spaniels in North America, but not in Scotland (see Chapter 5), suggesting that the breed predisposition is not universal.

Comparison of the types of study

A comparison of cohort, case-control and cross-sectional studies is given in *Table 15.3*.

Case-control studies can be conducted relatively quickly and are a useful means of initially 'trawling' for risk factors. Cohort studies, in contrast, may have a long duration (particularly those of diseases with lengthy incubation and latent periods such as cancer) and often focus on a specific risk factor.

A logical requirement of demonstration of cause is that an animal is exposed to a causal factor *before* disease develops (see Chapter 3). The design of cohort studies, which resembles an experiment, ensures that this temporal sequence is detected. However, cross-sectional and case-control studies may not detect the sequence. For example, if the association between spaying and PUI in female dogs were being

investigated using a cross-sectional study (spaying being the hypothesized risk factor), then spayed bitches with PUI may be identified; however, incontinence may have developed *before* spaying in some of the cases, in which instance spaying could not have been a component cause in those animals. For this reason, and the reason that a cohort study measures incidence, the cohort study therefore is a better technique for assessing risk and identifying causes than the other two types of study.

Measures of association

A hypothesis of association between disease and a factor can be tested using the χ^2 test (see Chapter 14). However, this test cannot be used to measure the *degree* of association. This is because χ^2 is a function of the proportions in the various cells and of the total sample size, whereas the degree of association is only really a function of the cell proportions; the sample size has a role to play in detecting significance but not in determining the extent of association. It also is desirable to provide a more informative *measure* of the impact of a factor on disease occurrence. This can be expressed by the absolute *difference* between disease occurrence in exposed and unexposed groups, estimated by determining the difference between the two proportions (see 'Attributable risk' later in this chapter). Alternatively, the *ratio* of disease occurrence between the two groups can be calculated. Ratios are relative measures, and two are widely used: the relative risk and odds ratio.

Relative risk

The **relative risk**, *RR*, is the ratio of the incidence of disease in exposed animals to the incidence in unexposed animals². Using the notation of *Table 15.1*:

$$\text{incidence}_{\text{exposed}} = a/(a + b),$$

$$\text{incidence}_{\text{unexposed}} = c/(c + d);$$

$$\text{therefore, } RR = \{a/(a + b)\} / \{c/(c + d)\}.$$

A *RR* greater than one indicates a positive statistical association between factor and disease. Thus, a *RR* of two indicates that the incidence of disease in exposed animals is twice that in unexposed animals. A *RR* less than one indicates a negative statistical association:

² This is more fully termed the relative risk in **exposed** animals, RR_{exp} , to distinguish it from the less frequently derived **population** relative risk, $RR_{\text{pop}} = \{(a + c)/n\} / \{c/(c + d)\}$. (See footnote 5 in this chapter.)

Table 15.3 Comparison of the advantages and disadvantages of cohort, case-control and cross-sectional studies. (Based on Schlesselman, 1982; Clayton and McKeigue, 2001; Mann, 2003; Carlson and Morrison, 2009; and Pugh *et al.*, 2014.)

	<i>Advantages</i>	<i>Disadvantages</i>
Cohort studies	1. Incidence in exposed and unexposed individuals can be calculated 2. Permit flexibility in choosing variables to be systematically recorded 3. Can investigate multiple outcomes and potential risk factors 4. Cohort studies are good for studying rare exposures 5. Disease progression can be studied 6. Temporal cause and effect relationships can be examined 7. Method of choice when randomized controlled trials are unethical	1. Exposed and unexposed proportions in target population cannot be estimated 2. Large numbers of subjects are required to study rare diseases 3. Potentially long duration for follow-up (unless retrospective) 4. Relatively expensive to conduct 5. Maintaining follow-up is difficult 6. Control of extraneous variables may be incomplete
Case-control studies	1. Well suited to the study of rare diseases or of those with long incubation/latent periods 2. Relatively quick to mount and conduct 3. Relatively inexpensive 4. Require comparatively few subjects 5. Existing records can be used 6. No risk to subjects 7. Allow study of multiple potential risk factors for a disease 8. Suited to the study of interaction between genotype and environmental factors	1. Exposed and unexposed proportions in target populations cannot be estimated 2. Often rely on recall or records for information on past exposures 3. Validation of information is difficult or sometimes impossible 4. Control of extraneous variables may be incomplete 5. Selection of an appropriate comparison group may be difficult 6. Incidence in exposed and unexposed individuals cannot be estimated 7. Temporal sequence of cause and effect cannot necessarily be determined 8. Unsited to rare exposures 9. Only one outcome generally can be investigated
Cross-sectional studies	1. When a random sample of the target population is selected, disease prevalence, and proportions exposed and unexposed in the target population, can be estimated 2. Relatively quick to mount and conduct 3. Relatively inexpensive 4. Current records occasionally can be used 5. No risk to subjects 6. Allow study of multiple potential causes of disease 7. Allow study of multiple outcomes	1. Unsited to the study of rare diseases and rare risk factors 2. Unsited to the study of diseases of short duration 3. Control of extraneous variables may be incomplete 4. Incidence in exposed and unexposed individuals cannot be estimated 5. Temporal sequence of cause and effect cannot necessarily be determined, and therefore causality inferred 6. Often rely on recall or records for information on past exposures

possession of the factor may be said to have a protective effect against the disease. A *RR* of one suggests no association.

The *RR* can be derived either from cumulative incidence, when it is also termed the **risk ratio**, or from incidence rates, when it is also called the **rate ratio**³.

³ Another parameter, the **hazard ratio**, also can be thought of as the ratio of two incidence rates, and is commonly employed when comparing survival in two groups (Altman *et al.*, 2000; Hernán, 2010).

(Some authorities use relative risk as a synonym only for risk ratio.) The *RR* can only be estimated directly in a cohort study.

Calculation of confidence intervals

The *RR* is estimated from a sample of the study population. Therefore, the significance of the result needs to be assessed. The hypothesis that the *RR* is significantly greater (or less) than one can be tested (Fleiss *et al.*, 2003). Alternatively, confidence intervals can be estimated. The latter approach is adopted in this

chapter because it also indicates the precision of the measure of association (see Chapter 14). However, care needs to be exercised because the relative risk statistic is not Normally distributed.

Logarithmic-based method An approximate 95% confidence interval for the relative risk for large samples can be calculated, based on a transformation of the limits for the natural logarithm ($\log_e$) of the RR (Katz *et al.*, 1978).

Using the data in *Table 14.6a* relating to PUI; first, the sample (point) estimate of the $\widehat{RR}$ is calculated:

$$\begin{aligned}\widehat{RR} &= \{a/(a+b)\} / \{c/(c+d)\} \\ &= (34/791) / (7/2434) \\ &= 14.95.\end{aligned}$$

The variance (var) of $\log_e \widehat{RR}$ is approximately equal to:

$$\begin{aligned}&\{(b/a)/(a+b)\} + \{(d/c)/(c+d)\} \\ &= \{(757/34)/(34+757)\} + \{(2427/7)/(7+2427)\} \\ &= 0.028 + 0.142 \\ &= 0.170.\end{aligned}$$

The 95% confidence interval is:

$$\begin{aligned}&\widehat{RR} \exp(-1.96\sqrt{\text{var}}), \widehat{RR} \exp(+1.96\sqrt{\text{var}}) \\ &= 14.95 \exp(-0.8081), 14.95 \exp(0.8081) \\ &= 14.95 \times 2.72^{-0.8081}, 14.95 \times 2.72^{0.8081} \\ &= 14.95 \times 1/(2.72^{0.8081}), 14.95 \times 2.72^{0.8081} \\ &= 14.95 \times 0.45, 14.95 \times 2.24 \\ &= 6.73, 33.49.\end{aligned}$$

Thus, the relative risk is significantly greater than one at the 5% level, suggesting an association between spaying (the risk factor) and PUI. Note that this result is in accord with the result of the χ^2 test conducted in Chapter 14, but also gives a *measure* of the association with defined *precision*.

Test-based method An alternative method for calculating approximate confidence intervals for the relative risk utilizes the appropriate text statistic: χ^2 .

A 95% confidence interval for the RR can be derived thus:

$$\widehat{RR}^{1 \pm 1.96/\chi}.$$

(Note that χ , not χ^2 , is used.)

Using the data in *Table 14.6a*:

the estimated $RR = 14.95$.

The value of χ^2 has been derived in Chapter 14, and is 73.35[†].

Thus $\chi = \sqrt{73.35} = 8.564$, and the 95% confidence interval is:

$$\begin{aligned}&14.95^{1-1.96/8.564}, 14.95^{1+1.96/8.564} \\ &= 14.95^{0.771}, 14.95^{1.229} \\ &= 8.05, 27.80.\end{aligned}$$

Compare this with the logarithmic approximation, 6.73, 33.49, which is less precise.

Other confidence intervals can be constructed using appropriate multipliers (see Appendix VI).

Exact intervals can be computed⁴, and point and interval estimates based on animal- (person-) years at risk, also can be calculated (Kahn and Sempos, 1989).

Odds ratio

The **odds ratio (relative odds)**, ψ (psi), is another relative measure based on 'odds': the ratio of the probability of an event occurring to the probability of it not occurring (a ratio little used outside betting circles). Thus, the probability of throwing a head with a coin is 0.5 (1/2), whereas the odds are 'even', 1:1, that is (0.5/{1 - 0.5}). Similarly, the probability of throwing a specified number with a six-sided dice is 1/6 (0.167), whereas the odds are 1:5, that is (0.167/{1 - 0.167}). Note that the odds are larger than the probability.

In a cohort study, a disease odds ratio, ψ_d , is estimated; this is the ratio of the odds of disease in exposed individuals to the odds in those unexposed (*Table 15.4*). This simplifies to ad/bc , the **cross-product ratio**, which therefore is a synonym for the odds ratio.

[†] This value is computed using a continuity correction. There is debate as to whether this should be applied, and some authors suggest that another derivation of χ^2 – the Mantel–Haenszel χ^2 – is used in computing confidence intervals; see Sahai and Kurshid (1995) for a discussion. For large samples, the differences are trivial.

⁴ The availability of computers has fostered the development of alternative methods for calculating confidence intervals for many parameters, and for computing P values, when the exact method proves to be too lengthy. The main methods involve **bootstrap estimation** (see Chapter 12) or **Monte Carlo simulation** (see Chapter 23). One method is not uniformly better than the other, each having merit, depending on the problem to be solved. Consideration of these methods is beyond the scope of this text; Efron and Tibshirani (1993), Chernick (2008) and the User Manual to *StatXact* (see Appendix III) should be consulted for a full discussion.

Table 15.4 Derivation of odds ratios in observational studies.

Cohort study	
Probability of disease _{exposed}	$= a/(a+b)$
Probability of no disease _{exposed}	$= b/(a+b)$
Odds of disease _{exposed}	$= [a/(a+b)]/[b/(a+b)]$
$(a+b)$ cancels out from the numerator and denominator, leaving a/b	
Probability of disease _{unexposed}	$= c/(c+d)$
Probability of no disease _{unexposed}	$= d/(c+d)$
Odds of disease _{unexposed}	$= [c/(c+d)]/[d/(c+d)]$
$(c+d)$ cancels out from the numerator and denominator, leaving c/d	
The <i>disease odds ratio</i> , ψ_d , is the ratio of the odds of disease _{exposed} to the odds of disease _{unexposed} : $(a/b)/(c/d) = ad/bc$	
Case-control study	
Probability of exposure _{diseased}	$= a/(a+c)$
Probability of no exposure _{diseased}	$= c/(a+c)$
Odds of exposure _{diseased}	$= [a/(a+c)]/[c/(a+c)]$
$(a+c)$ cancels out from numerator and denominator, leaving a/c	
Probability of exposure _{controls}	$= b/(b+d)$
Probability of no exposure _{controls}	$= d/(b+d)$
Odds of exposure _{controls}	$= [b/(b+d)]/[d/(b+d)]$
$(b+d)$ cancels out from the numerator and denominator, leaving b/d	
The <i>exposure odds ratio</i> , ψ_e , is the ratio of the odds of exposure _{diseased} to the odds of exposure _{controls} : $(a/c)/(b/d) = ad/bc$	
Cross-sectional study	
Both ψ_d (based on prevalence) and ψ_e can be computed $= ad/bc$ (prevalence odds ratio, ψ_p)	

In a case-control study, a different odds ratio, the exposure odds ratio, ψ_e , is determined. It is the ratio of the odds of exposure to the factor in cases to the odds of exposure in controls. Note, however, that this also simplifies to ad/bc .

A prevalence odds ratio, ψ_p , is derived in a cross-sectional study, thus: ad/bc .

In a cohort study, when disease is rare, the incidence of disease in exposed animals approximately equals the odds of disease because a is small relative to b ; thus $a/(a+b) \approx a/b$. Similarly, $c/(c+d) \approx c/d$. Thus, the values of the odds ratio and relative risk are similar. Moreover, since ψ_e , ψ_d and ψ_p are equivalent (ad/bc), odds ratios derived in case-control and cross-sectional studies give indirect estimates of the relative risk (Cornfield, 1951). Additionally, with appropriate sampling protocols, an estimate of the relative risk

can be obtained from case-control studies without the assumption of rarity of disease (Rodrigues and Kirkwood, 1990)⁵.

Calculation of confidence intervals

Logarithmic-based method An approximate 95% confidence interval for the odds ratio can be calculated, based on a transformation of the limits for the natural logarithm ($\log_e$) of ψ (Woolf, 1955).

The method is exemplified using the data in *Table 15.5a* relating to the association between type of ventilation and respiratory disease (specifically enzootic pneumonia) in pigs. In this example, herds – not individuals – are the sampling units. ‘Cases’ are herds with a high prevalence of pneumonia (a three-year average > 5%), whereas ‘controls’ are herds with a low prevalence.

First, the sample estimate of ψ is calculated:

$$\begin{aligned}\hat{\psi} &= ad/bc \\ &= (91 \times 60)/(73 \times 25) \\ &= 2.99.\end{aligned}$$

The variance (var) of $\log_e \hat{\psi}$ is approximately equal to:

$$\begin{aligned}&(1/a + 1/b + 1/c + 1/d) \\ &= 0.01099 + 0.01370 + 0.04000 + 0.01667 \\ &= 0.08137.\end{aligned}$$

The 95% confidence interval is:

$$\begin{aligned}&\hat{\psi} \exp(-1.96\sqrt{\text{var}}), \hat{\psi} \exp(+1.96\sqrt{\text{var}}) \\ &= 2.99 \exp(-0.5591), 2.99 \exp(0.5591) \\ &= 2.99 \times 2.72^{-0.5591}, 2.99 \times 2.72^{0.5591} \\ &= 2.99 \times 1/(2.72^{0.5591}), 2.99 \times 2.72^{0.5591} \\ &= 2.99 \times 0.572, 2.99 \times 1.749 \\ &= 1.71, 5.23.\end{aligned}$$

⁵ Strictly, case-control and cross-sectional studies produce estimates of the ‘relative risk’ of *being* diseased, whereas cohort studies estimate the relative risk of *becoming* diseased. The former relative risk is more properly termed the **prevalence ratio** (Kleinbaum *et al.*, 1982). This also can be calculated directly in a cross-sectional study, using the formula for the relative risk. Cross-sectional studies also allow calculation of a **population prevalence ratio**, which is commonly termed the population ‘relative risk’, $RR_{pop} = \{(a+c)/n\}/\{c/(c+d)\}$. This adjusts the standard prevalence ratio (‘relative risk’) for the prevalence of the factor in the population (Martin *et al.*, 1987). A **population odds ratio**, ψ_{pop} , also can be calculated in cross-sectional and case-control studies (if controls are representative of non-diseased animals in the study population), and is interpreted in the same way as RR_{pop} , $\psi_{pop} = \{d/(a+c)\}/\{c(b+d)\}$ (Martin *et al.*, 1987).

Table 15.5 The relationship between type of ventilation system and porcine enzootic pneumonia. (Data from Willeberg, 1980b.)

<i>(a) Crude relationships</i>			
	<i>Cases*</i> (No. of herds)	<i>Controls**</i> (No. of herds)	<i>Totals</i>
Fan ventilation	91 (a)	73 (b)	164
No fan ventilation	25 (c)	60 (d)	85
<i>Totals</i>	116	133	249

* Herds with a high prevalence of porcine enzootic pneumonia.

** Herds with a low prevalence of porcine enzootic pneumonia.

(b) Relationships according to herd size

	<i>Herd size</i>									
	≤ 200		201–300		301–400		401–500		>500	
	+	–	+	–	+	–	+	–	+	–
Fan ventilation	2	7	15	30	13	19	7	5	54	12
No fan ventilation	4	27	8	18	7	10	2	4	4	1

+ = high prevalence of porcine enzootic pneumonia (disease 'present');

– = low prevalence of porcine enzootic pneumonia (disease 'absent').

$\hat{\psi}_i = 1.93$	$\hat{\psi}_i = 1.13$	$\hat{\psi}_i = 0.98$	$\hat{\psi}_i = 2.80$	$\hat{\psi}_i = 1.13$
$n_i = 40$	$n_i = 71$	$n_i = 49$	$n_i = 18$	$n_i = 71$
$w_i = 0.70$	$w_i = 3.38$	$w_i = 2.71$	$w_i = 0.56$	$w_i = 0.68$
$v_i = 0.93$	$v_i = 0.28$	$v_i = 0.37$	$v_i = 0.45$	$v_i = 1.35$
$w_i^2 = 0.49$	$w_i^2 = 11.42$	$w_i^2 = 7.34$	$w_i^2 = 0.31$	$w_i^2 = 0.46$
$w_i^2 v_i = 0.46$	$w_i^2 v_i = 3.20$	$w_i^2 v_i = 2.72$	$w_i^2 v_i = 0.14$	$w_i^2 v_i = 0.62$

The odds ratio is therefore significantly greater than one at the 5% level, suggesting an association between fan ventilation and pneumonia, based on these crude data.

A more precise method of estimating confidence intervals is described by Cornfield (1956), but in large studies the difference between this and Woolf's method is trivial.

Test-based method The test-based formula for an approximate 95% confidence interval is simply:

$$\hat{\psi}^{1 \pm 1.96/\chi}$$

Exact confidence intervals can be calculated (Mehta *et al.*, 1985); the method requires an appropriate computer program.

An odds ratio or relative risk cannot be calculated when a contingency table cell contains the value zero. This problem can be overcome by the addition of $\frac{1}{2}$ to the values in each cell of the table before calculating

the odds ratio and its associated confidence interval (Fleiss *et al.*, 2003). However, if a study involves 2×2 tables with cell totals of zero, or with cell totals so small that adding $\frac{1}{2}$ will substantially affect the calculation, then its precision is likely to be too low to contribute much to knowledge. Alternatively, if a cell has a zero value, the χ^2 test can be applied, or a confidence interval can be calculated for the difference between the two proportions (see Chapter 14). Zou (2010) discusses other approaches.

Attributable risk

The terms 'attributable risk' and 'attributable proportion' have been used to denote a number of different concepts, often with several inconsistently used synonyms (Porta, 2014). The first describes absolute differences; whereas the second expresses differences in relative terms. Each can relate either to animals exposed to the risk factor or to the total population.

Attributable risk (exposed)

Table 14.6a shows that although the incidence of urinary incontinence in spayed (exposed) dogs, $a/(a+b)$, is greater than the incidence in entire (unexposed) dogs, $(c/c+d)$, the spayed dogs are still susceptible to a 'background risk', corresponding to $(c/c+d)$. Put another way, if some of the spayed dogs had not been neutered then they may still, as entire dogs, have developed urinary incontinence. The extent of the risk associated with spaying is the **attributable risk (risk difference or attributable rate) in exposed animals**, δ_{exp} (delta_{exp}): the difference between the incidence of disease in exposed animals and the incidence in unexposed animals:

$$\delta_{exp} = \{a/(a+b)\} - \{c/(c+d)\}.$$

Thus, in this example:

$$\begin{aligned}\delta_{exp} &= (34/791) - (7/2434) \\ &= 0.043 - 0.003 \\ &= 0.040.\end{aligned}$$

This represents an incidence of incontinence in spayed dogs, attributable to spaying, of 4.0 per 100 during the period of observation. The attributable risk therefore indicates the extent to which the incidence of disease in exposed animals would be reduced if they had not been exposed to the risk factor, *assuming that the risk factor is causal*.

This attributable risk can be expressed in terms of the relative risk:

$$\begin{aligned}\delta_{exp} &= \{a/(a+b)\} - \{c/(c+d)\} \\ &= [\{a/(a+b)\} / \{c/(c+d)\} - 1] \times \{c/(c+d)\}.\end{aligned}$$

Since $\{a/(a+b)\} / \{c/(c+d)\} = RR$, then

$$\delta_{exp} = (RR-1) \times \{c/(c+d)\}.$$

Note that the incidence in unexposed animals is required to calculate δ_{exp} .

An attributable risk, based on prevalence values, can be estimated in cross-sectional studies because the prevalence in unexposed animals is known. However, this is not known in a case-control study, and so δ_{exp} cannot be determined in this type of study, unless information from other sources is available on the baseline incidence.

It is clear that ratios $\{a/(a+b)\} / \{c/(c+d)\}$ of 0.02/0.01 and 0.0002/0.0001 give the same relative risk, even though they represent vastly different incidence ratios. The attributable risk, however, includes the baseline incidence rate, and therefore gives an indication of the magnitude of the effect of a causal factor in the population. Thus, the attributable risk gives a better indication than the relative risk of the effect

of a preventive campaign that removes the factor (MacMahon and Trichopoulos, 1996). However, the use of 'attributable' in the former term is dangerous because it implies a *causal* relationship, whereas the parameter is based only on *statistical associations*. Its practical application in reducing disease incidence therefore depends on strong evidence of a causal relationship between risk factor and disease, which may need to be determined by other means (see Chapter 3).

The main advantage of the relative risk is the empirical finding that the relative risk for a particular disease/factor relationship is fairly consistent in a wide range of populations (Elwood, 2007) and, in contrast to the attributable risk, is therefore independent of the background incidence. This property of consistency makes the relative risk more valuable than the attributable risk in evaluating whether a relationship is likely to be causal. Moreover, the relative risk, or an approximation of it, can be derived from any of the main types of study.

An approximate confidence interval for δ_{exp} can be calculated using the formula for the difference between two unrelated proportions (see Chapter 14). Rothman and others (2008) give a formula based on incidence rates. (These authors also argue a distinction between attributable risk and attributable rate, depending on whether cumulative incidence or incidence rate, respectively, are used.)

Population attributable risk

The **population attributable risk (population attributable rate)**, δ_{pop} , is the difference between the incidence of disease in the total population and the incidence of disease in the unexposed group:

$$\begin{aligned}\delta_{pop} &= \{(a+c)/n\} - \{c/(c+d)\} \\ &= \{(a+b)/n\} \times \delta_{exp}.\end{aligned}$$

This gives a direct indication of the amount of disease in the total population attributable to the risk factor.

Using the data in Table 14.6a:

$$\begin{aligned}\delta_{pop} &= (41/3225) - (7/2434) \\ &= 0.013 - 0.003 \\ &= 0.010.\end{aligned}$$

Thus, the incidence of incontinence in the total population during the period of observation, attributable to spaying, is one per 100, and, if bitches were not spayed, the incidence in the population could be expected to be reduced by one per 100.

The population attributable risk can be calculated only when disease morbidity in the total population is known.

Again, a population attributable 'risk', based on prevalence values, can be estimated directly in a cross-sectional study.

Kahn and Sempos (1989) describe methods for calculating a confidence interval for δ_{pop} .

Attributable proportion

Attributable proportion (exposed)

The **attributable proportion (exposed)**, λ_{exp} (λ_{exp}) (Elwood, 2007), also termed the **aetiological fraction (exposed)** (Porta, 2014), **attributable fraction (exposed)** (Martin *et al.*, 1987), **attributable risk (exposed)** (Kahn and Sempos, 1989) and **relative attributable risk** (Porta, 2014), is the proportion of incidence in exposed animals attributable to exposure to a risk factor.

$$\lambda_{exp} = (RR - 1) / RR.$$

Using the data in Table 14.6a:

$$\begin{aligned}\lambda_{exp} &= (14.95 - 1) / 14.95 \\ &= 0.93.\end{aligned}$$

That is, 93% of cases of incontinence in spayed bitches are attributable to spaying, assuming that spaying is causal.

Alternatively:

$$\begin{aligned}\lambda_{exp} &= (\text{incidence}_{\text{exposed}} - \text{incidence}_{\text{unexposed}}) / \\ &\quad (\text{incidence}_{\text{exposed}}), \\ &\quad \text{i.e., } \delta_{exp} / \text{incidence}_{\text{exposed}} \\ &= \{a / (a + b) - c / (c + d)\} / \{a / (a + b)\}.\end{aligned}$$

It is estimated directly in a cohort study, and can be estimated indirectly in case-control studies, using the odds ratio approximation to the relative risk:

$$\lambda_{exp} = (\psi - 1) / \psi.$$

Population attributable proportion

The **population attributable proportion**, λ_{pop} (Elwood, 2007), also termed the **population aetiological fraction** (Schlesselman, 1982), **attributable fraction** (Ouelett *et al.*, 1979), **population attributable fraction** (Martin *et al.*, 1987), **attributable risk** (Lilienfeld and Lilienfeld, 1980), **attributable risk percent** (Cole and MacMahon, 1971), **population attributable risk** (Kahn and Sempos, 1989) and **population attributable risk proportion** (Porta, 2014), is the proportion of the incidence in the population

attributable to exposure to a risk factor. Therefore, it represents the proportion of disease occurrence that would be eliminated if the group exposed to a causal factor has its incidence reduced to the level of the unexposed group.

$$\lambda_{pop} = \{(RR - 1) / RR\} \times f,$$

where f is the proportion of diseased individuals exposed to the causal factor, $a / (a + c)$.

Using the figures in Table 14.6a:

$$RR = 14.95$$

$$f = 34 / 41$$

$$= 0.829.$$

Thus:

$$\begin{aligned}\lambda_{pop} &= \{(14.95 - 1) / 14.95\} \times 0.829 \\ &= 0.77.\end{aligned}$$

Thus, if spaying is causal, 77% of cases of PUI in the population are attributable to spaying.

The population attributable proportion, like the population attributable risk, therefore is an indication of the impact that removal of a causal factor would have on overall reduction in incidence in the population⁶. In terms of causal model 1 (see Chapter 3), the sum of the population attributable proportions of all sufficient causes is one (100%).

Other formulae for calculating λ_{pop} are:

- $\lambda_{pop} = (\text{incidence}_{\text{population}} - \text{incidence}_{\text{unexposed}}) / (\text{incidence}_{\text{population}});$
i.e., $\delta_{pop} / (\text{incidence}_{\text{population}})$
 $= \{(a + c) / n - c / (c + d)\} / \{(a + c) / n\};$
- $\lambda_{pop} = \{p(RR - 1)\} / \{p(RR - 1) + 1\},$
where p = proportion of population exposed, $(a + b) / n$;
- $\lambda_{pop} = \delta_{pop} / \{(a + c) / n\};$
- $\lambda_{pop} = (RR_{pop} - 1) / RR_{pop}.$

The population attributable proportion can only be calculated directly when disease morbidity in the total population is known. However, it also can be estimated indirectly in case-control studies (if controls are representative of the healthy population):

⁶ For example, it has been applied to assess the impact of endemic helminth infections on disease morbidity, and therefore the extent to which helminth control will improve community health (Booth, 1998).

$$\lambda_{pop} = 1 - [\{c(b+d)\} / \{d(a+c)\}].$$

Again, a value of λ_{pop} , based on prevalence values, can be estimated directly in cross-sectional studies.

The derivation of confidence intervals for λ_{exp} and λ_{pop} is described by Kahn and Semplos (1989).

Forrow and others (1992), Bucher and colleagues (1994) and Jenick (2013) discuss the influence of the type of parameter quoted (absolute versus relative) on clinical decisions.

Interaction

Interaction was introduced in Chapter 5 as occurring between two or more factors when the frequency of disease is either in excess of or less than that expected from the combined effects of each factor. If there is a plausible biological mechanism for the interaction, then **synergism** is said to occur when the frequency is in excess of the anticipated value, and **antagonism** when the frequency is less than the anticipated value.

Two models of interaction were also introduced in Chapter 5: **additive** and **multiplicative**. The choice of model depends on the measure of disease frequency. Generally, the additive model is most appropriate in indicating the impact of interaction on disease incidence (Kleinbaum *et al.*, 1982; Rothman *et al.*, 2008; Kendler and Gardner, 2010)⁷. Therefore, an additive model will now be described (details of both types of model are given by Kleinbaum and others (1982) and Schlesselman (1982)). The model is based on additivity of excess risks.

The additive model

Consider two factors, x and y . If p_{00} is the incidence rate when neither factor is present, p_{10} is the incidence rate when x alone is present, p_{01} is the incidence rate when y alone is present, and p_{11} is the incidence rate when both are present, then:

$$p_{10} - p_{00} = \text{risk attributable to } x,$$

$$p_{01} - p_{00} = \text{risk attributable to } y.$$

If the combined effect of x and y equals the sum of their individual effects, then:

$$(p_{11} - p_{00}) = (p_{10} - p_{00}) + (p_{01} - p_{00}),$$

and there is no interaction.

Lack of interaction can be expressed in terms of the excess relative risk, by dividing the formula above by p_{00} :

$$(p_{11}/p_{00} - 1) = (p_{10}/p_{00} - 1) + (p_{01}/p_{00} - 1).$$

The relative risk when both factors are present is denoted by $RR_{xy} = p_{11}/p_{00}$; the relative risks when x or y is present alone are denoted by $RR_x = p_{10}/p_{00}$ and $RR_y = p_{01}/p_{00}$, respectively.

Thus:

$$(RR_{xy} - 1) = (RR_x - 1) + (RR_y - 1),$$

that is:

$$RR_{xy} = 1 + (RR_x - 1) + (RR_y - 1).$$

If more than two factors, j say, are being considered, then the combined relative risk, RR_m , for these, is given by:

$$RR_m = 1 + \sum_{i=1}^j (RR_i - 1),$$

where $RR_1, \dots, RR_j$ denote the individual relative risks.

Examples of positive interaction, based on an additive model, are given in Table 15.7 and Figure 15.2, using the raw data in Table 15.6 relating to the feline urological syndrome in male cats (Willeberg, 1976).

Hypothesized causal factors in males are feeding high levels of dry cat food, castration, and low levels of outdoor activity. The background risk ($RR = 1$)

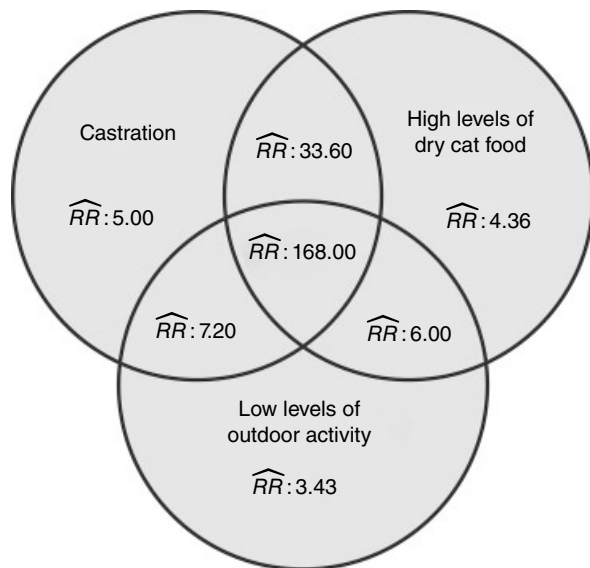


Fig. 15.2 Venn diagram of three high-risk factors for the feline urological syndrome in male cats: castration; high levels of dry cat food; and low levels of outdoor activity. $\widehat{RR}$ = odds ratio approximation. (Adapted from Willeberg, 1977.)

⁷ For a discussion of the appropriateness of additive and multiplicative models, see Thompson (1991) and Ahlbom and Alfredsson (2005).

Table 15.6 Number of cases of the feline urological syndrome and controls, and estimated relative risk values for combinations of categories within the factors, sex, diet and activity in male cats. (From Willeberg, 1976.)

Sex	Categories		No. of cats		Estimated relative risk ($\widehat{RR}^\dagger$)
	Level of dry cat food	Level of outdoor activity	Cases	Controls	
Entire male	Low	High	1	12	1
	Low	Low	2	7	3.43 (<i>a</i>)
	High	High	4	11	4.36 (<i>b</i>)
	High	Low	2	4	6.00 (<i>d</i>)
Castrated male	Low	High	5	12	5.00
	Low	Low	3	5	7.20
	High	High	14	5	33.60***
	High	Low	28	2	168.00***
Total no. of male cats			59	58	12.2**

** Significant at the 1% level; *** significant at the 0.1 % level, by the χ^2 method.

[†] Odds ratio approximation, relative to unexposed group = entire cats with high levels of outdoor activity and receiving low levels of dry cat food.

Table 15.7 Comparison of estimated and expected relative risk values of the feline urological syndrome for male cats for multiple excess risk category combinations, based on an additive interaction model. (Adapted from Willeberg, 1976.)

Sexual status	Categories		Estimated relative risk ($\widehat{RR}$)	Expected relative risk based on additive model ($\widehat{RR}_m$)
	Level of dry cat food	Level of outdoor activity		
Entire	High	Low	6.00 (<i>d</i>)	6.79 (<i>c</i>)
Castrated	Low	Low	7.20	7.43
Castrated	High	High	33.60	836
Castrated	High	Low	168.00	10.67

therefore is represented by entire male cats consuming low levels of dry cat food, and with high levels of outdoor activity.

Consider the two factors: low levels of outdoor activity and high levels of dry cat food intake. The estimated relative risk associated with low levels of outdoor activity and low levels of dry cat food intake in entire males is 3.43 (*a* in Table 15.6). The relative risk associated with high levels of dry cat food and high levels of outdoor activity in entire males is 4.36 (*b* in Table 15.6). Therefore, applying the additive model for these two factors (Table 15.7):

$$\widehat{RR}_m = 1 + (3.43 - 1) + (4.36 - 1) = 6.79 (\text{c in Table 15.7}).$$

The estimated combined relative risk for these two factors is 6.00 (*d* in Tables 15.6 and 15.7).

Thus, there is no evidence for interaction between high levels of dry cat food and low levels of outdoor activity because the estimated combined relative risk (6.00) is similar to the expected combined relative risk, assuming no interaction (6.79: *c* in Table 15.7). Similarly, there is no evidence of interaction between castration and low levels of outdoor activity. However, there is evidence of positive interaction between castration and high levels of dry cat food, and between castration, high levels of dry cat food, and low levels of outdoor activity: in each case the estimated combined relative risk is greater than the expected combined relative risk, using the additive model.

A plausible biological mechanism for the interaction should be posited; that is, synergism should be explained. Thus, castration and high levels of dry cat food intake (usually associated with overfeeding)

may both result in inactivity, thereby reducing blood flow to the kidneys, impairing kidney function, and therefore possibly promoting changes in the urine that are conducive to the formation of uroliths; this constitutes a possible common causal pathway.

Estimation of confidence intervals for interaction is discussed by Hosmer and others (2013).

Bias

Observational studies are subject to **bias** (see Chapter 10). Although many types of bias can occur in observational studies (Sackett, 1979; Delgado-Rodríguez and Llorca, 2004), three are particularly pertinent:

1. selection bias;
2. misclassification;
3. confounding.

Selection bias

Selection bias results from systematic differences between characteristics of the study population and the target population from which it was drawn. Most observational studies use data gathered from convenient populations such as veterinary clinics, abattoirs and particular farms. Willeberg's investigation of the feline urological syndrome in Denmark (Willeberg, 1977), for instance, utilized data collected at a veterinary school's clinic. Ideally, a sample should be selected from the target population (all cats in Denmark in this example), but this is rarely possible. It was stated in Chapter 13 that the inferences from investigations that might be biased by selection need to be made with care if they are to be extrapolated to the target population. Consideration should be given to the likelihood of the study population being biased with respect to the disease and factors that are being investigated. Selection bias is unlikely if:

- exposure to a factor does not increase the likelihood of an animal being present in the study population;
- the likelihood of inclusion of cases and controls in the study population is the same.

For example, Darke and others (1985) investigated the association between the presence of an entire tail (the hypothesized causal factor) and tail injuries (the disease) in a veterinary clinic population to determine whether docking reduces the risk of tail damage. It is improbable that docking or otherwise affects attendance at a veterinary clinic and so selection bias was unlikely in this study.

Misclassification

Misclassification is a type of measurement bias; it occurs when either diseased animals are classified as non-diseased, or animals without a particular disease are classified as possessing it. The likelihood of misclassification depends on the frequency of disease, the frequency of exposure to the hypothesized causal factor, and the sensitivity and specificity of the diagnostic criteria used in the study (see *Table 10.4* and *Figure 10.5*). For example, Thrusfield and others (1985) studied the association between breed, sex and degenerative heart valve disease in dogs. Animals were classified as being diseased if they had either audible cardiac murmurs or signs of congestive cardiac failure. However, murmurs and cardiac failure can be produced by lesions other than heart valve incompetence – cardiomyopathy and anaemia are examples. Therefore, in order to prevent dogs with the latter two lesions being incorrectly classified as having heart valve incompetence (in which case they would constitute false-positives) their case records were scrutinized in detail to ascertain the exact nature of their murmurs. Similarly, early degenerative heart valve disease may not produce audible murmurs, and clinicians may miss the murmurs, in which circumstance animals would be classified incorrectly as disease-free (i.e., false-negatives).

Two types of misclassification can occur: **non-differential** and **differential**. The former occurs if the magnitude and direction of misclassification is similar in the two groups that are being compared (i.e., either cases and controls, or exposed and unexposed individuals). Non-differential misclassification produces a shift in the estimated relative risk and odds ratio towards zero (Copeland *et al.*, 1977) depicted in *Figures 15.3a* and *15.3b*, respectively⁸. *Figure 15.3a* illustrates that specificity is more important than sensitivity in determining bias in the estimate of relative risk. Even when sensitivity and specificity are seemingly acceptable (90% and 96% respectively, exemplified in *Figure 15.3a*) the relative risk can be severely biased. However, sensitivity plays a more important part as a source of bias in estimation of the odds ratio (*Figure 15.3b*).

Differential misclassification occurs when the magnitude or direction of misclassification is different between the two groups that are being compared. In this case, the odds ratio and relative risk may be biased in either direction (see Copeland *et al.*, 1977 for numerical examples). Therefore, misclassification

⁸ In extreme cases, the direction of the association can be reversed (Chyou, 2007). However, this only occurs when measurement is so bad that the sum of the sensitivity and specificity is less than 100%.

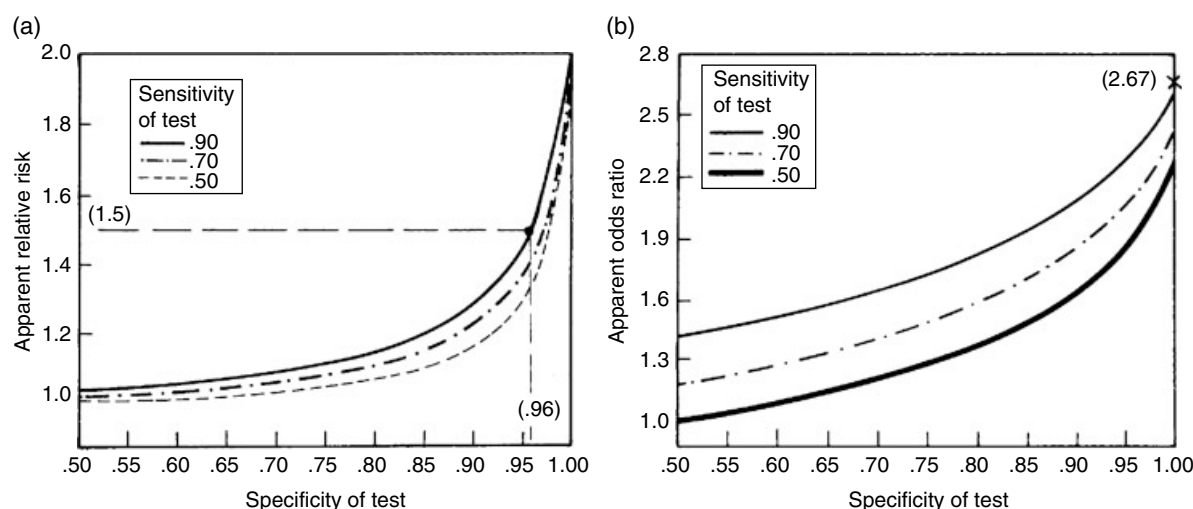


Fig. 15.3 (a) Cohort study: bias in the estimation of the relative risk as a function of sensitivity and specificity. Disease incidence (cumulative) in exposed and unexposed cohorts is 0.10 and 0.05, respectively. True relative risk (exposed and unexposed) equals 2.0. (b) Case-control study: bias in the estimation of the odds ratio as a function of sensitivity and specificity. Exposure in cases and controls is 40% and 20%, respectively. The true odds ratio equals 2.67. (Source: Copeland, *et al.*, 1977.)

can not only weaken an apparent association but also strengthen it.

If a simple, valid (i.e., highly specific and sensitive) test is not available, there can be difficulty in defining a case in the absence of a rigorous definition. For example, in an investigation of the relationship between enzootic bovine leukosis (EBL) and human leukaemia (Donham *et al.*, 1980), cattle were defined as being exposed to EBL virus when post-mortem examination revealed alimentary lymphosarcoma, even though this lesion may develop without exposure to the virus, and exposure to the virus may not produce alimentary tumours.

Similarly, it may be difficult to define and quantify a hypothesized causal factor to which an animal is exposed. For example, if 'inadequate feeding' were the factor, then the investigator may have to rely on an opinion based on owners' descriptions of diet, rather than using the more rigorous results of an examination by a nutritionist.

Confounding

Confounding was introduced in Chapter 3, where its effect on the inferring of causal associations was exemplified. In reiteration, a confounding variable (confounder) is any factor that is either positively or negatively correlated with both the disease and hypothesized causal factors that are being considered. For example, size of herd is a confounding variable in relation to porcine respiratory disease (see Figure 3.5b). If fan ventilation were being

considered as the factor under study, then the results would be confounded (biased, confused, rendered unrepresentative) if the herds that were fan ventilated and that had respiratory disease comprised all large herds (which are likely to develop the disease), and the herds that were not fan ventilated and were non-diseased comprised all small herds (which are much less likely to develop the disease). The uneven proportion of large and small herds in each group therefore will confound the association between fan ventilation and disease, therefore distorting the estimation of the odds ratio and relative risk.

Confounding is particularly important in case-control studies because animals are chosen according to presence and absence of disease: therefore cases may have a whole range of factors in common, some of which may be causal, and some of which may be statistically significant but non-causal because of an association with a confounder.

Confounding is not an 'all-or-none' event, but occurs to varying degrees. Tests for confounding are discussed by Schlesselman (1982); assessment of the extent of confounding is described by Miettinen (1972), Ejigou and McHugh (1977) and Rothman and others (2008). A pictorial representation of confounding is given by Rothman (1975).

These three causes of bias (selection bias, misclassification and confounding) should not be considered in isolation but as an interconnected complex that can distort results.

Controlling bias

Selection bias

It is often not possible to control selection bias; this is the result of inherent characteristics of the study population, and a less biased study population may not be available.

Control can be attempted during either the design or analysis of the investigation. The former essentially involves avoiding the bias by selecting animals from a population that will not produce the bias. This may be impractical and obviously depends on the investigator being aware of the potential bias.

Control during analysis requires knowledge of the probability of selection in the study population and the target population. Kleinbaum and others (1982) provide formulae for this correction.

Misclassification

The control of non-differential and differential misclassification are described by Barron (1977) and Greenland and Kleinbaum (1983), respectively. Essentially control is effected by algebraic manipulation during analysis, although this is never as satisfactory as using a highly sensitive and specific test to determine diseased and non-diseased cases.

Confounding

There are two simple methods of dealing with confounding:

1. by **adjusting** for the confounding variable in the analysis; for example, by using adjusted rates specific to the confounder (see Chapter 4), or by producing a summary odds ratio for the combined odds ratios of each confounder (Mantel and Haenzel, 1959);
2. by **'matching'** the two groups during the design of the study.

Additionally, more complex multivariable methods may be employed (see Chapter 22). The control of confounding is discussed mainly in the context of case-control studies in this chapter. Breslow and Day (1987) discuss control in cohort studies.

The Mantel-Haenszel procedure This technique produces a summary odds ratio, which is the weighted average of individual odds ratios derived by stratifying data with respect to potential confounders. This approach is exemplified using the data in *Table 15.5*. The crude estimate of the odds ratio has already been calculated earlier (2.99). The 95% confidence interval is 1.71, 5.23, suggesting an association between fan ventilation and pneumonia. However, herd size also is known to be associated with the prevalence of

pneumonia and type of ventilation (Aalund *et al.*, 1976). Thus, herd size could be confounding the association between fan ventilation and pneumonia that was identified in the crude data (*Table 15.5a*). The first step in adjusting for this potential confounding is construction of a series of sub-tables, according to herd size (*Table 15.5b*).

The Mantel-Haenszel summary odds ratio, ψ_{mh} , is given by:

$$\psi_{mh} = \{\Sigma(a_i d_i / n_i)\} / \{\Sigma(b_i c_i / n_i)\},$$

where a , b , c , d and n follow the same notation as *Table 15.1*.

$\Sigma(a_i d_i / n_i)$ and $\Sigma(b_i c_i / n_i)$ are calculated by summing the values of ad/n and bc/n , respectively, for each stratum. Thus, for the 'herd size ≤ 200 ' stratum, $a_i d_i / n_i = (2 \times 27) / 40 = 1.35$, and $b_i c_i / n_i = (7 \times 4) / 40 = 0.70$, and the stratum-specific odds ratio, ψ_i , is 1.93, and so on.

Thus:

$$\begin{aligned}\hat{\psi}_{mh} &= \frac{1.35 + 3.80 + 2.65 + 1.56 + 0.76}{0.70 + 3.38 + 2.71 + 0.56 + 0.68} \\ &= 10.12 / 8.03 \\ &= 1.26.\end{aligned}$$

An approximate 95% confidence interval is given by:

$$\hat{\psi} \exp(-1.96\sqrt{\text{var}}), \hat{\psi} \exp(+1.96\sqrt{\text{var}}),$$

where $\text{var} = \text{the variance of } \log_e \hat{\psi}_{mh}$

$$= \Sigma(w_i^2 v_i) / (\Sigma w_i)^2,$$

where $w_i = b_i c_i / n_i$,

and $v_i = (a_i + c_i) / a_i c_i + (b_i + d_i) / b_i d_i$.

Thus, for the 'herd size ≤ 200 ' stratum:

$$w_i = (7 \times 4) / 40$$

$$= 0.70,$$

$$\text{and } v_i = (2 + 4) / (2 \times 4) + (7 + 27) / (7 \times 27)$$

$$= 0.75 + 0.180$$

$$= 0.93,$$

and so on.

$$\begin{aligned}\Sigma(w_i^2 v_i) &= 0.46 + 3.20 + 2.72 + 0.14 + 0.62 \\ &= 7.14.\end{aligned}$$

$$\begin{aligned}(\Sigma w_i)^2 &= (0.70 + 3.38 + 2.71 + 0.56 + 0.68)^2 \\ &= 8.03^2 \\ &= 64.48.\end{aligned}$$

Thus,

$$\begin{aligned}\text{var } \log_e \hat{\psi}_{mh} &= 7.14/64.48 \\ &= 0.1107.\end{aligned}$$

Therefore:

$$\begin{aligned}\hat{\psi} \exp(-1.96\sqrt{\text{var}}), \hat{\psi} \exp(+1.96\sqrt{\text{var}}) \\ &= 1.26 \exp(-0.6521), 1.26 \exp(0.6521) \\ &= 1.26 \times 0.521, 1.26 \times 1.920 \\ &= 0.66, 2.42.\end{aligned}$$

The adjusted odds ratio, 1.26, is much less than the crude odds ratio, 2.99, indicating that confounding may be occurring.

The calculation of a summary, adjusted odds ratio implies that it adequately describes the data; that is, that the odds ratios in the different strata are *similar* (homogeneous). If there are major discrepancies in the stratum-specific values, either in the same or opposite direction (i.e., some values being greater than one, and others clearly less than one) then the Mantel-Haenszel procedure should *not* be used. The summary statistic may disguise important real variation, suggesting that *interaction* is present; that is, the effect of the hypothesized causal factor is modified by the potential confounder⁹. This may indicate a biological mechanism for the interaction (i.e., synergism; see the example earlier relating to the feline urological syndrome) and so should be identified, quantified, reported and explained. Some means of distinguishing between confounding and interaction therefore is needed, and this is fulfilled by testing for homogeneity of the odds ratios across the strata. A simple method¹⁰, which can be conducted on a pocket calculator, computes **Woolf's statistic** (Woolf, 1955):

$$\sum_{i=1}^k \left[\frac{\{\log_e(\hat{\psi}_i) - \log_e(\hat{\psi})\}^2}{\text{var}\{\log_e(\hat{\psi}_i)\}} \right],$$

where, following the notation in Table 15.5:

$$\begin{aligned}\hat{\psi}_i &= \text{odds ratio in the } i\text{th stratum,} \\ \hat{\psi} &= \text{summary odds ratio} = \hat{\psi}_{mh}, \\ \text{var}\{\log_e(\hat{\psi}_i)\} &= v_i.\end{aligned}$$

⁹ **Effect modification** therefore is also sometimes used to describe this interaction between factors (Miettinen, 1974; Porta, 2014), although a distinction sometimes is made between the two terms (see Chapters 16 and 18, and VanderWeele (2009)).

¹⁰ The method was devised in the context of the 'logit method' for estimating the summary odds ratio (which is not described in this book; see Sahai and Kurshid (1995) for details), but generally performs acceptably with other methods of estimation.

Thus, for the first stratum (herd size ≤ 200):

$$\begin{aligned}\frac{\{\log_e(\hat{\psi}_{i=1}) - \log_e(\hat{\psi})\}^2}{\text{var}\{\log_e(\hat{\psi}_{i=1})\}} &= \frac{(\log_e 1.93 - \log_e(1.26))^2}{0.93} \\ &= \frac{(0.6575 - 0.2311)^2}{0.93} \\ &= \frac{0.1818}{0.93} \\ &= 0.196;\end{aligned}$$

and for the second to fifth strata, the values are 0.042, 0.055, 1.417 and 0.009, respectively.

Therefore:

$$\sum_{i=1}^k \left[\frac{\{\log_e(\hat{\psi}_i) - \log_e(\hat{\psi})\}^2}{\text{var}\{\log_e(\hat{\psi}_i)\}} \right] = 0.196 + 0.042 + 0.055 + 1.417 + 0.009 = 1.719.$$

Woolf's statistic has a χ^2 distribution, with $k - 1$ degrees of freedom, where k = the number of strata. There are five strata, and so, consulting row 4 of Appendix IX, the value of 1.719 is less than the tabulated value for $P = 0.05$ (9.488); thus, there is *insufficient* evidence, at the 5% level of significance¹¹, that the stratum-specific odds ratios are not homogeneous, and so it is concluded that the difference between the crude and adjusted odds ratios is the result of the confounding effect of herd size, rather than interaction. The sub-tables thus may be collapsed legitimately to produce a summary adjusted odds ratio.

Note that the confidence interval for the adjusted odds ratio (0.66, 2.42) includes one, indicating that there is not a significant association between fan ventilation and pneumonia when the confounding effect of herd size has been removed.

A widely-used alternative test for homogeneity, which is too cumbersome to compute on a pocket calculator, is the **Breslow-Day test** (Breslow and Day, 1980)¹². Fortunately, it can be undertaken by some statistical packages (e.g. *OpenEpi*: Appendix III). The Breslow-Day statistic also has a χ^2 distribution, with $k - 1$ degrees of freedom. Using appropriate software, the Breslow-Day statistic has a value of 1.02 – somewhat lower than Woolf's statistic. However, consulting

¹¹ Tests for homogeneity have low power, and so some authorities advocate conservative interpretation; say, at the 10% level of significance.

¹² Some authors (e.g., Woods, 2013b) recommend a modification of this test, **Tarone's test**, but the differences between the results of each method are usually trivial.

Appendix IX, the same conclusion is reached: $P > 0.05$, and the summary odds ratio therefore may be quoted.

Alternatively, a 'heterogeneity index', **Higgin's & Thompson's H**, can be computed (Higgins and Thompson, 2002). A value of this index greater than 1.5 provides strong evidence of heterogeneity of the stratum-specific odds ratios, in which circumstance the sub-tables should be maintained and reported separately. A value less than 1.5 suggests that they are homogeneous, in which circumstance collapsing the sub-tables to produce a summary odds ratio is allowable. Using appropriate software (e.g., *WinPepi*: see Appendix III), Higgin's & Thompson's H has a value of 1.0, which is consistent with Woolf's statistic and the result of the Breslow–Day test, again indicating that collapsing the sub-tables to produce a summary odds ratio is legitimate.

Several other tests of homogeneity are compared by Paul and Donner (1989) and the topic is also discussed in Chapter 19.

In summary, assessment of confounding therefore includes these steps:

- calculate the crude odds ratio;
- calculate the adjusted odds ratio;
- decide whether the difference between the crude and the adjusted values is 'big' (this is somewhat arbitrary); if the answer is 'yes' then there is confounding, and the adjusted odds ratio should be used, if *there is no interaction*;
- assess if the stratum-specific odds ratios are homogeneously distributed across the strata (e.g., using Woolf's statistic, the Breslow–Day statistic, or Higgin's & Thompson's H); if not, there is interaction, and the sub-tables should not be collapsed.

The Mantel–Haenszel procedure is applicable to estimation of summary odds ratios for all three types of study, and there is also a modification for summary relative risk estimation in cohort studies based on animal- (person-) years at risk (Kahn and Sempos, 1989).

Matching Matching is the process of making the groups that are being compared comparable with respect to a potential confounder. It can be undertaken in case-control and cohort studies, and can be performed in two ways:

1. **frequency matching**, in which the groups to be sampled are divided so as to contain the same proportion of the potentially confounding variable; for example, if there are four times as many males as females in the case group, then the control group also should be selected to contain four times as

many males as females; this technique therefore is a form of stratification (see Chapter 13);

2. **individual matching**, a more precise form of matching in which each case is matched with a control with respect to the variable; for example, a six-year-old dog with bladder cancer is paired with a six-year-old dog without bladder cancer (matching for age).

Matching is useful when potential confounders are complex and difficult to measure or define (e.g., farm environments). Additionally, it can ensure comparability in terms of the information that is collected. However, it is cumbersome to match for many possible confounding variables. It is usual to match for the main possible ones: age, sex and breed (i.e., common determinants: see Chapter 5). As a general rule, if the confounding variable is unevenly distributed in the population (e.g., age in relation to chronic nephritis) then it is better to match cases or controls when the study is designed, rather than correct for them in the analysis (all young control animals would, in a sense, be wasted when considering chronic nephritis). Unnecessary matching ('overmatching') should be avoided (Miettinen, 1970). If the effect of a factor is in doubt, then it is best not to match but to control it in a subsequent analysis; *when a factor is matched, it cannot be studied separately*.

Matching is used to prevent confounding in cohort studies by making groups directly comparable with respect to potential confounders, and presents fewer intricacies than with case-control studies. For example, it is not necessary to take the matching into account for estimation of the relative risk (Costanza, 1995). In case-control studies, the main value of matching is the enhanced efficiency in controlling confounding in subsequent stratified analysis (Rothman *et al.*, 2008). (Matching can in itself be a source of confounding in case-control studies; notably, when the matched factor is correlated with the exposure factor but not with disease.)

Matched studies should be analysed as such. The simplest matched study involves 1:1 matching, where one matched control is chosen for each case (Table 15.8). Matching results in the two groups being *related*; thus, McNemar's change test (see Chapter 14), rather than

Table 15.8 Format for a 1:1 matched case-control study: numbers of pairs.

	Controls exposed	Controls unexposed
Cases exposed	<i>r</i>	<i>s</i>
Cases unexposed	<i>t</i>	<i>u</i>

the χ^2 test, can be used to assess the significance of observed differences between exposure status in cases and controls. The formula for the odds ratio uses just discordant pairs:

$$\hat{\psi} = s/t.$$

The variance (var) of $\log_e \hat{\psi}$ is approximately equal to $(1/s + 1/t)$, and an approximate 95% confidence interval for large samples is again:

$$\hat{\psi} \exp(-1.96\sqrt{\text{var}}), \hat{\psi} \exp(+1.96\sqrt{\text{var}}).$$

Calculation of exact intervals is described by Schlesselman (1982).

The analysis of studies where the matching ratio is not 1:1, and where further stratification is also practised during analysis, is more complex (Elwood, 2007).

What sample size should be selected?

Appropriate sample sizes can be determined for cohort and case-control studies. The principles relating to sample-size determination, outlined in Chapter 14, are followed. The examples below are conservatively based on two-tailed tests.

Cohort studies

Four values should be specified to determine optimum sample size in a cohort study:

1. the desired level of significance (α : the probability of a Type I error – claiming that exposure to a factor is associated with a disease when, in fact, it is not);
2. the power of the test ($1 - \beta$: the probability of claiming correctly that exposure to a factor is associated with disease, where β is the probability of a Type II error);
3. the anticipated incidence of disease¹³ in unexposed animals in the target population;
4. a hypothesized relative risk that is considered important enough, from the point of view of the health of the animal population.

The formula, for an unmatched study in which exposed and unexposed cohorts of equal size, and the hypothesis is conservatively two-tailed, is:

$$n = \frac{(p_1 q_1 + p_2 q_2) K}{p_1 - p_2},$$

¹³ Cumulative incidence must be specified. There is no simple method for sample-size calculation using incidence rates (i.e., based on animal-years at risk).

where:

- n = number required in *each* cohort;
- p_1 = anticipated incidence in unexposed animals;
- $q_1 = 1 - p_1$;
- p_2 = minimum incidence to be detected in exposed animals;
- $q_2 = 1 - p_2$;
- $K = (M_{\alpha/2} - M_{\beta})^2$, where $M_{\alpha/2}$ and M_{β} are the respective multipliers associated with α and β .

For example, if a relative risk of three or more is to be detected, the anticipated incidence in unexposed animals during the period of the study is one per 100, and significance level and test power are set at 0.05 and 0.80 ($\beta = 0.20$), respectively, then:

$$\begin{aligned} p_1 &= 0.01, \\ q_1 &= 0.99, \\ p_2 &= 0.03 \text{ (because the relative risk is set at three),} \\ q_2 &= 0.97. \end{aligned}$$

From Appendix XV:

$$\begin{aligned} M_{\alpha/2} &= 1.96, \\ M_{\beta} &= 0.84. \end{aligned}$$

Therefore:

$$\begin{aligned} K &= (1.96 + 0.84)^2 \\ &= 7.84. \end{aligned}$$

Thus:

$$\begin{aligned} n &= \frac{(0.01 \times 0.99 + 0.03 \times 0.97) \times 7.84}{(0.01 - 0.03)^2} \\ &= 0.306 / 0.0004 \\ &= 765. \end{aligned}$$

Therefore, a total of 1530 animals are needed for the study.

If a disease is rare, a cohort study requires a considerable number of animals in the exposed and unexposed groups to detect a significant difference, especially when the relative risk is small.

If the cohort sizes are different, a matched study is conducted, or a confounding factor is to be addressed, modifications of this formula are used (Breslow and Day, 1987; Elwood, 2007).

Case-control studies

Four values should be specified to determine optimum sample size in a case-control study:

1. the desired level of significance,
2. the power of the test,

3. the proportion of controls exposed to the risk factor,
4. a hypothesized odds ratio that is considered important enough, from the point of view of the health of the animal population.

The formula used to determine cohort sample sizes is used, but:

p_1 = proportion of cases exposed to the risk factor,
 p_2 = proportion of controls exposed to the risk factor.

The value of p_1 is estimated from p_2 , given the specified odds ratio:

$$p_1 = \frac{p_2 \times \psi}{1 + p_2(\psi - 1)}.$$

For example, if an odds ratio of four or more is to be detected, the anticipated proportion of controls exposed to the risk factor is 0.05 (5%), and significance and test power are set at 0.05 and 0.80, respectively (two-tailed hypothesis), then first estimate p_1 :

$$\begin{aligned} p_1 &= \frac{0.05 \times 4}{1 + 0.05(4 - 1)} \\ &= 0.2/1.15 \\ &= 0.174. \end{aligned}$$

Again, $K = 7.84$.

Thus:

$$\begin{aligned} n &= \frac{(0.174 \times 0.826 + 0.05 \times 0.95) \times 7.84}{(0.174 - 0.05)^2} \\ &= 1.499/0.015 \\ &= 100. \end{aligned}$$

Therefore, a total of 200 animals are needed for the study.

If exposure to the risk factor is rare in the target population, a case-control study requires a considerable number of animals in the case and control groups to detect a significant difference, especially when the odds ratio is small.

If the case and control group sizes are different, a matched study is conducted, or a confounder is to be addressed when the study is designed, modifications of this formula are used (Breslow and Day, 1987; Elwood, 2007). Matching criteria and stratum sizes are discussed by Greenland and others (1981).

Tables of the smallest and largest detectable relative risks and odds ratios, for different values of specified parameters, are given by Walter (1977). Sample-size estimations that include cost functions are described by Pike and Casagrande (1979).

Calculating the power of a study

The number of animals available for inclusion in a study may be limited (e.g., by financial constraints, availability or time). Additionally, a study may not detect a significant association between a risk factor and disease. In such circumstances, the investigation may have had insufficient **power**, and it may be useful to know the value of the study's power in detecting various levels of increased risk.

First, the value of M_β appropriate to the study is calculated. For a case-control study with groups of equal size, and a two-tailed test:

$$M_\beta = \sqrt{\frac{(p_1 - p_2)^2 n}{p_1 q_1 + p_2 q_2}} - M_{\alpha/2},$$

where p_1, q_1, p_2 and q_2 follow the same notation as that used in the formula for calculation of sample sizes in case-control studies, and n = number of animals studied in each group.

For example, suppose that a case-control study was conducted to investigate the association between urinary incontinence and spaying in bitches, and that only 50 dogs were studied in each group. If 40% of control dogs were spayed, then $p_2 = 0.40$. If a twofold increase in risk in spayed bitches is to be detected, then $\psi = 2$, and:

$$\begin{aligned} p_1 &= \frac{p_2 \times \psi}{1 + p_2(\psi - 1)} \\ &= \frac{0.40 \times 2}{1 + 0.40(2 - 1)} \\ &= 0.80/1.40 \\ &= 0.57. \end{aligned}$$

Thus:

$$\begin{aligned} q_1 &= 1 - 0.57 \\ &= 0.43; \\ q_2 &= 1 - 0.40 \\ &= 0.60; \\ n &= 50. \end{aligned}$$

If the level of significance is 0.05, $M_{\alpha/2} = 1.96$, and:

$$\begin{aligned} M_\beta &= \sqrt{\frac{(0.57 - 0.40)^2 \times 50}{(0.57 \times 0.43) + (0.40 \times 0.60)}} - 1.96 \\ &= \sqrt{\frac{1.455}{0.481}} - 1.96 \\ &= 1.73 - 1.96 \\ &= -0.23. \end{aligned}$$

Appendix XV is now consulted. Note, though, that the value of M_β is *negative*¹⁴ in this example, but, for brevity, the appendix only tabulates β for *positive* values of M_β . However, the appendix can still be used because the value of β for a negative M_β is one minus the value of β for a positive M_β . Thus, when M_β is negative, using the appendix as if M_β is positive, directly provides the power (because power = $1 - \beta$). The value of β corresponding to $M_\beta = 0.23$ is 0.4090, and this is therefore the power of the study. Thus, if the odds ratio in the target population were two, a study comprising 50 cases and 50 controls would only have a 41% chance of finding that the sample estimate will be significantly ($\alpha = 0.05$) greater than one.

Schlesselman (1982) gives an appropriate formula for determining power when case and control groups are of unequal size.

A similar calculation of power can be made for cohort studies based on cumulative incidence, with:

p_1 = anticipated incidence in unexposed animals,
 p_2 = minimum incidence to be detected in exposed animals.

The formula can also be applied for power calculations in clinical trials (see Chapter 17) when outcome is measured as a proportion.

Elwood (2007) gives an appropriate formula for determining power when exposed and unexposed cohorts are of unequal size.

If incidence rates are used in calculating the relative risk, then the power, with a two-tailed hypothesis, is calculated thus (Thrusfield *et al.*, 1998):

$$M_\beta = \frac{(N_2 \times RR \times p_1) - (N_1 \times p_1) - (M_{\alpha/2} \times \sqrt{N_1 \times p_1}) - 1}{\sqrt{N_2 \times RR \times p_1}},$$

where:

p_1 = incidence rate in the unexposed cohort;
 N_1 = animal time at risk in the unexposed cohort;
 N_2 = animal time at risk in the exposed cohort;
 RR = relative risk to be detected.

Lipsey and Hurley (2009) discuss power calculations for continuous response variables.

Calculating upper confidence limits

Some authors suggest that calculation of power after a study has been completed is uninformative because it does not utilize information acquired during the study

Table 15.9 Association between progestin treatment and pyometra in nulliparous dogs in Finland: odds ratio ($\hat{\psi}$) and its 95% confidence interval. (From Niskanen and Thrusfield, 1998.)

	Cases	Controls	Odds ratio $\hat{\psi}$	95% confidence interval or ψ
Progestin treated	6	47	1.36	0.47, 3.20
Untreated	925	9865		

$P = 0.457$ (Fisher's two-tailed test);

$\alpha = 0.05$;

$\psi = 2$; power = 0.41; likelihood = 0.19;

$\psi = 3$; power = 0.83; likelihood = 0.04.

(notably, an estimate of the relative risk/odds ratio, and its variance), and can be misleading (Smith and Bates, 1992; Goodman and Berlin, 1994).

Consider the results of a case-control study of the relationship between progestin treatment and canine pyometra (Table 15.9). A significant association at the 5% level was not detected ($P > 0.05$), and the 95% confidence interval for the odds ratio therefore included one. Post-study power calculation (as described previously) revealed that the power of the study to detect an odds ratio of two and three was 0.41 and 0.83, respectively ($\alpha = 0.05$). However, this does not incorporate the information that has been gleaned about the likely value of the odds ratio, which the sample estimate of 1.36 reveals. It may therefore be argued that there is little value in computing the power of the study to detect an odds ratio of three when the study suggests that the value is somewhat lower.

An alternative approach involves calculation of the upper confidence limit that just 'touches' the value of interest, and therefore the likelihood of the odds ratio exceeding this value. The following formula is used:

$$\hat{z}_\alpha = \frac{-\log_e(U/\hat{\psi})}{\text{var}^{1/2}},$$

where:

$\hat{z}_\alpha$ = estimate of the standardized Normal deviate;

U = the value of the odds ratio of interest;

$\hat{\psi}$ = study estimate of the odds ratio;

var = variance of the Natural logarithm of this estimate.

For example, considering the data in Table 15.9:

$$\hat{\psi} = 1.36;$$

$$\text{var} = 1/6 + 1/47 + 1/925 + 1/9865 = 0.1891.$$

¹⁴ Negative values of M_β indicate a power of less than 50%, whereas positive values point to a value greater than 50%.

If the odds ratio of interest is two, $U = 2$, and:

$$\begin{aligned}\hat{z}_\alpha &= \frac{-\log_e(2/1.36)}{0.1891^{1/2}} \\ &= \frac{-0.3857}{0.4349} \\ &= -0.8869.\end{aligned}$$

Appendix XV is then consulted. The negative sign is ignored¹⁵, and the nearest tabulated value of z_α is 0.89, with a related P value of 0.1867. This corresponds to a confidence interval of $\{1 - (2 \times 0.1867)\}\%$ (because the tabulated probabilities are one-tailed); that is, a 62.66% confidence interval. Therefore, the upper 62.66% confidence limit just touches an odds ratio of two. The interval is symmetrical, and so the likelihood of the odds ratio being greater than two is $(1 - 0.6266)/2 = 0.1867$. (Note that, for negative values of $\hat{z}_\alpha$, the likelihood can therefore be read directly from the tabulated P value.)

Further reading

- Breslow, N.E. and Day, N.E. (1980) *Statistical Methods in Cancer Research, Volume 1: The Analysis of Case-Control Studies*. IARC Scientific Publications No. 32. International Agency on Cancer Research, Lyon
- Breslow, N.E. and Day, N.E. (1987) *Statistical Methods in Cancer Research, Volume 2: The Design and Analysis of Cohort Studies*. IARC Scientific Publications No. 82. International Agency on Cancer Research, Lyon
- Elwood, J.M. (2007) *Critical Appraisal of Epidemiological Studies and Clinical Trials*, 3rd edn. Oxford University Press, Oxford
- Fleiss, J.L., Levin, B. and Paik, M.C. (2003) *Statistical Methods for Rates and Proportions*, 3rd edn. John Wiley, Hoboken
- Kelsey, J.L., Whittemore, A.S., Evans, A.S. and Thompson, W.D. (1996) *Methods in Observational Epidemiology*, 2nd edn. Oxford University Press, New York and Oxford
- Kleinbaum, D.G., Kupper, L.L. and Morgenstern, H. (1982) *Epidemiologic Research. Principles and Quantitative Measures*. Lifetime Learning Publications, Belmont
- Knol, M.J. and VanderWeele, T.J. (2012) Guidelines for presenting analyses of effect modification and

If the procedure is repeated for $U = 3$, the likelihood is 0.0351.

Thus, although the power of the study to detect an odds ratio of two or greater is 0.41 (i.e., approaching 50%), the likelihood of the odds ratio actually being two or more is only 0.19 ('19%'). More strikingly, the power to detect an odds ratio of at least three is impressive (0.83), but the likelihood of the odds ratio actually being that high is very low (0.04).

The same formula can be applied in cohort studies, with the relative risk replacing the odds ratio.

This chapter has focussed primarily on the relationship between disease and a single risk factor. Additionally, a second putative risk factor has been explored in the context of confounding, and the effects of three risk factors have been introduced in relation to interaction. Examination of the contribution of multiple risk factors to disease occurrence requires multivariable statistical techniques, which are discussed in Chapter 22.

- interaction. *International Journal of Epidemiology*, 41, 513–520
- Newman, S.C. (2001) *Biostatistical Methods in Epidemiology*. John Wiley and Sons, New York
- Pearce, N. and Checkoway, H. (2008) Cohort studies. In: *Encyclopedia of Quantitative Risk Analysis and Assessment*, Vol. 1. Eds Melnick, E.L. and Everitt, B.S., pp. 243–254. John Wiley and Sons, Chichester
- Rothman, K.J., Greenland, S. and Lash, L.T. (2008) *Modern Epidemiology*, 3rd edn. Lippincott, Williams and Wilkins, Philadelphia
- Rothman, K.J., Greenland, S. and Lash, T.L. (2008) Case-control studies. In: *Encyclopedia of Quantitative Risk Analysis and Assessment*, Vol. 1. Eds Melnick, E.L. and Everitt, B.S., pp. 192–204. John Wiley and Sons, Chichester
- Sahai, H. and Kurshid, A. (1995) *Statistics in Epidemiology: Methods, Techniques, and Applications*. CRC Press, Boca Raton. (A comprehensive discussion of the variety of methods for deriving parameters in observational studies)
- Schlesselman, J.J. (1982) *Case-Control Studies: Design, Conduct, Analysis*. Oxford University Press, New York and Oxford
- Vandenbroucke, J.P., von Elm, E., Altman, D.G., Gøtzsche, P.C., Mulrow, C.D. et al. (2007) Strengthening the reporting of observational studies in epidemiology (STROBE): explanation and elaboration. *Annals of Internal Medicine*, 147, W163–W194

¹⁵ If the value of $\hat{z}_\alpha$ is positive, one minus the tabulated probability is used: negative values yield likelihoods less than 0.5, whereas positive values give likelihoods greater than 0.5, but Appendix XV only tabulates P for negative values.

16

Design considerations for observational studies

The most common observational study designs employed in epidemiological research were introduced in Chapter 15. This chapter discusses these study designs in more detail, and provides an overview of some less-commonly-used study designs.

The key theory underpinning the design of observational epidemiological studies is that disease experience in a population varies between sub-groups within that population, where sub-groups are defined on the basis of exposure to risk factors. This enables variation in disease experience and risk factor exposure to be explored, and **associations** between disease patterns and risk factor exposure to be assessed.

Observational studies differ from experimental studies in that the investigator is not free to allocate factors (disease and hypothesized risk factors) to individuals. The inability to control the circumstances of exposure in observational studies reduces the validity of these studies, compared with experimental studies. Whereas randomization of exposure is key to the validity of experimental studies, observational studies must rely on minimization of bias in selection of subjects through careful design and, where possible, by the use of appropriate analytical methods that correct for potential biases. Issues of validity in epidemiological studies are described in detail in Chapter 18, whereas issues specific to the main study types are discussed in this chapter.

There are two main categories of observational studies: **descriptive** and **analytical**. Descriptive studies aim to explore patterns and characteristics of disease and/or exposure, without formally assessing the relationship between them. In contrast, analytical studies explore hypotheses regarding the cause(s) of a disease.

Descriptive observational studies

Descriptive studies have no comparator group, but rather they observe and record outcomes (such as diseases) or exposures in order to describe their

characteristics or patterns. Descriptive studies may be important in the *generation* (rather than in the *testing*) of hypotheses or to evaluate the burden of disease or frequency of exposure. The main types of descriptive studies are case studies, case series and descriptive cross-sectional studies.

Many texts do not consider **case studies** and **case series** as epidemiological studies. These studies provide a description of aspects of a single or group of cases, respectively, and may include data from an entire region's register of cases (**population-based case series**). Even though these studies may describe exposures or interventions, they have no comparator group and provide weak empirical evidence of causation (see also *Table 17.6*). However, they may provide information useful for generating hypotheses, particularly where a condition is poorly understood. Case studies and series may describe the case(s) at a single point in time (such as at the point of diagnosis) or may take a longitudinal perspective and describe the case(s) over time (e.g., before, during and/or after diagnosis or treatment).

The objective of **descriptive cross-sectional studies** is to determine what proportion of a defined population of interest has a particular characteristic or characteristics. These studies are sometimes called **prevalence studies** to reflect their key role. Cross-sectional studies can be repeated to determine whether changes are occurring over time (for monitoring or surveillance: see Chapter 21). The best way to achieve representativeness (other than by selecting the entire population, which may be appropriate in some circumstances) is to sample randomly from the population, and more complex random sampling strategies, such as multistage sampling, may be appropriate (see Chapter 13). Where complex sampling strategies are used this should be taken into account during analysis. Appropriate selection of individuals is important to enable use of the results generated from the study sample to make inferences about the population of interest. Ideally, the population is geographically defined and may be limited to sub-groups

within a larger population. Key design issues are similar for descriptive and analytical cross-sectional studies and are described further in the section on analytical cross-sectional studies. Sample selection is discussed in more detail in Chapter 13 and issues of selection bias are discussed in Chapter 18.

Often, cross-sectional studies are conducted at a **fixed point in time**. For example, a study to determine the prevalence of mastitis on a dairy farm might sample from the entire milking herd on a single day. Sometimes, descriptive studies are conducted retrospectively or prospectively.

Descriptive cross-sectional studies (in comparison with analytical cross-sectional studies) do not make comparisons between sub-sets of the populations and hence do not explore the relationship between disease and exposure. They cannot be used to answer questions of causality. Only analytical studies should be used to address issues of causality. Also note that analytical studies can provide considerable descriptive data.

Analytical observational studies

The three main types of analytical observational studies are **cohort studies**, **case-control studies** and **analytical cross-sectional studies**. Additional study

designs and design variants, discussed later in this chapter, include ecological studies; case-case studies, time-series studies, nested case-control studies, case-cohort studies, case-crossover studies, case-time-control studies, before-and-after studies and case-chaos studies.

Design of cohort studies

The starting point of a cohort study is identification of sub-sets of a defined population that have been, currently are, or in the future may be, exposed to risk factors of interest (or to different levels of these risk factors). These individuals are then followed over time and the relative frequency of the outcome(s) of interest is assessed (*Figures 16.1, 16.2a and 16.3a*). Cohort studies are often referred to as **prospective** as they look forward from exposure to the development of disease. Many cohort studies are also prospective in the sense that they are initiated before the follow-up period commences, and therefore collect future data. Retrospective cohort studies rely on records, but nevertheless commence with identification of (historical) exposure groups and compare historical, current or future frequencies of the outcome of interest. The advantage of a prospective design is that data collection can be tailored to the needs of the study, whereas

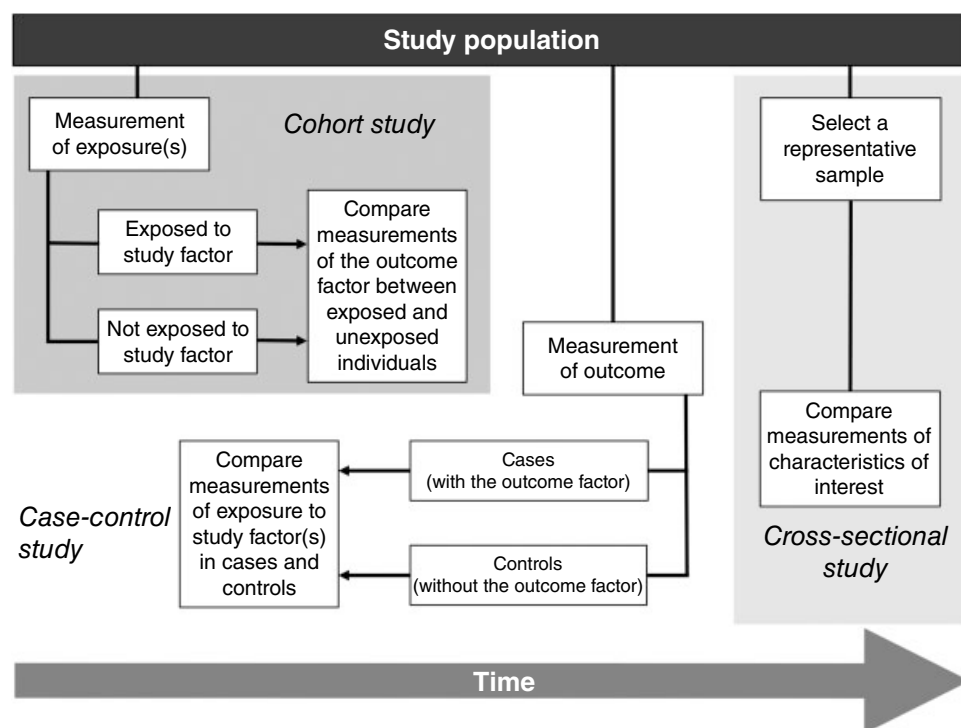


Fig. 16.1 Schematic representation of the processes of selection and measurement in cohort, case-control and cross-sectional studies.

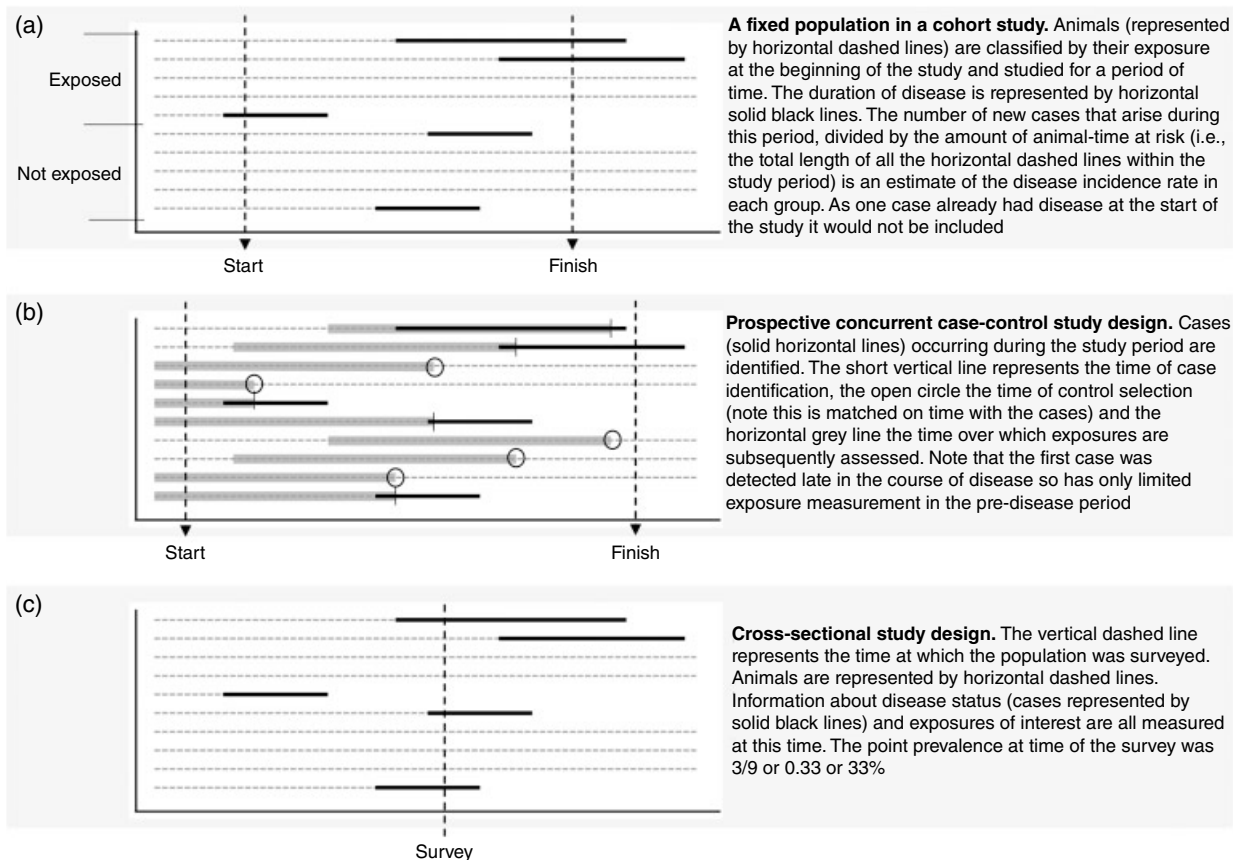
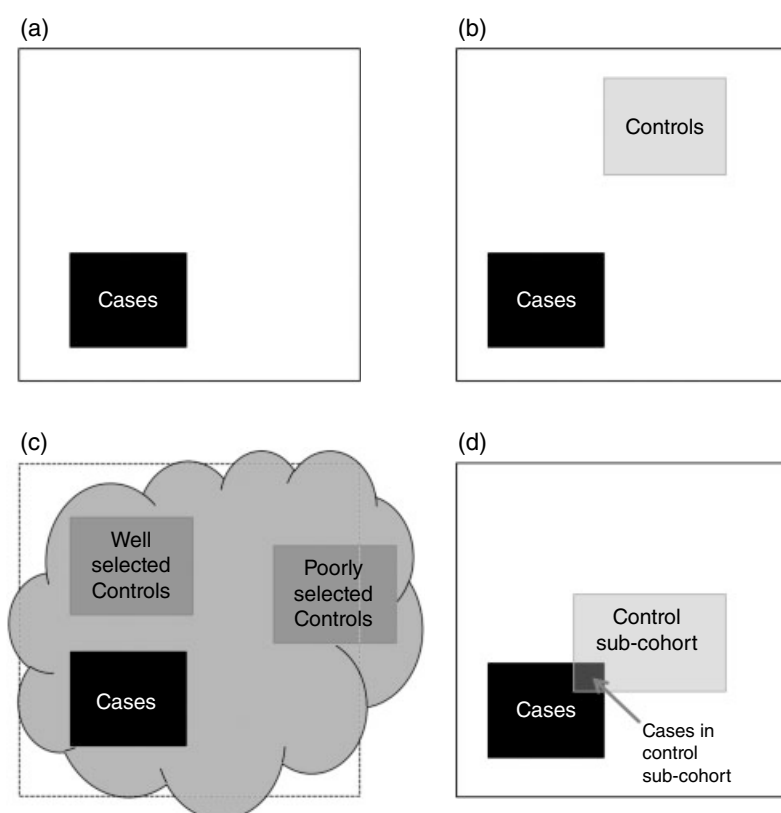


Fig. 16.2 Schematic representation of observations made on individuals selected for inclusion in (a) cohort, (b) case-control and (c) cross-sectional studies.

Fig. 16.3 Diagrammatic representations of cohort, nested case-control, case-control and case-cohort studies. (a) Cohort study, in which cases are identified from within the cohort. (b) Nested case-control study, in which cases are identified within the cohort and compared with controls selected from within the cohort. (c) Case-control study with a poorly-defined study base. A well-selected control set arises within the base population whereas a poorly-selected control set may arise (at least in part) from outside the base population of the controls. (d) Case-cohort study. (Adapted from Kulathinal *et al.*, 2007.)



a retrospective design relies on recorded or recalled information, which may be less complete and accurate. However, in a retrospective design some or all events have already occurred and conclusions often can be made more rapidly and for lower cost.

Cohort studies and intervention (experimental) studies (see Chapter 17) are longitudinal analytical studies in which all members of the study population are either exposed or unexposed to the putative risk factors being studied for some or all of the study period, and are followed over time to determine the outcome. This enables estimation of the disease incidence and confirms the temporal relationships between exposure and outcome. The critical difference between an intervention study and a cohort study is that, in an **intervention study**, a method of allocation (often random) is used to decide which individuals receive the intervention (e.g., a drug or husbandry method – i.e., the study factor) and which do not. In a cohort study, exposure to the study factor is not randomly allocated but, rather, natural exposure is measured at the start of, or during, the study for each subject.

In the simplest cohort study, disease-free animals would be classified into exposed and unexposed groups (cohorts) at the commencement of the study and would remain in those exposure groups throughout the study period (so-called **fixed cohorts**). Animals may leave the study through death (including slaughter), sale or loss to follow-up (e.g., ear tag loss), but no further animals are added to the study after the start date. **Closed cohorts** are fixed cohorts in which no animals are lost prior to the end of the study, except as a result of the outcome of interest. However, cohort study designs often demonstrate greater complexity. Often animals enter and leave the study at different times and many exposures are not permanent, resulting in an animal's exposure status changing during a study. The terms 'dynamic' and 'open' are used to describe such a population, in which individuals contribute varying amounts of time as exposed and unexposed. In studies based on dynamic populations, time at risk is the most appropriate denominator for calculations of incidence, as subjects contribute animal-time to the study until either the outcome event occurs, the study ends or they die, migrate or are lost to follow-up. The terms 'open cohort' or 'dynamic cohort' are best avoided as the term cohort implies a fixed register of individuals; the terms **open population** or **dynamic population** are preferred. Furthermore, many cohort studies begin by measuring numerous exposures. In this case, individuals' cohort membership cannot be defined by reference to a single exposure, and disease experience is compared with multiple exposures.

Sample-size calculation for cohort studies is discussed in Chapter 15.

Identifying the research question

A key step in any study is to identify the research question(s) of interest because this will guide the exposures and outcomes that need to be measured and help to determine the most suitable study design. For a cohort study, the appropriate research question is framed in terms of whether subjects with one or more exposures have different subsequent disease experience to those subjects without these exposures. Ideally, the research question is framed as a clearly-defined hypothesis (or series of hypotheses) to be tested. For example, a research question might be: 'Are cattle that are fed silage more likely to develop listeriosis than those not fed silage?' The null hypothesis would be that there is no difference in the incidence rates of listeriosis in cattle fed silage compared with those not fed silage. This hypothesis may have been generated from information from other observational studies (e.g., evidence from cross-sectional surveys) and strengthened by other sources of information (e.g., knowledge that silage contains *Listeria* spp.). This example will be used in this section to illustrate further aspects of the design and analysis of a cohort study.

Selection of study subjects

The study population should include both exposed and unexposed individuals. The exposure could be either present or absent (fed silage/not fed silage) or degrees of exposure (fed silage all year round/fed for part of the year/never fed silage). Often, cohort study subjects are selected (randomly) from the population and exposure measurements made on this sub-group. In such cases it may not be possible (or desirable) to divide study subjects into exposed and unexposed groups, but rather each individual is classified according to its status for a number of exposures. However, separate exposure cohorts may be selected at the outset, particularly if the study is exploring a specific hypothesis. Careful selection of exposure groups will allow the investigation of rare exposures. For example, if only a small proportion of the population were exposed, and individuals were selected at random, the sample would contain only a few exposed individuals. However, in this situation, selecting a larger proportion (larger sampling fraction) of those exposed, and comparing them with a relatively small proportion of those unexposed, will improve the statistical power of the study. This is sometimes referred to as **boosted sampling** (also referred to as **oversampling**).

In many cohort studies selection of study subjects relies to some extent on purposive sampling (see

Chapter 13). It is important that the selection of exposed and unexposed individuals is not biased by disease or exposure status. For example, it would be wrong to select the exposed group solely from farms that have reported cases of listeriosis in the recent past; this could produce a spurious association between exposure and disease. One way of selecting exposed and unexposed individuals would be to compile a comprehensive list of all the cattle within a defined area and divide them into cattle that were fed silage and cattle that were not fed silage. The next step would be to obtain a random sample of exposed and unexposed cattle from each group (the sample size would be determined by power-based sample-size calculations) and then to follow each group of animals over time (e.g., a period of one year).

Exposure measurement

Choice of exposure variables needs to be carefully considered in light of the research question and relevant knowledge, as choices may affect validity and interpretation of study results. Different measurement instruments may vary in terms of reliability and validity and in terms of their underlying meaning. Observations made by owners may differ in reliability and validity to related measurements made directly on animals. For example, owner estimates of an animal's weight may be less accurate than measurements made using calibrated scales. The meaning afforded by each measurement must also be considered. A study addressing the research question 'Is pathogen X associated with disease Y?' could consider measuring the risk factor (pathogen X) by ELISA, polymerase chain reaction, culture and so on. However, these measurement tools consider different components of exposure (host response, pathogen antigen, pathogen genetic material, viable pathogen) and results based on different forms of measurement may require different interpretations.

Measurement of exposures may be extremely difficult. Assessment of exposure is made more difficult where exposure is not fixed and where the distinction between exposure and non-exposure is not well defined. Examples of fixed and well-defined exposures may include sex and breed. However, even these may not be straightforward. Depending on how it is defined, an animal may change sex category from male to neutered male during a follow-up period. Furthermore, variants such as freemartins may complicate definition of categories. Although an animal's ancestry and hence its breed/genetics will not change over time, 'breed' may be difficult to categorize when crossbreeding and uncontrolled breeding is common.

Many exposures of interest in cohort studies could be expected to vary over time (so called **time-varying**

exposures) and this adds complexity to their measurement and to analysis of the study. Continuing the example from before, how could exposure to silage be defined? It could simply be defined as having at least some access to silage, or not (that is, a binary exposure). If so, a cow that spent one day in a loose yard in which silage was available would be in the same exposure category as a cow that was fed a diet consisting entirely of silage, *ad libitum*, for a year. Some level of quantification of exposure could be used, through measurement either as an ordinal or continuous variable. Ordinal categories could be defined as so to reflect the length of time over which animals were exposed to silage, or another measure of the quantity fed.

Continuous exposure data may be summarized in numerous ways. For example, exposure to silage could be measured as the total weight or volume consumed during the study period. This could be analysed as a continuous variable, but also may be categorized into an ordinal variable. In many cases, other summary measures are used, such as the average exposure per unit time; for example, weather information may be defined in terms of the average daily temperature and so on. Alternatively, maximum, minimum, median or other summary measures may be used. Continuous exposure data may be manipulated in other ways for analysis, such as to measure the total time where the level of exposure was above (or below) a particular level.

In addition to the presence or intensity of exposure, the timing of exposure(s) may be important when considering time-varying exposures. For example, if intake of colostrum is the exposure of interest, it is important to know the timing of consumption/administration relative to the time of birth. Similarly, the time of vaccination relative to the waning of maternal antibodies may affect the efficacy of this exposure.

As measurement of exposure is complex, decisions regarding the most appropriate measures need to be made following careful consideration of the research question and current understanding of the topic.

Outcome measurement

One of the major advantages of cohort studies is that they offer the opportunity to examine more than one outcome (this is in contrast to case-control studies where only one outcome can be investigated, albeit with multiple exposures). In our example we have chosen only one: listeriosis. However, we could have decided to record a number of different outcomes; for example, the occurrence of metabolic diseases or lameness. Care must be taken, however, as increasing the number of outcome variables will increase the likelihood of finding a statistically significant association

between an exposure and an outcome by chance (i.e., increasing the Type I error rate due to multiple testing; see Chapter 14).

Whatever the outcome, it must be clearly defined and the method of ascertainment should be identical for exposed and non-exposed individuals. For example, cases of listeriosis should have a high probability of being detected (i.e., high sensitivity; see Chapter 20), and there should be few disease-free cattle that are incorrectly classified as having the disease (i.e., high specificity). Furthermore, the frequency with which exposed and non-exposed groups are examined for evidence of disease should be identical. The greater the validity and reliability of the method of identifying cases, the lower the chance of introducing bias into the study. Blinding during outcome assessment is helpful to reduce the risk of inadvertent measurement bias associated with knowledge of exposure status.

In addition to ascertainment of the occurrence of the outcome, measurement of its timing may also be important. However, accurately measuring the time the outcome occurs can be difficult if disease onset is insidious. Furthermore, if the study is actually a panel study (see later in this chapter), the interval between repeat measurements will influence the accuracy of timing of outcome occurrence. The timing of loss to follow-up and censoring (see Chapter 4) may be similarly difficult to assess. An alternative to accurately measuring the timing of an outcome is to determine whether or not it has occurred by one or more specified times. Although this approach offers practical advantages there is a cost associated with loss of detail.

Follow-up

The duration of follow-up may vary considerably between cohort studies. Many cohort studies have follow-up periods that last months or years. For example, van Hagen and others (2005) investigated risk factors for hip dysplasia in boxers by recruiting purebred puppies at birth and following them for seven to eight years. In contrast, a study by Pinchbeck and colleagues (2004a) investigating risk factors for falls during horse racing, recruited horses on race day; the follow-up period was from the time of recruitment to the end of the race; that is, only a few hours. Prolonged follow-up periods increase the cost of cohort studies and may cause logistic problems. In prospective studies subjects may be lost to follow-up if owners/keepers can no longer be contacted or choose not to continue participating in the study. In retrospective cohort studies, records may be difficult to locate. Random loss to follow-up will reduce the precision of the study. Non-random loss to follow-up may induce selection bias

and hence reduce the validity of the study results (see next section and Chapter 18).

Bias in cohort studies

Selection and information bias can occur in cohort studies for many reasons and bias is discussed in more detail in Chapter 18. **Selection biases** occur when those subjects included in the analysis do not adequately represent the target population. Cohort studies may be particularly susceptible to certain types of selection bias, including: **biased selection of cohort members**, **loss to follow-up bias** and **non-response bias**. In addition, the **healthy-worker effect** (see Chapter 18) can induce bias. Ideally, the exposed subjects included in a cohort study should be similar in all important respects to the unexposed subjects, apart from the exposure(s). Bias will occur if factors associated with the probability of selection are also associated with the risk of exposure and of the outcome. This is a major disadvantage of cohort studies compared with randomized clinical trials (see Chapter 17) for evaluation of interventions. For example, in a cohort study investigating the efficacy of a new drug compared with an existing drug, allocation of the drugs is non-random and may be influenced by many factors. Bias will occur if clinicians favour either the existing or new drug for more severe cases, as exposure status (whether or not the animal receives the new drug) is related to the chance of the outcome (which may be defined as resolution of disease, survival or in other ways). Whilst such biases are often called selection biases, they actually result from confounding (see Chapters 15 and 18); if disease severity could be adequately measured, the effect on the association between the drugs and outcome could be controlled for by design or during analysis.

Information biases may arise when measurements on study subjects are made with error and may be particularly problematic when there is differential measurement error. Several features of cohort studies may influence the occurrence of bias. Error in measurement of exposure at the commencement of the study is unlikely to be influenced by outcome status, as the outcome has not yet occurred. However, knowledge of exposure status may lead to differential misclassification of outcome status, resulting in **outcome identification bias**. For example, subjects known to be exposed may receive more intensive follow-up, have test results differently interpreted (**diagnostic suspicion bias**), be questioned differently in an interview (**interviewer bias**) or answer differently (**responder bias**). Similarly, subsequent measurements of time-varying exposures may be biased by knowledge of previous exposure status.

Blinding of observers and/or study subjects to exposure status can minimize this effect, but may not always be possible.

As discussed in Chapters 15 and 18, a **confounding** variable is one that is related to the exposure and independently affects the risk of disease. For example, if certain age groups of cattle were more likely to be fed silage, and they were independently predisposed to listeriosis, then this effect clearly needs to be recognized and controlled for through design or analysis.

The need to measure and control confounders in this way underlines a major difference between observational studies and intervention studies. In an intervention study, the exposure can be randomly allocated to individuals in the study population. This randomization helps to ensure that the only non-random difference between the exposed and non-exposed groups is their exposure status. The corollary of this is that there should not be an association between the exposure and other confounding variables and is an example of controlling for confounding by design. (Note, however, that this is only true in the long run, over many such trials. Confounding variables may be unevenly distributed between treatment groups within a single trial, despite random allocation of an intervention.) In contrast, control of confounding in observational studies requires information about the confounding variables and it is important, therefore, to identify potential confounders at the start of the study and to record them accurately.

Where likely confounders can be identified at the outset, design considerations can be used to control confounding in cohort studies. **Restriction** may be used to limit selection of animals to only one level of a potential confounding variable. For example, only animals of a particular breed, age group or sex may be included in the study. **Matching** may be used to ensure equality between the exposed and unexposed groups with regard to the potential confounding variables. For example, for each exposed male animal, an unexposed male would be selected. The use of matching requires clearly-defined exposure categories. Restriction may be used in a study based on a dynamic population, but may affect generalizability of the results beyond the categories of animals included in the study. Provided important information on potential confounders is accurately measured and recorded, analytical methods can be used to control for their effects, for example by multivariable regression analysis (see Chapter 22). Each of these methods requires careful consideration of issues of confounding and selection during the design phase of the study.

Analysis and interpretation of cohort studies

The results can be analysed using either the incidence risk (calculated if subjects are the unit of the denominator) or incidence rate (calculated if animal-time is the unit of the denominator) of disease in the exposed and unexposed groups. Comparison of these will produce either relative risks (risk ratios) or relative rates (rate ratios) (see Chapter 15). If the exposure had more than one category then the risks or rates for each category could be compared, allowing for trends with increasing exposure to be evaluated (*Box 16.1*).

The situation becomes more complicated when there are several confounding factors, especially if they have several categories or are measured on a continuous scale. In such circumstances, statistical modelling techniques such as multivariable logistic regression, Poisson regression or proportional hazards regression may be indicated. Approaches to statistical modelling are discussed in Chapter 22.

Another problem may arise if the original sample was clustered into groups. Using the example of listeriosis in cattle, it may be impossible to get a list of individual cattle, subdivided by exposure, from which to draw up a simple random sample. Instead, it may only be possible to obtain lists of farms, some of which feed silage. In such cases, the farm may be used as the sampling unit, and all animals within the farm may be included in the study. The individual animals in such a sample are therefore clustered into groups and no longer statistically independent of one another. Such a lack of independence should be taken account of in the analysis (see Chapter 22).

Having estimated the relationship between the exposure and the disease in question, this needs to be interpreted. The first issue is that of generalizability; does the estimate of effect apply to the target population? In our example, can we assume that all cattle in the country that eat silage are 1.5 times more likely to develop listeriosis? If the study population was chosen at random from the whole population of cattle in the country, and the effects of biases can be reasonably expected to be minimal, then it may be reasonable to assume that this estimate can be generalized to the rest of the country. However, if the cattle were chosen from a small geographical area, it may be unreasonable to generalize beyond a more localized population. There also may be variables that are effect modifiers (see Chapter 18). For example, the risk ratio for the relationship between silage and listeriosis may vary according to breed.

Problems with interpretation also may arise if confounding variables are poorly defined, inaccurately measured or not measured at all. In our example, we classified cattle into two age categories (0–3 and >3 years old). It is likely that both the risk of listeriosis

Box 16.1 Example of analysis of cohort studies

Consider a study that included 2000 cattle that were exposed to silage during a period of one year and compared these with 2000 cattle not exposed to silage during the same period, and which generated the following results:

	Exposure status	
	Fed silage	Not fed silage
Number of incident cases of listeriosis	75	25
Number of cattle	2000	2000
Incidence risk of listeriosis	0.0375	0.0125

The relative risk would therefore be $75/2000 \div 25/2000 = 0.0375/0.0125 = 3.0$.

Alternatively, the total length of time that each cow was at risk (of becoming an incident case of listeriosis) during the study period may be summed to produce an estimate of the amount of cow-time contributed by exposed and non-exposed cattle. Each cow would have been at risk of becoming an incident case until either it developed the disease, died, left the herd or was lost to follow-up (e.g., identification tag loss) or it was the end of the study period. In this case, the following results may be obtained:

	Exposure status	
	Fed silage	Not fed silage
Number of incident cases of listeriosis	75	25
Number of cow-years at risk	1250	1600
Incidence rate of listeriosis/cow-year	0.06	0.0156

The relative rate would therefore be $75/1250 \div 25/1600 = 0.06/0.0156 = 3.84$.

In order to control for the possible confounding effect of age, the relationship between silage and disease could be stratified by age group. For example, if there were just two age-group categories: 0–3 and >3 years old, and younger cows were not only more likely to be fed silage, but also more likely to contract listeriosis, the following results may be obtained after stratification and evaluating the risk ratios for each stratum:

Stratum 1: 0–3 years old

	Exposure status	
	Fed silage	Not fed silage
Number of incident cases of listeriosis	70	15
Number of cattle	1500	500
Incidence risk of listeriosis	0.0467	0.03

Relative risk = $0.0467/0.03 = 1.56$.

Stratum 2: >3 years old

	Exposure status	
	Fed silage	Not fed silage
Number of incident cases of listeriosis	5	10
Number of cattle	500	1500
Incidence risk of listeriosis	0.01	0.0067

Relative risk = $0.01 / 0.0067 = 1.50$

Without allowing for the confounding effect of age it appeared that cattle exposed to silage were three times more likely to develop listeriosis. However, this relationship was distorted by the fact that younger cattle were more likely to eat silage. Examination of each stratum shows that compared with animals of the same age group, eating silage increased the risk of developing listeriosis by a factor of 1.5.

and the exposure to silage will vary within these two age categories (e.g., a one-year-old may have a significantly higher risk than a two-year-old). Failure adequately to account for confounding variables may result in residual confounding and therefore produce a biased result.

Design of case-control studies

In a case-control study, individuals are initially classified by outcome status (i.e., case or control) and then

past exposure is measured for each group (see *Figures 16.1 and 16.2b*). Hence, case-control studies differ from intervention or cohort studies, in which animals are initially classified by exposure status, following which disease is measured in each group. Case-control studies are well suited to investigation of rare diseases, but may also be applied to the study of common diseases. Where the disease is common, case-control studies may have more limited benefits compared with cohort studies in terms of the sample size required for

a given precision, but may still have advantages in terms of ethical considerations, as disease has already occurred.

Case-control studies are often relatively quick and cheap to perform, but they are prone to bias from many sources. They are, however, extremely useful for identifying exposures that occur more or less commonly in either cases or controls and thereby in identifying potential risk factors for disease. This type of study is not suitable for the estimation of either prevalence or incidence, as only a portion of the non-diseased (control) population is sampled.

In a case-control study, the cases should be a representative sample of all animals that became diseased in the population under study, during the period of investigation. Likewise, the controls should be a representative sample of the population from which the cases arose. The sampling fractions often differ for cases and controls (e.g., all of the cases in a population may be selected and compared with a small sample of all possible controls). However, it is crucial that both cases and controls are sampled without regard to their exposure status, otherwise bias will be introduced into the study. A suitable control group is best thought of as a sample (ideally random) of the population from which the cases came.

Vandenbroucke and Pierce (2012) provide an overview of case-control studies; sample-size calculation for such studies is discussed in Chapter 15.

Identifying the research question

The essential elements of conducting a case-control study are similar to those of a cohort study. Attention should be paid to identifying the purpose of the study; will the study explore a range of potential risk factors or concentrate on a specific, well-defined hypothesis? Great care should be taken to ensure that disease and exposure variables are clearly defined and potential confounders are identified and measured accurately. The analysis should take into account the role of confounding variables, and the interpretation of the results should allow for all potential sources of bias.

For example, we can investigate the relationship between silage and listeriosis using a case-control design. The initial null hypothesis would be that there is no difference in the prevalence of exposure to silage for animals with listeriosis (cases) compared with animals without listeriosis (controls). Instead of classifying cattle by exposure to silage and evaluating the occurrence of disease in the two groups, we would identify cattle with listeriosis (cases) and compare their exposure to silage with the exposure status of a group of disease-free cattle (controls).

Defining the study population

Where a study is conducted in a clearly-defined population, particularly one with an accurate sampling frame, and the cases and controls included in the study are representative of all the cases and non-cases in the underlying population, the study is referred to as a **primary-base** or **population-based study**. Such studies are most feasible when a population register exists, such as may occur for case-control studies conducted on farms, where cases are obtained from all the cases arising on the participating farms, and the controls are randomly selected from these farms.

In contrast, in many situations it may be difficult to define precisely the underlying population from which the cases are identified. A common instance where case-controls studies may have a poorly-defined study base is when the cases are selected from admissions to hospitals, particularly referral hospitals. Such cases may be a poorly-defined sub-set of cases occurring over a wide geographical area, with a range of factors affecting the probability that the case will be presented to the hospital. In such situations it may be very difficult to define the underlying study population, which may be referred to as a **secondary-base** or **secondary-source population**. Studies using a secondary base provide weaker evidence than those with a primary base. Hillyer and others (2002) utilized a two-step approach to try to select relatively unbiased controls from a secondary base. They undertook a case-control study of simple colonic obstruction and distension colic in horses, with cases being identified from two referral hospitals. Controls were selected by randomly selecting owners/carers who had brought a horse to the same hospitals in the previous year. These people were then contacted and a horse was randomly selected from among the horses in their care. However, as a final check, a horse so selected was excluded as a control if the owner indicated it 'would not be taken to the Veterinary School as a referral for colic if it was suggested by the attending veterinary surgeon'.

Comparability principles

Wacholder and others (1992a) define three key principles that should underpin decisions about selection of controls: study base, comparable accuracy and deconfounding.

Study-base principle This principle states that the control group should be representative of the population from which the cases arose. Hence, if a primary base is used, the controls should be representative of this well-defined population. However, if a secondary base is used it may be difficult to determine the extent to which the selected controls accurately reflect the

exposure experience of the population from which the cases arise. For the study-base principle to apply, controls must be selected from the same underlying population as the cases, and exposure status should not affect the probability of selection.

Comparable accuracy principle This asserts that the information obtained about cases and controls be equally reliable, so that any bias that does occur is non-differential. This may be difficult to achieve in case-controls studies. For example, in studies that rely on recall of exposure, a knowledge of case or control status may affect recollection.

Deconfounding principle This underpins the use of control selection to limit the effect of confounding variables. This may be achieved through methods such as matching (see later).

Selection of cases

Prior to case selection, it is important that a case definition be clearly articulated. Case definitions should be as precise as possible with a clear policy for exclusion and inclusion. Several factors should be considered when developing a case definition. The ideal case definition would result in correct identification of all true cases and the exclusion of all non-cases (i.e., with 100% sensitivity and specificity). Whilst this may be straightforward in some circumstances, multiple case definitions could be considered in many situations. Narrow case definitions may result in some true cases being classified as non-cases, whereas more inclusive definitions may result in misclassification of non-cases. Furthermore, narrow case definitions may lead to the study results only being applicable to a sub-set of all cases of a disease. Whatever the case definition selected, it should be able to be equally applied to all cases in the study population.

Ideally, only incident cases should be used in case-control studies. When using prevalent cases, exposures may be related both to the rate at which disease occurs and survival time, resulting in biased selection of cases with (or without) specific exposures. In addition, the temporal relationship (cause or effect) between exposure and disease may be more difficult to determine if prevalent cases are used. The problems of disentangling these effects mean that incident cases are preferred to prevalent cases.

Cases should be recruited into the study independently of their exposure status. Ideally all cases (or a random sub-set of all cases) in the study population would be included in the study. However, in reality, some cases may be less likely to be included in the study, perhaps because they are not detected, or because owners

decline to participate. For example, if farmers were aware of possible links between silage and listeriosis, they may be more likely to report cases of listeriosis from cattle exposed to silage. If the cases for the case-control study were selected in this way, there will be a clear bias towards cases with the exposure. This should be avoided by ensuring that cases are chosen without reference to their exposure.

Selection of controls

Selection of appropriate controls is a fundamental goal in the design of case-control studies and relies on identification of the appropriate underlying study population.

There are three sampling schemes for controls in case-control studies (Rodrigues and Kirkwood, 1990). The choice of sampling scheme determines the appropriate measures of association (relative risk, incidence rate ratio or odds ratio: see Chapter 15) and can have practical implications (*Box 16.2*).

The **traditional** or **exclusive design** (now referred to as **cumulative sampling**; Pearce, 2012) is the archetypal case-control study. In this design, controls are selected from among those individuals still at risk in the study population at the end of the study (i.e., after all the cases have been identified). In studies utilizing a traditional design, the ratio of the odds of exposure in the cases to that in the controls is equivalent to the **odds ratio** of disease of exposed to non-exposed animals.

In a **concurrent design** (now referred to as **density sampling**; Pearce, 2012) controls are sampled from the population still at risk at the time the case was diagnosed; that is, cases are matched to one or more controls at the time of case identification. In some cases, this time matching may be very precise. For example, Parkin and others (2005) matched case horses (those that suffered fatal metacarpal or metatarsal condylar fractures during a race) with controls selected from among the other horses in the same race. In contrast to the exclusive design, controls may become cases at a later date (indeed, if such controls are excluded this study design reverts to the traditional design). If the analysis accounts for the matching on time (and it should, particularly when exposure varies in time), the odds ratio of exposure of cases to controls is an unbiased estimate of the incidence rate ratio of disease of exposed to non-exposed animals (provided that the rate of developing disease does not change over time in either the exposed or unexposed animals).

For the **inclusive design**, controls are sampled at defined time points throughout the study from all non-case individuals in the population, irrespective of whether or not they have been, or later become,

Box 16.2 Understanding case-control studies

If we consider the cases and controls as a proportion of all possible cases and controls in a population then¹:

p_1 = proportion of all cases,

p_0 = proportion of all controls.

These are the **sampling fractions** for cases and controls. If we conduct a **traditional** or **exclusive** case-control study and consider one exposure where:

a = the total number of **exposed cases** in the population,

b = the total number of **unexposed cases** in the population,

c = the total number of **exposed controls** in the population,

d = the total number of **unexposed controls** in the population,

we have:

	<i>Exposed</i>	<i>Unexposed</i>
Cases	p_1a	p_1b
Controls	p_0c	p_0d
<i>Totals</i>	$p_1a + p_0c$	$p_1b + p_0d$

The proportion of exposed animals that are cases is $p_1a/(p_1a + p_0c)$ which, unless $p_1 = p_0$, will not equal the prevalence or **risk** of disease amongst exposed animals,

¹ In cross-sectional and longitudinal studies $p_1 = p_0$, as the sampling fraction is the same for both diseased and non-diseased animals.

namely $a/(a + c)$. The same applies for the proportion of non-exposed animals that are cases. This explains why prevalence cannot be directly measured from a case-control study (unless the sampling fractions are known). It also explains why, with a **traditional design**, the case-control estimate of risk ratio would not equal the population risk ratio: $[a/(a + c)]/[b/(b + d)]$.

However the case-control estimate of **odds ratio**:

$$(p_1a/p_0c)/(p_1b/p_0d)$$

$$\text{or } (p_1a \times p_0d)/(p_1b \times p_0c)$$

does equal the population odds ratio $(a \times d)/(b \times c)$ because p_1 and p_0 cancel each other out.

Hence, provided p_1 and p_0 are not biased by exposure status (i.e., they estimate the true prevalence of exposure among cases and controls), the case-control estimate of odds ratio will be a good estimate of the population odds ratio.

For an inclusive design

The estimate of case-control odds ratio will be:

$$[p_1a/p_0(c + a)]/[p_1b/p_0(d + b)],$$

which is equivalent to the risk ratio because p_1 and p_0 cancel each other out, leaving:

$$[a/(a + c)]/[b/(b + d)].$$

For a concurrent design

In a concurrent design the cases and controls are matched on time so a **matched analysis** must be performed. Details of how this design can produce an unbiased estimate of the **rate ratio** are given in the appendix of the paper by Rodrigues and Kirkwood (1990), although there is an error in the table (the numerators for the non-exposed cases and controls should be $N_U(t)\lambda_U(t)$ and $N_U(t)$, respectively).

cases. In a closed population this is equivalent to sampling from all individuals in the population at the start of the study (this results in a case-cohort study, which is further discussed later). The odds ratio of exposure of cases to controls is an estimate of the relative risk of disease of exposed to non-exposed animals.

Choice of control sampling scheme may be based on several considerations, including disease-related factors such as the rarity of the disease, whether or not the disease is recurrent or non-recurrent, and whether or not the exposed group is at uniform risk of disease (Rodrigues and Kirkwood, 1990).

The *methods used to select controls* (regardless of the sampling scheme adopted) often depends on whether the study has a primary or secondary base (Gail, 2005).

When sampling from a primary base with a complete sampling frame, **population controls** can be selected at random from the list of study subjects. More complex sampling strategies may be used, such as multi-stage sampling, but this sampling structure should be taken into account in the analyses. This approach may be used for case-control studies using animals in managed settings where records are accurate (such as often occurs for farms, catteries, kennels, etc.). Where controls are selected at random from the study frame, it is important that all cases also be in the sampling frame, and that cases that are not listed are excluded from the study. A major advantage of this approach is that it provides greater confidence that the study-base principle has been met. In practice,

sampling from the study population may sometimes be less convenient than sampling from more readily available animals, such as location-matched animals. Also, population controls (or owners of population controls) may be less motivated to participate and may have greater recall bias, compared with other types of controls.

For studies with a primary base but without a complete sampling frame, alternative selection procedures may be required in order to select controls from the study population. Although not ideal, this situation still benefits from detailed information about the study base, such as its geographical range. In contrast, for studies reliant on a secondary study base, it may be difficult to determine the criteria on which the study population can be defined. Methods of control selection may be adopted in each of these situations, but are more limited and more prone to bias where a secondary base is used.

Neighbourhood controls are selected on the basis of proximity to cases, and are therefore matched on location. Hence, neighbouring farms may be used as controls, as may animals kept in adjacent cages, pens or stables. In studies with a secondary base, it may be less clear that neighbourhood controls would have been identified as cases had they developed the disease of interest. This approach may result in overmatching and may introduce bias.

Random digit dialling (see Chapter 13) has not been widely used in veterinary case-control studies. If this approach is used, cases should only be included if their owners have a telephone. Bias may occur due to a range of factors, such as variation in telephone ownership and variable time spent at the location of the phone. Increasing use of mobile phones, which tend not to be listed, and other advances in communication may increase such problems.

Hospital-based case-control studies are a common type of study utilizing a secondary-base population, but may also be used in primary-base studies, particularly those without a comprehensive sampling frame. In these studies controls are selected from animals registered at the hospital, but which were seen for different reasons; as such, these could be called **case-other disease studies** (see later). Similar approaches may be used for cases identified through laboratories or other disease registries. **Hospital-based controls** are most appropriate when cases and controls would have been seen at the hospital if they had had either the case or control conditions and when the condition used to define the controls is not associated with the exposure of interest. If both these criteria are true, then the exposure experience of the hospital-based controls is representative of that in the study population.

However, in many situations, these criteria may not be met. For example, many animals are presented to veterinary hospitals because of conditions associated with either young or old age, or with certain breeds. Selection of controls with these conditions would result in a different age or breed distribution to that in the underlying population, resulting in Berkson's bias (see Chapter 18). For example, Doherr and others (1997) used three different diseases (colic, cuts and fractures) as sources of controls in a case-control study of *Corynebacterium pseudotuberculosis* abscesses in horses. They found that these control groups were poorly representative of the study population with regard to numerous potential risk factors. Similarly, cats selected from veterinary hospital appointment lists have important differences to random samples of cats from the same hospital's patient register (Murray *et al.*, 2012), and cats registered with a veterinary practice differ to cats not registered (Murray and Gruffydd-Jones, 2011). Which of these groups (general population, registered or hospital-visiting animals), if any, would be the most appropriate controls for cases selected from a hospital would depend on a number of factors, including issues to do with case ascertainment.

Friend controls may be selected from a list of friends or other known people obtained from the case. For example, a farmer or dog owner may be asked to name other farmers or dog owners, respectively. Such lists may be easily generated (although ethical concerns may apply) and this approach may help to match socio-economic backgrounds. However, this approach is unlikely to generate controls that meet the study-base principle and is best avoided.

Historical controls are selected from a time period prior to that in which the cases occur. The use of historical controls assumes that, other than being temporally separated, the control population is representative of the cases. This in turn requires a range of assumptions, including that the underlying exposure experience of the study population has not changed, that the methods of measurement have not changed, and that the population from which the controls arose is equivalent to the population base for the cases. As a general rule, this type of control group should be avoided.

It is possible to choose more than one control group for a case-control study. This may be done if the different control groups enable control of different potential confounders (either through design, or at the analysis stage). Alternatively, additional control groups may be used when there is uncertainty as to the most appropriate method of control selection. Whilst this approach may provide reassurance when the results using each control group are similar, interpretation of the study is more difficult when the

results are discordant. Furthermore, similar exposure between the different control groups may simply reflect similarities in bias. Some authors (Wacholder *et al.*, 1992b; Dohoo *et al.*, 2003) have recommended against this approach because of the difficulty in the interpretation of conflicting results, and recommend making a decision as to the most appropriate control group and to use that as the source of controls. Where there is doubt about the choice of control group, this should be fully documented alongside the results. Furthermore, the use of another plausible control group, and presentation of discordant results, may serve to emphasize the potential impacts of these uncertainties in a study and hence may aid communication of the potential impact of biased control selection.

Matching

Although random selection of controls from the study population is the simplest method of control selection (where it is possible), controls also may be matched with cases on the basis of one or more variables (see Chapter 15). Matching has advantages and disadvantages, and the decision to use matched or unmatched controls should be made after careful consideration. Controls can be matched to the cases by known confounding variables (e.g., sex or age). Matching may also be undertaken on the basis of **time** (for example, for comparability where exposure varies over time or where concurrent control selection is used) and for **pragmatic** reasons; for example, it may be simpler and less time consuming to match cases and controls by location (e.g., farm). An important motivation to use matching is that it can increase study power and facilitate identification of effect modification (see Chapters 15 and 18), for example, by ensuring sufficient numbers of exposed controls where exposure is rare in the population.

Disadvantages of matching include: additional cost and difficulty associated with identifying suitable controls; exclusion of cases for which suitable controls cannot be found; and loss of the ability to assess the effect of the matched variable. Therefore, controls should never be matched on variables of interest, although effect modification due to matching variables can still be assessed. Matching should be considered for variables that are not of interest in themselves, but that may confound the relationship between the exposure of interest and the outcome.

Some variables, such as location, may act as proxies for underlying variables that may be difficult to measure. For example, matching cases and controls by farm of origin may help to control for many unknown and unmeasurable confounders associated with, for example, management practices and local weather and

ecology. However, care must be taken to avoid **over-matching** when cases and controls are so similar in their exposure status that important differences become undetectable. This would occur if cases and controls were matched on a variable that was closely associated with exposure, but not disease (other than through their association with the exposure in question). Matching on a variable that is on the causal pathway may also introduce bias.

There are two basic types of matching used in case-control studies. **Frequency**, or **stratum**, matching is where the cases and controls are selected to produce a similar distribution of a matching variable in both groups. For example, if 70% of cases of listeriosis are more than three years old, the controls are selected to produce a sample with a similar age distribution. **Individual** or **pairwise** matching is where each case is matched with a control that is identical with respect to the matching variable.

Matching is a way of stratifying the data by known confounding variables. Controls are selected to be similar to cases with respect to these variables, and hence, if these variables are truly confounders, the matching variables and the exposure variable will be associated (Rothman and Greenland, 1998). A matched case-control study design requires a stratified or matched analysis because the exposure frequency in the controls will be systematically different to that of the underlying population and, consequently, matching has introduced a selection bias. This occurs whether or not the matching variable is associated with the outcome.

Case-control studies and rare diseases

Although this type of study design is suitable for common diseases, it is particularly useful for rare diseases. Generally, the sample size for a case-control study is lower than the equivalent sample size for a cross-sectional or cohort study aimed at addressing the same epidemiological question, and this difference becomes greater for rarer diseases. The most economical ratio of case to control is 1:1; however the power of a case-control study can be considerably increased by choosing more controls per case (although the advantage per extra control animal diminishes considerably beyond four controls per case). This is particularly useful if only small numbers of cases can be ascertained.

Case-control studies and common diseases

Although case-control studies can be used to study common diseases, care is required, particularly for common infectious diseases for which, as a result of high levels of exposure, there is a high level of immunity in the population. For example, new cases of

Campylobacter enteritis are more likely to arise in the sub-set of non-immune people who do not have a history of exposure (Havelaar *et al.*, 2009). Hence both cases and controls will tend to be similar in terms of exposure to risk factors, reducing the measure of effect towards the null.

Bias in case-control studies

For case-control studies the biases of most concern are those that lead to violation of the study-base principle or the comparable accuracy principle. Appropriate selection of controls is required in order to fulfil the study-base principle. In many case-control studies, control selection is problematic and researchers must make decisions as to the most appropriate methods for control selection. This process is more difficult in situations in which less is known about the study base, and the potential for **non-representative selection of controls** is a major disadvantage of case-control studies. Loss of eligible cases or controls may result in **non-response bias** if exposure is systematically different among those included in the study, compared with those that are not.

As case status is known at the time of case and control recruitment, differential measurement biases (so-called **exposure suspicion biases**) such as differential recall bias may invalidate the principle of comparable accuracy. Compared with control owners, who may have no particular interest in the disease under study, case owners may invest greater time and effort considering reasons for their animal's illness. Case owners may also erroneously recall exposures they believe increase the risk of disease or may act to deflect perceived responsibility from themselves. Differential recall bias may be difficult to prevent in some case-control studies; steps that may minimize this include the use of consistent phrasing of questions and blinding of interviewers to case or control status (although this may be difficult to maintain).

Case-control studies may be subject to other forms of differential information bias. **Diagnostic suspicion bias** occurs when knowledge of exposure to the risk factor of interest results in more detailed diagnostic investigation, leading to greater identification of exposed, compared with unexposed, cases.

Analysis and interpretation of case-control studies

In the absence of confounding and effect modification, traditional unmatched case-control studies may be analysed using simple 2×2 tables to estimate the odds ratio by comparing the odds of exposure for cases with the odds of exposure for controls (see Chapter 15). For rare diseases, this odds ratio is equivalent to the risk ratio for disease amongst exposed animals compared

with unexposed animals. For common diseases, the meaning of the odds ratio estimated in a case-control study depends on the sampling scheme used for controls (see earlier section on choosing a control group). Stratifying the data by other variables will help to adjust for confounding in a similar way to that described for cohort studies. In matched case-control studies, the data may be analysed by stratifying the data according to each case-control matched group. Often, multivariable generalized linear models are used to analyse data collected from case-control studies. Most commonly, this involves (unconditional) logistic regression for unmatched studies and conditional logistic regression for matched studies. For more information about the statistical modelling of epidemiological data see Chapter 22.

Design of cross-sectional analytical studies

In this type of study, the association between the study base and outcome factors is examined as they exist at one point in time (or over a short period) and thus provide a snapshot of the population (see *Figures 16.1* and *16.2c*). When conducted at one point in time, the proportion of individuals with a disease is an estimate of the **point prevalence** of that disease. When conducted over a short period, the number of individuals with the disease at any time during the study period (i.e., the point prevalence at the start of the study, plus new cases arising during the study period) is expressed as a proportion of the (mean) number at risk during that period. This is an estimate of the **period prevalence**.

Cross-sectional analytical studies provide only relatively weak evidence of causality because it is often not possible to be sure about the temporal relationship between the study factor and outcome factor. For example, the results of a cross-sectional analytical study inquiring into the association between obesity (study factor) and osteoarthritis (outcome factor) in dogs might suggest that obesity is a cause of this disease. However, it is equally possible that osteoarthritis leads to decreased physical activity, which results in the obesity.

Cross-sectional surveys may be repeated. In a **repeat survey**, a different study population is selected for the subsequent survey, whereas in a **panel survey** the same subjects are included in each survey. These are further described later. In a **census study** the entire study population is sampled at a point in time. Although census studies may include very large populations, a census may be more commonly carried out on relatively small population, such as a farm or cattery. The UK Annual Survey of Agricultural and Horticultural Activity was conducted as a census of all farms until 1995 when it changed to a cross-sectional

survey; the sample still included approximately 80% of farms, although not all responded.

Identifying the research question

Cross-sectional studies may be used to collect information on exposures and outcomes. As well as being used to estimate the prevalence of exposure or outcome, these data may also be used to explore patterns of morbidity or exposure (e.g., by location or by exposure group). The choice of study or sampling unit will depend on the objective of the study but in most cases it will be either the individual animal or the group (e.g., farm, household and so on).

Often cross-sectional studies do not have a defined hypothesis, but rather are used to gather information about a range of exposure and/or outcome variables of interest. These studies can provide useful information, but may be better viewed as hypothesis-generating, rather than hypothesis-testing, studies. Where a well-defined hypothesis is formulated care should be taken to ensure that the outcome and exposures of interest, as well as potential confounders, are defined and accurately measured. As for any epidemiological study, the analysis should take into account the role of confounding variables, and the interpretation of the results should allow for all potential sources of bias.

For example, we can use a cross-sectional study to investigate the association between the proportion of cattle that are fed silage (or that receive different 'quantities' in their feed) and the prevalence of listeriosis (perhaps as farm-level prevalence) or, perhaps, the prevalence of shedding of *Listeria monocytogenes* in faeces. Hence, the initial null hypothesis could be that there is no difference in the prevalence of exposure to silage among farms with cases of listeriosis in the past 12 months and those with no cases in the same period. Information about the occurrence of listeriosis and about silage feeding would be collected simultaneously.

Selection of study subjects

Cross-sectional studies usually are undertaken in order to enable inference from the study results to the population of interest. Hence, some form of random sampling of subjects is usually required. The sample may be a simple random sample of the population, or may be stratified by a variable such as region.

In an unstratified cross-sectional study, the sampling fraction is the same for all diseased, not-diseased, exposed and non-exposed animals in the study population (allowing for some random variation). Alternatively, stratified or other forms of sampling, such as multistage sampling, may be used. In such cases the

sampling structure should be accounted for in the analysis (see Chapter 22). Sampling strategies are discussed in more detail in Chapter 13.

Exposure and outcome measurement

It is often possible to collect a large amount of information on exposures and outcomes in a cross-sectional study. Whilst this is often advantageous, overly onerous data collection (such as the use of long questionnaires) may reduce participation rate. Furthermore, care may be needed when associations between outcomes and potential risk factors are assessed, as multiple statistical tests will increase the chance of a Type I error (see Chapter 14).

Cross-sectional studies can be used to collect a wide variety of data, ranging from tissue and fluid samples for further laboratory assessment to clinical data and information derived from questionnaires. They may be used to investigate people's practices, knowledge, beliefs and attitudes and hence may be useful for the design of interventions.

Bias in cross-sectional studies

Cross-sectional studies may be subject to a wide range of selection biases, particularly if the probability of selection is related to one or more of the study or exposure variables under study. As cross-sectional studies rely on existing (rather than new) cases they may be subject to **incidence-prevalence bias**. This bias arises because the prevalence of a disease in a population is related both to the rate of occurrence of the disease (i.e., its incidence) and the duration of disease (see Chapter 4). Therefore, factors identified in cross-sectional studies as being associated with the prevalence of disease may affect the risk of disease occurrence, the disease duration, or both. For example, in our study of silage and listeriosis, cattle exposed to greater amounts of spoiled silage may be more likely to develop listeriosis, but also may be more likely to die rapidly and perhaps, therefore, more likely to be lost to the study. Although loss to follow-up bias is not a concern in cross-sectional studies, **non-response bias** may occur.

Cross-sectional studies may also be subject to information bias depending on the type of data to be collected and the methods of collection. As exposure and outcome status is generally not known at the time of data acquisition, some forms of differential misclassification may be unlikely. However, differential bias may occur due to respondents' knowledge of exposure or outcome status, or to acquisition of this information by the observer during the study (e.g., during conduct of a questionnaire, information gained from responses

to initial questions may induce **interviewer bias** in subsequent questions).

Confounding (see Chapters 15 and 18) may also be a problem in cross-sectional studies. It may be possible to restrict the study to only include one stratum of a particular confounder; however, in analytical cross-sectional studies, it is more common to control for potential confounders during analysis.

Analysis and interpretation of cross-sectional studies

In the absence of confounding and effect modification, cross-sectional studies may be analysed using simple 2×2 tables to estimate the prevalence of disease in the exposed and unexposed groups, and the relative risk (more correctly termed the prevalence ratio in this context: see Chapter 15) can be calculated as the ratio of these prevalences.

Where confounding may exist, stratifying the data by other variables will help to adjust for confounding; however, it is more common to adopt an appropriate statistical modelling approach (see Chapter 22). Where data collection utilizes more complex sampling strategies, data may be clustered by one or more variables. This data structure should also be taken into account in the analysis (see Chapter 22).

When interpreting the results of the study, care needs to be taken to assess the generalizability of the results to the populations of interest. Generalizability may be an issue whenever the study population is not representative of the target population (or other population of interest).

Sample-size calculation for cross-sectional studies

The required sample size for simple cross-sectional studies (i.e., without clustering) can be estimated using the methods described for calculation of the sample size required for a cohort study (see Chapter 15) or the calculation used to detect a difference between two proportions (see Chapter 14). Where the cross-sectional study employs clustered sampling, it is necessary to correct the sample-size estimate to account for the impact of clustering. One approach is to multiply the required sample size in each group (assuming random sampling) by the design effect (see Chapter 13). More advanced methods are available using simulation-based approaches (for example, see the software *MLPowSim*: Appendix III).

Overview of other study designs

Cohort, case-control and cross-sectional studies are the most common observational study designs in veterinary epidemiology. These designs are applicable to

many situations and are well understood. However, many other study designs exist and may be well suited to specific situations. These designs are often variants of cohort, case-control or cross-sectional studies, and may be hybrid designs that incorporate features of two or more of these.

Ecological studies

In most of the study designs described in this chapter, it is necessary to measure the exposure and disease status at the level of the unit of interest; that is, the measurements are made at the level at which inference will be made. Generally, the unit of interest is the **individual** animal, so measurements are made on individuals and the results are used to make inferences about the causal factors of disease in individuals. Sometimes this information is not available. Characteristics of **groups** may then be studied, although an inference may still be required at the level of the individual. Such studies are ecological studies¹, in which exposures and outcomes are measured at a geographical scale (e.g., regions) and comparisons made at that scale – that is, the relationship is not assessed at the level of the individual, but rather at the group level. For example, in The Netherlands, the highest human lung cancer mortality rate between 1969 and 1984 was found in the North Brabant region. Most pet bird keepers and bird clubs are also located in this region. Moreover, people who keep birds inhale allergens and dust particles, which impair the function of lung macrophages, resulting in reduced protection of the bronchial epithelium (Voisin *et al.*, 1983). It is therefore tempting to speculate that bird keeping is a risk factor for lung cancer, although the exposure and disease status of individuals is not known. This inference is logically defective because it is based on the erroneous assumption that group and individual characteristics are always the same; this logical error is termed the **ecological fallacy** (Selvin, 1958). The correlation between group (i.e., ecological) variables is often considerably different to that of the individual correlation in the same populations (Robinson, 1950); although the lung cancer rate was highest in the region where bird keeping was predominant, the cases may have occurred more frequently among people who did not keep birds.

Ecological studies therefore should be treated with care when interpreted at the individual (or sub-unit) level, but are useful preliminary indicators to causal hypotheses that should be tested more thoroughly.

¹ Some authors use the term **spatial correlation study** when the relationship between geographical variation in morbidity and putative explanatory variables, measured at the area level, is assessed (Durr *et al.*, 2000).

Thus, a subsequent case-control study demonstrated an association between bird keeping and lung cancer (Holst *et al.*, 1988), supporting a causal hypothesis at the level of the individual. Piantadosi and others (1988) discuss the ecological fallacy in detail, and Kleinbaum and colleagues (1982) describe ways of strengthening causal inferences from ecological studies.

However, the unit of interest in veterinary epidemiological research is often the group (e.g., the herd of cattle, the pens of pigs, dog kennels) and hence the group may be the sampling unit and the unit of interest. Unless inference is subsequently made at the level of the individual (sub-group), this is not an ecological study. The study of disease at the level of the group is often appropriate, particularly when the aim is to understand the cause of incidence, rather than the cause of individual cases. Dohoo and others (2009) provide an excellent discussion of the benefits of group-level studies for veterinary medicine. They note that whilst there is considerable concern regarding the ecological fallacy, very little is written about the **atomistic fallacy**, which arises when inference is made about the group-level based on individual-level data. The atomistic fallacy arises when factors important at the group level differ from those at the individual level.

Time series studies

Time series studies investigate the relative or absolute frequency of an event in a population over time, with measurements made at *appropriate* and usually (but not necessarily) equal time intervals over a *sufficient* time period (see Chapter 8). For example, within-year (seasonal) patterns may require measurements to be aggregated to monthly or shorter intervals over at least two years (in order to demonstrate pattern cyclicity). Time series data have a natural temporal ordering that distinguishes time series studies from most other studies. Before-and-after studies (see later) can be thought of as simplified time series studies in which measurements are obtained for only a few times, and the majority of data remain unobserved.

Time series studies typically aim to investigate underlying long-term trends, identify repeated (e.g., seasonal) variation, explore associations with time-varying risk factors, including change points, and/or examine unexplained variation. For example, a time series study by Archer and colleagues (2006) identified seasonal patterns in the presentation to a referral hospital of certain types of equine colic. Time series studies can also incorporate spatial data to explore spatio-temporal patterns in order to identify anomalies in space-time that could be outbreaks or the result of a temporary, localized change in one or more risk

factors. For example, French and others (1999) examined spatial and temporal patterns to identify outbreaks of sheep scab.

Change points are times at which the relative or absolute frequency of an event changes, either by a sudden step-change or as a change in the long-term trend. For example, Leslie and others (2009) identified a long-term reduction in the rate of hip fractures among people in Canada between 1985 and 2005, and that the rate of reduction was greater after 2006.

Time series studies usually utilize group-level data and hence, as for ecological studies, they can be subject to the ecological fallacy if results are used to make inferences at the level of the individual.

Panel studies

Panel studies are repeated cross-sectional studies in which the same study subjects (the **panel**) are included each time. Hence, such studies combine features of cross-sectional and cohort studies. Whereas a cohort study collects information on events as they occur, panel studies collect information only at (pre-defined) time points and so the distinction between cohort and panel studies increases when the interval between observation times in the panel study increases. Panel studies enable investigation of the temporal relationship between exposure variables and disease. Unlike standard time series studies, panel studies collect information at the individual level and hence provide stronger evidence of associations at that level. Panel studies may be used to generate individual-level time series data.

Repeated surveys

Repeated surveys involve repeated application of a cross-sectional study within a population with different samples of study subjects on each occasion. Ideally, some form of random sampling should be used to identify each study sample and the measurements made should be sufficiently precise to ensure that observed variation between surveys is due predominantly to variation in the population of interest (rather than due to selection bias or lack of measurement precision). Repeated surveys can be used to investigate population-level trends over time.

Before-and-after studies

Before-and-after studies evaluate the effect of an exposure or intervention by measuring the characteristics of a population before and after the onset of that event. Typically, before-and-after studies employ at least two cross-sectional studies and aim to ascribe any observed changes to the intervening event. For example, the impact of vaccination for Johne's disease was

assessed in 231 cattle herds using cross-sectional studies prior to and following initiation of vaccination on the relevant farms (Wilesmith, 1982). Although it may be tempting to attribute the reduction in clinical Johne's disease to the intervention, a range of other control measures, including improved hygiene practices and culling of affected and high-risk animals, accompanied the vaccination campaign. Therefore, the contribution of the vaccine to the reduction in clinical disease could not be assessed.

Before-and-after studies may involve different samples for each cross-sectional study, and are thus forms of *repeated surveys*. Alternatively, a before-and-after study could utilize the same study subjects for each cross-sectional survey, in which case it would be a *panel study*. Observations may be made at the level of the individual or the group. In the latter case, before-and-after studies may be subject to the ecological fallacy if results are used to make inferences at the level of the individual.

The evidence provided by before-and-after studies can be enhanced by: identification and consideration of other factors that may account for the observed effects; examination of trends over a longer time; repeating the study in different populations; staggering the introduction of the intervention over time; or by conducting a controlled before-and-after study.

Controlled before-and-after studies are non-randomized studies that compare two groups: one that is exposed (or given the intervention) and a control group that is not exposed. The control group is selected to be comparable to the exposure group on key characteristics. Measurements are made before and after the introduction of the exposure and the effect in the exposure group is compared with the effect in the control group in order to evaluate the impact of the exposure while controlling for the effects of time. However, unmeasured differences between the exposure and control groups may bias the results.

Nested case-control studies

When a case-control study is undertaken within a fully enumerated population the study is described as being **nested** within that well-defined population (see *Figure 16.3b*). The term is generally used to describe a case-control study that is undertaken within a cohort study, but any case-control study conducted within a well-defined and observed population can be thought of as being nested. As for a standard case-control study, case animals are identified as those that have occurred (in a **retrospective nested case-control** study) or as they occur (in a **prospective nested case-control** study) within the cohort. Usually all cases are used, but sometimes a (random) sample of cases may be chosen. Control animals are selected from the non-

cases in the cohort through one of several approaches. Controls may be sampled at random from the at-risk cohort. This may be achieved by sampling from the entire at-risk population, or following identification of grouped-time risk sets, with controls being randomly selected from within the same grouped-time risk set as the cases. Alternately, continuous-time risk sets may be used, in which controls are sampled from the risk set at the time that the case occurs, resulting in selection of (time) matched controls (Langholz and Clayton, 1994; Langholz, 2005).

Nested case-control studies achieve some of the economy of case-control studies and reduce the risk of selection bias associated with control selection in case-control studies conducted in poorly-defined populations (see *Figure 16.3c*). Furthermore, recall bias will not occur for those exposures that can be measured at the start of the study. In theory, the only disadvantages of a nested case-control study compared with a study utilizing the full cohort are a reduction in precision and power (due to sampling a sub-set of all possible controls) and possible flaws in the design of conduct of the process of control (and perhaps case) selection (Wacholder, 2009). That is, nested case-control studies may be thought of as cohort studies with missing data.

Rothman and Greenland (1998) contend that it is useful to think of all case-control studies as being nested within underlying cohorts of individuals. The definition of the cohort in which this nesting occurs is the population that gives rise to the cases and hence is the source population for the controls. Difficulty identifying this cohort suggests that biased selection of controls may arise due to failure to select cases and controls from the same underlying populations.

French and others (1998) conducted a prospective nested case-control study to investigate risk factors for faecal soiling in lambs. The cohort included lambs on eight farms and was observed for almost five months. Cases were incident cases and controls were randomly selected for each case from a continuous-time risk set and were matched on group. Hence cases and controls were matched on time and farm. The results of this nested case-control study were compared with an analysis of the full cohort. Although the results showed some consistency between the studies, some variables were only significant in one or other model. The greater statistical power of the cohort study may account for some of this variation, particularly where the odds ratios were similar in both studies but with wider confidence intervals in the nested study. However, some variables were strongly associated with faecal soiling in the nested study only, probably due to chance, bias or confounding. As the cohort and nested studies

were each subject to different biases and because some exposures were only measured in the animals included in the nested study, it is unclear which study's results best reflect the underlying exposure risks. In this case the benefits of conducting the nested case-control study include the ability to include some exposure variables that could not be measured for the entire cohort and minimization of some bias due to loss to follow-up. However, whilst the controls were selected from a well-enumerated cohort there was some loss of statistical power and the control population may not have been fully representative of the underlying cohort.

Case-cohort studies

As in the nested case-control study, the case-cohort study is undertaken within a fully enumerated cohort and all cases (or a random sub-set of cases) arising during the follow-up period are included in the study (see Figure 16.3d). Controls are selected as a random sample of the starting population, often at the time of enrolment in the cohort. Hence all members of the cohort have an equal chance of being selected as a control, regardless of the amount of time spent in the cohort. The union of the cases and the control sub-cohort is called the **case-cohort set**. It is possible that some animals selected as controls will later become cases and will appear in both groups; that is, they will appear twice in the case-cohort set. Because some cases will appear in both the case group and the control sub-cohort, the size of the control sub-cohort will need to be larger than the control group of an equivalent case-control study if it is to have the same statistical power. This has little effect if the disease is rare. However, if the disease is likely to occur in, say, 50% of the control sub-cohort, then the size of this group should be doubled in order to obtain the appropriate number of non-case controls.

An advantage of case-cohort studies is that the control sub-cohort can be used to investigate risk factors for several disease end-points. Also, as the control sub-cohort is drawn randomly from the full cohort it can be used to measure the distribution of the measured exposures in the full cohort. Therefore, provided the sampling of the control sub-cohort is independent of exposure, the *pseudo-risk* of disease in the exposed and unexposed case-cohort set can be calculated. Although these pseudo-risks cannot be directly interpreted, the ratio of the exposed to unexposed pseudo-risks provides an estimate of the risk ratio.

Case-case studies

In case-case studies, cases with a specific characteristic are compared with cases without that characteristic in

order to try to identify aetiological factors specific to cases with the characteristic. For example, a comparison of more and less severe cases may be undertaken to identify factors associated with severity. For infectious diseases, comparisons could be made between cases due to different subtypes of organism (McCarthy and Giesecke, 1999). Gillespie and others (2002) used a case-case study to compare *Campylobacter coli* and *C. jejuni* infection. They identified some different risk factors for disease due to each species of *Campylobacter* and concluded that aggregation of cases due to these species may mask risk factors for one or both species. The advent of new modelling tools to attribute particular pathogen subtypes to individual reservoirs now permits conduct of 'source-assigned' case-case studies. For example, Mullner and colleagues (2010) identified risk factors for human campylobacteriosis due to different probable sources (e.g., poultry, ruminants) by comparing information on exposures among campylobacteriosis cases.

Case-case-control studies

The case-case-control study uses two separate case-control studies to evaluate risk factors specifically associated with each of the (related) case diseases. For example, Kaye and others (2005) used a case-case-control design to identify risk factors for isolation of vancomycin-resistant *Enterococcus* that were distinct from those factors associated with isolation of vancomycin-susceptible *Enterococcus*. To do this they assessed two case populations, one infected with vancomycin-resistant *Enterococcus* and the other with vancomycin-susceptible *Enterococcus*. These were compared in separate analyses with a control population drawn from patients without *Enterococcus* infection. Comparison of the two models identified risk factors specific to isolation of vancomycin-susceptible *Enterococcus* and those common to both organisms. Again, the advent of methods to assign pathogen subtypes to probable reservoirs enables 'source-assigned' case-control studies to be conducted. For example, Mughini Gras and others (2012) identified risk factors for human campylobacteriosis by distinguishing, within a case-control framework, cases caused by *Campylobacter* attributed to different animal reservoirs (based on multilocus sequence typing profiles), thereby permitting separate case-control studies to be conducted for the different source-attributed cases of campylobacteriosis. Mughini-Gras and others (2014) used a similar approach to identify risk factors for human salmonellosis due to different source attributed *Salmonella*.

The data collected for a case-case-control study enables concurrent analysis of the cases only, in a case-case study.

Case-other disease studies

The case-other disease comparison study is a hybrid of the case-control study design. Whereas as controls in a case-control study are selected from the population from which the cases arose, the comparator group in a case-other disease study is made up of individuals with another disease. An advantage of this approach is that careful selection of the comparator disease may result in a similar recall bias between the case and comparator groups (Smith *et al.*, 1988). However, recall could still be differentially biased and the exposures of the comparator group might not represent the exposure experience of the study base. Many hospital-based studies referred to as 'case-control' studies use as controls other animals attending the hospital with specific diseases and hence may be more accurately defined as **hospital-based case-other disease studies**. The use of this term may be preferable as it highlights that exposures leading to the comparator disease, and factors associated with these exposures, will be over-represented in this group compared with the study base. Furthermore, selection bias due to case ascertainment may be different for the different diseases.

Case-other disease studies are different to case-case studies (see earlier) as in the latter both disease outcomes are of interest. In contrast, in case-other disease studies, the other disease is selected with the aim that cases of that disease are representative of the study base.

Case-only studies (or genetic case-only studies)

Case-only studies are hybrid case-control studies in which the only study subjects are cases. In these studies the population distribution of exposure experience is estimated theoretically and used instead of real control subjects. For example, in genetic studies it may be possible to determine the population distribution of genotypes and the genotypes of case animals can be compared with the expected population distributions. Case-only studies also can be used to identify interactions between genetic factors and other exposures (but not the independent effects of each). This approach is only valid if these factors are independent in the population. Whilst this may be true in many cases, there are some genes that may be expected to be associated with different levels of exposure. For example, variation in alcohol dehydrogenase genes may be associated with altered alcohol metabolism that may in turn be associated with a person's (chosen) level of alcohol consumption. Case-only studies are less useful for investigation of interactions between non-genetic risk factors, perhaps because many non-genetic factors do not meet the assumption of independence. For more details see Khoury and Flanders (1996).

Case-crossover studies

The case-crossover study has been applied to investigation of exposures that have a transient effect on the risk of rapid-onset events. Variants of this design include the case-time-control study and the case-case-time-control study (see below). In these studies the *case window* refers to the period just before the onset of the outcome of interest whereas the *control window* is a period of the same duration and prior to the case window that is not closely associated in time to an outcome event. Case and control windows do not overlap.

Case-crossover studies include only cases (Figure 16.4). They are hybrid case-control studies in which control information for each case derives from its own previous exposure experience; that is, each case contributes a *case window* and a *control window*. Control windows serve to provide an estimate of each case's expected exposure, which can be compared with the exposure observed in the case window. In a study investigating the potential for physical exertion to trigger acute myocardial infarction, the level of physical exertion in the one to two hours prior to the onset of myocardial infarction was compared with two sets of control data; the same patients' usual level of exertion over the past year and their actual level of exertion in the same one- to two-hour period the day before the onset of myocardial infarction (Mittleman *et al.*, 1993).

Case-crossover studies avoid issues of control selection and control for between-individual confounding. However, they may be biased if exposure varies systematically over time and due to differential recall. The case-crossover odds ratio is calculated as the ratio of the number of cases exposed only during the case window to the number exposed only during the control window (Hernández-Díaz *et al.*, 2003).

Case-time-control studies

The case-time-control study is an extension of the case-crossover study. Both cases and controls are included (as for a case-control study), but each individual is included twice; once during the *case window* and once during the *control window* (Figure 16.4). The case window is the at-risk period immediately before the event of interest. The control window is the same length as the case window, but occurs at a time to assess the 'expected' exposure. The case-time-control study avoids bias due to time trends in exposure, but does require the selection of appropriate controls and may offer little or no benefit over the standard case-control approach.

The case-time-control study enables calculation of the case-crossover odds ratio (see earlier). The case-time-control odds ratio can be calculated by dividing the case-crossover odds ratio by the ratio of the

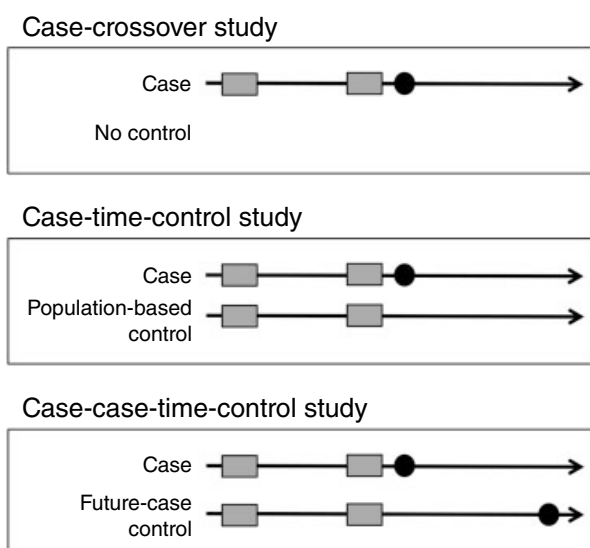


Fig. 16.4 Diagrammatic representations of case-crossover, case-time-control and case-case-time-control studies. Horizontal black arrows represent time. Solid black circles represent the occurrence of the outcome of interest, and grey rectangles represent the case and control time windows (i.e., the periods for which exposure is assessed). For example, in a case-crossover study, exposure during the case time window, which occurs immediately before the occurrence of the outcome of interest, is compared (for the same case) with a control time window, which is a specified period prior to the case window.

number of controls exposed only during the case window to the number exposed only during the control window (Hernández-Díaz *et al.*, 2003)

Case-case-time-control studies

The case-case-time-control study is another extension of the case-crossover study but, rather than use an external control group (as for the case-time-control study), it uses time windows for future cases as controls for current cases (so-called **future case controls**), in order to avoid bias due to temporal

trends in exposure (Wang *et al.*, 2011). Exposure is measured for the case window and control window for the case subject and for equivalent calendar time for the matched control subject (i.e., the future case control). The key difference between case and future case-control subjects is that the case window occurs immediately prior to the event for the case, but occurs at some distance prior to the event for the control (Figure 16.4). Dividing the exposure odds for the cases by the exposure odds for the controls provides an estimate of the case-case-time odds ratio.

Case-chaos study

The case-chaos study has recently been proposed as an alternative (or addition) to case-control studies, particularly where control selection is problematic and rapid analysis is required, such as may occur with point-source infectious disease outbreaks (Gillespie *et al.*, 2012). The study compares information on exposures observed for case subjects with matched data created by randomly permuting each case's exposure data. The method identifies exposures among the cases that occur commonly relative to the average among the exposures evaluated. Hence, the exposures identified by the method are likely to be either risk factors or just common exposures. Therefore, interpretation of the results requires judgements about the likely frequency of each exposure in the underlying population.

The method has only been used with binary exposure data and requires further validation. However, it may prove to be a useful tool in certain situations, particularly for outbreaks associated with a few exposures. It is less likely to be useful for complex, multi-factorial diseases.

Further reading

- Breslow, N.E. and Day, N.E. (1980) *Statistical Methods in Cancer Research, Volume 1: The Analysis of Case-Control Studies*. IARC Scientific Publications No. 32. International Agency on Cancer Research, Lyon
- Breslow, N.E. and Day, N.E. (1987) *Statistical Methods in Cancer Research, Volume 2: The Design and Analysis of Cohort Studies*. IARC Scientific Publications No. 82. International Agency on Cancer Research, Lyon
- Collett, D. (2003) *Modelling Binary Data*, 2nd edn. Chapman and Hall, London. (Describes the application of the logistic model to observational studies)
- Elwood, J.M. (2007) *Critical Appraisal of Epidemiological Studies and Clinical Trials*, 3rd edn. Oxford University Press, Oxford
- Fleiss, J.L., Levin, B. and Paik, M.C. (2003) *Statistical Methods for Rates and Proportions*, 3rd edn. John Wiley, Hoboken
- Frankena, I. and Graat, E.A.M. (2001) Multivariate analysis: logistic regression. In: *Application of Quantitative Methods in Veterinary Epidemiology*, revised reprint. Eds Noordhuizen, J.P.T.M., Frankena, K., Thrusfield, M.V. and Graat, E.A.M., pp. 131–161. Wageningen Pers, Wageningen. (A concise introduction to the logistic model)

- Green, M.D., Freedman, D.M. and Gordis, L. (2011) Reference guide on epidemiology. In: *Reference Manual on Scientific Evidence*, 3rd edn. Federal Judicial Center/National Research Council of the National Academies, pp. 549–632. The National Academies Press, Washington DC. (*A general guide to analytical methods, with emphasis on interpretation*)
- Hosmer, D.W., Lemeshow, S. and Sturdivant, R.X. (2013) *Applied Logistic Regression*, 3rd edn. John Wiley, New York
- Kelsey, J.L., Whittemore, A.S., Evans, A.S. and Thompson, W.D. (1996) *Methods in Observational Epidemiology*, 2nd edn. Oxford University Press, New York and Oxford
- Kleinbaum, D.G., Kupper, L.L. and Morgenstern, H. (1982) *Epidemiologic Research. Principles and Quantitative Measures*. Lifetime Learning Publications, Belmont
- Newman, S.C. (2001) *Biostatistical Methods in Epidemiology*. John Wiley, New York
- Pearce, N. (2012) Classification of epidemiological study designs. *International Journal of Epidemiology*, **41**, 393–397
- Rothman, K.J., Greenland, S. and Lash, L.T. (2008) *Modern Epidemiology*, 3rd edn. Lippincott, Williams and Wilkins, Philadelphia
- Sahai, H. and Kurshid, A. (1995) *Statistics in Epidemiology: Methods, Techniques, and Applications*. CRC Press, Boca Raton. (*A comprehensive discussion of the variety of methods for deriving parameters in observational studies*)
- Schlesselman, J.J. (1982) *Case–Control Studies: Design, Conduct, Analysis*. Oxford University Press, New York and Oxford
- Vandenbrouke, J.P. and Pearce, N. (2012) Case-control studies: basic concepts. *International Journal of Epidemiology*, **41**, 1480–1489

17

Clinical trials

The effects of some treatments are so marked that they are obvious; for instance, the intravenous administration of calcium to cases of acute bovine hypocalcaemia. However, this is not true for many prophylactic and therapeutic procedures, where, for example, the advantages of a new drug over an established one may be small. Moreover, the observations of individual veterinarians provide insufficient evidence for determining efficacy¹. In the past, treatment often was based on beliefs that had never been scientifically assessed; and anecdotal or unattested cures entered the veterinary and medical literature. Indeed, by as late as the end of the 20th century, only about 20% of human medical procedures were estimated to have been evaluated properly (Konner, 1993), and many veterinary procedures also have been poorly evaluated (Shott, 1985; Smith, 1988; Bording, 1990; Elbers and Schukken, 1995)². Although observational epidemiological studies (see Chapter 15) can be used to investigate treatments (Carlson and Morrison, 2009), the best evidence of efficacy is provided by a well-conducted **clinical trial** (Rubin, 2008). In such trials, treatments are commonly termed 'interventions'.

Clinical trials date back to the 18th century, when they provided clues to the cause of disease³. The

provision of citrus fruits to English sailors prevented scurvy, indicating that the cause (subsequently shown to be vitamin C deficiency) was nutritional (Lind, 1753)⁴. Similarly, at the beginning of the 20th century, improvement in the diet of inmates of some American orphanages and asylums cured and prevented pellagra, and suggested a nutritional cause of the disease, which hitherto had been considered to be infectious (Goldberger *et al.*, 1923).

Definition of a clinical trial

A clinical trial is a systematic study in the species, or in particular categories of the species, for which a procedure is intended (the target species) in order to establish the procedure's **prophylactic** or **therapeutic** effects. In veterinary medicine, effects may include improvements in production as well as amelioration of clinical disease. The procedure may be a surgical technique, modification of management (e.g., diet), or prophylactic or therapeutic administration of a drug. If the latter is being assessed, a clinical trial would also include studies of its pattern of absorption, metabolism, distribution within the body and excretion of active substances.

With these aims in mind, the assessment of drugs can be classified chronologically into four phases,

¹ A distinction is sometimes made between *effectiveness* and *efficacy* (Porta, 2014). The former is the extent to which a procedure does what it is intended to do when applied in the day-to-day routine of medical or veterinary practice. The latter is the extent to which a procedure produces beneficial results under ideal conditions.

A procedure may be efficacious but relatively ineffective if it is not taken up widely (e.g., if the owner's compliance with the advice of the veterinarian is low).

² See Wynne (1998) and Verdier and others (2003) for a discussion of assessment of veterinary homeopathic remedies.

³ The principle of making medical inferences from comparisons is much older. The earliest recorded example is probably the study of the diet of captive Jewish children in Babylon in the 6th century BC (documented in the Old Testament: *Daniel* 1, 3–16).

Nebuchadnezzar, the Chaldean ruler of Babylon, instructed Ashpenaz, his chief eunuch, to take some of the captive children into his court. They were offered the king's meat but refused it, eating vegetables instead. After 10 days *'they looked healthier and were*

better nourished than all the young men who had lived on the food assigned them by the king'.

⁴ From 1795, lemon juice was added to the watered rum ration that was issued regularly on ships that were at sea for more than six weeks (Price, 1979). The number of cases of scurvy in the naval Royal Hospital Haslar (Hampshire, England) fell from 1754 cases in 1760 to one case in 1806 (Drummond and Wilbraham, 1957). The beneficial effect of lemon juice in combatting scurvy had been identified initially over a century earlier by the Elizabethan merchant sailor, James Lancaster, during voyages to the Spice Islands (Milton, 1999).

according to the purpose of the assessment (Friedman *et al.*, 2015):

1. **phase I: pharmacological and toxicity trials**, conducted on the target species to study the safety of a drug, often after preliminary information has been gathered from *in vitro* studies or laboratory-animal models⁵;
2. **phase II: initial trials of therapeutic effect and safety**, conducted on the target species on a small scale, and often with the object of selecting the potentially most attractive drugs from those that are available;
3. **phase III: clinical evaluation of efficacy**, undertaken on a larger scale **in the field**; that is, under **operational conditions**, where management and environment can affect the result of the trial⁶;
4. **phase IV: post-authorization surveillance** of a drug after it has been licensed, to monitor adverse drug reactions.

This chapter focusses on the phase III trial: assessment of efficacy in the field, in which circumstance the term **field trial** commonly is used.

Hitherto, some authors have distinguished between a field trial and a clinical trial on the grounds that the former possesses two characteristics:

1. it is conducted under operational conditions;
2. it is frequently prophylactic, and therefore relies on natural challenge to the treatment that is being assessed (e.g., assessment of the efficacy of a bacterial pneumonia vaccine would rely on vaccinated animals being naturally exposed to infection with the relevant bacterium during the period of the trial).

However, these characteristics only represent related circumstances under which clinical trials may be conducted (2 is a logical consequence of 1), and so a distinction between clinical and field trials is not considered necessary on these grounds.

Some authors (e.g., Rothman *et al.*, 2008) draw the distinction more forcibly on the grounds that a clinical trial is undertaken on patients that are ill, and therefore is always therapeutic (this may include prevention of sequelae, though; see Chapter 27, footnote 2:

⁵ Recently, the term 'phase 0 (pre-phase 1)' has been coined to describe trials (commonly of anti-cancer drugs) aimed at accelerating the discovery and development of potential therapies before traditional investigation of safety (Kummar *et al.*, 2008; Kummar and Doroshow, 2011).

⁶ Some authorities have considered hybrids of phase II and phase III trials, to increase efficiency (Schaid *et al.*, 1988; Storer, 1990), noting that the purposes of phases can overlap (ICH, 1997).

secondary and tertiary prevention); whereas a field trial is invariably conducted on clinically-healthy individuals to determine prophylactic effect (primary prevention). Such a strong distinction is also not made in this chapter, where in addition, for brevity, all types of procedure are described as 'treatment'.

Randomized controlled clinical trials

The clinical condition of sick animals can be compared before and after treatment. This is sometimes termed an **open trial** (Toma *et al.*, 1999). However, interpretation of such an **uncontrolled trial** may be difficult because any observed changes could result either from the intervention or from natural progression of the disease. An essential feature of a well-designed clinical trial therefore is comparison of two 'treatment arms': (1) a group receiving the treatment, with (2) a **control** group not receiving it; this is a **controlled clinical trial**.

The control group may be selected at the same time as the group receiving the treatment (a **concurrent** control group) or generated using historical data (an **historical** control group). Early clinical trials such as Lister's assessment of antiseptic surgery (Lister, 1870)⁷, and the evaluation of diphtheria antiserum (Lancaster, 1994)⁸, utilized historical controls, but this approach is open to criticism because of the various factors, unrelated to treatment, that can produce observed differences over a period of time (e.g., improvements in husbandry, changes in diagnostic criteria, disease classification and selection of animals, alterations in virulence of infectious agents, and reduction in the severity of disease). The net result of these problems is that studies with historical controls tend to exaggerate the value of a new treatment (Pocock, 1983). Concurrent control groups are therefore usually advocated⁹.

The control group may receive another (standard) treatment with which the first treatment under trial is being compared (a 'positive' control group), no treatment (sometimes called a 'negative' control group), or a **placebo** (a substance with no therapeutic effect that is visually similar to the treatment under trial, and therefore cannot be distinguished from the treatment by those administering and those receiving the treatment and placebo).

⁷ The use of disinfectants already had been pioneered in hospitals by Semmelweis (see Chapters 1 and 3).

⁸ Diphtheria was a major problem in Victorian industrial England (Briggs, 1982) and antiserum was made available in 1894–1895.

⁹ A notable early example of the use of concurrent controls was Pasteur's trial of an anthrax vaccine in sheep (Descour, 1922).

Placebo effect The 'placebo effect' is the expectation that a treatment will have an effect that is sufficient to produce an improvement. A placebo was originally a substance with no known therapeutic effect, which was administered in the hope that the power of suggestion, alone, would alleviate symptoms (Latin: *placebo* = 'I shall be pleasing'). In human medicine, this effect may be demonstrated in patients receiving treatment¹⁰. Additionally, it may be demonstrated 'by proxy', when clinicians and family members of patients receiving treatment either report an improvement or influence a patient's perceptions (Grelotti and Kaptchuk, 2011; Whalley and Hyland, 2013)¹¹. It also can occur in veterinary medicine (the 'caregiver placebo effect': Conzemius and Evans, 2012).

Bias (see Chapter 10) can occur in a trial if there is preferential assignment of subjects to treatment or control groups, differential management of the groups, or differential assessment of the groups. For example, a veterinarian may allocate only animals with a good prognosis to the treatment group. A central tenet of a controlled clinical trial is that subjects are assigned to treatment and control groups **randomly** (see Chapter 13) so that the likelihood of bias due to preferential allocation is reduced. This process of **randomization** should also balance the distribution of other variables that may be outcome-related (e.g., age), and guarantees the validity of the statistical tests used in the analysis of the trial (Altman, 1991b).

Randomized controlled clinical trials therefore adopt the experimental approach (see Chapter 2) and closely resemble cohort studies (see Chapter 15)¹².

Confirmatory and exploratory trials

Trials are also classified as **confirmatory** or **exploratory** (EMA, 2012). The former include randomized controlled clinical trials and controlled trials to determine appropriate dosage levels of drugs, both of which

require strict protocols (discussed later in this chapter). These are often preceded by exploratory trials, the initial nature of which allows a less stringent protocol, which may be modified as analyses are undertaken; however, exploratory trials cannot be the sole basis of proof of efficacy. A trial that has both exploratory and confirmatory aspects is a **composite trial**.

Cluster randomized trials

A **cluster (group) randomized trial** is one in which the experimental unit (see later) is a group of individuals (Murray *et al.*, 2004a; Puffer *et al.*, 2005), and the groups, rather than the individuals in them, are randomized. These trials are undertaken when treatments are most conveniently or usually applied to groups (e.g., disease-preventive measures applied to a shed of chickens, rather than to individual birds).

Community trials

A **community trial** is one in which the experimental unit is an entire community. Community trials have been undertaken in human medicine; for example, the fluoridation of the public water supply to prevent dental caries (Lennon, 2003)¹³. Similarly, in trials of policy or educational interventions aimed at influencing animal health, the experimental units may be communities (e.g., Stringer *et al.*, 2011).

Multicentre trials

Pharmacological and toxicity trials, initial trials of therapeutic effect and safety, and exploratory trials are usually conducted either in a single laboratory or at a single field site. Confirmatory field trials, however, are often conducted at several sites, following a standard protocol. Such **multicentre trials** are conducted for two reasons:

1. they may be the only means of accruing sufficient animals within a reasonable period of time;
2. they allow a widely representative cross-section of the population of animals to be exposed to the therapy, increasing the validity of the trial (see 'The experimental population' later in this chapter).

Superiority, equivalence, and non-inferiority trials

Superiority trials are designed to detect a difference between a treated and a control group. Efficacy is established most convincingly in such trials. Sometimes, however, two treatments are compared without the goal of demonstrating superiority (e.g., comparing an inexpensive new drug with an established costly drug, with

¹⁰ The first placebo-controlled clinical trial was probably undertaken by the English physician, John Haygarth, at the end of the 18th century (Haygarth, 1800). At that time, a popular treatment for many diseases was the application of metal rods, called 'Perkins' tractors', to the body to relieve symptoms by the supposed electromagnetic influence of the metal. Haygarth made wooden imitation tractors, and found that they were as efficacious as the metal ones. In so doing, he demonstrated the placebo effect.

¹¹ The anthropologist, Claude Lévi-Strauss, alluded to this when he described how medical treatments, including those where ritual alone provides the cure, occur in a social context and convey a 'sense of security' to the social group (Lévi-Strauss, 1963).

¹² In the UK, randomized controlled trials were introduced in the mid-1940s by Bradford Hill: the first to test the efficacy of pertussis vaccine, and the second to test the efficacy of streptomycin in the treatment of pulmonary tuberculosis (Doll, 1992).

¹³ The application of a therapy to a whole community without its consent does, of course, raise ethical issues (Cross, 2003).

the objective of replacing the latter by the former). This may involve demonstrating that the effects of each treatment are, within certain limits, the same (**equivalence trials**: Christley and Reid, 2003). A particular category of equivalence study is the **bioequivalence study**. This assesses the pharmacological kinetics of a new substance (e.g., the extent to which an active substance reaches the tissues, and its tissue concentration) relative to a reference substance. This is the basis for concluding that preparations are therapeutically equivalent. Equivalence trials that assess if the effects of two treatments are the same may be more specifically termed 'full equivalence trials'.

A special case of an equivalence trial is the **non-inferiority trial**, in which the goal is to demonstrate that a therapy is no worse than an established one (Head *et al.*, 2012). This is common in initial trials of therapeutic effect, where it is often desirable to demonstrate that a new drug is no less effective than (i.e., is non-inferior to) an established preparation, although it could also be equivalent or better.

Design, conduct and analysis

The trial protocol

The goal and design of a clinical trial should be documented in a **trial protocol**. This is required by regulatory organizations, which assess the value and validity of the proposed trial, and also provides background information to veterinarians and owners who are asked to participate in the trial. The main components of a protocol are listed in *Table 17.1*¹⁴.

A sound trial protocol is consistent with well-focussed clinical questions, which are central to evidence-based medicine. Such questions are often cast in a specialized framework, termed **PICO** (Richardson *et al.*, 1995; Huang *et al.*, 2006):

- **P**: the patient/population being addressed;
- **I**: the intervention being considered;
- **C**: the comparison/control;
- **O**: the outcome¹⁵.

¹⁴ A similar protocol is the basis of the standard for reporting trials in livestock with production, health and food-safety outcomes (O'Connor *et al.*, 2010), and medical clinical trials (Schulz *et al.*, 2010).

¹⁵ The PICO framework can be extended to **PICOTT**, to include the Type of question being asked (e.g., relating to therapy, diagnosis, prognosis or harm) and the best Type of study design to answer the question (e.g., randomised controlled trial; diagnostic test evaluation) (Schardt *et al.*, 2007).

The primary hypothesis

The first step in writing a protocol for a clinical trial is determination of its **major objective**, so that a **primary hypothesis** can be formulated. Thus, a primary hypothesis could be 'evening primrose oil has a beneficial effect against canine atopy' (Scarff and Lloyd, 1992). Several principal criteria of response might be assessed, but it is helpful if one particular response variable can be identified as the main criterion for testing the primary hypothesis; this is the **primary end-point**. The following topics must be addressed in determining this end-point:

- which end-points are the most *clinically* and *economically* important?
- which of these can be measured in a reasonable manner?
- what practical constraints (e.g., budgetary limits) exist?

Thus, the primary end-point in the evening primrose oil trial might be the level of pruritus. Other end-points could include the levels of oedema and erythema; these constitute **secondary end-points**.

The response variables that are used to measure the end-points (the **primary** and **secondary end-point variables**) should adequately represent the effect that is being studied in the trial, and therefore address the primary hypothesis (**construct validity** and **content validity**: see Chapter 10). Thus, there is a relationship between plasma essential fatty acid levels and the inflammatory response (Horrobin, 1990), and so changes in plasma phospholipid levels could be monitored, but these are less clinically relevant than the actual clinical signs, which therefore may be more appropriate response variables for ensuring construct validity. However, clinical signs often are measured subjectively on an ordinal or visual analogue scale (see Chapter 10), whereas fatty acid levels can be measured on the ratio scale. Thus, a compromise between strength of measurement and relevance (construct validity) may be necessary. If complex or subjective measures are used, their reliability should be assessed (see Chapter 10).

In exploratory trials, it may be desirable to use more than one primary end-point variable, to cover the potential range of effects of a therapy, one of which may then be subsequently selected for a confirmatory trial.

Defining efficacy

The primary end-point defines the **outcome** that is assessed, and therefore the nature of the trial's response variables (*Table 17.2*), and efficacy is determined in terms of differences between treatment and control

Table 17.1 Main components of a protocol for a clinical trial. (Adapted and reduced from Noordhuizen *et al.*, 1993; and Chan *et al.*, 2013: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3541470/table/tbl1/>.)

General information	Strategies to reach target sample size Owners' informed consent
Title of trial:	Therapeutic or prophylactic intervention:
Study design	Dosage
Population	Product formulation and identification
Interventions	Placebo/standard treatment formulation and identification
Names and addresses of investigators	Method of administration (in sufficient detail to allow replication)
Name and address of sponsor(s)/source of funding	Operators' safety
Identity of trial site(s)	Definition of stage at which administration stops (stopping guidelines)
Ethical approval (plans for seeking research ethics committee/institutional review board approval)	Blinding technique (including details of who will be blinded: owners, outcome assessors, data analysts, etc.)
Justification and objectives	Compliance monitoring
Reason for execution of the trial (including summary of relevant studies – published and unpublished)	Randomization
Primary hypothesis to be tested	Stratification variables
Primary end-point	Implementation of allocation process
Secondary hypotheses to be tested	Protocol amendments (communication of changes in eligibility criteria, outcomes and analyses to investigators, participants and regulators)
Design	Data collection:
Type of trial (e.g., parallel-group, cross-over, sequential, factorial)	Data to be collected
Response variables:	Frequency of data collection
Nature of response variables (level of measurement)	Method for recording adverse drug reactions/harm
Scoring system (for ordinal variables)	Identification of experimental units
Definition of efficacy (magnitude of the difference to be detected between treatment and control groups)	Training/standardization of data collection and recording
Explanation of relevance (e.g., to clinical outcome)	Confidentiality
Duration:	Communication between participants
Participant timeline (date of beginning, time schedule for enrolment of cases, date of end)	Data management:
Duration of disease under study	Data entry
Duration of treatment	Coding
Drug withdrawal period (for food-producing animals)	Security
Decision rules for terminating a trial	Data checks
Experimental population:*	Data storage
Study setting (e.g., veterinary practices, farms)	Data analysis:
The experimental unit	Technique for 'unblinding'
Composition (e.g., age, sex, breed)	Description of statistical methods
Inclusion/exclusion criteria	Interpretation of significance levels/confidence intervals
Post-admission withdrawal criteria	Interim analyses
Definition of cases/diagnostic criteria	Approach to withdrawals and animals 'lost to follow-up'
Case identification	Dissemination:
Selection of controls	Plans to sponsor and communicate trials results to participants, the veterinary profession, the public and other relevant groups
Sample-size determination (method, including statistical and clinical assumptions)	Plans (if any) to grant public access to the full protocol, participant-level dataset and statistical code

* Some authorities refer to the experimental population as the study population. The latter term is not used, to avoid confusion with study population defined as the population from which a *sample* of animals is drawn (see Chapter 13).

groups. The differences may be measured either absolutely or relatively (e.g., by the relative risk¹⁶).

¹⁶ Caution should be exercised in using the odds ratio in clinical trials. Recall (in Chapter 15) that the odds ratio overestimates the relative risk, and this overestimation can be considerable if disease (outcome) is not rare. In clinical trials, the outcome of interest (e.g., recovery rate) may not be rare, in which circumstance the odds ratio will substantially overestimate the effect of treatment.

Several parameters can be used to express the effect of therapeutic or prophylactic treatment, exemplified here using the results of a clinical trial of the efficacy of a *Dichelobacter nodosus* (previously *Bacteroides nodosus*) vaccine against footrot in sheep (Table 17.3).

Relative risk reduction The difference between disease risk in untreated and treated animals, in relative terms,

Table 17.2 Response variables assessed in clinical trials.

Response variable		Efficacy	
Level of measurement	Examples	Definition	Description of method and sample size determination
Nominal	Mortality Incidence Prevalence	Difference between two proportions Relative risk	Chapter 14 Chapter 15
Ordinal	Scores of clinical severity Condition scores	Difference between two medians	Chapter 14
Interval and ratio and the visual analogue scale	Liveweight gain Milk cell counts Visual analogue assessment of clinical severity	Difference between means (if the variables are Normally distributed)	Chapter 14

Table 17.3 Efficacy of a *Dichelobacter nodosus* vaccine against footrot (84 days after vaccination). (Raw data derived from Hindmarsh *et al.*, 1989.)

	Footrot present	Footrot absent	Total
Non-vaccinated sheep	94 (<i>a</i>)	328 (<i>b</i>)	422 (<i>a</i> + <i>b</i>)
Vaccinated sheep	21 (<i>c</i>)	296 (<i>d</i>)	317 (<i>c</i> + <i>d</i>)

is expressed as the relative risk reduction (*RRR*). Using the notation and values in *Table 17.3*:

$$\begin{aligned}
 RRR &= [a/(a+b) - c/(c+d)]/[a/(a+b)] \\
 &= (94/422 - 21/317)/(94/422) \\
 &= (0.223 - 0.066)/0.223 \\
 &= 0.157/0.223 \\
 &= 0.704.
 \end{aligned}$$

Thus, the risk of footrot in vaccinated (treated) animals is 70.4% less than the risk in unvaccinated (untreated) animals; that is, 0.066 is 70.4% less than 0.223 (i.e., is only 29.6% of 0.223). Alternatively, 70.4% of footrot in unvaccinated sheep is attributable to not being vaccinated; equally, this is the percentage of disease prevented by the vaccine in vaccinated animals.

If a therapy is totally ineffective, disease risk in treated and untreated animals would be the same, and the *RRR* = 0%. In contrast, if a therapy is completely effective, there would be no disease in treated animals, and the *RRR* = 100%.

The *RRR* is algebraically identical to the attributable proportion (exposed), λ_{exp} (see Chapter 15), in which untreated animals are defined as 'exposed' to the risk factor, and so confidence intervals can be calculated in a similar way. This parameter is commonly quoted for **vaccinal efficacy**, but has the same disadvantage as the relative risk in not reflecting baseline levels of disease occurrence (see Chapter 15). It therefore is not possible to discriminate between large treatment effects and small ones.

Absolute risk reduction Baseline disease occurrence is maintained by calculating the absolute risk reduction (*ARR*), which was expressed in Chapter 15 as the attributable risk (exposed). In the current context, the exposed group, again, is the untreated group. For the data in *Table 17.3*:

$$\begin{aligned}
 ARR &= a/(a+b) - c/(c+d) \\
 &= 94/422 - 21/317 \\
 &= 0.223 - 0.066 \\
 &= 0.157.
 \end{aligned}$$

Therefore, if sheep are vaccinated, footrot will be reduced by 15.7 cases per 100 animals that are vaccinated; this is the difference between footrot occurrence if sheep are not vaccinated (22.3 cases per 100 sheep) and footrot occurrence if they are vaccinated (6.6 cases per 100 sheep).

Confidence intervals for the *ARR* are estimated in the same way as those for the attributable risk (exposed).

Number needed to treat The number of animals that need to be treated to prevent one additional case of

disease is the 'number needed to treat' (*NNT*). This is probably easier to grasp than the *ARR*. It is simply the reciprocal of the *ARR*:

$$NNT = 1/ARR.$$

Using the values in *Table 17.3*:

$$\begin{aligned} NNT &= 1/0.157 \\ &= 6.37. \end{aligned}$$

Thus, rounding up, seven sheep need to be vaccinated to prevent one additional case of footrot, and about one in every seven sheep will benefit from vaccination.

A low value for the *NNT* is therefore better than a high value: a treatment that will lead to one less case for every 10 individuals treated is clearly better than a competing treatment that leads to one less case for every 50 treated. A treatment that is highly efficacious, targeted against a disease that is common, will result in a low *NNT*. In contrast, a treatment with only modest efficacy, targeted against a rare disease, will result in a high *NNT*. The *NNT* therefore depends on both efficacy and disease frequency.

Confidence intervals can be calculated for the *NNT* by taking the reciprocals of the confidence intervals for the *ARR* (Cook and Sackett, 1995; Daly, 1998). This can produce enigmatic results; notably, when treatment effects are not statistically significant, in which circumstance the related confidence intervals can produce a negative value for the lower limit, which conflicts with the possibility that the *NNT* can only be positive. Consequently, when treatments are not significantly different, the *NNT* is sometimes given without a confidence interval. Altman (1998) provides a solution to this problem.

Some authors discourage the use of the *NNT*, mainly because of the assumptions made when converting rate differences into numbers of individuals; and, in some situations, it may be preferable to quote the *ARR*, multiplied by a constant – say 100 – as the main summary measure of effect from a clinical trial (Hutton, 2000).

In situations where treatments have adverse effects, it also is possible to calculate the **number needed to harm**, and, similarly, the **relative risk increase** (Sackett *et al.*, 2000).

Standards for efficacy

There is not always a fixed standard for acceptable therapeutic effect or efficacy. In the European Union, for example, the therapeutic effect of a veterinary medicinal product is generally understood by the

relevant regulatory body to be the effect 'promised by the manufacturer' (Beechinor, 1993). European regulatory guidelines have attempted to define efficacy of ectoparasitic preparations (CVMP, 1993):

$$\% \text{ efficacy} = \frac{C - T}{C} \times 100,$$

where:

C = mean number of ectoparasites/animal in the control group;¹⁷

T = mean number of ectoparasites/animal in the treated group.

An approximate confidence interval can be calculated for this parameter by computing a confidence interval for the difference between the two means, *C* and *T* (as described in Chapter 14), and then dividing each limit by *C*. (Note that this approximate approach ascribes no sampling variation to *C* in the denominator.)

The European Union recommendations are an overall efficacy of 90–100% for permanent ectoparasites of cattle, and treatment is aimed at eliminating the parasitic stages of those ectoparasites that only complete part of their life-cycle on an animal (EMEA, 2014). Specific levels include approximately 100% for all lice and *Sarcoptes scabiei*; more than 90% for *Psoroptes bovis*, *Chorioptes bovis* and ticks; about 100% (always more than 90%) for *Haematobia* and *Stomoxys* spp.; and more than 90% for *Musca* and *Hydrotea* spp. Note, however, that the value of a therapeutic effect lies ultimately in its clinical and economic impact.

The experimental unit

The **experimental unit** is the smallest *independent* unit to which the treatment is randomly allocated. It may be elementary units (see Chapter 13) – usually individual animals – or aggregates such as pens or herds. Most companion-animal and human clinical trials involve allocation to individuals. Some trials in livestock, in contrast, may involve allocation of treatments to groups (cluster randomized trials, previously described; e.g., Gill, 1987). In contrast, the experimental unit may be the udder quarter when locally-administered intramammary preparations are being

¹⁷ The mean may be the arithmetic mean, geometric mean, or other appropriate transformation (see Chapters 12 and 20); noting, however, that transformations have less meaning to clinicians than the original scales. Dobson and others (2009) discuss the appropriateness of using the arithmetic mean and the geometric mean.

assessed; the elementary unit is then the quarter, not the animal.

The experimental unit may be a group because events at the individual level cannot be measured, even though they are of interest. For instance, in trials of in-feed compounds likely to affect weight gain in poultry and pigs, either the amount eaten by, or the weight increase of, individuals within a house or pen is not recorded. This often arises because it is not practical to identify individual animals at weighing. Consequently, liveweight gain per house or pen is the response variable.

Thus, the efficacy of in-feed antibiotic medication in reducing the incidence of streptococcal meningitis in pigs could be assessed by dividing a herd into pens containing a specified number of animals (Johnston *et al.*, 1992). The treatment is then randomly allocated to the pens, and medicated and placebo diets supplied to pigs in the respective treatment and control pens. Additionally, when animals are penned together, external factors (e.g., farm hygiene) may affect the groups, and such 'group effects' cannot be separated from individual treatment effects (Donner, 1993; Speare *et al.*, 1995). In these circumstances, sample-size determination for the trial and subsequent analyses must take into account the clustering of individuals within pens, because the clustering results in non-independent observations.

A particular problem arises with trials involving some infectious diseases. If the treatment could reduce excretion of infectious agents (e.g., vaccination in poultry houses or anthelmintic trials on farms), then treated and control animals should not be kept together because any reduction in infection 'pressure' will benefit treated and control animals; similarly, control animals constitute a source of infection to treated animals. This can lead to similar results in both categories (Thurber *et al.*, 1977), therefore reducing the likelihood of detecting beneficial therapeutic effects. The practice of mixing animals in each group is therefore unacceptable when herd immunity or group immunity is being assessed. In these circumstances, an appropriate independent unit must be identified. Thus, separate houses could be used on an intensive poultry enterprise, or separate tanks on a fish farm. Dairy farms, in contrast, usually have a continuous production policy with mixing of animals, and so the herd may become the experimental unit.

The experimental population

The population in which a trial is conducted is the **experimental population**. This should be representative of the target population (see also Chapter 13).

Differences between experimental and target populations may result in the trial not being generalizable (**externally valid**); that is, unbiased inferences regarding the target population cannot be made. For example, findings from a trial of an anaesthetic drug conducted only on thoroughbred horses may not be relevant to the general horse population because of differences in level of fitness between thoroughbreds and other types of horse (Short, 1987). External validity (which is facilitated by conducting trials 'in the field') contrasts with **internal validity**, which indicates that observed differences between treatment and control groups in the *experimental population* can be legitimately attributed to the treatment. Internal validity is obtained by good trial design (e.g., randomization). The evaluation of external validity usually requires much more information than assessment of internal validity.

Prophylactic trials may require selection of an experimental population that is at high risk of developing disease so that natural challenge can be anticipated during the period of the trial. Previous knowledge of disease on potential trial sites may be sufficient to identify candidate populations (Johnston *et al.*, 1992). However, the period of natural challenge may vary, reflecting complex patterns of infection. Many infections are seasonal (see Figure 8.15); others may be poorly predictable (Clemens *et al.*, 1993).

Admission and exclusion criteria

Criteria for inclusion of animals in a trial (**admission criteria, eligibility criteria**) must be defined. These should be listed in the protocol, and include:

- a precise **definition of the condition** on which the treatment is being assessed;
- the **criteria for diagnosis** of the condition.

For example, in the trial of the efficacy of evening primrose oil in the treatment of canine atopy, chronically pruritic dogs were included only if they conformed to a documented set of diagnostic criteria (Willemse, 1986) and reacted positively to the relevant intradermal skin tests. Similarly, specific types of mastitis may need to be defined in bovine mastitis trials; other admission criteria could include parity and stage of lactation.

Exclusion criteria are the corollaries of admission criteria. Thus, dogs with positive reactions to flea allergens were excluded from the trial of evening primrose oil. Cows might be excluded from a mastitis trial if they had been previously treated for mastitis during the relevant lactation, if they had multiple mammary infections, or if they also had other diseases that

could affect treatment. Trials of non-steroidal anti-inflammatory drugs would require exclusion from the treatment group of animals to which corticosteroids were being administered. However, too many exclusion criteria should be avoided; otherwise, external validity may be compromised. It may be prudent to accommodate factors either in the trial design by stratification, or during the analysis.

Informed consent The objectives and general outline of a trial should be explained to owners of animals that are included in the trial, and then their willingness to participate recorded before the trial begins. This is **informed consent**. This has been poorly documented in veterinary studies in the past (Lund *et al.*, 1998). Although research institutions and veterinary schools often have ethical review boards that are able to assess informed consent, such reviews may not be available to veterinary practitioners and others who may be involved in clinical trials. However, ethical guidelines for the conduct of research in general veterinary practice, including clinical trials, have been suggested by a number of official veterinary organizations (e.g., Trees *et al.*, 2013).

Blinding

Blinding (masking) is a means of reducing bias. In this technique, those responsible for measurements or clinical assessment are kept unaware of the treatment assigned to each group. The traditional classification of blinding into **single** or **double (full)** is based on whether the owner or attendant (patient in human medicine) or investigator is 'blinded' (Table 17.4). 'The investigator' can be more than one category of person; for example, participating veterinary practitioners and the principal investigators that analyse the results (the term **treble blinding** or **triple blinding** has been advocated in this situation).

A problem with this classification is that there is considerable variability in clinicians' interpretations and epidemiology-textbook definitions of these terms

(Devereaux *et al.*, 2001). The blinding status of all people for whom blinding may influence the validity of a trial thus should be explicitly reported, to avoid any ambiguity, and some therefore advocate discontinuation of the traditional blinding terms (Moher *et al.*, 2010).

Blinding should be employed wherever possible, and is facilitated by the use of a placebo in the control group. However, there may be circumstances in which blinding is not feasible; for example, if two radically different treatments are being compared (e.g., comparing infiltration of local anaesthetic with bloodless castrators to reduce pain associated with castration and tail-docking of lambs: Kent *et al.*, 2004), or if formulation of visually identical 'trial' and 'standard' drugs is impracticable. Such unblinded studies are sometimes termed **open-label** trials (Everitt, 2006). Open-label trials can be avoided by partial blinding through denying personnel involved in clinical assessment access to details of treatment. If full blinding is infeasible, those that are blinded (sponsor, investigator or owner) should be clearly documented, as should any intentional or unintentional breaking of blinding.

Randomization

Simple randomization

Simple randomization is the most basic type of randomization. When there are only two treatments, tossing a coin is an elementary method. However, it is usually more rigorous to randomize in advance using random numbers (using Appendix X or appropriate software), allocating units identified by odd numbers to one group, and evenly numbered units to the other. Randomization should be applied *after* eligible units have been identified.

When comparing a new treatment with an established one, and there is evidence that the new treatment is superior, it can be allocated to twice the number of units as the established one (Peto, 1978). This can increase the benefit to participating animals. For example, if a new treatment was expected to reduce mortality by 50%, 2:1 randomization would be expected to produce an equal number of deaths in the two groups. This randomization ratio can be obtained by using twice as many random numbers for allocation of the new treatment as those used to allocate the established one. There is no advantage in increasing the ratio further, because of the resultant loss of statistical power, which can only be counteracted by increasing the total sample size.

Table 17.4 Summary of traditional types of blinding to assignment of treatment.

Type of blinding	Knowledge of assignment of treatment	
	Owner	Investigator
None	Yes	Yes
Single	No	Yes
Double (full)	No	No

Block randomization

Simple randomization can produce grossly uneven totals in each group if a small trial is undertaken. This problem can be overcome using **block (restricted) randomization**. This limits randomization to blocks of units, and ensures that within a block equal numbers are allocated to each treatment. For example, if randomization is restricted to units of four animals, receiving either treatment A or treatment B, the numbers 1–6 are attached to the six possible treatment allocations in a block: AABB, ABBA, ABAB, BBAA, BAAB and BABA. One of these numbers is then selected randomly for the next block of four individuals entering the trial, and given its treatment allocation.

Stratification

Some factors (e.g., age, parity or severity of disease) may be known to affect the outcome of a trial and may bias results if they are unevenly distributed between the treatment and control groups. This can be taken into account during initial randomization by **stratifying** both groups according to these confounding factors. The experimental units are then allocated to treatment and control groups within the strata, using simple or block randomization¹⁸.

Stratification leads to related samples and therefore decreases the number of units that are required to detect a specified difference between treatment and control groups (see Chapter 14).

These and other methods of randomization are described in detail by Rosenberger and Lachlin (2002), Berger and Antsygina (2015) and Baghbaninaghadehi and others (2016).

Alternatives to randomization

Some alternatives to randomization include allocation according to date of entry (e.g., treatment on odd days, placebo on even days), clinic record number, wishes of the owner, and preceding results. An example of the last method is the 'play-the-winner' approach (Zelen, 1969): if a treatment is followed by success, the next unit receives the same treatment; if it is followed by failure, the next unit receives the alternative treatment. This limits the number of animals receiving an inferior treatment. All of these techniques have disadvantages

¹⁸ The best way to make individuals in treatment and control groups as alike as possible with respect to characteristics that may affect outcome is by individual matching (see Chapter 15). In a trial with two treatment groups, this would necessitate finding pairs of individuals matched on all factors selected for matching and then randomizing the pairs; however, this is usually impracticable.

and should never be considered as acceptable alternatives to randomization (Bulpitt, 1996).

Minimization is a largely non-random method, which aims to ensure that treatment groups are balanced with predefined patient factors, as well as with the number of individuals in each group (Pocock and Simon, 1975; Taves, 2010). This can provide better balanced groups than block randomization, and can incorporate more factors than stratification (Scott *et al.*, 2002).

Trial designs

There are four main trial designs:

1. parallel-group (standard);
2. cross-over;
3. sequential;
4. factorial.

Parallel-group-design (standard) trials

The **parallel-group (standard)** design is commonly used in confirmatory trials. Experimental units are randomized to a single treatment group using either simple or block randomization, and each group receives a single treatment. A specified number of units enter the trial and are followed for a predetermined period of time, after which the treatment is stopped. The basic design can be refined by stratification.

The analytical techniques employed in a parallel trial involving two unstratified groups are listed in *Table 17.2*. Estimation of parameters with associated confidence intervals is preferred to hypothesis testing, for the reasons given in Chapter 14. Confidence intervals also should be quoted for negative, as well as positive, results.

Stratification may require complex analysis (Meinert, 2012), but this approach is seldom used in veterinary product development.

Cross-over-design trials

In a **cross-over trial**, subjects are exposed to more than one treatment consecutively, each treatment regimen being selected randomly (Hills and Armitage, 1979). Experimental units therefore serve as their own controls, and treatment and control groups are therefore individually matched. This design is useful when treatments are intended to alleviate a condition, rather than effect a cure, so that after the first treatment is withdrawn the subject is in a position to receive a second. Examples are comparisons of anti-inflammatory drugs in arthritis, and hypoglycaemics in diabetes. Moreover, a comparison on the same

individuals is likely to be more precise than a comparison between subjects because the responses are paired (see Chapter 14). The cross-over trial is therefore valuable if the number of experimental units is limited. However, analysis of results is complex if a treatment effect carries over into the next treatment period.

If treatment effects do not carry over into subsequent treatment periods, the techniques described in Chapter 14 for the analysis of related samples can be used. However, the absence of a carry-over effect may be difficult to prove. If there is any doubt, conclusions should be based only on the first period, using analyses of independent samples. Alternatively, more complex methods that identify interactions between treatment effect and period of treatment can be applied (Jones and Kenward, 2015).

Sequential-design trials

A **sequential trial** is one whose conduct at any stage depends on the results so far obtained (Whitehead, 1997). Two treatments are usually compared, and experimental units (usually individuals) enter the trial in pairs; one individual being given one treatment, and one the other. Results are then analysed sequentially according to the outcome in the pairs, and boundaries are drawn to define levels at which specified differences are obtained at the desired level of statistical significance. The trial may be terminated when these levels are reached. If the desired level is not reached, the investigator may decide to increase the sample size indefinitely until the former is reached; this is an **open**¹⁹ trial. Alternatively, the trial may be terminated if a specified difference is not reached by a certain stage; this is a **closed** trial.

Sequential trials facilitate early detection of beneficial treatment effects and can require fewer experimental units. However, they may be difficult to plan because their duration is initially unknown. They also are unsuited to trials in which treatment response times are long because responses need to be analysed quickly so that a decision can be taken to enlist more subjects, if necessary.

A key feature of sequential trials therefore is that significance tests are conducted repeatedly on accumulating data. This tends to *increase* the overall significance level (see Chapter 14: 'Multiple significance testing', and Armitage *et al.*, 1969). For example, if five interim analyses, rather than one, are conducted, the chance of at least one analysis showing a treatment

difference at the 5% level ($\alpha = 0.05$) increases to 0.23 (i.e., $1 - [1 - \alpha]^5$); if 20 interim analyses are undertaken, it increases to 0.64 (i.e., $1 - [1 - \alpha]^{20}$). The overall Type 1 error therefore increases if, for any single interim analysis, $\alpha = 0.05$ is used as the trial's stopping criterion. If data are analysed frequently enough, a value of $P < 0.05$ is likely, regardless of whether there is a treatment difference.

This problem can be overcome by choosing a more stringent *nominal* significance level for each repeated test, so that the overall significance level is kept at a reasonable value such as 0.05 or 0.01 (Pocock, 1983). Table 17.5 can be used for this purpose under two-tailed conditions. For example, if the overall significance level is set at $\alpha = 0.05$, and if a maximum of three analyses is anticipated, $P < 0.022$ is used as the stopping rule for a treatment difference at each analysis; similarly, if a maximum of five analyses is anticipated, $P < 0.016$ is used. Suitable values for one-sided tests are given by Demets and Ware (1980). Sample-size calculations should therefore be modified if more than one significance test is planned (Wittes, 2002).

Trials also can be adapted during their remaining time to allow for modification of particular design elements (e.g., sample size, randomization ratio) at an interim analysis, with full control of Type I error, in which circumstance they are termed **adaptive-design trials** (Pong and Chow, 2010).

Sequential trials are considered in detail by Armitage (1975), Whitehead (1997) and Ellenberg and others (2002).

Table 17.5 Nominal significance level required for repeated significance testing with an overall significance level, $\alpha = 0.05$ or 0.01, and various values of N , the maximum number of tests. (From Pocock, 1977.)

N	$\alpha = 0.05$	$\alpha = 0.01$
2	0.0294	0.0056
3	0.0221	0.0041
4	0.0182	0.0033
5	0.0158	0.0028
6	0.0142	0.0025
7	0.0130	0.0023
8	0.0120	0.0021
9	0.0112	0.0019
10	0.0106	0.0018
15	0.0086	0.0015
20	0.0075	0.0013

¹⁹ This should not be confused with open, uncontrolled trials (mentioned earlier in this chapter) in which the same animals are compared before, and after, treatment.

Factorial-design trials

If two factors, A and B, are to be investigated at a levels and b levels, respectively, this gives rise to ab experimental conditions, corresponding to all possible combinations of the levels of the two factors; this is a complete $a \times b$ **factorial-design** study (Zar, 1996). Thus, in a 2×2 factorial design where one factor is the absence or presence of treatment A ($a = 2$) and the other factor is the absence or presence of treatment B ($b = 2$), animals are randomly allocated to one of the four combinations of two treatments thus: A alone, B alone, A and B together, and neither A nor B. This is a powerful method of testing the effect of two factors in the same study, using the same experimental units. It can be used to explore any interactions that might occur between the two treatments and, in the absence of interaction, enables groups to be combined to increase the power to detect the effects of treatment A and treatment B. The approach can be extended to any number of factors, and with each factor having a different number of levels.

What sample size should be selected?

Superiority trials

The number of experimental units in treatment and control groups in a superiority trial should be determined using the techniques outlined in others chapters (Table 17.2). In summary, the following four parameters should be considered:

1. the acceptable level of Type I error, α (the probability of erroneously inferring a difference between treatment and control groups);
2. test power, $1 - \beta$ (the probability of correctly inferring a difference between treatment and control groups) where β = the probability of Type II error (the probability of erroneously missing a true difference between treatment and control groups);
3. the magnitude of the treatment effect (i.e., the difference between proportions, medians or means);
4. the choice of alternative hypothesis: 'one-tailed' or 'two-tailed'.

There is no rule for defining parameters 1–3. Type I error is traditionally set at 0.05, but a value as low as 0.01 can be justified if a trial is unique and its findings are unlikely to be repeated in the future. Power can vary considerably (values between 0.50 and 0.95 have been quoted in human clinical trials; 0.80 is common when $\alpha = 0.05$, and 0.96 when $\alpha = 0.01$: see Chapter 14). The magnitude of the treatment effect depends on its clinical and economic relevance.

(In clinical trials in which treatment and control groups are matched, the formulae for sample-size

determination listed in previous chapters will tend to overestimate the number of units required.)

If a placebo or no treatment has been administered to the control group, and there is therefore intuitive evidence that the treatment can cause only an improvement in comparison with the control group, then a one-tailed test (see Chapter 14) is justifiable, and the sample size can be determined accordingly. However, the use of placebos or 'negative' control groups is now ethically debatable; consequently, many contemporary clinical trials use a 'positive' control group and it is therefore prudent to assume two-tailed conditions (i.e., the treatment under test may be either better, or worse, than the standard treatment). Additionally, the magnitude of the difference between treatment and 'positive' control groups may be small; thus, large sample sizes may be specified. These may be unattainable in practice. However, knowledge of sample-size determination is necessary to appreciate the inferential limitations that may be imposed by the number of experimental units included in a trial.

Wittes (2002) presents a general discussion of sample-size calculation in clinical trials, and Machin and others (1997) tabulate sample sizes. Sample-size determination for cross-over trials is discussed by Senn (2002). Sample-size determination for sequential trials is discussed by Armitage (1975) and Whitehead (1997); the estimated sample size for a given Type I and Type II error is smaller than for a non-sequential trial. General guidelines are provided by Shuster (1992). Hayes and Bennett (1999) review methods of sample-size calculation for cluster randomized trials. Hallstrom and Trobaugh (1985) provide formulae that incorporate diagnostic sensitivity and specificity (see Chapters 10 and 20). Norman and his colleagues (2012) discuss determination of sample size in the absence of good data for parameter values.

Equivalence and non-inferiority trials

Determination of sample size to demonstrate equivalence focusses on the maximum difference that is tolerated – termed the **margin of clinical equivalence** (M). This is the largest difference that is clinically acceptable, larger differences having unwelcome consequences; for example, a difference in mean blood glucose levels induced by a new hypoglycaemic, relative to an established drug, such that signs of diabetes recur. The common context is therefore of demonstrating non-inferiority.

Thus, for a dichotomous response variable, the sample-size formula to demonstrate a difference between two proportions (see Chapter 14) is applied, but $p_1 - p_2$ is now the margin of clinical tolerance, rather than the difference to be detected. Moreover, the values of α

and β are reversed because attention now focusses on the power of the comparison to detect any difference that may be present.

For example, a new footrot vaccine may be compared with the one listed in *Table 17.3*, which prevents disease in 296 of 317 sheep (93%). In determining equivalence of the two vaccines, it may be considered acceptable not to detect a difference as trivial as 5% or less *in favour* of the established vaccine (in which circumstance the vaccines are deemed to be equivalent), but desirable to detect a difference greater than 5% (in which circumstance they are identified as not being equivalent); thus $M = 5\%$. Note that this is a one-tailed situation, because if non-equivalence is demonstrated it is only in the direction of the new vaccine being inferior to the established one – not *either* inferior *or* superior.

Assume that the two vaccines are equivalent, with the proportion of disease in vaccinated sheep = 0.07 (i.e., 100% – 93%: the estimate for the established vaccine). Thus, $p_1 = 0.07$. If $M = 5\%$, $p_2 = 0.07 + 0.05 = 0.12$, and $(p_1 + p_2)/2 = (0.07 + 0.12)/2 = 0.095 = p$. Set β at 0.05 (that is, power = 0.95), and set α at 0.20; thus, from Appendix XV, $M_\beta = 1.64$, and $M_\alpha = 0.84$ (because the hypothesis is one-tailed). The number of animals required in each vaccinated group, n , is then derived thus:

$$\begin{aligned} n &= \frac{[M_\alpha \sqrt{2p(1-p)} + M_\beta \sqrt{p_1(1-p_1)p_2(1-p_2)}]^2}{(p_2 - p_1)^2} \\ &= \frac{(0.84\sqrt{0.19 \times 0.905} + 1.64\sqrt{0.07 \times 0.93 + 0.12 \times 0.88})^2}{(0.12 - 0.07)^2} \\ &= [(0.3483 + 0.6776)^2]/0.0025 = 421. \end{aligned}$$

Therefore, a trial comprising 421 animals vaccinated with the established vaccine, and 421 animals vaccinated with the new vaccine, will detect any difference between the performance of the two vaccines as small as an absolute difference in disease occurrence of 5%, but no smaller, with probability 0.95.

Alternatively, the requirement may be to show that a new vaccine is neither *inferior* nor *superior* to an established one (i.e., assessment of full equivalence). In this circumstance, M_β is obtained from Appendix XV using a β value obtained by setting $1 - 2\beta$ equal to the overall power required for the two one-sided tests that need to be conducted. For example, if the overall power is to be 0.95, then M_β should be based on β being 0.025 (i.e., $M_\beta = 1.96$).

The same approach can be adopted for continuous and ordinal variables, using the relevant formulae for sample-size determination for differences between

two means or two groups of ordinal-ranked data (see Chapter 14).

Losses to follow-up

The outcome of a trial may not be recorded in some experimental units because they are lost to follow-up. For example, owners may move house or refuse to continue with the trial. The extent of this loss to follow-up needs to be assessed, and is frequently based on the experience of the investigator. The sample size then needs to be increased by multiplying the sample size by $1/(1 - d)$, where d is the anticipated proportion of experimental units lost. For example, if $d = 10/100$, the sample size would need to be multiplied by 1.11 ($1/0.9$) to compensate.

Compliance

The success of a trial depends on participants acting in accordance with the instructions of the trial's designers; that is, **complying** with treatment. For example, they may decide to switch from the treatment under trial to an alternative treatment. Poor compliance will decrease the statistical power of the trial because the observed difference in outcome between treatment and control groups will be reduced, but it will not produce spurious differences between groups. Reasons for poor compliance include:

- unclear instructions;
- forgetfulness;
- inconvenience of participation;
- cost of participation;
- preference for alternative procedures;
- disappointment with results;
- side-effects.

Participants cannot be forced to comply, and so regular contact should be maintained with them so that they can be encouraged to comply, and the degree of compliance should be regularly assessed. For example, if a treatment is formulated as a tablet, the number of tablets remaining can be counted regularly by the veterinarian. Assessment may be difficult (e.g., with in-feed medication) but should, nevertheless, be attempted. Other methods of improving compliance include:

- enrolling motivated participants;
- assessing the willingness of participants to comply;
- providing incentives (e.g., free treatment);
- supplying simple, unambiguous instructions;
- limiting duration of the trial.

If non-compliance is substantial, the required sample size should again be modified in the same way as adjustment for loss to follow-up. If both losses to follow-up and non-compliance are anticipated, a composite value for d is required.

The potential for loss to follow-up and incomplete compliance raises issues about how a trial is analysed. One option involves analysis by **intention-to-treat**, which compares individuals in the groups to which they were originally randomly assigned. This is generally interpreted as including all individuals, regardless of the treatment they actually received, and subsequent withdrawal or deviation from the trial protocol. This approach preserves the initial randomization; moreover, any deviations also are likely to occur when treatments are applied in routine clinical practice. Intention-to-treat analysis therefore is most suitable for trials of effectiveness (i.e., the effect of the treatment in all to whom it is offered), particularly in superiority trials, rather than investigations of efficacy, which it tends to underestimate (Armitage *et al.*, 2002).

A second option is analysis **per protocol**, in which departures from the trial protocol are excluded from the groups to which they were initially assigned, and which allows regrouping and subsequent analysis of participants according to the treatment that they actually received. This therefore explores the consequences of very specific treatment regimens, which are unlikely to be typical of the target population. As such, per protocol analysis may provide initial insights into the value of a treatment, but is generally no substitute for intention-to-treat analysis²⁰.

Terminating a trial

The number of experimental units entering a trial and the duration of treatment are specified during the design of a trial; therefore a trial will usually last as long as it takes to enlist the units and for the last unit to complete the trial. However, it may be necessary to terminate a trial (particularly a long-term one) prematurely if there are serious adverse side-effects in the treatment group or the number of patients recruited will not be adequate, and such **decision rules** should be written into the trial's protocol. In sequential trials, another decision rule may be that a trial will be terminated when the specified difference is detected to the

predetermined level of significance (see earlier in this chapter).

Decision rules, and the advantages of early and late termination of trials, are discussed in detail by Bulpitt (1996).

Interpretation of results

In Chapter 14, the use of statistical hypothesis testing as an approach to interpreting data was discussed. Increasingly, however, this is being replaced by estimation – in particular, by calculating confidence intervals (introduced in Chapter 12). Significance testing and confidence interval estimation are two ways of interpreting the same data. However, an advantage of confidence intervals is that they encourage the investigator to express results in terms of the size of any treatment effect or difference. The following discussion will therefore place particular emphasis on the interpretation of confidence intervals.

Superiority trials

The goal of a superiority trial is to detect a difference between treated animals and controls. Evidence of a difference is provided, at the 5% level of significance, if the probability of a Type I error is less than 5% (exact values of P should always be quoted). The 95% confidence interval for the difference between the treatment effects will then exclude the null value (zero for differences; but one for ratio measures such as the relative risk and odds ratio, and ratios of geometric means). This is illustrated in *Figure 17.1a*. A 95% confidence interval with a lower bound clearly above the null value, and with a related value of P substantially lower than 0.05, provides strong evidence for superiority of the treated group over the control group. A 95% confidence interval with the lower limit touching the null value ($P = 0.05$) provides adequate evidence of superiority at the 5% level of significance. In contrast, if the 95% confidence interval includes the null value ($P > 0.05$), there is insufficient evidence to demonstrate superiority.

Equivalence and non-inferiority trials

A full equivalence trial is intended to confirm the absence of a clinically-relevant difference between treatments. This is best explored using confidence intervals. First, the margin of clinical equivalence, M , is selected. This margin should be chosen before a trial is undertaken to prevent bias, and therefore has been specified in the sample-size calculation undertaken before the study was conducted (see earlier). Two treatments (say, control and new treatment) are considered equivalent if the 95% confidence

²⁰ Some authors recommend per protocol analysis for equivalence and non-inferiority trials (Dasgupta *et al.*, 2010), although the relative merits of it and the intention-to-treat approach are less clear for non-inferiority trials (Sanchez and Chen, 2006).

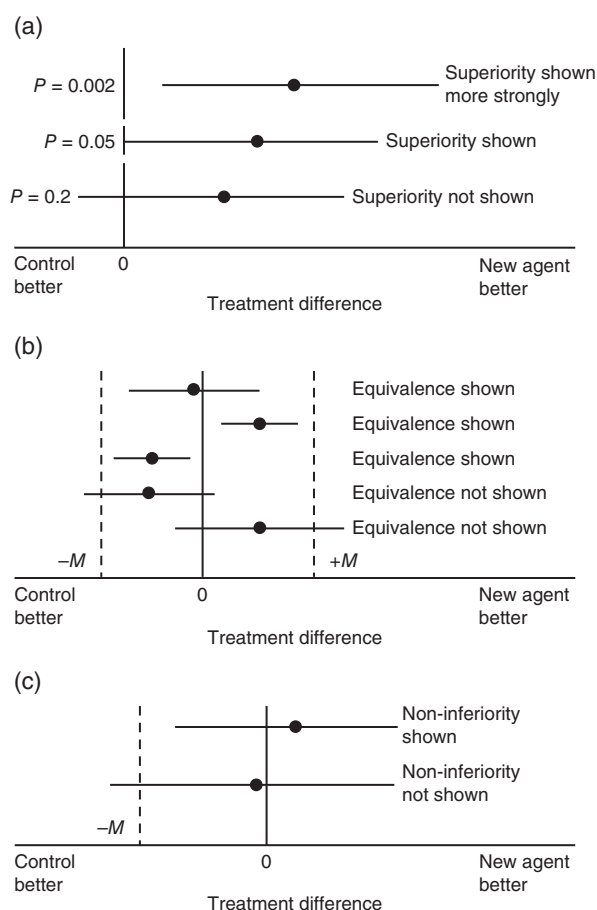


Fig. 17.1 Significance levels and confidence intervals in clinical trials. (a) Superiority trials; (b) equivalence trials; (c) non-inferiority trials. Null value for treatment difference between treated animals and controls = 0. ●: Point estimate of treatment difference; —: 95% interval estimate of treatment difference.

interval²¹ lies entirely within the interval $-M$ to $+M$ (Figure 17.1b).

A non-inferiority trial aims to demonstrate that a new therapy is no less efficacious than (i.e., is non-inferior to) an established preparation, although it also could be equivalent or better. Thus, concern lies with a

difference only in one direction. The new preparation therefore is considered not to be inferior to the established (control) preparation only if the confidence interval lies entirely to the right of $-M$ (Figure 17.1c). For example, a new footrot vaccine may be compared with an established one, with M set at a difference of 5% in the proportion of disease in sheep between the two groups in favour of the established vaccine (i.e., 5% less disease in sheep vaccinated with the established preparation than in sheep given the new vaccine). If the 95% confidence interval for the difference was -7% , -3% , then it would cross the $-M$ boundary (i.e., does *not* fall entirely to the right of -5%), and so it could *not* be concluded that the new vaccine was non-inferior to the established one.

Sometimes, the goal of a comparison may switch from a non-inferiority trial to a superiority trial, or vice versa. Results then have to be interpreted cautiously (EAEMP, 2000).

Meta-analysis

Meta-analysis is the statistical analysis of data pooled from several studies to integrate findings²². The technique has its recent origins in psychotherapeutic and educational research (Glass, 1976; Hunt, 1997)²³ and has been widely applied in the social sciences, where key texts have been published (Glass *et al.*, 1981; Card, 2011; Schmidt and Hunter, 2015). More recently, it has been used in economics (Van den Bergh *et al.*, 1997), marketing (Farley and Lehmann, 1986), biology (e.g., to investigate parasite-induced behavioural changes: Poulin, 1994) and ecology (Osenberg *et al.*, 1999). In human and veterinary medicine, meta-analysis has been applied in several areas (Stangle and Berry, 2000), including the evaluation of diagnostic tests²⁴ (e.g., Greiner *et al.*, 1997), genetics (Guerra and Goldstein, 2009), observational studies²⁵ (Willeberg, 1993; Fourichon *et al.*, 2000; Stroup *et al.*, 2000), health

²¹ In bioequivalence studies involving drug kinetics, 90% intervals are the accepted standard (Ocaña *et al.*, 2008). This exemplifies the **two one-sided tests (TOST) procedure** (Schuirmann, 1987). Using TOST, equivalence is established at the α significance level if a $(1 - 2\alpha) \times 100\%$ confidence interval for the difference in efficacies is contained within the interval $-M$ to $+M$. The reason the confidence interval is $(1 - 2\alpha) \times 100\%$, and not the usual $(1 - \alpha) \times 100\%$, is because the method is tantamount to performing two one-sided tests. Thus, using a 90% interval yields a 0.05 level of significance for testing equivalence. The TOST procedure can be directly extended to testing equivalence in other parameters such as means and odds ratios.

²² The term is derived from the Greek preposition, *μετα-* (*meta-*) = 'alongside', 'among', or 'in connection with'. A subsidiary meaning is 'after'. Meta-analysis is therefore either one that is done alongside/ in conjunction with the normal analysis, or one that is done after the normal analysis, that is, at a later stage in the process.

²³ The first identifiable meta-analysis was undertaken on typhoid vaccination at the beginning of the 20th century by the English mathematician, Karl Pearson (1904).

²⁴ Whiting and others (2003) give guidelines for the quality assessment of studies of diagnostic tests included in systematic reviews.

²⁵ There are formal recommendations for reporting such studies (*MOOSE: Meta-analysis Of Observational Studies in Epidemiology*: Stroup *et al.*, 2000).

policy (Tangl and Berry (2000), cost–benefit analysis of diagnostic techniques and treatments, assessment of the magnitude of health problems (e.g., Chesney, 2001; Dohoo *et al.*, 2003; Trotz-Williams and Trees, 2003) and drug resistance (Falzon *et al.*, 2014). However, it has been used most extensively in the area of clinical trials (e.g., Srinand *et al.*, 1995; Peters *et al.*, 2000; Whitehead, 2002; Steffan *et al.*, 2006) and, for that reason, is introduced in this chapter.

Meta-analyses frequently are statistical components of **systematic reviews**, which developed in medicine and veterinary medicine towards the end of the last century, and which assemble and critically appraise all relevant studies on a specific topic, using systematic methods to limit bias (Porta, 2014). They therefore mark a shift away from traditional ‘authoritative reviews’ by experts (Oxman and Guyatt, 1993). Moreover, the quality of their content can be assessed rigorously (Balslem *et al.*, 2011; Guyatt *et al.*, 2011a–h).

The key characteristics of a systematic review (Higgins and Green, 2011) are:

- a clearly-stated set of objectives with pre-defined eligibility criteria for studies;
- an explicit, reproducible methodology;
- a systematic search that attempts to identify all studies that would meet the eligibility criteria;
- an assessment of the validity of the findings of the studies that are included (e.g., through assessment of the risk of bias);
- a systematic presentation, and synthesis, of the characteristics and findings of the studies that are included.

Many, but not all, systematic reviews include meta-analyses. Systematic reviews are discussed in detail in Chapter 19, which also further considers meta-analysis.

Goals of meta-analysis

The aims of meta-analysis (Sacks *et al.*, 1987; Dickersin and Berlin, 1992; Marubini and Valsecchi, 1995) are to:

- increase statistical power for primary end-points;
- resolve uncertainty if there are conflicting results;
- improve estimates of therapeutic effect, and their precision²⁶;
- answer questions not posed at the beginning of individual trials;
- give a ‘state-of-the-art’ literature review;

²⁶ This includes not only revealing beneficial effects that are not identified in isolated studies, but also identification of false-positive effects in individual studies: meta-analysis is designed to produce *accurate* results – not necessarily positive ones.

- facilitate analysis of sub-groups when the power of individual analyses is low;
- guide researchers in planning new trials;
- offer rigorous support for generalization of a treatment (i.e., external validity);
- balance ‘overflow of enthusiasm’ which might accompany introduction of a new procedure following a single beneficial report.

Correctly conducted meta-analyses therefore offer strong evidence for efficacy of treatment (Table 17.6). The statistical procedures used are also applicable to the analysis of multicentre trials.

However, there are disadvantages, as well as advantages, to the technique (Table 17.7). Perhaps the major disadvantage is the seductive notion that combination

Table 17.6 Hierarchy of strength of evidence concerning efficacy of treatment. (From Marubini and Valsecchi, 1995.)

1. Anecdotal case reports
2. Case series without controls
3. Series with literature controls
4. Analysis using computer databases
5. Case-control observational studies
6. Series based on historical control groups
7. Single randomized controlled clinical trials
8. Meta-analyses of randomized controlled clinical trials

The table lists the types of study used in medicine, suggested by Green and Byar (1984). The table can be considered as an eight-tiered pyramid. In the context of clinical trials, the base on which conclusions about efficacy can be built becomes broader as one moves downwards. Some authors place initial assessment of efficacy in laboratory animals at the apex of the pyramid because of weaknesses associated with inter-species extrapolation (see Chapter 9); and the order of single randomized controlled clinical trials relative to meta-analyses is arguable (Berlin and Golub, 2014). Similar hierarchies (levels of evidence) also are available for more general application in evidence-based medicine (Burns *et al.*, 2011).

Table 17.7 Advantages and disadvantages of meta-analysis. (Based on Meinert, 1989.)

Advantages

Focusses attention on trials as an evaluation tool
Increases the impact of trials in clinical practice
Encourages good trial design and reporting

Disadvantages

Current fashion for meta-analysis may discourage large definitive trials
Tendency to unwittingly mix different trials and ignore differences
Potential for tension between meta-analyst and conductors of clinical trials

of several small trials is a substitute for a well-designed large one²⁷.

In this section, the main issues associated with meta-analysis are outlined. For details of specific statistical procedures, the reader is directed to the standard texts mentioned above, and to Abramson (1991), Dickersin and Berlin (1992) and Deeks and others (2001, 2011).

Components of meta-analysis

There are both *qualitative* and *quantitative* components to meta-analysis, listed in a scheme for meta-analysis of clinical trials (Naylor, 1989):

- selection of trials according to inclusion and exclusion criteria;
- evaluation of the quality of the trials;
- abstraction of key trial characteristics and data;
- analysis of similarity in design, execution and analysis, and exploration of differences between trials;
- aggregation of data, testing various combinations and interpretations;
- drawing of careful conclusions.

Note that the conventional, qualitative, review article has traditionally been accepted as the means of summarizing research data – usually by listing the individual results of several studies – and lacks objective rigorous analysis. A properly-designed meta-analysis, in contrast, goes further, and uses quantitative analytical procedures to combine results from several sources, where possible, to produce an overall conclusion.

Sources of data

Data for meta-analyses are usually obtained from published material, most of which is presented in refereed journals. This has the advantage of guaranteeing (at least theoretically) minimum standards with respect to the design, conduct and analysis of the component studies. However, there is a tendency for positive findings (beneficial treatment effects) to be more readily accepted for publication than results that either do not show significant effects or reveal only minor effects; this constitutes **publication bias** (Rothstein *et al.*, 2005). This is a complex matter, though, and, although published trials can show larger intervention effects than ‘grey trials’ (i.e., reports that are produced

by all levels of government, academics, business and industry, in print and electronic formats, but that are not controlled by commercial publishers: Hopewell *et al.*, 2007), unpublished results also can show larger effects than published ones (Detsky *et al.*, 1987). Assessment of the quality of *all* potential data therefore is desirable, so that useful material does not escape the analyst. Approaches to correcting for publication bias are detailed by Rothstein and others (2005), Moreno and others (2009), Ahmed and others (2011), Sterne and others (2011), and Simonsohn and others (2014).

Other related biases also can occur during the process of publication. These include **time-lag bias**, where studies with unfavourable findings take longer to be published than those with favourable results (Ioannidis, 1998); **language bias**, where non-English-language articles are more likely to be rewritten in English if they report significant results (Egger *et al.*, 1997); and **selective-outcome reporting**, where non-significant study outcomes are entirely excluded on publication (Kirkham *et al.*, 2010).

Comparability of sources

A key feature of component trials is the **variability (heterogeneity)** in their results. Trials may seem to measure the same outcome but, nevertheless, may be inconsistent or contradictory; this is **statistical heterogeneity** (Fletcher, 2012). This is generally due to differences in the design, conduct or analysis of the studies (Horwitz, 1987); for example, contradiction appears to be more prominent between studies with small sample sizes, and between those with non-randomized designs (Ioannidis, 2005).

In contrast, different trials may be looking at different concepts, albeit with the same intention (e.g., management of conditions using advisory leaflets, CD roms or consultations with veterinarians or veterinary nurses); this is **clinical heterogeneity** (Fletcher, 2012).

Additionally, different trials may be measuring different response variables on different scales (e.g., ordinal data or visual analogue measurements). Differences between old and recent studies may be ascribed to underlying health trends unrelated to the therapy in question – somewhat akin to the use of historical controls.

If a meta-analysis intends to address general policy or efficacy of a class of drugs, then incorporation of trials with obvious differences can be condoned. However, a specific question will require selection of a relatively homogeneous set of trials.

Differences between the different studies that are included in an analysis, and reflected in statistical heterogeneity, prevent interpretation of pooled estimates

²⁷ This may appear particularly attractive in the current academic climate where financial support is in short supply, and there is pressure to generate publications.

as being precise²⁸, and 99% confidence limits therefore may be more prudent than the conventional 95% limits.

Data analysis

Analytical techniques treat each incorporated clinical trial as a **stratum**. The single treatment effects are estimated within each trial, and are then combined to produce a suitable summary, weighted treatment effect. Methods of weighting, and addressing variability in study results, vary. However, the tendency to simply pool the results of the trials and compute an average effect should be avoided. This could be dangerously misleading; for example, a mean mortality rate computed from a series of separate mortality rates does not address differences in sample size between trials, and therefore the different precision of each trial's estimate.

A common approach for categorical data is to provide a weighted estimate, for example, of the odds ratio or relative risk. Standard methods include the Mantel–Haenszel procedure (see Chapter 15); Westwood and others (2003) give an example relating to the effects of monensin treatment on lameness in dairy cattle. More sophisticated procedures allow pooling of parameters that have been adjusted for confounding (Greenland, 1987; McCandless, 2012).

An approach for continuous response variables involves calculation of an **effect size**²⁹. This may be expressed as the difference between the mean values of the treatment and control groups divided by the standard deviation in the control group, when the standard deviations in the treatment and control groups differ (**Glass's delta**: Glass *et al.*, 1981). Alternatively, if the two standard deviations are roughly the same then they can be pooled to calculate **Cohen's d** (Ellis, 2010); and if the groups are of different sizes then each group's standard deviation can be weighed according to its size to calculate **Hedges' g** (Hedges, 1981).

The effect size can be interpreted with reference to tables of probabilities associated with the upper tail of the Normal distribution (see Appendix XV). For example, an effect size of 2.9 means that 99.8% of controls have values below the mean value of treated

individuals. Consulting Appendix XV, this percentage is obtained by identifying the one-tailed probability, P , in the body of the table for which the effect size equals z . The percentage then equals $(1 - P) \times 100$. Thus, if the effect size = 2.9, $P = 0.0019$, and $(1 - P) \times 100 = (1 - 0.0019) \times 100 = 99.81\%$. Similarly, for an effect size of 1.0, the corresponding percentage is 84% ($\{1 - 0.1587\} \times 100$).

Effect size has no units, and so allows the combination of results expressed in different units. However, it should be interpreted with caution because it depends not only on differences in the effect itself, but also on differences in standard deviations. The use of effect size is therefore particularly dubious if sample sizes are small. McGough and Faraone (2009) discuss the clinical relevance of the various effect sizes.

Heterogeneity

The statistical heterogeneity between studies must always be addressed. Commonly, tests for heterogeneity are based on χ^2 or F statistics for categorical (Schlesselman, 1982) and continuous (Fleiss, 1986) data, respectively (see Chapter 15). These are usually interpreted liberally at the 10% level because of the relatively low power of such tests (Breslow and Day, 1980). A sensitivity analysis (see Chapter 23) also can be conducted to determine if exclusion of one or more trials materially affects the heterogeneity. If the heterogeneity is larger than can be inferred from the results of significance tests, a summary measure is questionable, and the reason for the heterogeneity should be explored³⁰. Note, however, that a high P value does not unequivocally indicate that the results are homogeneous, and the data should be explored by other means, such as graphical representation. Examples include a vertical two-tiered plot of results (e.g., odds ratios, relative risks³¹ or effect size) with their 95% and 70% confidence intervals, for ease of comparison around the point estimates (Pocock and Hughes, 1990).

Higgins and Thompson (2002) derive other statistics to assess heterogeneity, as an alternative to significance testing (see also Chapter 15).

Fixed-effect and random-effects models

Many of the analytical procedures that have been employed in meta-analyses are based on a **fixed-effect (common effect)** model (Hedges and Vevea, 1998),

²⁸ The confidence limit is strictly a limit on the expected results, based on what was done in the studies, rather than on future trials.

²⁹ The term 'effect size' also is used generally to refer to ways of quantifying the size of differences between two groups. It therefore encompasses parameters such as the odds ratio, relative risk and number needed to treat. See Kelley and Preacher (2012) for a detailed discussion of effect size, including its different and conflicting definitions.

³⁰ For example, different dosage levels (analogous to different exposure levels in observational studies: e.g., Frumkin and Berlin, 1988) may induce heterogeneity.

³¹ Odds ratios and relative risks are best plotted on a logarithmic scale.

which assumes that all trials included in the meta-analysis are estimating the *same* treatment effect. They therefore assume that the only differences in treatment effect are as a result of sampling variation, and ignore any treatment variability between different studies when producing a summary estimate. Therefore, when assigning weights to the different studies, the information in the smaller studies can be largely ignored because better information is available about the same treatment effect in the larger studies.

An alternative approach, based on a **random-effects** model (DerSimonian and Laird, 1986), assumes that the treatment effect may be *different*, and each study represents a random sample of a (theoretically infinite) number of studies; that is, there is a *distribution* of true effect sizes. The variability between studies is then an integral part of the analysis (Bailey, 1987), the aim of which is to estimate the mean treatment effect in a range of studies. Since each study provides information about a different treatment effect, small studies therefore cannot be discounted (large studies lose influence, whereas small studies gain influence), the variations in the observed treatment effects resulting from two sources: (1) the sampling variation in each study (the within-study variance); and (2) the variation of the true study effects about their mean (the between-study variance). The net result of such an analysis is that the interval estimate of treatment effect is generally widened relative to the fixed-effect estimate, particularly if there is clear heterogeneity between studies (Dickersin and Berlin, 1992).

In reality, whether or not the studies are all estimating the same treatment effect is not known. The results of tests for heterogeneity therefore frequently have formed the basis for deciding on the appropriate model. If the result of a test is non-significant, the fixed-effect model is generally employed; whereas significant results prompt a random-effects model.

Some authorities, however, argue that tests for heterogeneity should not form the basis of model selection (Borenstein *et al.*, 2009, 2010)³², concluding that the fixed-effect model should be used if: (1) the studies included in the analysis are functionally identical (i.e., any variables that have an impact on outcome are the same in all studies); and (2) the aim is to compute a common treatment effect in a particular population, rather than generalizing it to other populations. In contrast, if data have accumulated from a series of studies conducted independently then it is unlikely that the studies are functionally identical,

and so a random-effects model should be applied. This also is appropriate if the analysis aims to generalize results beyond a narrowly-defined population.

The random-effects model needs to be interpreted with caution (Marubini and Valsecchi, 1995). First, the degree of heterogeneity may be such that a random-effects model may greatly modify the inferences made from a fixed-effect model. This will tend to nullify the summary statistic for both models, and there is then a need to investigate the variability further. Secondly, specific statistical distributions of the random-effects model cannot be justified either empirically or by clinical reasoning. Finally, the random-effects model cannot be interpreted meaningfully at the level of the target population; it is merely the mean of a distribution that generates effects. The random-effects model therefore '*exchanges a questionable homogeneity assumption for a fictitious distribution of effects*' (Greenland, 1987).

Debate continues over the relative merits of the fixed-effect and random-effects approaches. Some of the biases can be reduced by excluding poorly-designed trials and including all relevant results (e.g., results from germane unpublished studies). With this goal in mind, Meinert (1989) has suggested that meta-analyses should be planned prospectively, with the component trials enlisted into a meta-analysis when they start, rather than being retrospectively identified. This should promote good individual trial design, and therefore consistent quality. Moreover, cumulative meta-analyses may allow both fixed-effect and random-effects models to demonstrate efficacy in the presence of heterogeneity of estimates³³.

Presentation of results

Any pooled results, and the results of each study, should be reported as point estimates with 95% confidence intervals, and presented graphically next to each other. *Figure 17.2* is an example. The results of the individual studies show considerable variability, with some results (*Figure 17.2a*: Studies 3, 4, 5, 7, 8, 9, 11 and 13) providing no evidence of a treatment effect (the upper 95% confidence limits for the odds ratios being greater than one). This variability is masked in the analysis of the accumulating data (*Figure 17.2b*), which consistently demonstrates a beneficial effect. The pooled results of the random-effects model and the final analysis of accumulating data, in this example, generate similar point and interval estimates of the

³² Application of such tests in these circumstances is an example of 'HARKing' (Hypothesizing After the Results are Known), which is considered to be bad practice (Kerr, 1998).

³³ There is, of course, danger of an increase in Type I error such as that which can occur in sequential trials. Yusuf and others (1991) suggest methods of significance-level adjustment in this circumstance.

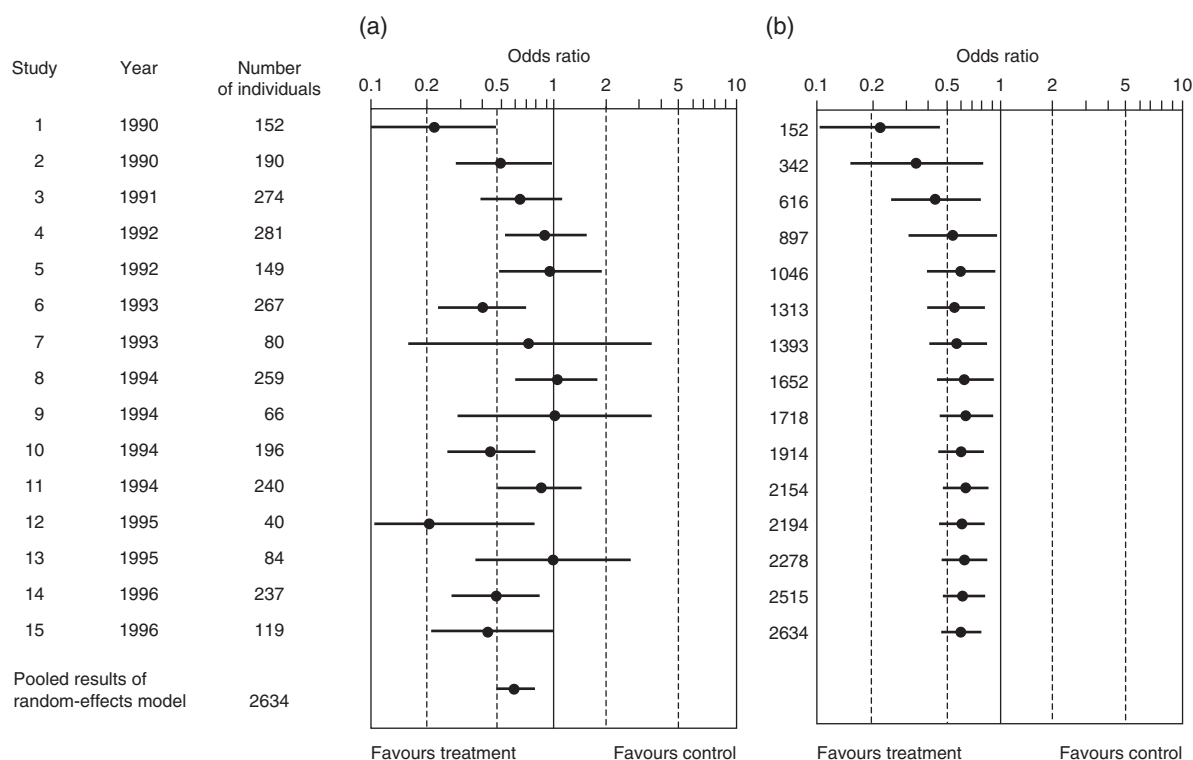


Fig. 17.2 A typical meta-analysis. (a) Individual results and pooled results using a random-effects model; (b) results of sequential analyses of accumulating data. ●: point estimate of the odds ratio; —: 95% interval estimate of the odds ratio. (Source: Based on Ionnidis, 2000. Reproduced with permission from Elsevier.)

odds ratio, demonstrating a significant treatment effect.

Although there has always been some controversy about the validity of meta-analysis (e.g., Liberati, 1995; LeLorier *et al.*, 1997; Borenstein *et al.*, 2009), it is becoming

increasingly popular as the number of studies with similar protocols has grown. By systematically combining studies, a rational attempt is made to overcome limits of size or scope in individual studies to obtain more reliable information about treatment effects.

Further reading

- Brody, T. (2012) *Clinical Trials: Study Design, Endpoints and Biomarkers, Drug Safety, and FDA and ICH Guidelines*. Academic Press, London
- Bulpitt, C.J. (1996) *Randomised Controlled Clinical Trials*, 2nd edn. Kluwer Academic Publishers, New York
- Card, N.A. and Casper, D.M. (2013) Meta-analysis and quantitative research synthesis. In: *The Oxford Handbook of Quantitative Methods. Volume 2: Statistical Analysis*. Ed. Little T.D., pp. 701–717. Oxford University Press, New York
- Chow, S.-C. and Liu, J.-P. (2014) *Design and Analysis of Clinical Trials*, 3rd edn. John Wiley, New York
- Cleophas, T.J., Zwinderman, A.H., Cleophas, T.F. and Cleophas, E.P. (2009) *Statistics Applied to Clinical Trials*, 4th edn. Springer, Dordrecht
- Dent, N. and Visanji, R. (Eds) (2001) *Veterinary Clinical Trials from Concept to Completion*. CRC Press, Boca Raton
- Donner, A. and Klar, N. (2000) *Design and Analysis of Cluster Randomized Trials in Health Research*. Arnold, London/Oxford University Press, New York
- Duncan, J.L., Abbott, E.M., Arundel, J.H., Eysker, M., Klei, T.R. *et al.* (2002) World association for the advancement of veterinary parasitology (WAAVP): second edition of guide-lines for evaluating the efficacy of equine anthelmintics. *Veterinary Parasitology*, **103**, 1–18
- Edwards, P. (2010) Questionnaires in clinical trials: guidelines for optimal design and administration. *Trials*, **11**, 2; doi 10.1186/1745-6215-11-2
- Elbers, A.R.W. and Schukken, Y.H. (1995) Critical features of veterinary field trials. *Veterinary Record*, **136**, 187–192

- Ellis, P.D. (2010) *The Essential Guide to Effect Sizes*. Cambridge University Press, Cambridge. (Includes a simple guide to meta-analysis)
- Friedman, L.M., Furberg, C.D., DeMets, D.L., Reboussin, D.M. and Granger, C.B. (2015) *Fundamentals of Clinical Trials*, 5th edn. Springer Cham, Heidelberg
- Gigerenzer, G., Gaissmaier, W., Kurz-Milcke, E., Schwartz, L.M. and Woloshin, S. (2008) Helping doctors and patients make sense of health statistics. *Psychological Science in the Public Interest*, **8**, 53–96. (A comprehensive discussion of the interpretation of the quantitative results of clinical trials and other medical studies)
- Gomberg-Maitland, M., Frison, L. and Halperin, J.L. (2003) Active-control clinical trials to establish equivalence or noninferiority: methodological and statistical concepts linked to quality. *American Heart Journal*, **146**, 398–403. (Guidelines for equivalence and non-inferiority trials)
- Guidelines for Clinical Trials (1988) Questionnaire 1088/A. International Dairy Federation, Brussels. (Guidelines with specific reference to mastitis)
- Guidelines for the Conduct of Bioequivalence Studies for Veterinary Medicinal Products (2012) EMA/CVMP/EWP/81976/2010. European Medicines Agency, London
- Guideline for the Demonstration of Efficacy for Veterinary Medicinal Products Containing Antimicrobial Substances (2013) EMA/CVMP/261180/2012. European Medicines Agency, London
- Guideline for the Testing and Evaluation of the Efficacy of Antiparasitic Substances for the Treatment and Prevention of Tick and Flea Infestation in Dogs and Cats (2016) EMEA/XCVMP/EWP/005/2000-Rev.3. European Medicines Agency, London
- Guideline on Statistical Principles for Clinical Trials for Veterinary Medicinal Products (Pharmaceuticals) (2012) EMA/CVMP/EWP/81976/2010. European Medicines Agency, London
- Guidelines: Veterinary Medicinal Products: General, Efficacy, Environmental Risk Assessment (1999). Office for Official Publications of the European Communities, Luxembourg
- Hackshaw, A.K. (2009) *A Concise Guide to Clinical Trials*. Wiley-Blackwell, Chichester
- Haidich, A.B. (2010) Meta-analysis in medical research. *Hippokratia*, **14**, Suppl. 1, 29–37
- Hennesy, D.R., Bauer, C., Boray, J.C., Conder, G., Dauschies, A. et al. (2006) World Association for the Advancement of Veterinary Parasitology (WAAVP): second edition of guidelines for evaluating the efficacy of anthelmintics in swine. *Veterinary Parasitology*, **141**, 138–149
- Hunt, M. (1997) *How Science Takes Stock: The Story of Meta-analysis*. Russell Sage Foundation, New York. (Examples of meta-analyses across a range of disciplines)
- Jacobs, D.E., Arakawa, A., Courtney, C.H., Gemmell, M. A., McCall, J.W. et al. (1994) World Association for the Advancement of Veterinary Parasitology (WAAVP): guidelines for evaluating the efficacy of anthelmintics for dogs and cats. *Veterinary Parasitology*, **52**, 179–202
- McLean, A. (1998) Good clinical practice for the conduct of clinical trials on veterinary medicine products: a critical look at the E.U. note for guidance. *The Quality Assurance Journal*, **2**, 69–73
- Murad, M.H., Montori, V.M., Ionnidis, J.P.A., Jaeschke, R., Devereaux, P.J. et al. (2014) How to read a systematic review and meta-analysis. *Journal of the American Medical Association*, **312**, 171–179
- Noordhuizen, J.P.T.M., Frankena, K., Ploeger, H. and Nell, T. (Eds) (1993) *Field Trial and Error*. Proceedings of the international seminar with workshops on the design, conduct and interpretation of field trials, Bergen Dal, Netherlands, 27 and 28 April 1993. Epidecon, Wageningen
- O'Connor, A.M., Sargeant, J.M., Gardner, I.A., Dickson, J. S., Torrence, M.E. et al. (2010) The REFLECT Statement: methods and processes of creating guidelines for randomized controlled trials for livestock and food safety. *Journal of Veterinary Internal Medicine*, **24**, 57–64
- Perino, L.J. and Apley, M.D. (1998) Clinical trial design in feedlots. *Veterinary Clinics of North America, Food Animal Practice*, **14**, 343–365
- Pocock, S.J. (1983) *Clinical Trials: A Practical Approach*. John Wiley, Chichester and New York
- Russo, M.W. (2007) How to review a meta-analysis. *Gastroenterology and Hepatology*, **3**, 637–642
- Schukken, Y.H. and Deluyker, H. (1995) Design of field trials for the evaluation of antibacterial products for therapy of bovine mastitis. *Journal of Veterinary Pharmacology and Therapeutics*, **18**, 274–283
- Schulz, K.F., Altman, D.G. and Moher, D. (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *British Medical Journal*, **340**, 698–702
- Senn, S. (2002) *Cross-over Trials in Clinical Research*, 2nd edn. John Wiley, Chichester
- Senn, S. (2007) *Statistical Issues in Drug Development*, 2nd edn. John Wiley, Chichester. (Includes the design and interpretation of clinical trials)
- Spriet, A. and Dupin-Spriet, T. (1997) *Good Practice of Clinical Drug Trials*, 2nd edn. Karger, Basel
- Sutton, A.J. and Higgins, J.P.T. (2010) Recent developments in meta-analysis. *Statistics in Medicine*, **27**, 625–650

Wood, I.B., Amaral, N.K., Bairden, K., Duncan, J.L., Kassai, T. *et al.* (1995) World Association for the Advancement of Veterinary Parasitology (WAAVP) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). *Veterinary Parasitology*, **58**, 181–213

Yazwinski, T.A., Chapman, H.D., Davis, T.J., Letonja, R.B., Pote, L. *et al.* (2003) World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anthelmintics in chickens and turkeys. *Veterinary Parasitology*, **116**, 159–173

18

Validity in epidemiological studies

The goal of epidemiology is to generate and interpret information about disease and health in populations in order to aid decision making. In an ideal world each research question would be addressed by a particular study, the study would provide an exact representation of the relevant domain, and the study results would provide the information needed to truthfully answer the question. Unfortunately, this is never the case. In truth, all studies provide flawed depictions of 'reality'. Maclure and Schneeweiss (2001) imagine epidemiological studies of causation to be like a telescope used to observe populations – they call this the **Episcope**. The Episcope is made up of a number of filters and lenses, each of which is imperfect and therefore distorts the image to a greater or lesser extent. A simplified version of Maclure and Schneeweiss' Episcope is shown in *Figure 18.1*. This has eight lenses representing the key issues affecting the validity of epidemiological studies (although these could be thought of as compound lenses, each containing an array of imperfect lenses):

1. background factors;
2. interpretation biases;
3. selection biases;
4. statistical interaction and effect-measure modification;
5. information biases;
6. confounding;
7. errors in analysis;
8. communication biases.

Each of these lenses will induce an amount of error and the resulting image of reality will be imperfect. An unavoidable problem is that we can only compare this image with other imperfect images, and hence we cannot say exactly where, how and to what extent the image differs from reality. The role of the epidemiologist is to minimize the error associated with each of these lenses as best as possible and then to understand where residual errors remain and the potential impact of these on the results. By doing this we can get closer

to knowing the true situation. Although it is often useful to think about these main sources of error in isolation, in reality they are an interconnected complex that may independently impact estimates of association between an exposure and an outcome, but also may do so by impacting one another. Hence, information biases may lead to selection biases and may affect measurement of, and therefore the ability to control for the effect of, confounders. Examples are described below.

Types of epidemiological error

Broadly, error can be defined as any belief, conclusion or action that is not correct. In epidemiology, the term error may be used in numerous ways. It is common to distinguish between random error (*Box 18.1*) and non-random error. **Random error** is variation that is due to chance. That is, random errors in one variable are not associated with other variables. In contrast **non-random errors** vary systematically between groups and are often called **biases**. (The concept of bias was introduced in Chapters 10 and 15.) Hence, bias occurs when an error affects some groups more than others. The term *bias*, when used in epidemiology, does not necessarily suggest prejudice on the part of the experimenter; many forms of bias may be inadvertent.

Conceptualization of random error depends, to some extent, on one's worldview. Some people view chance variation as an intrinsic part of reality that prevents full prediction, whilst others take a more deterministic view in which full understanding of all relevant variables can enable prediction without error, with random error simply resulting from incomplete knowledge. However, even with this latter view, in most situations it is unlikely that we will acquire sufficient knowledge to enable full prediction, and the unpredictability that results from incomplete knowledge has the same effect as that due to random variation.

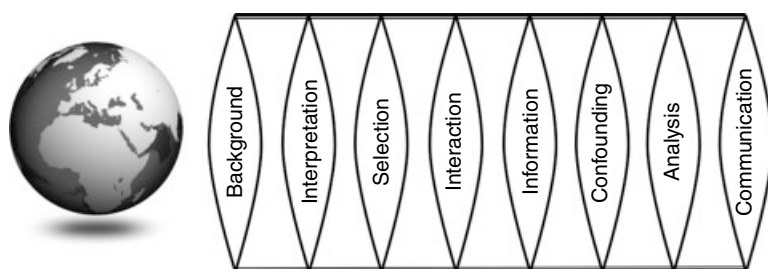


Fig. 18.1 Maclure and Schneeweiss (2001) imagine epidemiological studies of causation to be like a telescope (an Episcope) made up of imperfect lenses. The observer (epidemiologist) makes observations through this series of lenses, within each of which particular biases and errors may occur.

Box 18.1 An example of random error, selection bias and information bias.

A survey is carried out to assess the prevalence of lameness in a dairy herd of 100 milking cows. Because of time constraints, only a sample of the herd can be examined. If the entire herd were examined, it would have been found that 10 were lame. In other words, the true prevalence of lameness in this herd is 10% and, on average, if we examine a random sample of 10 cows, we may expect to find one lame. This last statement implies that, if we took many random samples of 10 cows, the average number of lame cows observed in the samples would be one.

Imagine a veterinarian examines a random sample of 20 cows from this herd and finds four lame; the prevalence estimate is therefore 20%. If another survey was conducted choosing another random sample of 20 animals, the prevalence estimate may have been 5%. If the study is repeated many times, the average prevalence estimate will be very similar to the true prevalence. The differences in prevalence estimates between studies occur because, by chance, more or fewer lame cows may appear in each selected group. This is known as

random error. The larger the sample size, the lower the error and the more precise the estimate of prevalence.

However, this assumes there is no systematic error (bias) in lameness assessment. Selection bias may have occurred if the cows selected were the first 20 to enter the yard. This method of selection may underestimate the prevalence of lameness, as the lame cows may tend to be toward the back of the herd. Importantly, repeating the study, with the same selection procedure, would lead to similarly biased estimates of the prevalence of lameness, with some variation due to random error.

There may also be measurement bias. For example, the investigator may be inexperienced and not identify subtle lameness. In this situation, the prevalence may be underestimated. Alternatively, the inspection may be carried out on very rough ground, resulting in the overdiagnosis of lameness and an overestimation of the prevalence. Again, repeating the study under these same circumstances would lead to repeated estimation of biased prevalence estimates.

The results or interpretation of an epidemiological study may, therefore, be 'wrong' due to random error (chance) and systematic error (bias). When conducting studies, epidemiologists should attempt to reduce both sources of error. When interpreting studies, a reader should, ideally, be aware of both types of error, be able to identify potential sources and impacts of these errors, and assess whether they have been adequately addressed in the study.

Accuracy, precision and validity in epidemiological studies

The terms accuracy, precision and validity are often applied to measurements (see Chapter 10). However,

these terms can equally be applied to epidemiological studies. A study that is able to estimate a parameter (e.g., prevalence or relative risk) with little error is said to have a high level of **accuracy**. In such cases the parameter estimates will be close approximations of the true values. Just like error, accuracy can be divided into two component parts. Studies in which parameters are estimated with little random error are said to have high **precision**, whereas studies with little systematic error (bias) are said to have high **validity** (see Chapter 10). Hence, for a study to be accurate, it must be both precise and valid.

Validity may further be divided into two types: **internal validity** and **external validity** (see Chapter 17). A study is said to be internally valid if the study results provide unbiased information about the individuals included in the study (i.e., the study sample or

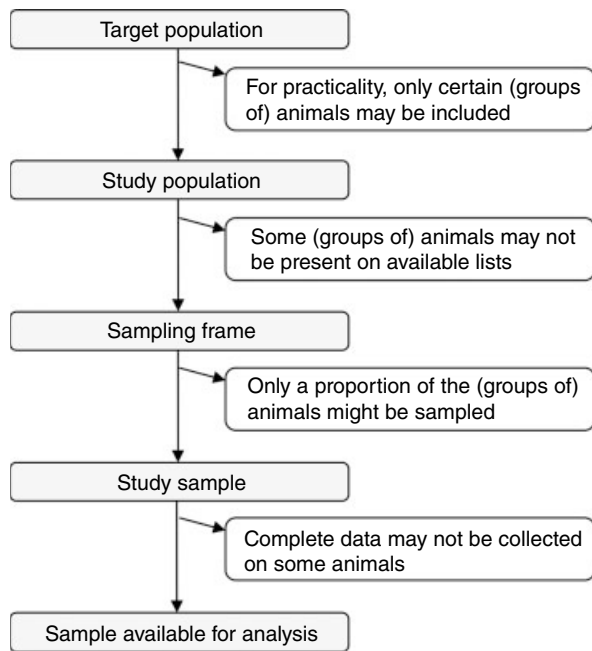


Fig. 18.2 Schematic representation of the process of selection of the sample available to the analyst from the target. At each stage only a sub-set may proceed to the subsequent stage. The aim of the selection process should be to ensure that all study units present at higher levels have equal probability of selection into the subsequent level, and failure to achieve this may result in selection bias.

experimental population) (Figure 18.2). Much of the practice of epidemiology is concerned with minimizing bias in order to maximize internal validity. External validity refers to the ability to extrapolate from the results of a study to a target population. In designing a study there may be tension between attempts to maximize internal validity and external validity. Selection of a relatively narrow study population may increase the potential to sample representatively from that population, but may make the study population unrepresentative of the target population. Although such situations may not be ideal, internal validity should be preferred over external validity, as there is little value in generalizing incorrect (biased) results.

Background factors

Random variation can be thought of as part of the 'background noise' within which an epidemiological study takes place. **Random error** is the resultant distortion in the results of a study. Random error often occurs because we usually study a sample of the population, rather than the entire population, but it can occur at any stage in an epidemiological study. In

the absence of bias, increasing the size of the sample will reduce the random error. When the prevalence, relative risk or other measure is calculated, what is really reported is an estimate, based on the available data. Ideally, this will approximate the true value, and confidence intervals (see Chapters 12 and 15) provide a guide to the precision of the estimate. Wide confidence intervals suggest poor precision (large random error), whereas narrow confidence intervals suggest high precision (small random error).

Epidemiological studies are conducted against the background of **current scientific knowledge**. Although science (and epidemiology) is often portrayed as an empirical endeavour in which the scientist is an impassive observer, in reality scientists must make a multitude of decisions and assumptions during the scientific process (e.g., Gilbert and Mulkay, 1984). These actions are taken, at least in part, on the basis of cultural understandings and within the existing scientific paradigm (see Chapter 1). That is, scientific research is less objective and more contingent than may be suggested in formal reports of research. Error and bias in current scientific knowledge may lead to error and bias in choice of research question and hypotheses. Similarly, incomplete scientific knowledge (the normal state of affairs) may restrict the range of possible research questions available to researchers. For example, prior to recognition, in 1984, of the association between *Helicobacter pylori* and peptic ulcers, hypotheses relating to management of this infection in order to treat gastric ulcers were simply not postulated. Subsequently, the discovery of this link has led to studies that have also identified a range of other effects of *H. pylori*, including as risk factors for gastric malignancies (Fock *et al.*, 2013).

Interpretation bias

Interpretation of scientific data is never completely independent of scientists' pre-existing beliefs and expectations. Errors resulting from the effects of scientists' (or other users of scientific results) pre-existing beliefs and expectations are called **interpretation biases**, and Kaptchuk (2003) defines six forms of these biases. Most interpretation biases occur after data are collected or during review of other scientists' work. **Confirmation bias** arises when evidence that supports a scientist's preconceptions is interpreted differently (more favourably) to that which challenges these notions. This also may involve **rescue bias**, in which evidence at odds with the scientist's convictions is discounted through selectively applied critical scrutiny.

Similarly, **mechanism bias** may result in reduced scepticism of the quality of evidence which is supported by the scientist's beliefs about the underlying processes. Where unanticipated evidence cannot be explained in such ways, **auxiliary hypothesis bias** may lead the scientist to suggest *ad hoc* modifications to the original hypothesis in order to explain why the unexpected results have occurred. Scientists also vary in the level of evidence required to make a judgement – so-called **time-will-tell bias**. Some may rapidly accept new data as evidence or proof (particularly where they have a vested interest, such as when it is they who have conducted the study), whereas others remain unconvinced by the data (perhaps employing one of the previous forms of interpretation bias). **Orientation bias** occurs prior to data collection and results in researchers' preconceptions affecting the design of the study or the collection of data in a way that influences the results. This may include decisions concerning which exposures to study, and how these and the outcome(s) are defined. It may also result in information biases, such as **expectation bias** (see information biases, later in this chapter).

Other forms of interpretative bias may be defined. For example, Gilbert and Mulkay (1984) describe the **truth-will-out device** in which scientists, faced with evidence that challenges their preconceptions, propose that despite such evidence their own beliefs will be shown to be correct *in the long run*. **Cognitive dissonance bias** (Sackett, 1979) occurs when belief in a mechanism increases in the face of contradictory evidence.

Interpretative biases can result from the **heuristics** (that is, 'rules of thumb') used when making decisions under uncertainty. Tversky and Kahneman (1974) provide a fascinating description of many such heuristics that may influence judgements by scientists and non-scientists alike.

Selection bias

Selection bias (introduced in Chapter 15) results from systematic differences between characteristics of the subjects available for analysis and the population from which they were drawn. Selection bias may arise between the study population and the study subjects during the selection process itself, during the period when the study is being conducted, or after collection of the data. Hence, there are many potential sources of selection bias and many types of selection bias have been named. Some of these are discussed in more detail below.

In any study, the aim is to draw conclusions about the target population based on the sample available for analysis. Therefore, the aim of any study is to ensure the latter group is truly representative of the former. Ideally, the study sample would be randomly drawn from all subjects in the target population and all sampled subjects would have complete data available for analysis. However, this is rarely the case and individuals with certain characteristics may be more or less likely to be present in the sample available for analysis. It is useful to think of the sampling process in terms of a flow diagram in which individuals may be more or less likely to leave at certain stages (*Figure 18.2*). The different levels of the sampling hierarchy were introduced in Chapter 13, but are repeated here because a clear understanding of the sampling process is needed in order to appreciate the range of potential sources of selection bias. The **target population** is the population about which the study is designed to make conclusions. The **study population** (also called the **study base**) is the population that the researchers aim to include in the study and from which a sample is drawn. Ideally, the target and study population should be the same. However, it may be impractical to sample from the entire target population. The **sampling (or study) frame** is a list of **study units** (often individual animals) that the researchers believe are in the study population. The **study sample** includes those study units selected from the study frame. The **sample available for analysis** consists of those study units about which sufficient data are collected for them to be included in the study analysis.

At each stage of the selection process only a sub-set of study units may be included into the subsequent level. The aim of the selection process should be to ensure that all study units present at higher levels have equal probability of selection into the subsequent level. Selection bias may arise due to the way in which study units are selected from the sample population and/or due to selective loss from the sample population prior to analysis. Examples of selection bias arising at each stage of the selection process are described here.

Selection of the study population from the target population

Often it is not possible to select all the animals in the target population for inclusion in a study, so the study population includes only a sub-set of the target population. Consider a study that aims to make conclusions about the dogs in a particular geographical area, and that defines dogs attending small-animal veterinary practices in that area as the study population (i.e., the veterinary practices are the source of the study population). It is possible that the dogs treated at these

practices may not be representative of the general dog population in the region (i.e., the target population). For example, these practices may see disproportionately more of certain breeds or types of animals. This may be a consequence of dealing with particular breeders, having a specialist interest in racing greyhounds, or being associated with a rescue shelter and seeing many young animals for vaccination and neutering, and so on. Alternatively, they may have a limited geographical scope (e.g., all urban dogs). In such cases the study population is unlikely to be representative of the proposed target population and thought should be given to developing a more representative study population or to redefining the target population. For example, consider a study that aims to determine the national prevalence of a particular disease. If, for practical reasons, the study only can select animals from just one region of the country it may be best to redefine the target population to include only that region. In this way it is clear to the researchers and to the readers that the study results apply directly to this region, and that extrapolation to the rest of the country relies on assumptions about the generalizability of the results to these other areas.

Identification of the sampling frame

In some situations the study population and the study sampling frame may be identical, such as when the study population is the dogs attending a clinic and the sampling frame is the patient records of the clinic. However, in other circumstances, an accurate sampling frame does not exist or cannot be accessed or constructed, such as when the study population is the dogs in a particular town and the sampling frame is the patient records of one clinic in that town (which, even if the only clinic in the town, may be unlikely to have records for all dogs in the town). In such situations researchers must rely on alternative sampling methods and, although probability sampling methods (Chapter 13) may be applied, a random sample may not be generated as not all units within the study population have an equal chance of being selected into the study sample.

Selection of the study sample from the sampling frame

A complete sampling frame (i.e., one that includes every individual identified with a unique identifier) often permits selection of the study sample by probability sampling methods. The absence of a suitable sampling frame, or the use of a non-probability method to select from a sampling frame, may result in biased selection into the study samples. For example, biased selection is likely to occur when only a

portion of animal owners asked to volunteer to be involved in a study agree to do so, even where those owners have been selected by probability sampling methods.

Omission of study subjects from the analysis

Not all individuals selected for study may provide data for the analysis. Hence, selection bias may occur not only when the study sample is selected, but also due to the presence of 'missing' data from the study sample (see Chapter 11). This type of selection bias occurs due to events during the implementation of the study. The most common causes include non-response bias, losses to follow-up and missing data in multivariable analyses.

Examples of selection biases

There are very many types of selection bias defined. Some are described in the section below. This list, which is adapted from Sackett (1979), is not exhaustive, but serves to illustrate some of the wide variety of ways in which selection bias can occur.

Competing risks bias may arise when there is 'competition' between mutually exclusive events and is most frequent when dealing with causes of death; as an animal can only die once, the risk of a specific cause of death can be affected by the risk of an earlier cause of death. As euthanasia and culling are widespread in veterinary medicine, this bias greatly impacts upon estimates of death due to particular disease. For example, a veterinary practice with a tendency towards early (pre-operative) euthanasia of horses with severe colic may have a higher post-operative survival rate compared with a practice that tends to operate irrespective of severity. Interpretation of the results from such studies should take into account the competing causes of death.

Healthcare access bias occurs when the study animals are those that are attended by an institution or clinic and they do not represent the general population. This may arise because: (1) personnel at the institution may have a special interest towards particular cases (**popularity bias**); for example, an orthopaedic surgeon may have a particular interest in greyhounds and attract a high proportion of this breed to his or her practice, hence conditions affecting greyhounds may be over-represented; (2) difficult or unusual cases are referred to referral centres (**referral filter bias**); for example, recurrent airway obstruction in horses often can be diagnosed and managed by field clinicians and only difficult, protracted or atypical cases tend to be referred; (3) geographical, cultural or economic factors may limit access by some subjects to diagnostic

facilities (**diagnostic access bias**); for example, owners of pleasure horses may be more or less likely to seek veterinary attention for particular types of health problems in their horses, compared with owners of performance horses.

Incidence-prevalence bias (also called **survival bias** or **Neyman bias**) may occur if 'survivors' of a disease are studied, and the exposure is related to prognostic factors or to survival. Such exposures will affect the probability that an individual will survive long enough to participate in a study. This bias can occur in cross-sectional and case-control studies using prevalent cases. For example, a study may wish to examine the association between exposure to a particular factor and the level of milk production in dairy cows. However, in a cross-sectional study, the cows most affected by the exposure may have already been culled from the herd prior to the survey, due to low milk production.

Case ascertainment bias arises when there is a systematic difference in the probability that particular types of cases will be detected and included in a study. For example, in order to be included as a case in a hospital-based study, an animal must attend a veterinary clinic. However, this may depend on the affluence of the owner, which may be related to a number of risk factors. If the control population is not selected from the equivalent population from which the cases arose (i.e., among those animals that would have attended a clinic if ill), bias could result. This example could also be considered an example of healthcare access bias. Case ascertainment bias may occur due to **surveillance bias** when there is more intensive surveillance or screening among those individuals or groups known to be exposed compared with those unexposed.

Exclusion bias arises when individuals in one group are excluded if they have conditions related to an exposure, whereas they are included in the other group. For example, an intervention study was designed to assess the effect of post-partum administration of a non-steroidal anti-inflammatory drug (NSAID) on calving-to-conception interval in cattle. One group of post-partum cows was randomly selected to receive the NSAID (i.e., the treatment group). However, if animals in the non-treatment group required treatment with NSAIDs for another reason (lameness, mastitis, etc.), they were excluded from the study. Hence, the treatment group was more likely to include cows with a range of other diseases, which may increase the calving-to-conception interval and alter the apparent efficacy of the NSAIDs. This is often dealt with during the analysis by 'intention-to-treat' (i.e., non-exclusion) (see Chapter 17). Exclusion

bias may be a particular problem in some case-control studies. For example, cases may be selected from all animals with a particular condition, whereas controls are selected only from the healthy population (i.e., those without the case disease or other diseases). As the healthy population may have a lower exposure to a range of risk factors that also relate to the disease in question, the effect of these risk factors may be overestimated.

The **healthy-worker effect** may cause a lower rate of disease in individuals in particular population sub-groups compared with the general population as a result of biased recruitment or retention of individuals into those sub-groups. Classically, this effect is observed in human occupational epidemiology where active workers are typically healthier than the general population. This may occur because very unhealthy people may be less likely to be employed or more likely to leave employment. Hence, real increased disease due to, say, exposure to toxins at work, may be partly or wholly masked. This effect is not well described in veterinary epidemiology but may arise whenever subgroup membership is related to health. For example, performance horses and show animals may be healthier than the general populations from which they arise.

Samples obtained by **non-probability sampling** (see Chapter 13) may not be representative of the target population and the use of such methods may induce a range of biases. The use of volunteers may introduce **volunteer bias** as these people may wish to participate for reasons associated with exposure or outcome. For example, farmers with a herd mastitis problem may be more willing to participate in a study of mastitis, as they may perceive it will benefit them in some way. Conversely, farmers with poor management may be less willing to be involved in studies if they perceive they will be criticized. Volunteers arising from advertisements, for example in a dog-fancier magazine, may not represent the general population of dog owners. Membership lists are often attractive sources for sampling as they may be accessible and well maintained, and the people listed may be enthusiastic participants. However, **membership bias** may arise when samples are drawn from groups whose members are systematically different to the target population. This may include, for example, breed societies and interest groups (such as farmer groups). **Telephone random sampling bias** arises because not everyone has a telephone or is listed in telephone directories and may spend differing amounts of time at or near the phone. This has also become a problem because of a greater reliance on mobile phones, which tend not to be listed, and other modern modes of

communication. In intervention trials, selection bias may occur if **non-random allocation** methods are used. For example, French and others (1994) report a study investigating the effect of tail amputation on lamb health and productivity. In this study, lambs were allocated to the treatment or control groups on the basis of odd or even ear tag number. The research team carried out ear tagging on all but one farm, where the farmer performed this procedure. On this farm more female lambs were docked than male lambs; as only female lambs were retained for breeding on this farm, the authors suggested that the farmer might have preferentially allocated ear tags to females that would have ensured that only docked adult ewes remained on the farm.

Procedure selection bias occurs when certain treatments or procedures are preferentially applied to different risk groups. For example, medical management of a disease may be offered in preference to surgical treatment to milder cases.

Inclusion bias occurs in hospital-based case-control studies (or case-other disease studies) when one or more diseases in the controls are related to the exposure in question. For example, a study of colic in horses admitted to veterinary hospitals compared these cases with controls drawn from horses presented for all other problems (Reeves *et al.*, 1996a). Many of the control horses were racehorses and performance horses, with almost half of the control group presenting for orthopaedic problems. Hence, the apparent reduction in risk of colic associated with consumption of concentrate feed may have arisen because horses undertaking performance-level physical activity were more likely to receive concentrate and more likely to suffer orthopaedic injury. Such situations can lead to **admission rate (Berkson's) bias**, which arises in hospital-based (and similar) case-control studies when the rate of hospitalization (or of veterinary attention), and hence the probability of inclusion in the study, differs between the cases and controls and is also influenced by exposure. Berkson suggested that the relative frequency of disease in a group of patients that has entered a hospital (or otherwise been identified within a health system) is inherently biased when compared with the whole population served by the hospital (Roberts *et al.*, 1978). Jelinski and others (1996) identified that an observed association between abomasal hairballs and abomasal perforating ulcers in calves was most likely spurious due to a Berkson's bias affecting control selection. The authors postulated that two factors, age at death and cause of death, may have been involved in inducing the bias. Among the controls (non-ulcer group), 55% died in the first two weeks of life, compared with just 12.5% of the cases. Hence,

the control calves had less time in which to develop an abomasal hairball compared with the general population. Also, most control calves died of enteritis or septic processes. Both conditions have a long clinical duration, compared with fatal ulcers, during which time the lethargic and sick calves may be less likely to engage in normal (self- and allo-) grooming and nursing (that involves licking the udder and under belly) – behaviours that may encourage ingestion of hair.

Diagnostic suspicion bias usually induces information bias, but in case-control studies, knowledge of putative causal exposures may influence identification of cases and controls. **Matching bias** may arise in matched case-control studies. Matched controls are selected to be similar to cases with respect to the matching variables and hence, if these variables are truly confounders, the matching variables and the exposure variable will be associated. Therefore, the exposure frequency in the controls will be systematically different to that of the underlying population and, consequently, matching has introduced a selection bias, unless the matching is accounted for in the analysis.

Omission of study subjects from the analysis can result in selection bias. **Loss to follow-up bias** may occur in both cohort and intervention studies if losses are associated with exposure and outcome. For example, owners may withdraw their animals from the study and refuse further participation. Alternatively, animals may be moved (livestock between farms, or pets with their owners) so that they are difficult to trace. For example, performance horses that do not recover from a particular injury sufficiently to race again may be either more difficult to trace (if they are sold for other purposes) or more readily traced, if they are repeatedly seen by their veterinary surgeon, compared with those that do recover. **Non-response bias** occurs when those people that respond to requests to participate in a study are different to non-responders. For example, people whose animals do not suffer from a particular disease may be less interested in participating in a study of that disease. In such instances, data will be missing for some study subjects. **Multivariable analyses** only include subjects with complete records for all of the variables included in the analysis; unless data are imputed (see later) those with **missing data** in any of these variables will be excluded. If the individuals with complete records do not represent the target population, selection bias may occur. For example, complete data may be more likely to be obtained from serious cases and the sample analysed may under-represent less serious cases. In each of these three situations (loss to follow-up, non-response and missing data) bias only occurs if the risk of missing

data is associated with both the exposure and the outcome; for example, missing data may be more or less likely in exposed cases. Multiple imputation methods may be used during analysis in an effort to account for the effects of missing data (Sterne *et al.*, 2009).

Information bias

Gathering of information is essential to all epidemiological studies. Data must be collected on the exposures and outcomes of interest, as well as on potential confounders and effect-measure modifiers. Information bias arises when errors in measurement result in biased estimates of the parameters of interest, such as measures of frequency or association. Information bias may occur if errors in the measurement of an exposure, outcome or confounder are associated with the value of that variable, the value of other variables, or to the errors in the measurements of other variables (Rothman *et al.*, 2008). Information bias is often referred to as **misclassification bias** when the error is in a categorical variable and **measurement bias** when the error is in a quantitative variable.

Misclassification

Misclassification (introduced in Chapter 15) is a type of information bias due to error in the measurement of a categorical exposure or outcome variable. As noted in Chapter 15, two types of misclassification can be defined: differential and non-differential. **Differential misclassification** occurs when the magnitude or direction of misclassification is different between the two groups that are being compared. **Non-differential misclassification** occurs if the magnitude and direction of misclassification are similar in the two groups that are being compared (i.e., either cases and controls, or exposed and unexposed individuals).

Emphasis is often placed on minimizing unpredictability due to differential bias through steps designed to ensure misclassification occurs non-differentially, for example through blinding. However, Rothman and others (2008) point out that these approaches do not rule out the possibility of unpredictable effects on estimates of relative risks or odds ratios. A reduction of continuous variables measured with non-differential error to categories may often result in differential misclassification (Flegal *et al.*, 1991). Similarly, collapsing of categorical exposure variables into fewer levels may change non-differential misclassification to differential misclassification, and

Wacholder and others (1991) argue that this may occur regardless of whether the categories are collapsed at the analysis stage or at the exposure assessment stage. Dosemeci and colleagues (1990) illustrate that non-differential misclassification of polychotomous variables (i.e., categorical variables with more than two levels) can induce bias both towards and away from the null, depending on the form of the non-differential error. Bias towards or away from the null may also occur when both an exposure and outcome are measured with non-differential error when these errors are correlated (Chavance *et al.*, 1992; Kristensen, 1992). An example of where this may occur is when the threshold for reporting both the exposure and the outcome varies between subjects. Finally, if the non-differential misclassification is of a confounder, the ability to control for the effect of the confounder is reduced and the observed results for each stratum will lie somewhere between the uncorrected and the corrected measurement of effect, and may misleadingly suggest effect-measure modification. However, if the confounder is measured with differential error, the estimate of the effect may lie outside the range of the corrected and uncorrected estimates. This effect is further discussed in Rothman and others (2008).

The widespread belief that non-differential misclassification predictably results in biased estimation of effect towards the null has led to conclusions that detection of a (statistically and clinically) significant effect in the presence of non-differential bias is sufficient to claim that a larger effect could be expected in the absence of misclassification. However, as noted above, non-differential misclassification only consistently results in bias towards the null under quite specific circumstances. Hence, even when steps have been taken to try to ensure non-differential misclassification, the effect of misclassification can be unpredictable.

Measurement

Measurement error occurs when a quantitative outcome, exposure or confounder is measured with error. Where this occurs randomly, the **precision** of the measurement is reduced. **Measurement bias** occurs when this error is systematic and this affects the **validity** of the study. As with misclassification, measurement error may be differential or non-differential.

Examples of information biases

As with selection bias, many types of information bias have been identified. Some important sources of information bias are described here (adapted from Sackett,

1979; and Choi and Pak, 2005). The list is not exhaustive and different classifications exist, but it does illustrate some of the many ways in which information bias can arise.

Outcome identification bias and **exposure identification bias** may occur due to many types of bias that result in error in measurement of an outcome or exposure, respectively. **Expectation bias** results from systematic errors leading to measurement or recording of information in the direction of the observer's prior expectations. **Interviewer bias** may arise when interviewers' conscious or subconscious preconceptions affect the way in which an interview is conducted (see also Chapter 11). Interviewers may phrase questions to individuals from different groups (cases vs non-cases, exposed vs not exposed) sufficiently differently to systematically elicit different results, thus resulting in differential information bias. Even when the interviewer only uses exactly worded questions, the question may tend to be repeated more often to one group than another. Where possible, interviewer bias may be controlled by blinding the interviewer to the status of the interviewee. If the location of an interview can influence the information observed, **interview setting bias** may occur (e.g., if owners of cases are interviewed in a veterinary consulting room and owners of non-cases are interviewed in the community). Where interviewees respond differently due to knowledge of exposure or outcome status, **responder bias** occurs.

Recall bias is a form of responder bias that may occur when information gathering relies on the recollection of the study subjects (or, in the case of animals, their owners or keepers). For example, in a case-control study investigating risk factors for horse falls in the cross-country phase of eventing, Murray and others (2004b) found that riders' ability to recall dressage penalty scores was influenced by the time between the event and the administration of the questionnaire, and by their level of performance, with riders who performed well reporting more accurately their scores compared with those who performed poorly. Recall bias is a particular issue in case-control studies because the recall of cases and controls may vary (both in amount and in accuracy) due to their knowledge of their outcome status. Hence, recall bias is usually differential. Recall bias may also arise in other types of studies (e.g., cross-sectional studies) when they rely on recall.

Obsequiousness bias may result from subjects altering their responses to better match those they perceive to be desired by the researcher. **Unacceptability bias (social undesirability bias)** may arise when collection of information results in discomfort or

embarrassment and hence may result in such measurements being avoided or under-reported. This may be further classified as **unacceptable disease (or exposure) bias** where the disease (or exposure) being measured may have social (such as embarrassment or stigmatization) or legal consequences (such as illegal activities or notifiable diseases). Such effects also may be called **faking good bias** where socially undesirable responses are incorrectly reported or under-reported. **Faking bad bias** may occur if respondents report greater levels of disease in order to appear worthy of assistance or support. Faking bad bias may occur prior to treatment only to be replaced by faking good bias following treatment. The behaviour of study subjects (be they human or animal) may alter when they are aware they are being observed, resulting in **attention bias**.

Where an individual's past exposure is known, **diagnostic suspicion bias** (also called **diagnostic bias**) may result in differential application, intensity or outcome of diagnostic procedures. Similarly, **exposure suspicion bias** may occur when knowledge of an individual's outcome status affects the application, intensity or outcome of ascertainment of exposure information.

Measurements using instruments may be affected by **instrument bias** if incorrect calibration or maintenance results in systematic error, or by **apprehension bias** if stress associated with the procedure affects the measurement (e.g., measurement of heart rate). When measurement requires the use of scales (e.g., Likert scales: see Chapter 11) respondents may avoid the extremes and tend to provide answers towards the middle of the scale (**end aversion bias**). Long questionnaires may result in **response fatigue bias** leading to inaccurate or incomplete completion of (often the latter) parts of the questionnaire.

Data capture bias and **data entry bias** may occur when practices for the acquisition or database entry of data vary between different locations. This may result in spurious differences between these locations. For example, different countries may have different systems to capture or enter national data relating to the occurrence of certain diseases which may influence the assessment of the relative occurrence of diseases between countries.

Controlling information bias

Many forms of information bias may be prevented through careful planning, or their affects may be measured and accounted for when conclusions are drawn from a study. The diversity of sources of potential information biases highlights the need for their identification and consideration as part of study planning and design. Useful strategies include: blinding of

interviewers and observers to the subjects' exposure or outcome status (see Chapter 16); the use of standardized questionnaires and measurements (see Chapter 11); ensuring use of explicit and objective criteria of exposure and outcome assessment, which are equally applied across all subjects and all study sites; and validation of data (or sub-sets of data), particularly where they may be affected by biased responses by study subjects (such as recall bias or unacceptability bias) or instruments.

It may be possible to correct for the effects of information bias following data collection if information on the probabilities of misclassification are available (e.g., sensitivities and specificities) or if validation studies have been performed. Hill and Kleinbaum (2005) and Dohoo and others (2009) summarize some methods to correct for misclassification. Approaches have been also described to attempt to correct for the effects of measurement error and are described in more detail in Dohoo and others (2009).

Statistical interaction and effect-measure modification

The term **statistical interaction** is often referred to simply as **interaction** although this may lead to confusion with biological interaction (see Chapter 5); statistical interaction also is often used interchangeably with **effect-measure modification**. However, VanderWeele (2009) highlights a distinction between these (related) terms. According to VanderWeele (2009), the definition of statistical interaction (see Chapters 5 and 15) does not privilege one variable of interest (e.g., referred to as x in Chapter 15) over the other (referred to as y) and it is the causal effect of the two exposures together that is of interest. In contrast, VanderWeele (2009) refers to **effect modification** when the causal effect of one exposure (say, x), within strata of the other exposure (y), is of interest. That is, there is asymmetry in the roles of x and y in that only the effect of x on the outcome is evident; the role of y simply concerns whether the effect of x varies across strata of y . For example, age may modify the effect of vaccination against canine parvovirus (Godsall *et al.*, 2010). Young unvaccinated dogs have a greater risk of parvovirus infection compared with young vaccinated dogs, but this difference is less evident in older dogs, possibly due a protective effect in older dogs of past low-level environmental exposure. Effect-measure modification is often also referred to as heterogeneity of effects, sub-group effects or simply as effect modification. However, *effect-measure modification* is preferred over *effect modification* as it is possible to distinguish

modification on two types of measures of effect: **risk-ratio modification** and **risk-difference modification**.

Importantly, VanderWeele (2009) demonstrates that effect-measure modification and interaction only co-occur under specific circumstances, such as when the effects on the outcome of the effect modifier, or one of the interacting variables of interest, are not confounded by another (measured or unmeasured) variable.

The validity of conclusions about the causal effects of one or more exposure variables is dependent on consideration of relevant statistical interactions and/or effect-measure modification and clear presentation of this information. However, Knol and VanderWeele (2012) contend that many authors do not provide sufficient information to enable readers to adequately interpret effect modification and interaction, and they provide recommendations for the presentation of the results of these analyses.

Confounding

Confounding (introduced in Chapters 3 and 15) is defined as occurring when the measure of effect of an exposure on the risk of the outcome is distorted due to the association of that exposure with other, extraneous, variable(s) that influence the outcome, with such extraneous variables referred to as **confounders** or **confounding variables** (Porta, 2014). In order for the results of a study to be valid, it is therefore essential that the effects of confounding be adequately controlled for within the study and/or the analysis of the results.

As confounding results from the confusion of the effects of extraneous variables with those of the exposure of interest, it is a logical requirement that to confound the relationship between the exposure and the outcome, the extraneous variable must (1) be a risk factor for the outcome, and (2) be associated with the exposure in the study population. A third requirement is (3) that the confounder must not be affected by the exposure or the outcome. In the example provided in Chapter 3 (see *Figure 3.5b*), pig herd size (the confounder) is: (1) a risk factor for respiratory disease because, for example, larger herds may be kept at higher density, with poorer biosecurity or be more likely to have pathogens introduced than in smaller herds; (2) associated with fan ventilation because larger pig herds are more likely to require ventilation due to poorer natural ventilation in larger sheds; and (3) is not affected by the exposure (having fan ventilation does not change the size of the herd) or outcome (if it is reasonable to assume that respiratory disease does not affect the size of the herd).

Criteria for confounding

The three criteria noted in the previous section must be met in order for a variable to be a confounder. However, it is possible for a variable to satisfy these criteria and still not confound the association between an exposure and an outcome. Hence, these criteria are necessary, but not sufficient, characteristics of a confounder. Careful consideration of these criteria is needed in order to determine if a variable should be considered as a potential confounder and hence whether steps should be taken to control its effects. This process is important, but is not straightforward. Whilst control of a confounder will make the estimate of the association of interest less biased, control of a variable that is not a confounder may add additional bias to the estimate. It may be that, following deliberation, it is unclear whether a variable is a confounder or not, and both assumptions may need to be explored in separate analyses.

1. **A confounder must be a risk factor for the outcome.** To be a confounder, a variable must be a risk factor for the outcome independent of the exposure of interest. Hence, the confounder must alter the risk of the outcome among the unexposed population and this criterion is based on the actual relationship between the confounder and outcome, not on whether or not an association is present in the data. Nevertheless, the data may provide useful information about this relationship, particularly where the data are likely to provide precise (i.e., a large study) and valid (i.e., selection, information and other biases are small) estimates. Where good external evidence exists, information derived from the data should be tempered by existing knowledge. Strong external evidence as to whether or not a variable is an independent risk factor for the outcome usually takes precedence over information derived from the data. However, as is often the case, external evidence may be limited (or have only limited generalizability to the particular population under study) and the use of evidence from the data may be warranted (either in isolation, or in combination with other evidence).
2. **A confounder must be associated with the exposure in the study population.** To produce confounding, the potential confounder must be associated with the exposure within the study population. In a cohort study, in the absence of selection and information bias, the cohort is representative of the study population and hence the association should be present, and can be identified, within the cohort. Similarly, in the absence of bias, the sample population in a cross-sectional

study should be representative of the study population. In a case-control study, the association should exist within the population from which the cases occurred. Ideally, the control group will be representative of this underlying population and the presence or absence of this association can be assessed using these individuals. However, for any of these study types, if there is selection and/or measurement bias, the estimate of this association may also be biased and judgements may need to rely on external knowledge or advanced methods, such as bias analysis (see Greenland and Lash, 2008). It is often assumed that randomization of an intervention in a trial ensures that the exposure is not associated with other variables. However, whilst on average there should be no association across a large number of randomizations, this does not necessarily hold for each individual randomized study, particularly small studies. Hence, confounding can occur in randomized trials due to random differences between the intervention groups. Furthermore, if many study subjects do not complete the study confounding can occur even in large trials.

3. **A confounder must not be affected by the exposure or the outcome.** A confounder cannot be caused by the exposure or the outcome. This criterion is not met when the potential confounder is on the causal pathway of interest between the exposure and the outcome. For example, a study may investigate the role of obesity as a cause of laminitis in horses. Potential confounders of this relationship may include age, diet, housing, level of exercise, concurrent disease and so on. Is insulin resistance a potential confounder? Insulin resistance is a risk factor for laminitis (including among non-obese horses) and is associated with obesity, and mechanical application of statistical methods such as regression is likely to identify insulin resistance as a confounder. However, much of the increased risk of laminitis in obese horses is mediated through the effects of insulin resistance. Assessments of the total impact of obesity on the risk of laminitis should not control for insulin resistance. If insulin resistance is controlled in the analysis only the impact of obesity that is *not* mediated by insulin resistance will be calculated. If the risk of laminitis from obesity was entirely mediated by insulin resistance, the controlled analysis would suggest that obesity does not affect the risk of laminitis. It is worth noting that factors which precede (in time) the exposure and disease cannot lie on the causal pathway and hence will satisfy this criterion.

Confounding and causal diagrams

It is useful to represent potential confounding relations using **causal diagrams** (also called path diagrams, see Chapter 3). Hernán and others (2002) and Glymour and Greenland (2008) provide excellent explanations of the use of causal diagrams to aid thinking about confounding and other forms of bias. Causal diagrams provide pictorial representations of relationships between an exposure of interest (represented here by E), the outcome, or disease, of interest (represented by D) and other measured variables (represented by C). Figure 18.3a depicts C as a confounder of the association between E and D . The direction of

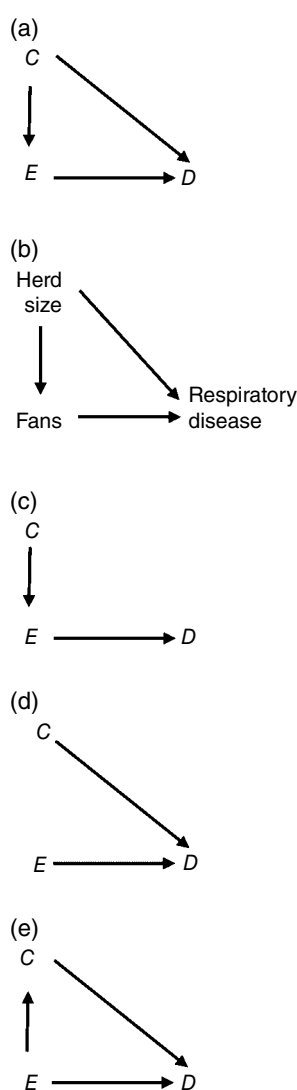


Fig. 18.3 Causal diagrams representing different relationships between an exposure of interest (E), the outcome, or disease, of interest (D) and other measured variables (C). C represents the potential confounders of the association between E and D .

the arrows is used to indicate that it is believed that C is a risk factor for D (criterion 1, above) and C is associated with E (criterion 2). Furthermore, as we cannot follow the arrows from E to D via C , C is not on the causal pathway of interest. Returning to the example of the impact of fan ventilation on porcine respiratory disease (see Chapter 3), Figure 18.3b indicates the belief that herd size is an independent risk factor for respiratory disease (including among those sheds without fan ventilation), that herd size is associated with the presence of fans and that herd size does not lie on the causal pathway between the use of fan ventilation and respiratory disease. Figure 18.3c–e illustrates situations in which: (c) C is not a risk factor for D ; (d) C is not associated with E in the study population; and (e) C is on the causal pathway between E and D , respectively. In each of the cases represented, C is not a confounder of the association between E and D because it fails to meet criteria 1 to 3, respectively. As noted in Chapter 22, in the situation depicted in Figure 18.3e, the inclusion of C in an analysis assessing the association between E and D would identify the *direct* effect of E , rather than its *total* effect, as the effect of E due to one causal pathway (that via C) has been controlled for within the analysis (Westreich and Greenland, 2013).

Controlling confounding

The effects of confounding can be controlled at two stages: during study design and during analysis.

Controlling confounding at the design phase

At the study design phase, confounding can be controlled by restriction, randomization and matching.

Restriction may be used to limit the study to only one level of a potential confounder. For example, only one sex or a particular breed might be included for study. As all members of the restricted study population have the same status with regard to the potential confounder, there can be no association between the confounder and the exposure within this population, and criterion 2, is not satisfied. This approach limits direct interpretation of the results to the restricted population and hence may affect the generalizability of the study.

Randomization (i.e., in intervention trials) may be used to attempt to have the potential confounders evenly distributed amongst the study population. This is the only method that can take in to account potential confounders that have not been explicitly identified (and measured). However, as noted earlier, randomization of an intervention in a trial does not guarantee that there will be no association between the

intervention and other variables in individual randomized studies, particularly where the study is small. Randomization may be done within strata (e.g., age- or sex-specific groups) to better ensure comparability between the intervention and control groups for known potential confounders.

Matching is the process of making the study groups comparable with respect to an extraneous variable, such as a potential confounder. Methods of matching are described in Chapters 15 and 16. Whilst matching can be used to control confounding, this process will induce matching bias in case-control studies (see earlier in this chapter) unless specific steps are taken during analysis to account for the matching, such as using McNemar's change test (see Chapter 15) or conditional regression procedures.

Controlling confounding during analysis

Measured confounding variables may also be controlled (adjusted) for during **analysis**. Methods include: **demonstrating an absence of association** between the potential confounder and the exposure in the study population (i.e., demonstrating that criterion 2 is not met), using adjusted rates specific to the confounder (see Chapter 4); producing a summary odds ratio for the combined odds ratios of each confounder (using the **Mantel-Haenszel procedure**; see Chapter 15); and through the use of **multi-variable statistical models** (see Chapter 22). The latter approach is now generally preferred. Effective control of confounding during analysis requires accurate measurement of all potential confounders and careful planning and implementation of the analysis based on a firm understanding of the underlying causal web (and of uncertainties in this understanding).

Errors in analysis

Analysis strategy bias occurs due to problems in the plans for the analysis (Choi and Pak, 2005). This may arise due to the many choices that must be made during the planning and implementation of analytical procedures. These include, but are not limited to: **reverse causation**, where an association between two factors, A and B, is analysed and interpreted as if A causes B, whereas in fact the reverse is true; poor **selection of variables** for consideration in statistical models (e.g., inclusion of variables on the causal pathway between the exposure and outcome of interest and failure to include confounders); selection of an **inappropriate statistical model** (e.g., using a linear model for count

data, where alternatives, such as a Poisson model, would be more appropriate; see Chapter 22); assumption of an **inappropriate form for the relationship** between an exposure and outcome (e.g., the relationship may be assumed to be linear when a quadratic or other form is more appropriate; see Chapter 22); **choice of category levels** when collapsing categorical variables or creating categorical variables from continuous variables; and failure to account for **non-independence of the data** (e.g., failure to account for clustering of observations on cows within farms; see Chapter 22).

Post hoc analysis bias occurs when incorrect estimates are calculated due to investigation of questions only considered after data collection and, perhaps, in light of the results of initial data analysis. This may arise due to sub-group analyses and 'data dredging'. **Sub-group analyses** (particularly those planned only after data collection) increase the risk of Type I error (due to multiple testing) and Type II error (due to the reduced sample size in the sub-groups). Generally, sub-group analysis should be limited (at most) to a few pre-defined analyses based on plausible biological hypotheses, and results should only be used for hypothesis generation, rather than to affect the study's main conclusions. Despite this, Assmann and others (2000) identified that many clinical trials undertook sub-group analyses that were often overinterpreted. Data dredging is another form of secondary data analysis that can result in **data-dredging bias** (Sackett, 1979). Under this approach, multiple (often unplanned) analyses are conducted using the study data. This may include exploration of exposures about which data happen to be available and re-analysis with other outcomes. *Post hoc* analysis bias may contribute to publication bias (see next section).

Tidying-up bias occurs when outlying or other awkward data are excluded from the study (Sackett, 1979). **Missing data bias** (Sackett, 1979) may occur if missing data are inappropriately imputed or given a specific response (e.g., missing data in a questionnaire which are treated as if the respondents meant 'no').

Communication bias

Publication bias is an important source of communication bias. **Publication bias** (or **positive results bias**; see also Chapter 19) arises when positive results are more likely to be submitted and accepted for publication, and are published more rapidly by more 'highly

respected' journals (Dubben and Beck-Bornholdt, 2005). Only about half of the abstracts presented at biomedical conferences are subsequently published in peer-reviewed scientific literature, and they are more likely to be published if they include positive results (Scherer *et al.*, 2007). Hence, the published literature may represent a biased sub-set of all studies of a particular question, and interpretations based only on these publications may be misleading. Publication bias also may occur due to the topic of study or due to the slant of the paper. **All's well literature bias** occurs when publishers preferentially publish reports that omit or downplay controversies, whereas **hot-topic bias** occurs when studies of novel, controversial and emotive topics are preferentially published (Sackett, 1979). **Language bias** may affect retrieval or consideration of reported studies written in (or not written in) specific languages.

Outcome reporting bias arises due to selective reporting of outcomes other than those originally planned (Dwan *et al.*, 2008) and may be related to data-dredging bias. A review of studies that have assessed outcome reporting bias in medical randomized trials identified that between 40% and 62% of trials changed, introduced or omitted at least one outcome (Dwan *et al.*, 2008). This also may be a common practice in veterinary epidemiological studies, but is difficult to detect unless study protocols are published prior to the study being undertaken.

Other forms of communication bias also exist. **Significance bias** occurs when the clinical or biological significance of the results of a study are confused with

the statistical significance. **Magnitude bias** occurs when the scale on which results are communicated affects interpretation. For example, a large risk ratio may be misinterpreted as implying an exposure has an important population-level impact when, in fact, the additional risk (attributable risk) and the population attributable proportion (see Chapter 15) due to that exposure may be small.

The *Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)* and the *Consolidated Standards of Reporting Trials (CONSORT)* statements provide guidelines aimed at improving the reporting of observational epidemiological studies and clinical trials, respectively (von Elm *et al.*, 2007; Schulz *et al.*, 2010). These aim to minimize communication bias and make clearer the existence of other biases (such as selection bias and measurement bias) within studies and have been found to improve reporting quality in the medical literature (Moher *et al.*, 2001). However, some authors have warned that using these statements as checklists may do little to ensure and promote honest and adequate reporting of research and have suggested inclusion in publications (or online supplementary material) of a narrative section that provides details of the strengths and weaknesses of the study (Schriger, 2005). However, as noted by Greenland and others (2004), pressures on researchers to demonstrate the scientific or policy importance of their results may lead to overinterpretation of results. These pressures may also reduce researchers' willingness to highlight potential flaws in their studies.

Further reading

- Dosemeci, M., Wacholder, S. and Lubin, J.H. (1990) Does nondifferential misclassification of exposure always bias a true effect toward the null value? *American Journal of Epidemiology*, **132**, 746–748
- Glymour, M.M. and Greenland, S. (2008) Causal diagrams. In: *Modern Epidemiology*, 3rd edn. Eds Rothman, K.J., Greenland, S. and Lash, L.T., pp. 183–209. Lippincott, Williams and Wilkins, Philadelphia
- Greenland, S., Gago-Dominguez, M. and Castela, J.E. (2004) The value of risk-factor ('black-box') epidemiology. *Epidemiology*, **15**, 529–535
- Hernán, M.A., Hernández-Díaz, A., Werler, M.M. and Mitchell, A.A. (2002) Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. *American Journal of epidemiology*, **155**, 176–184
- Kaptchuck, T.J. (2003) Effect of interpretative bias on research evidence. *British Medical Journal*, **326**, 1453–1455
- MacLure, M., and Schneeweiss, S. (2001) Causation of bias: the episcopo. *Epidemiology*, **12**, 114–122
- Rothman, K.J., Greenland, S. and Lash, T.L. (2008) Validity in epidemiological studies. In: *Modern Epidemiology*, 3rd edn. Eds Rothman, K.J., Greenland, S. and Lash, L.T., pp. 128–147. Lippincott, Williams and Wilkins, Philadelphia
- Schriger, D.L. (2005) Suggestions for improving the reporting of clinical research: the role of narrative. *Annals of Emergency Medicine*, **45**, 437–443

19

Systematic reviews

Evidence synthesis

This chapter focusses on evidence synthesis (i.e., development of techniques to combine multiple sources of evidence) and, in particular, systematic reviews, including meta-analysis. A major challenge for decision making is how to effectively garner information from the many research results that are published. One way to address this challenge is to use summaries that combine scientific evidence from original research studies that have been performed by others. Numerous approaches to summarizing evidence from multiple sources exist (Grant and Booth, 2009). Narrative literature reviews, critical reviews, expert elicitation, systematic reviews and meta-analyses, combined or separate, are examples of evidence-synthesis approaches.

The clear advantage of any evidence summary (a product of evidence synthesis) is that it is faster and, therefore, more time and cost-efficient for the end-user than identifying, obtaining and reading all of the relevant original research on a topic. However, such advantages come at a cost. The disadvantages of using evidence summaries are that they are sometimes not easy to adapt to individual cases or policy questions, and one can often find conflicting results between summaries. This problem is common to all of the evidence-summary approaches. Another disadvantage of evidence-synthesis summaries is the faith that the end-user must place in the final product. If the evidence summary is itself biased, then biased information will inform decisions. It is therefore important to be able to assess the potential for bias in the evidence summary. Assessing the extent of bias in traditional evidence summaries such as narrative reviews, expert elicitations and meta-analyses without systematic reviews can be difficult. These methods do not explicitly report the methods used to identify, assess and synthesize the evidence. This absence of transparency means the end-user is unable to assess the potential for bias, the validity of conclusions drawn

by the reviewer, and the impact of those biases on the decision-making process. Although many evidence-synthesis approaches may aim to be unbiased, systematic reviews are the only approach to date that has mechanisms that explicitly require the reviewer to evaluate biases in individual studies and to provide the end-user with the opportunity to assess the bias in the evidence summary.

Overview of systematic reviews

Systematic reviews (introduced in Chapter 17) have been used to summarize disease prevalence and incidence estimates, aetiology and risk factors, diagnostic test characteristics, and efficacy of preventive or therapeutic interventions. The focus of this chapter will be on systematic reviews of interventions. An 'intervention' refers to treatments used to prevent, reduce or treat an adverse health outcome or event, and encompasses strategies such as use of antimicrobials and biologicals, and dietary or management manipulation.

A well-executed systematic review is a rigorous and replicable approach to identifying, evaluating and summarizing scientific evidence relevant to a specific clinical or policy question (EFSA, 2010). Integral to the systematic review methodology is the emphasis on employing systematic methods to reduce bias in the identification and evaluation of studies to be included in the review, and an appraisal of the risk of bias in the primary research studies included in the review. Therefore, systematic reviews can provide clinicians and other decision makers with a scientifically defensible summary of the current state of knowledge about a specific question, without the need to read the vast amount of primary research. The findings of the review can then be incorporated into decision making¹.

¹ It follows that systematic reviews, themselves, should be published; some are not (Tricco *et al.*, 2009).

Table 19.1 Structured steps used to conduct systematic reviews. (Based on O'Connor *et al.*, 2014.)

Step	Main purpose of steps	Brief summary
1	Define the review question and the approach to conduct of the review	Determine the need, identify a review team, identify the type of question and the relevant acronym (PICO, PECO, PO, PIT) and refine the review question, prepare and publish the protocol
2	Comprehensive search for studies	Identify the sources of information relevant to the review and within the resources (time or money) available. Document all decisions made. The search should be designed around some or all of the components (PICO, PECO, PO, PIT) of the review question
3	Select relevant studies from the search results	Use several questions designed around the components of the review question (PICO, PECO, PO, PIT) to identify relevant studies captured by the search
4	Collect data from relevant studies	Extract information about sources of contextual and methodological heterogeneity and the outcomes from the relevant studies
5	Assess the risk of bias in relevant studies	Assess the risk of bias in the individual studies and the entire body of work
6	Synthesize the results	Conduct a meta-analysis if possible, and assess sources of heterogeneity. Use narrative synthesis if meta-analysis is not feasible
7	Present the results	Present the results of steps 2 to 6 using an appropriate combination of text, figures and tables
8	Interpret the results and discussion	Interpret the results and discuss limitations of the individual studies and the approach to conducting the review

PICO(S): P, population; I, intervention; C, comparator; O, outcome; and, optionally, S, study design.

PECO: P, population; E, exposure; C, comparator; O, outcome.

PO: P, population; O, outcome.

PIT: P, population; I, index test(s); T, target condition or disease.

Systematic reviews include explicit steps that other evidence-synthesis approaches do not. The steps of a systematic review are described in *Table 19.1*. The approach to all steps is documented in a review protocol prior to the start of the review. Guidelines for reporting a protocol are available (Moher *et al.*, 2015; Shamseer *et al.*, 2015).

Differences between systematic reviews and narrative reviews

Approaches and reasons for summarizing research vary, and the terminology is not consistent. For example, Grant and Booth (2009) describe 14 types of published reviews, although they are not mutually exclusive: critical review, literature review, mapping review/systematic review, meta-analysis, mixed studies review/mixed methods review, overview, qualitative systematic review, rapid review, scoping review, state-of-the-art review, systematic review, systematic search and review, systematized review, and umbrella review. Regardless of the terminology, all reviews are characterized by the combining of information from primary research and do not themselves generate primary research results (Cooper *et al.*, 2009). The term narrative review is used to describe the product that most veterinarians are familiar with from textbooks

or journals; that is, a review that combines information from multiple sources. Some authors use the terminology 'literature review', 'narrative review', 'critical review', etc. To understand how a systematic review differs from a narrative review, the terminology is less important than the features. There is a common but erroneous belief that systematic reviews are the same as narrative reviews, only more comprehensive. However, '*systematic reviews are not just big reviews, and their main objective is not simply to search more databases*' (Sargeant and O'Connor, 2014). Rather, a systematic review addresses a specific question of interest in a way that reduces bias in the selection and inclusion of studies, appraises the potential for bias in the included studies, and summarizes the results objectively. Systematic reviews also differ from other types of review in the measures taken to reduce bias, such as using several reviewers working independently to screen papers for inclusion and to assess the risk of bias.

Questions that are suitable for systematic reviews

A systematic review is not the solution to every evidence-synthesis need. It is important to clarify the types of questions that should be addressed by a

systematic review (EFSA, 2010). For example, if the goal is to understand the current state of knowledge about the epidemiology, pathology, diagnosis and treatment and control options for a particular disease, then a narrative review may be more appropriate. However, incorporation of some components of a systematic review (e.g., a comprehensive search to identify relevant literature and an assessment of sources of bias in relevant studies) would increase the transparency of such reviews.

The systematic review approach should be used when there is a specific question to be answered, rather than a need for broad understanding. Furthermore, a systematic review should be used when it could be envisioned that the specific question would result in a parameter that has a sampling distribution. For example, systematic reviews could be used to address the following questions:

‘What is the difference in mortality between vaccinated and unvaccinated pigs receiving a PCV II vaccine?’

‘What is the sensitivity and specificity of the Rose Bengal test for detecting *Brucella abortus* in cattle?’

For each of these questions it is possible to envision a primary research study that could be designed to estimate the parameter of interest (i.e., the difference in mortality, or sensitivity and specificity, respectively). Moreover, it is possible to envisage multiple studies that would obtain multiple estimates of the parameter.

In contrast, systematic reviews would be unsuitable to address these questions:

‘What vaccines can reduce mortality in a swine herd?’

‘What are the diagnostic tests that can be used to detect brucellosis infection in cattle?’

This is because these questions seek to generate a list, rather than a parameter estimate. Although a comprehensive search may be important in ensuring that the list is comprehensive, questions such as these do not naturally relate to a parameter with a sampling distribution.

Types of review questions suitable for systematic reviews

Systematic review questions can be classified as questions about interventions, causes, disease burden (prevalence/incidence) and detection. The steps of the systematic review are the same for each of these question types. However, within each question type, the approach to searching for data, the appropriate study designs to include, the type of data to be extracted, sources of bias, the data analysis and the

method of presentation differ. Due to the focus of most health agencies on interventions, the systematic review methodology is most developed for questions about interventions.

Extensive search of the literature

An extensive literature search is a cornerstone of the systematic review. The aim is to conduct an extensive search to identify as many relevant results as possible. The rationale is to reduce the bias associated with the accessibility of studies based on their outcome, sometimes called **retrieval bias**. Retrieval bias is a subtype of publication bias (see Chapter 18), in that studies with more favourable or interesting outcomes are published in higher profile locations that are easier to access. It has been suggested, in human health, that inclusion of estimates from readily accessible studies could result in overestimation of the positive effects of interventions because positive findings are more likely to have a higher profile (e.g., in peer-reviewed publications compared with only appearing in conference proceedings: Scherer *et al.*, 1994; Krzyzanowska *et al.*, 2004).

In animal health, empirical evidence of bias towards publication of positive findings is limited. In one study that attempted to assess this question (for trials that reported assessment of vaccines for diseases of cattle and pigs), so few studies that had been identified from conference abstracts were subsequently published that the power to detect such a bias was limited (Brace *et al.*, 2010), the authors reporting a conference abstract to publication ratio of 5:89 for trials in pigs, and 6:65 for trials in cattle². Although the data were limited, the authors did calculate a positive-outcome proportion for conference proceedings and the matching peer-reviewed article as an approach to describing publication bias. The positive-outcome proportion has as the numerator the number of articles with a positive outcome, and the denominator is the number of articles. The positive-outcome proportion for pig conference proceedings and journal articles was 57/89 and 4/5, respectively (prevalence ratio = 1.25; 95% confidence interval (CI) = 0.78, 1.99; conference proceedings as referent). The positive-outcome proportion for bovine conference proceedings and journal articles was 34/65 and 4/6, respectively (prevalence ratio of journal articles:conference proceedings = 1.27; CI = 0.69, 2.35). For this group of

² The numerator for the abstract-to-publication ratio was the number of vaccine-related conference proceedings with matching journal articles; the denominator was the total number of vaccine-related conference proceedings.

studies, the positive-outcome proportion was higher in peer-reviewed studies compared with conference proceedings, although the precision of this estimate is poor. For food-safety outcomes there is evidence of bias: abstracts reporting at least one positive outcome were more likely to be published (odds ratio = 2.6; CI = 1.1, 6.2) and were published faster (hazard ratio³ = 2.3; CI = 1.1, 4.7). Time to publication decreased with the number of positive outcomes reported (hazard ratio = 1.1; CI = 1.0, 1.3) (Snedeker *et al.*, 2010a,b).

However, a comprehensive search does not solve the major component of publication bias, which is that studies with unfavourable outcomes are less likely to be publicly available. This issue can only be addressed indirectly in meta-analysis (see following and Chapter 17). Instead, an extensive search only addresses the issue that even among the published studies, those studies with less favourable, novel or interesting results may be harder to retrieve because they may be in journals without open access, or in poorly-indexed journals, conference proceedings and university research reports.

The other important aspect of an extensive search is that it should be well documented (i.e., the strategy employed, the sources searched and the date of the search). By providing the end-user with an explicit description of the search, he or she is able to make a judgement as to the potential impact of publication bias. For example, a review based only on a search conducted in PubMed might be skewed toward a population of studies with positive findings and consequently overestimate the efficacy of interventions. Numerous sources of literature are available, and several electronic databases of interest are provided in *Table 19.2*.

Assessment of risk of bias in a systematic review

For some study questions, particular study designs may have a very high potential for bias (i.e., to present erroneous associations). The results of such studies, if included in a systematic review, may introduce bias into the results and conclusions of that review. It is important therefore that the potential for bias to affect the conclusion of a review is discussed to ensure end-users are aware of this potential. Systematic reviews therefore include an explicit component that purposely describes the potential for bias in studies considered relevant to the review question.

³ The hazard ratio is similar, but not identical, to the relative risk: see Chapter 15 and Nurminen (1995) and Stare and Maucourt-Boulch (2016).

Studies may obtain a result different from the true value for two reasons: **random error** or **systematic error** (see Chapters 10 and 18, and Rothman, 2012). The potential for random error in a study finding is a function of sample size and underlying variation in the effect size of interest (see Chapter 17). Estimates of effect sizes from larger studies are likely to be closer to the true effect size's underlying value. The precision of the effect size is reported using a confidence interval. Greater levels of variance yield larger confidence intervals, and hence less precise estimates of the effect size. Smaller studies yield larger confidence intervals, and hence less precise estimates of the effect size.

The potential for systematic error is often less transparent. Systematic error (which causes bias) is a systematic deviation in the associations observed; for intervention assessment studies, a systematic error would result in a distortion in the association between an intervention and an outcome, which is created due to the execution of the study (Rothman, 2012). The association may be biased towards or away from the null hypothesis. Unlike random error, increasing sample size does not reduce the potential for systematic error. Systematic errors arise in many ways; three main categories include: confounding, information bias and selection bias (see Chapters 15 and 18).

Although all studies are likely to have some systematic error, the extent of bias introduced may differ between studies (see Chapters 15, 16 and 17). For example, cross-sectional studies, which measure the outcome and exposure at a single point in time, are particularly prone to selection bias because only prevalent cases are studied. Case-control studies, which may, for example, ask producers to recall biosecurity practices as they were *prior* to a disease outbreak, may suffer from information bias, because producers with and without disease may differentially recall practices that occurred weeks or months before the interview. For assessing interventions, the randomized controlled design (see Chapter 17) has the least potential for systematic bias. The potential for confounding is limited by the use of random allocation methods with or without additional restrictions, such as blocking or stratification. Other design features such as allocation concealment and blinding of the caregiver and outcome assessor can be incorporated into the design to reduce the potential for other biases.

Steps of a systematic review

Systematic reviews address a targeted question using a structured series of steps, listed in *Table 19.1*. The approach to each step should be documented in the

Table 19.2 Examples of electronic databases that may contain literature relevant to animal health, welfare and food safety.

Database	Content	Interface
<i>Science Citation Index (SCI)</i>	Bibliographical and citation information from over 3700 scientific and technical journals across 100 disciplines. Coverage is international and from 1900 to present. Updated weekly	Web of Knowledge, Thompson Reuters
<i>Conference Proceedings Citation Index – Science (CPCI-S)</i>	Bibliographical and citation information from the published literature of international science and technology conferences, symposia, colloquia and workshops. Coverage is from 1990 to present and the database is updated weekly	Web of Knowledge, Thompson Reuters
<i>CAB Abstracts</i>	Indexes the journal, book and conference proceeding literature in the applied life sciences from 1910 to present. Coverage is international and includes animal sciences, veterinary medicine and human food and nutrition	Web of Knowledge, Thompson Reuters, OvidSP and other interfaces
<i>BIOSIS Previews</i>	Provides information on international published research in biological sciences, life sciences and biomedical research. Comprises records of journal articles from 5000 journals indexed in <i>Biological Abstracts</i> with records of reports, reviews and conferences from Biological Abstracts/RRM (Reports, Reviews, Meetings)	Web of Knowledge, Thompson Reuters
<i>MEDLINE and MEDLINE In-Process</i>	Holds over 19 million references to journal articles in life sciences, including: nursing, dentistry, veterinary medicine, pharmacy, allied health and pre-clinical sciences. Coverage is from 1950 to present and the database is updated daily	OvidSP, PubMed and other interfaces
<i>AGRIS</i>	Database of international agriculture literature including forestry, animal husbandry, aquatic sciences and fisheries, and human nutrition. Includes both published and grey literature from 1975 to present	http://agris.fao.org/
<i>AGRICOLA</i>	Database of almost five million bibliographical records from the US Department of Agriculture's National Agricultural Library (NAL catalogue). The database indexes journal articles, book chapters, monographs, theses, patents, software, audio-visual materials and technical reports on all aspects of agriculture and allied disciplines	http://agricola.nal.usda.gov/
<i>TEKTRAN</i>	Contains articles accepted for publication (published or soon to be published) of recent research results from the Agricultural Research Service (ARS), the US Department of Agriculture's chief scientific research agency. Subject coverage includes food, nutrition, food safety, crops and livestock, natural resources and industrial products	http://www.ars.usda.gov/services/tektran.htm
<i>CRIS</i>	Provides documentation and reporting for ongoing agricultural, food science, human nutrition, and forestry research, education and extension activities for the US Department of Agriculture	http://cris.nifa.usda.gov/
<i>Science.gov</i>	Provides access to over 55 databases and over 2100 selected websites from 13 federal agencies, offering 200 million pages of US government science information including research and development results	http://www.science.gov/
<i>ScienceResearch.com</i>	A deep web search engine that uses federated search technology to search a range of other search engines and then collates, ranks and de-duplicates the results. It is multidisciplinary and can retrieve grey literature such as reports and government publications	http://scienceresearch.com/scienceresearch/
<i>OpenGREY</i>	Provides access to 700 000 multidisciplinary bibliographical references of grey literature including reports, conference papers and theses, all produced in Europe	http://www.opengrey.eu/

review protocol. The protocol should be developed by a team that includes expertise in information retrieval, the topic area, evidence synthesis and meta-analysis. Some members of the review team may cover more than one area of expertise. This team should, *a priori*,

describe the approach to each step of the review. By having a comprehensive, well-thought-out plan prior to starting the review, the review team ensures they limit deviations from protocol that could change the scope of the review and introduce bias. Deviations that

do occur should be reported in the final review publication.

Step 1: Define the review question and the approach to conduct of the review (i.e., create a protocol)

Preparing for the review has multiple steps including: (1) determining the need for the review – perhaps by conducting a scoping review; (2) creating a team; and (3) preparing and publishing the protocol.

Determining the need for a review may be simple because it may be requested by an agency or funding group. However, it is also possible, and may be prudent, to conduct a **scoping review**. A scoping review aims to identify the literature that is available relevant to the topic. The aim is not to extract the data or assess the risk of bias in the literature identified, but rather to characterize what is available. With this information it is often possible to determine if resources should be devoted to a full review.

A review team also needs to be assembled prior to the review. The review team may be formed before the scoping review (if one is conducted) or just for the systematic review. The review protocol is developed by a review team that consists of **content specialists** and **methods specialists**. Content specialists provide guidance related to the topic of the review question, such as what outcomes will be measured in relevant publications and which key conferences are likely to have relevant studies. The methods specialists should be familiar with research synthesis methods including information retrieval, risk of bias assessment, data extraction and meta-analysis. Some team members may serve both roles.

The development of a review protocol is a unique and critical aspect of a systematic review. Many narrative reviews do not start with an explicit question, but rather a theme or objective. Consequently, the focus of a narrative literature review can change over time, depending upon the available literature and incidental findings. For a narrative literature review this may be acceptable. However, a systematic review is designed to explicitly answer a question in the manner reminiscent of the way primary research studies are designed to test a hypothesis (see following). By developing a review protocol, the review team ensures that the review question is answered. The answer may be that there are insufficient data to answer the question, but this is, in itself, an important finding. After the protocol is completed it should then be peer reviewed (some journals do this) or, as a minimum, time-stamped so that modifications that occur after the start of the review can be assessed for their impact. The approach

to all steps is documented in a review protocol prior to the start of the review. The protocol, which is akin to a research plan for any primary research study, aims to explicitly record the intent of the project; doing so reduces the potential for bias to be introduced into the review and to maintain the focus on the specific review question.

For reviews about interventions, the format to create the review question is summarized by the acronym **PICO(S)**, which stands for **P** = population, **I** = intervention, **C** = comparator, **O** = outcome and, optionally, **S** = study design (see also Chapter 17). By defining each of these components, it will be clear to the end-user what studies are relevant to the review question. For livestock, the **population** is frequently defined by the species, production type and production system. For companion animals, the species, age or reproductive status might be characteristics used to define the population. The **intervention** refers to a therapeutic or preventive intervention applied by an investigator, clinician or policy group. As the aim often is to recommend such interventions, these should be well specified. The **comparator** can be either an **active** or **non-active** comparator. An active comparator would likely be the current recommended standard of care or a common standard of care. A non-active comparator may be a placebo or a non-treated group.

The **outcome** of interest also must be clarified. Phrases such as ‘effect on production’ or ‘impact on welfare’ are too vague for systematic reviews and must be refined. If the exact outcome(s) of interest are not pre-specified (e.g., by the funding organization), one approach to identifying the important outcomes is to survey a group of potentially relevant studies and identify the outcomes reported. Subsequently, a group of experts and stakeholders could be asked to rank the outcomes based on relevance to the end-user. This approach to identifying outcomes can identify differences between what is studied (and published) and what is considered important (Guyatt *et al.*, 2011b; Baltzell *et al.*, 2013). When policy makers are making decisions, it will likely be important to consider the balance between favourable and adverse outcomes associated with an intervention, so reviews should be conducted for all relevant outcomes. Outcomes can be graded as critical, important and not important (Guyatt *et al.*, 2011b). Routinely, outcomes ranked as not important are not reviewed because they do not influence the decision-making process.

Finally, it is sometimes reasonable to limit the review scope based on a particular study design. For example, the review team might only want to assess results from controlled trials with naturally-occurring disease. It is important to ensure that study design is considered at

the protocol stage. Later in the systematic review, biases are assessed but they cannot be 'adjusted' away. Therefore, if a particular study design is likely to be so biased as to provide invalid estimates, it would be important to exclude such designs from the scope of the review. In human and veterinary systematic reviews of interventions, the design is often limited to randomized trials; however, that may not always be the case (Higgins and Green, 2011). For example, sometimes a randomized trial is neither ethical nor possible and in such circumstances the review panel may decide to include non-randomized trials or cohort studies in the scope of the review. Having established each of the components of the review question, the exact scope of review should be clear.

An important starting point to narrowing the review question is to identify the demographic characteristics that might explain differences among studies, such as breed, age and production system. These are referred to as **sources of clinical heterogeneity**. Evaluating sources of heterogeneity is discussed later in this chapter, but when clarifying the review question, one way to narrow the question is to eliminate some sources of clinical heterogeneity based on the definition of the components. For example, time of administration is a likely source of heterogeneity for the effect of analgesia on castration in pigs. If it is of interest to report the impact of time of administration on the extent of analgesia, then the review question should be written in a manner that allows those sources of heterogeneity to be evaluated by the review, and not excluded at the screening stage of the review. For example, the following question may be too narrow:

'What is the change in the number of squeals during castration and change in play activity within 24 hours of the castration in piglets less than 28 days of age that received 0.5–1 ml of intratesticular 2% lidocaine *immediately at the time of castration* compared with no local anaesthetic?'

To enable assessment of impact of timing of administration as part of the review, the question should be:

'What is the change in the number of squeals during castration and subsequent play activity within 24 hours of the castration in piglets less than 28 days of age that received 0.5–1 ml of intratesticular 2% lidocaine compared to no local anaesthetic?'

Acronyms are available for the other types of review questions and the approach to refining the question is the same (EFSA, 2010). For the review questions about causation, the acronym used is **PECO**: **P** = population, **E** = exposure, **C** = comparator, **O** = outcome. For review questions about the prevalence or incidence

of a condition, the acronym used is **PO**: **P** = population, **O** = outcome. For review questions about the accuracy of diagnostic tests, the acronym used is **PIT**: **P** = population, **I** = index test(s), **T** = target condition or disease⁴.

Step 2: Comprehensive search for studies

The search should be designed based on some or all of the concepts included in the review question (i.e., the PICO(S), PECO(S), PO(S) or PIT(S) components). It is not always necessary to include all components in the search. For example, frequently the outcomes are not included in the search for reviews of interventions because these may not be explicitly reported in the abstracts of all studies. The aim is to design a search that will capture as many relevant studies as possible ('high sensitivity') with as few irrelevant studies as possible (Higgins and Green, 2011). This inevitably involves a trade-off to achieve as few 'false-negatives' as possible (relevant papers missed) even if this results in a large number of 'false-positives' (irrelevant papers included). It is not unusual to identify only one to five relevant papers per 100 citations retrieved. It would be highly unusual to have 20 relevant papers per 100 citations retrieved and this might suggest that the search was too precise. One approach to determining if additional search terms are useful is to conduct two searches, one with the additional search terms, and to screen a sub-set of the additional references retrieved for relevance. Based on a predetermined number, say 100, if no or few relevant studies are identified it may be decided to exclude the additional terms. Such explorations and decisions should be documented in the protocol and the final report, along with the details of the search strategies undertaken. An example of a simple search conducted in multiple databases is provided in *Table 19.3*.

Another important component of the search is the need to adapt it to the differences in indexing approaches employed by different bibliographic databases. *Tables 19.4* and *19.5* provide examples of the differences in the design of a search for the same review question in two different databases: *Science Citation Index* (*Table 19.4*) and *MEDLINE* (*Table 19.5*). Understanding indexing differences among databases and designing sensitive searches can be difficult and requires the expertise of an information

⁴ As noted in Chapter 17, alternative but overlapping acronyms have been suggested, such as **PICOTT**, which includes the Type of question being asked (e.g., relating to therapy, diagnosis, prognosis or harm) and the best Type of study design to answer the question (e.g., randomized controlled trial; diagnostic test evaluation) (Schardt *et al.*, 2007).

Table 19.3 Simple search strategies showing the impact of searching specific fields of a database record, including subject indexing fields. (Searches conducted on 27 June 2013.)

Database	Search string entered*	How the search is interpreted and run by the database	Number of results returned
PubMed	cattle AND otitis media AND antibiotics	("cattle"[MeSH Terms] OR "cattle"[All Fields]) AND ("otitis media"[MeSH Terms] OR "otitis"[All Fields] AND "media"[All Fields]) OR "otitis media"[All Fields]) AND ("anti-bacterial agents"[MeSH Terms] OR ("anti-bacterial"[All Fields] AND "agents"[All Fields]) OR "anti-bacterial agents"[All Fields] OR "antibiotics"[All Fields] OR "anti-bacterial agents"[Pharmacological Action]) <i>If no search field is specified then PubMed searches across all fields, and maps the terms to MeSH (the subject indexing language used by PubMed). This can have uncertain results as the searcher is not in control of the terms being used in the strategy. For example, the term 'antibacterial' is being searched for across all fields even though this is not included in the original search string</i>	7
PubMed	cattle [tiab] AND otitis media [tiab] AND antibiotics [tiab]	cattle[tiab] AND otitis media[tiab] AND antibiotics[tiab] <i>Specifying the fields in which the terms are found ensures that PubMed only searches the required fields; in this case the title and abstract field. This can increase the precision of the search</i>	2
PubMed	("Anti-Bacterial Agents"[Mesh] OR antibiotic [tiab] OR antibiotics[tiab]) AND ("Cattle"[Mesh: NoExp] OR "Cattle Diseases"[Mesh:NoExp] OR cattle[tiab]) AND ("Otitis Media"[Mesh] OR otitis media[tiab])	("Anti-Bacterial Agents"[Mesh] OR antibiotic [tiab] OR antibiotics[tiab]) AND ("Cattle"[Mesh: noexp] OR "Cattle Diseases"[Mesh:noexp] OR cattle[tiab]) AND ("Otitis Media"[Mesh] OR otitis media[tiab]) <i>To increase sensitivity, the strategy should specify both the title and abstract fields and the subject indexing field to be searched. In this example, additionally searching the subject indexing field has increased the number of results identified</i>	9
CAB Abstracts via Web of Knowledge (Thompson Reuters)	TS = (cattle) AND TS = (otitis media) AND TS = (antibiotics)	<i>TS indicates topic field. This searches across a number of individual fields including title, abstract and subject indexing. This approach will not find records where the subject index terms applied are different to the terms searched for in the topic field</i>	10
CAB Abstracts via Web of Knowledge (Thompson Reuters)	TS = (cattle) AND TS = (otitis media) AND (TS = (antibiotics) OR DE = (antibacterial agents) OR DE = (antiinfective agents))	<i>This more sensitive strategy searches the DE field, indicating a descriptor or subject indexing search, in addition to searching the topic field. This is important as the CAB Thesaurus contains potentially relevant subject index terms (antibacterial agents and antiinfective agents) which would not be found by the terms used in the topic field searches</i>	10

* Information about Boolean Logic terms (AND, OR, NOT) is available at https://www.nlm.nih.gov/bsd/disted/pubmedtutorial/020_350.html. Information about Mesh terms in Medline can be found at <https://www.nlm.nih.gov/bsd/disted/mesh/tutorial/introduction/>. Information about search operators in CAB can be found at https://images.webofknowledge.com/WOKRS511B5/help/CABI/hs_search_operators.html.

Table 19.4 Complex search strategy for *Science Citation Index* (Web of Science, Thompson Reuters) for studies reporting specific approaches to stunning animals at slaughter.

Search line number	Search terms entered	Explanation of the search line
#1	TS = ("stunning" or "stun" or "stunned" or "stuns" or "stunner" or "restun*" or "unstun*" or "unconscious*" or "euthan*" or "narcosis")	Searches for terms in the topic field which includes title, abstract and author keywords. Some terms are truncated using the * symbol to find zero or unlimited additional characters. Terms are enclosed in quotation marks to prevent the database automatically searching for related or variant terms
#2	TS = ("carbon dioxide" or "co2" or "co 2")	Searches in the topic field
#3	TS = ("gas" or "gases" or "gassing")	Searches in the topic field
#4	TS = (("electric*" or "electrified") near "waterbath*")	Near searches for the given terms within 15 words of each other, in either direction
#5	TS = (("electric*" or "electrified") near/3 ("bath" or "baths") or "voltage" or "electronarcosis" or "head-only" or ("wave" near/3 "frequenc*"))	Near/3 specifies that the given terms must appear within three words of each other, in either direction
#6	TS = (("captive" near/2 "bolt\$") or ("bolt" near/2 "pistol*") or "zephyr" or "bolt gun\$" or "boltgun\$" or "stun bolt\$" or "stunbolt\$" or "cattle gun\$")	\$ truncates the term to find none or one additional character
#7	TS = ("penetrating bolt\$" or "penetrative bolt\$")	Searches in the topic field
#8	TS = ("ritual" or "religious" or "kosher" or "halal" or "shechita" or "shehitah" or "shehita" or "shechitah" or "dhabihah" or "zabiha")	Searches in the topic field
#9	#8 OR #7 OR #6 OR #5 OR #4 OR #3 OR #2	Stunning methods search results are combined together using OR
#10	TS = ("bovine" or "cow" or "cows" or "cattle" or "beef" or "calf" or "calves" or "veal" or "bull" or "bulls" or "buffalo*" or "pig" or "pigs" or "sow" or "sows" or "pork" or "swine" or "porcine" or "finisher\$" or "boar" or "boars" or "sheep" or "murine" or "lamb" or "lambs" or "mutton" or "goat\$" or "poultry" or "chicken*" or "hen" or "hens" or "broiler*" or "turkey \$")	Searches in the topic field
#11	TS = ("animals" or "animal" or "livestock" or "ruminant\$")	Searches in the topic field
#12	TS = ("slaughter*" or "abattoir*" or "meat")	Searches in the topic field
#13	#12 OR #11 OR #10	Animal context search results are combined together using OR
#14	#1 AND #9 AND #13	The three concepts (stunning, stunning methods and animal context) are combined using AND

Table 19.5 Complex search strategy for *Ovid MEDLINE* for studies reporting specific approaches to stunning animals at slaughter.

Search line number	Search terms entered	Explanation of the search line
1	(stunning or stun or stunned or stuns or stunner or restun\$ or unstun\$ or unconscious\$ or euthan\$ or narcosis).ti,ab.	Searches for terms in the title and abstract fields. Some terms are truncated using the \$ symbol to find zero or unlimited additional characters
2	exp Unconsciousness/ve	Searches the subject indexing field.
3	1 or 2	Stunning search results are combined together using OR
4	(carbon dioxide or co2 or co 2).ti,ab.	Searches for terms in the title and abstract fields
5	(gas or gases or gassing).ti,ab.	Searches for terms in the title and abstract fields
6	Carbon Dioxide/	Searches the subject indexing field
7	((electric\$ or electrified) adj4 waterbath\$1).ti,ab.	Adj4 searches for the given terms with four words of each other in either direction. \$1 truncates the term to find none or one additional character
8	((((electric\$ or electrified) adj3 (bath or baths)) or voltage\$ or electronarcosis or electro-narcosis or head-only or (wave adj3 frequenc \$)).ti,ab.	Adj3 searches for the given terms with three words of each other in either direction
9	Electroshock/ or Electronarcosis/	Searches the subject indexing field
10	((captive adj2 bolt\$1) or (bolt adj2 pistol\$1) or zephyr\$1 or bolt gun\$1 or boltgun\$1 or stun bolt\$1 or stunbolt\$1 or cattle gun\$1).ti,ab.	Searches for terms in the title and abstract fields
11	(penetrating bolt\$1 or penetrative bolt\$1).ti,ab.	Searches for terms in the title and abstract fields
12	(ritual\$ or religious\$ or kosher or halal or shechita or shehitah or shehita or shechitah or dhabihah or zabiha).ti,ab.	Searches for terms in the title and abstract fields
13	or/4-12	Stunning methods results are combined together using OR
14	(bovine or cow or cows or cattle or beef or calf or calves or veal or bull or bulls or buffalo\$1 or pig or pigs or piglet\$ or sow or sows or pork or swine or porcine or finisher\$1 or boar or boars or sheep or murine or lamb or lambs or mutton or goat\$1 or poultry or chicken\$1 or hen or hens or broiler\$1 or turkey\$1).ti,ab.	Searches for terms in the title and abstract fields
15	(animal or animals or livestock or ruminant\$1).ti,ab.	Searches for terms in the title and abstract fields
16	cattle/ or exp goats/ or exp sheep/ or exp swine/ or chickens/ or turkeys/	Searches the subject indexing field.
17	(slaughter\$ or abattoir\$1 or meat).ti,ab.	Searches for terms in the title and abstract fields
18	Abattoirs/	Searches the subject indexing field
19	or/14-18	Animal context results are combined together using OR
20	3 and 13 and 19	The three concepts (stunning, stunning methods and animal context) are combined using AND

specialist (EFSA, 2010; Dudden and Protzko, 2011). In animal health and welfare reviews, the inclusion of *CAB Abstracts* is likely to be important because data suggest that it provides the most comprehensive coverage of animal health topics (Grindlay *et al.*, 2012).

Step 3: Select relevant studies from the search results

Once the citations have been retrieved, and the results from multiple sources combined and de-duplicated, it

is necessary to identify those relevant to the review question. This step is called **screening**. The aim is to quickly remove irrelevant studies captured by the search, and to retain relevant studies. To do this, a series of short questions are asked about each citation. The screening questions should be based on all the components (PICO) of the review question. Multiple levels of screening might be employed for efficiency. The *first level of screening* should be conducted on the citations from electronic databases, or the titles of conference proceedings if hand searching. When

using the title and/or abstract, the information available in order to exclude studies is limited, and so the first level of screening should not include questions about information that is only likely to be reported in the full text. Instead the first level of screening should focus on broad issues such as population, intervention and study design, which should be described in titles and abstracts.

For example, based on the review question 'What is the change in the number of squeals during castration and during play activity within 24 hours of the castration in piglets less than 28 days of age that received 0.5–1 ml of intratesticular 2% lidocaine compared with no local anaesthetic?', the first-level screening questions might be:

'Does the citation title and abstract describe a study where:

1. piglets are undergoing castration? (P = population);
2. lidocaine is administered as the intervention? (I = intervention);
3. a comparison group is included? (C = comparator);
4. at least one outcome assessed is a measure of pain? (O = outcome).'

For each of these questions possible responses are 'yes', 'no' or 'not determinable'. Citations that have a 'no' response to any of the questions are excluded.

Designing efficient screening questions is important. For example, in the above set of four questions, a 'no' response to any question removes the study from the review without further consideration. A time-saving technique is to make screening questions hierarchical; that is, a citation is excluded as soon as the first 'no' response is obtained. It is not usual to describe the reasons for exclusion at the first level of screening, therefore answers to all questions are not required.

At the first level of screening, the use of the third option 'not determinable' (or 'unclear' or 'need full text to determine') should be reserved for studies that are relevant based on other criteria and where there is a reasonable expectation that such information would be in the full text but not the abstract. As an illustration, in the example above, questions 3 and 4 are most likely to return 'not determinable' because such information may not be described in an abstract. A review team might decide to leave those questions for the *second level of screening* to be conducted on full papers. The disadvantage of moving these questions to the second level is that it limits the reviews team's ability to exclude studies that did report such information in the abstract and adds to the number of papers that must be obtained. Hence, it is often preferable to retain

questions 3 and 4 in the first level of screening but be aware that these questions are candidates for a high frequency of 'not determinable' responses, requiring these citations to be passed to the next level of screening.

For studies that do proceed to full-text retrieval, assessment of relevance will be more refined, ensuring that the study fits all of the criteria in the PICO(S), PECO(S), PO(S) or PIT(S) format. For example, it would be possible to ask the following screening questions for the smaller number of articles that pass the first level of screening:

'Does the full text describe a primary study where:

5. piglets less than 28 days of age are undergoing castration? (P = population);
6. 0.5–1 ml of intratesticular 2% lidocaine is administered as the intervention? (I = intervention);
7. a parallel non-active control group was used? (C = comparator);
8. one outcome is either the number of squeals during the castration or play activity within 24 hours (O = outcome).'

When the full text is assessed for relevance, the reason for the exclusion should be included in the final report. Having identified the relevant studies, the next step is to extract data from the relevant studies.

Step 4: Collect data from relevant studies

Following completion of screening, the next step in a systematic review is the extraction of data from the relevant studies. In this step, the results of the relevant studies are extracted, as are the potential sources of clinical heterogeneity.

Extraction of outcomes (results)

One of the key features of systematic reviews is the emphasis on extraction and reporting of the magnitude of outcomes and precision of estimates. Unlike some narrative reviews, the inference from either hypothesis testing (significant or not significant) or the authors' interpretation from the original research publication generally is not reported. For example, a narrative review might report that two studies found evidence against the null hypothesis at the $P < 0.05$ level, whereas one study did not. Such an approach is referred to as **vote counting** and is not valid because of the potential for bias in the studies to influence the observed P values, and because of the inability to weight studies (Deeks *et al.*, 2011). Instead, a systematic review would extract the numerical results for the outcome of interest, such as the mean difference or the

relative risk, and the level of precision of such estimates.

For data extraction, it is highly recommended that a statistician is consulted about the expected study designs and form of extracted data needed for meta-analyses. In anything other than the simplest of designs, substantial familiarity with statistical analysis will be needed to validly determine the data to be extracted.

Common group-level measures of disease frequency include risk, rate, prevalence, disease odds, prevalence odds or exposure odds (see Chapters 4 and 15). In other circumstances, authors may report the effect size that compared the outcome of the study groups, such as the risk ratio, rate ratio (relative risk), prevalence ratio, disease odds ratio, prevalence odds ratio or exposure odds ratio (see Chapters 15). Some authors may report both group-level data and effect size. The measures of variation for either the group-level measure or effect size also must be extracted or calculated. Knowledge of statistics is particularly helpful when extracting data from designs other than a two-arm parallel randomized controlled trial. For example, if a design involves a clustered population and blocking and stratification to control for confounding as well as random allocation, the authors may describe the group-level event risk and model-adjusted risk ratio. In such situations, the adjusted estimates of the risk ratio and standard error of the estimated risk ratio from models are more appropriate than descriptive group-level data because the model-derived standard error of the adjusted estimate has accounted for non-independence.

Some important outcomes in veterinary science are continuous data, such as milk yield or average daily weight gain. Production-related outcomes are often reported at the group level, such as the group-level average daily gain, or group-level average milk production. Alternatively, effect sizes such as the difference in average daily gain, difference in average daily milk yield, difference in mean piglets per litter and corresponding estimated standard error of the mean difference can be reported. Again when model-adjusted estimates are available that have accounted for confounding or non-independence, these might be more valid than group-level descriptive information (Peters and Mengersen, 2008). Time-to-event outcomes are also common in veterinary intervention trials. Knowledge of the statistical method often will be necessary to validly extract the results of such studies.

If the data were incompletely reported, as frequently occurs, it can be possible to calculate measures of dispersion from *P* values and the number of animals in each group to obtain the estimated standard error of

the mean or standard deviation. Formulae for such calculations for continuous variables are published elsewhere, however an awareness of the assumptions necessary for these calculations is required to ensure that the resulting values are valid (Deeks *et al.*, 2011). Software that uses these formulae (e.g., *Review Manager*: Appendix III) also is available.

Extraction of study characteristics

When identifying data other than the outcome to be extracted, a useful approach is to consider what the end-user will need to understand the results of the studies and place the results in context, such as the breed of animals, the age of animals studied, or the season the study was conducted. To place the results in context, end-users need to be aware of possible sources of heterogeneity in intervention effect among the relevant studies (the concept of heterogeneity was introduced in Chapters 15 and 17). The aim is to extract factors that are known or thought to be associated with a different intervention effect. Later, these factors may be included in a meta-analysis and formally evaluated as sources of **effect modification** (see Chapters 15 and 18). However, even if not used formally in meta-analysis, reporting possible sources of heterogeneity enables the end-user to understand how varied studies within the review were, and to make judgements about the applicability of these studies to their setting.

Differences in the intervention effect between the relevant studies might occur due to characteristics of the population, specific intervention protocols, or the comparator (**contextual diversity** or **contextual heterogeneity**) and study design and method of study execution (**methodological diversity** or **methodological heterogeneity**) (Deeks *et al.*, 2011; Khan *et al.*, 2012). **Statistical heterogeneity** is the difference in the intervention effect observed in studies beyond that expected by random chance; that is, as a consequence of clinical (contextual) heterogeneity, methodological heterogeneity, or both (Deeks *et al.*, 2011). **Contextual heterogeneity** refers to demographic characteristics that modify the effect size in studies. Examples of factors often investigated as potential sources of contextual heterogeneity include region, average age and production systems. Contextual heterogeneity also may be referred to as **clinical heterogeneity** in clinical settings. To capture the same concept for broader settings, such as food safety or animal welfare, the term contextual heterogeneity is preferable. The number of sources of heterogeneity will depend upon how refined the review question was. As with the assessment of effect modification in primary research, knowledge of sources of heterogeneity

increases understanding of the intervention. Therefore, assessment of heterogeneity is essential in research synthesis. In this context, the presence of heterogeneity should *not* be viewed as a nuisance to be removed by analyses (see Chapter 15). In this step of the review, sources of contextual heterogeneity are of interest. Methodological and statistical heterogeneity are relevant in later steps.

Sources of contextual heterogeneity

Population Differences in the population studied may contribute to differences in observed intervention effect. As no two studies are conducted in exactly the same population, the variation in the study populations should be communicated to the end-users. If the population was not very refined in the review question, there may be sources of heterogeneity such as age, sex or breed that need to be extracted. For example, consider a review to evaluate the impact of a vaccine on mortality in naturally-infected calves raised in commercial settings. Suppose the original review question did not limit the production system: therefore studies on beef and dairy calves are included in the review. However, the review team hypothesized that the vaccine might be less effective in populations with high stocking density because of increased organism transmission. As most studies will not report stocking density, the review team may consider that reporting the production systems will convey some information about production systems and stocking density to the end-user. Even if the production system were not used in a meta-analysis, the end-user will still benefit from knowing which studies were conducted in dairy calves and which in beef calves. Consequently, such information should be extracted and reported.

Intervention Differences in intervention also may explain differences in the intervention effect. Often interventions are similar, especially if the intervention is pharmaceutical or biological. If the intervention was explicitly described in the review question, then little or no variation in protocols may be present. For example, the description of an intervention as '0.5–1 ml of intratesticular 2% lidocaine within two minutes of castration' leaves little room for variation because the dose, the route, the concentration and timing were described. However, if the intervention was described as '0.5–1 ml of intratesticular 2% lidocaine' then timing of administration is a possible source of difference in intervention effect and should be extracted, so that the impact can be assessed and reported.

Not all interventions are biological or pharmaceutical; for interventions such as behaviour modification, the variation in what constitutes the intervention

between studies will likely be large and particular sub-groups may modify the effect of the intervention. Such detail is important and should be extracted. For example, if a review was evaluating the impact of biosecurity on the number of disease outcomes on farms, numerous studies may be relevant to the review; however, the biosecurity protocols will differ among the studies and these differences may explain differences in the effect of biosecurity on the outcome. The end-user would need to be aware of these differences and so they must be extracted and reported.

Comparator The comparator is often not a source of variation in veterinary intervention trials because many studies use non-active (negative) controls. However, if an active (positive) control is used, as with the intervention, it may also vary. In particular, when the comparator is the prevailing standard of care this can vary greatly over time or between countries. If the variation in comparator is large, this may preclude a simple pairwise meta-analysis and may require advanced methods of analysis.

Step 5: Assess the risk of bias in relevant studies

Sources of methodological heterogeneity

Study execution Methodological heterogeneity arises when the reported effect of the intervention differs due to the methods of study execution (i.e., the effect of the intervention is different in studies that are biased compared with unbiased studies). In this step of the review, the potential for bias to influence the outcome is assessed. This step also is commonly referred to as **quality assessment**, although **risk of bias** assessment is more accurate. Assessment of risk of bias aims not just to convey the presence or absence of design features that are associated with bias; instead, the rationale behind transparently reporting the potential for bias is to alert the end-user of potential concerns and uncertainties about the evidence summary, which should be considered in the decision-making process.

At the protocol development stage, the systematic review team will have considered the potential sources of systematic bias that could occur in studies relevant to the specific review question. The sources of bias may be subject-specific, and certainly are specific to the type of review question; for example, intervention, exposure, diagnostic test assessment, or prevalence estimate.

For PICO(S) and PECO(S) questions, the important sources of bias include confounding, selection bias and information bias (see Chapter 18). It is preferable to design a *risk of bias assessment tool* that explicitly addresses the type of bias, rather than to extract

information on a specific design tool that might have been used in the study to address that particular bias. For example, in an assessment of a controlled trial, rather than asking, 'Did the authors report randomization to treatment group', the tool should ask 'Were appropriate steps taken to ensure that the study groups are exchangeable?' The later question refers to the bias, whereas the former question only addresses a single design tool (randomization) to address that bias. In veterinary science, it is common that studies use restricted populations, stratification and blocking at the design stage to increase the exchangeability of the groups. For example, a challenge study with fewer than 10 animals per group may have chosen a restricted population, then stratified treatment allocation by sex, then blocked by weight. Such an approach to allocation may have reduced the risk of bias due to confounding even if randomization was not reported. The results of the challenge trial may have poor external validity but the risk of bias due to confounding is minimal. However, this low risk of bias due to confounding will not be conveyed if the first style of question is used, compared with the second style of question. This example illustrates the difference between assessing bias and assessing quality of reporting, topics that are frequently confused.

There is a temptation to use the reporting guidelines checklists such as the CONSORT statement (Moher *et al.*, 2010; Schulz *et al.*, 2010) or the REFLECT statement (O'Connor *et al.*, 2010; Sargeant *et al.*, 2010) as a tool for assessing the risk of systematic bias in studies, but this should be avoided. These documents are guidelines for *reporting* research studies and, as such, provide guidance on the nature and scope of information that should be reported. Reporting guidelines are designed for particular study designs but not for particular topic questions, where differences in the potential for bias may occur (O'Connor, 2010). For example, consider two studies, one conducted in a large poultry abattoir and another in a university laboratory. Because of the realities of working in a large production facility, the authors decide to apply an intervention to every second carcass in sequence, and the outcome is assessed on 4000 birds, with 2000 in each group. These authors were not able to randomly allocate to treatment, therefore the answer to the question 'Did the authors report randomizing to treatment group' is 'no'. However, this is unlikely to be a true assessment of the potential for confounding bias associated with the allocation method, which might be considered low because the assessors might think it unlikely that the population of 'second carcasses' has a different confounding variable distribution than the population of 'first carcasses'. Conveying this

perceived low risk of bias is more useful to the end-user. Alternately, if a small study was conducted in a research laboratory with only six birds per group and the authors similarly assigned every second bird captured to the same group, the potential for differences in groups would be higher and should be conveyed to the end-user.

Assessment of bias requires expertise in both sources of bias and content. The *Cochrane Handbook of Systematic Reviews for Interventions* has an entire section devoted to assessing the risk of bias (Higgins *et al.*, 2011). The approach used is to assess whether the risk of bias is high, low or unclear. Numerous tools have been designed to assess the impact of bias; the *Cochrane risk of bias tool* for randomized studies provides an excellent starting point, and should only be modified as needed. For example, some terms used in the *Cochrane risk of bias tool* for randomized studies need modification to work in the veterinary setting, but the general forms of bias for intervention trials (confounding, selection bias and information bias) are consistent between human and veterinary medicine trials. There are no international standards for bias terms, and the terms used in the *Cochrane Handbook of Systematic Reviews for Interventions* include 'selection', 'performance', 'detection' and 'attrition', which can be matched to confounding, selection bias and information bias. During protocol development, the potential impact of the absence of design features should be discussed with the entire review team and agreement reached about how the presence or absence of important design features such as randomization, allocation concealment, blinding of outcome assessment and multivariate analysis will be assessed with respect to the risk of bias.

One topic where new questions may be required is accounting for the impact of non-independence of populations in the analysis. Non-independent populations are quite common in veterinary settings: examples include, but are not limited to, shelter medicine, racing performance and production medicine. If relevant, this issue can be included in the 'other biases'⁵ section in the risk of bias assessment (Higgins *et al.*, 2011). Failure to control for non-independent observations is not a systematic bias that affects the magnitude of the effect. Instead, failure to adjust for non-independent populations affects estimates of uncertainty. As such, failure to control for non-independence might lead to smaller *P* values and narrower confidence intervals, and, because publication is

⁵ See the Cochrane Collaboration's tool for assessing risk of bias: Chapter 8 in <https://training.cochrane.org/handbook/pdf-versions> (accessed 23 August 2017).

associated with statistically significant findings, such studies might be over-represented in the literature. For example, if a study of vaccination allocated treatments to 10 pens of 60 animals but conducted the analysis as if there were 600 independent observations in each group, the potential for statistical significance is greater and so is the potential for publication.

The Cochrane *risk of bias tool* for randomized studies includes an assessment of **reporting bias**. Reporting bias is a sub-group of publication bias, and refers not to the exclusion of the study from the literature but to the exclusion of particular results from the study report (Higgins *et al.*, 2011). Reporting bias is extremely difficult to assess but worth examining because, as with publication bias, it probably biases towards positive effects. An example where reporting bias was considered important was a review that assessed vaccination to prevent the adverse reproductive effects of *Trichostrongylus axei* infection (Baltzell *et al.*, 2013). A common approach employed in the primary studies was to allocate a group of cows to vaccinated or unvaccinated groups prior to exposure of both groups to bulls known to be infected with *T. axei*, with subsequent measurement of reproductive outcomes. The review authors considered that outcomes likely to be available included early conception rate (<60 days), mid-term pregnancy rates and calving rates. After reviewing the data, positive and unexpected effects of the vaccine were found for calving rates but not for early conception rates. Moreover, it was found that more studies reported early conception rates than calving rates. The authors hypothesized that the primary studies that found no association between the vaccine and early conception rates or calving rates might have tended to report only the findings from the comparison of early conception rates, assuming the calving rate data were implied. However, studies that found no effect on conception rates but an effect on calving rates were perhaps more inclined to report both outcomes. The review authors hypothesized that the result could be a bias towards overestimating the effect of the vaccine on calving rates, because non-significant results appeared to be less likely to be reported within a particular study. Of course, the potential for this source of bias was based on the judgement of the review authors; however, bias assessment is always a judgement. The rationale for any judgement about bias should be explained, and the end-user can decide if he or she agrees with the interpretation. In the same review, the authors found a single study published 20 years earlier reporting a positive effect of the vaccine on reproductive outcomes associated with bulls. In the assessment of bias, the review authors concluded that

it seemed incongruous that no published study had replicated this positive effect in 20 years and therefore proposed that there was a strong potential for publication bias in this body of work for that bull-related outcome. A tutorial on assessing outcome reporting bias has been published (Dwan *et al.*, 2008, 2010). For non-randomized intervention studies, the *ROBINS-I (Risk Of Bias In Non-randomized Studies – of Interventions)* tool is available for assessing the risk of bias (Sterne *et al.*, 2016). This tool imagines every non-randomized study in the framework of an intervention study. It can therefore be difficult to use for some observational studies where the exposure is difficult to envision as an intervention (e.g., intrinsic determinants such as sex). There is currently a *ROBIS-E tool* under development, which asks about exposures rather than non-randomized interventions. Such tools require considerable input from content experts to identify important confounders that should be controlled in the study.

For other types of review questions (e.g., diagnostic test assessments, exposure assessment and prevalence estimates) the sources of bias differ. For diagnostic test evaluation, the STARD statement is a reporting guideline that can be used to learn more about sources of bias in diagnostic tests (Bossuyt *et al.*, 2003; Christopher, 2007). The QUADAS-2 statement is designed as a quality assessment tool of diagnostic test assessments (Whiting *et al.*, 2011). As it is a risk of bias tool, it requires tailoring to the specific review question and identifies four domains for bias in diagnostic tests. As the development and validation efforts that have been devoted to tools such as QUADAS-2 are rarely possible for in-house tools, authors of reviews in veterinary science are advised to use these tools with as little modification as necessary.

Finally, quantitative scores of quality or bias should not be used. Such scoring systems are arbitrary and do not convey the importance of bias to the topic. For example, a scoring system that assigns double the points to lack of randomization compared with lack of blinding, implies that the 'bias' due to randomization is double that due to lack of blinding – such effects cannot be so quantified. Concerns about quality scoring systems are discussed in more detail elsewhere (Whiting *et al.*, 2005; Higgins *et al.*, 2011).

Study design The role of study design, rather than bias within the design, as a potential source of heterogeneity is poorly understood in the assessment of interventions in veterinary science. The easiest solution is to limit systematic reviews to a single design at the start of the review (e.g., only randomized controlled trials may be deemed eligible). In this situation, methodological heterogeneity relates only to biases

that occur due to execution as described earlier. However, it is theoretically possible to identify relevant studies from different designs. The availability of challenge studies, where researchers are able to induce the disease experimentally and assess interventions, makes this issue particularly relevant to veterinary science. It is currently unclear if all the heterogeneity that might be expected to be due to the study designs should be classified as biases. Two good examples where differences in the intervention effect due to the study design might be expected are: (1) the assessment of intervention in trials with naturally-occurring disease and induced disease (challenge studies); and (2) the assessment of interventions with group-level or individual-level allocation to treatment. For example, estimates of vaccine efficacy obtained from trials with induced disease are likely larger than estimates of vaccine efficacy obtained from naturally-occurring disease in large commercial field settings. However, any difference may not be caused by bias due to study execution; that is, the designs may be well executed and so the risk of systematic bias within either design is minimal. However, because of the inherent differences in the design settings, the magnitude of the effect of the intervention may be different. Similarly, a review assessing the impact of a vaccine on average daily gain could contain trials that allocate pens of animals to receive the intervention or comparator, as well as trials that allocate individual animals to receive the intervention or comparator. The effect of the intervention may differ due to this design, even if both types of studies employ all the necessary design features to control for confounding, selection bias and information bias internally. Therefore, if multiple study designs are incorporated into the review, the design must be extracted and reported to the end-user. If multiple study designs are included, this variable often is used for sub-group analysis. Considerations should also be given to deciding, *a priori*, to conduct separate meta-analyses for different study designs (see later). Again, the importance of discussing this issue when formulating the PICO(S) question cannot be overemphasized.

Step 6: Synthesize the results

Having extracted the data about the results, study characteristics, and potential sources of contextual or methodological heterogeneity, the next step is to synthesize the results. If the data are amenable to quantitative analysis, this would enable a **meta-analysis**. This aims to combine, for each outcome, the observed result from the relevant studies into one estimate. Numerous terms are used to describe the single

estimate obtained from a meta-analysis such as: **summary effect measure**; **summary effect size**; **pooled effect estimate**; and **overall effect estimate**. Each study contributes an observation to the calculation of the summary effect size. The contribution of each study, however, is weighted. There is a tendency for larger studies to provide more data to the calculation of the estimate; however, the exact weighting formula depends upon the methods chosen for the meta-analysis and the effect size used. A common method is to weight studies by the inverse of the variance of the study-level effect size (van Houwelingen *et al.*, 2002). If a quantitative analysis is not possible, a narrative discussion of the results will be required.

Quantitative approaches to synthesis

The essential elements of a meta-analysis are planning, conduct and interpretation. Planning requires deciding which comparisons to make, what summary effect measure to use, which statistical model to use, and which sources of heterogeneity to assess. For example, when comparing two interventions, possible comparisons may include differences in mortality, morbidity and weight gain. Model choices are discussed later, and sources of heterogeneity have been discussed previously. Conducting a meta-analysis involves assessing heterogeneity and calculating the summary effect measure, if appropriate. Interpreting the meta-analysis requires the consideration of how to interpret findings about heterogeneity and the summary effect measure. The summary effect size may reflect no effect, a strong protective effect, a weak protective effect, a strong deleterious effect or a weak deleterious effect. Based on the same level of confidence (for example 95%), these may be informative (a narrow 95% confidence interval) or non-informative (a wide 95% confidence interval).

Exploring heterogeneity

The first step to synthesize the extracted outcomes is to assess whether there is evidence of heterogeneity that would suggest that a summary effect size does not appropriately describe the intervention effect (i.e., the intervention effect is different in different sub-groups of the population). To support and inform this decision, the review team can use statistical approaches to assess heterogeneity by examining forest plots (see later), Cochran's Q statistic and I^2 (Higgins *et al.*, 2003). Provided it is determined that the studies are homogeneous, it is reasonable to calculate the overall effect size using the relevant studies, interpret the findings, and conclude how much confidence should be placed in them. If the decision is made to not provide an overall effect estimate, the rationale

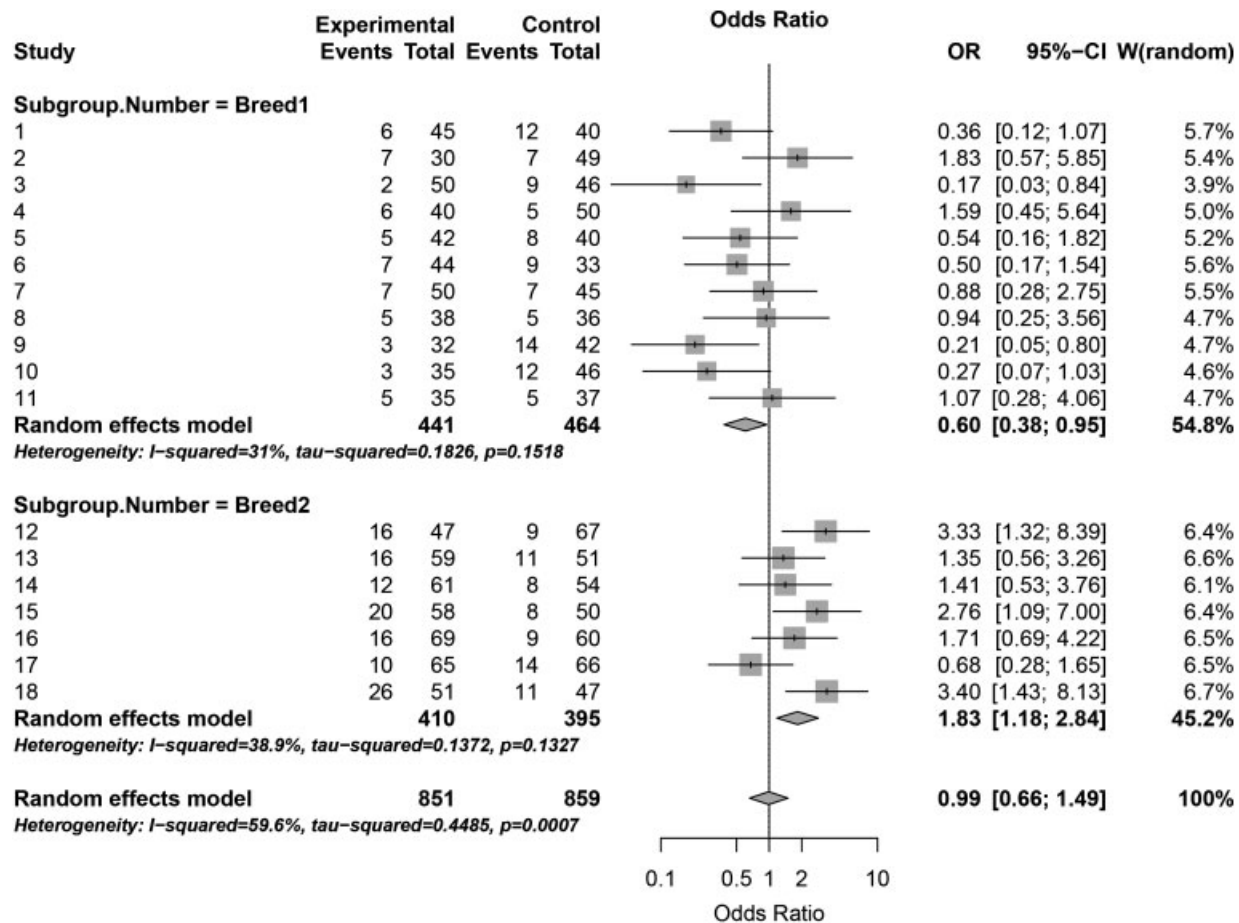


Fig. 19.1 Results and forest plots from an example of a pairwise meta-analysis with sub-group analysis to explore heterogeneity, using hypothetical data. The meta-analysis includes data from 18 studies, uses the odd ratio (OR) from each study as the effect size, and calculates a summary odds ratio using a random-effects model.

should be provided and the source of heterogeneity formally explored.

Exploring heterogeneity of effects between studies should begin with examination of descriptive tables and forest plots (see later: 'Pairwise meta-analysis') for variations in the study-level effects. Figure 19.1 gives an example of exploration of heterogeneity due to breed. Some difference in the effect of the intervention among studies should always be expected, and so the reviewer is looking for meaningful differences rather than no difference. Supplements to visual examination for heterogeneity are statistical tests of heterogeneity. A test for heterogeneity examines the null hypothesis that all studies are evaluating the same value of effect (i.e., that they are homogeneous). A commonly-used test for assessing heterogeneity is the Cochran Q χ^2 test of homogeneity (Q test), which tests the extent of deviation of observed effect sizes from an underlying overall effect size (Higgins and Thompson, 2002). (See also Chapter 15 for methods of assessing heterogeneity.)

The Q test is based on the Q statistic and is used to determine whether there are genuine differences underlying the results of the studies (heterogeneity), or whether the variation in findings is compatible with chance alone (homogeneity). P values for Q are obtained by comparing Q with a χ^2 distribution with $k - 1$ degree(s) of freedom, where k is the number of studies. The null hypothesis is that there is homogeneity of effect measures in the studies included in the analysis; therefore, rejection of the null hypothesis implies heterogeneity is present. If a small number of studies is included in the calculation, the power of the test to detect heterogeneity may be low, and the Q hypothesis test may be a poor indicator of true heterogeneity among relevant studies. It therefore is common that a P value cut-off of 0.10 or larger is used as a significance level, although this approach carries the risk that, when there are many large studies available, a clinically unimportant difference could be significantly heterogeneous. It is always preferable to report

the exact P value observed rather than report significance or non-significance based on an arbitrary (critical) cut off for the P value (see Chapter 14). As with any significance test, common sense should prevail when interpreting the meaning of the P value: a P value of 0.011 should not result in a markedly different inference compared with a P value of 0.09. In *Figure 19.1*, the P value for the Cochran Q χ^2 test of homogeneity for the entire group of 18 studies is 0.0007 (the actual Q statistic is not presented in the figure).

Another aspect of exploring heterogeneity is to quantify heterogeneity (see Chapter 15 and Higgins *et al.*, 2003). This approach quantifies the effect of heterogeneity, providing a measure of the degree of inconsistency in the studies' results, rather than just applying a test. Two quantities may be calculated, I^2 and τ^2 . I^2 represents the approximate proportion of total variability in point estimates that can be attributed to heterogeneity. Negative values of I^2 are converted to zero so that I^2 lies between 0% and 100%. A value of 0% indicates no observed heterogeneity and larger values show increasing heterogeneity. An advantage of I^2 is that it enables determination of an interval that communicates uncertainty; this conveys considerably more information than the P value of Q (Higgins and Thompson, 2002). In *Figure 19.1*, the I^2 value 59.6% is for the entire group of 18 studies. Tau-squared (τ^2) is the between-study variance in a random-effects model (see following section) measured in the same units as the outcome. If τ^2 equals zero, the random-effects and fixed-effect models are essentially the same (Borenstein *et al.*, 2009). In *Figure 19.1*, the τ^2 value of 0.4485 is for the entire group of 18 studies.

As heterogeneity will always exist, it is important to decide the point at which the level of heterogeneity becomes too great for valid calculation of a summary effect size. The current recommendation is that the magnitude of effects, the P value for Q and the size of I^2 should all be considered. However, these values should only be used for guidance, not fixed rules, and the heuristic nature of the guidance is indicated by the overlapping interpretation of the categories. For example, the following guidelines for I^2 values, with overlapping categories, have been suggested: 0%–40% is unimportant, 30%–60% may represent moderate heterogeneity, 50%–90% may represent substantial heterogeneity and 75%–100% may represent considerable heterogeneity (Deeks *et al.*, 2011).

If evidence of meaningful heterogeneity is identified, then possible sources should be explored. Heterogeneity might be attributable to differences in population, intervention or comparator characteristics (clinical or contextual heterogeneity), different study designs

or study execution (methodological heterogeneity). Approaches to formally investigating causes of heterogeneity include conducting sub-group analyses or using meta-regression techniques (Borenstein *et al.*, 2009). In a sub-group analysis, the data are stratified by a categorical factor and separate meta-analyses carried out in each of the strata. The disadvantage of stratification is that individual strata may contain relatively few studies and only categorical study characteristics can be explored as sources of heterogeneity. *Figure 19.1* shows an example of a sub-group analysis by breed. The results for each sub-group include a Cochran Q χ^2 test of homogeneity (Q test), I^2 and τ^2 . In this hypothetical example, it appears that breed is associated with a different summary effect size. Breed 1 has a summary odds ratio of 0.6 (95% CI = 0.38, 0.95), while Breed 2 has a summary odds ratio of 1.83 (95% CI = 1.18, 2.84).

A second approach to exploring heterogeneity is **meta-regression**. This is a weighted regression of the study results on the factors of interest. For the meta-regression, the unit of concern is the study, and the outcome of interest is the effect of the intervention at the study level. The explanatory variables are trial characteristics in the meta-regression. If the number of studies is limited, factors might be investigated one at a time in univariable analyses, or if there are sufficient data, a multivariable meta-regression model could be built. One advantage of meta-regression over stratified analysis is that it allows evaluation of continuous study characteristics such as herd size or weight at enrolment, as well as categorical characteristics. Several publications describe the use of meta-regression (Higgins and Thompson, 2002; Morton *et al.*, 2004). Meta-regression should generally not be considered when there are few studies; a rough guideline is that more than 10 studies should be available before considering meta-regression as an approach to exploring heterogeneity analysis (Higgins *et al.*, 2011).

As part of the meta-analysis, a formal analysis of publication bias or dissemination bias should be included (Song *et al.*, 2010). Simple approaches include the use of **funnel plots**, which plot an indicator of study size against the estimated effect; an asymmetrical distribution provides clues to the existence of publication bias. However, alternative explanations for asymmetrical funnel plots do exist: for example, smaller studies may have been conducted in populations that were uniquely different from larger populations, in which there is a different treatment effect (Sterne *et al.*, 2011). Thus, as previously discussed, estimates of vaccine effect obtained from challenge studies that enrol few animals are probably truly larger than estimates of vaccine effect obtained from naturally-occurring

disease in the large commercial field setting. In the larger setting, the ability to administer the vaccine properly is decreased and concurrent disease may affect response to the vaccine. In this case, asymmetry may be observed in a funnel plot as a function of study design and population effects, rather than publication bias. This also could be interpreted as a source of methodological heterogeneity. If publication bias is detected, advanced meta-analysis approaches to adjust the summary effect for publication bias are available (Carpenter *et al.*, 2009; Schwarzer *et al.*, 2010).

The impact of publication bias on the validity of reviews is critical. PRISMA, a reporting guideline for systematic reviews and meta-analysis, includes assessment of publication bias, and this should be a step in any meta-analysis (Liberati *et al.*, 2009; Moher *et al.*, 2009). If meta-analysis is not possible then the potential impact of publication bias should be discussed.

Pairwise meta-analysis

When a review assesses the effect of a single intervention on a single outcome, a pairwise meta-analysis can be conducted. The overall effect estimate will summarize the comparison over multiple studies. An example of pairwise meta-analysis is provided in Figure 19.2. The results are presented graphically, although a table could also be used. The example in Figure 19.2 is for a dichotomous outcome, and summarizes the data from each study with the odds ratio and calculates an overall odds ratio using random effects and fixed-effect models. This approach to the display of a meta-analysis is

called a **forest plot**. This displays the point estimate of the treatment-effect measure for each study, in this example the odds ratio, with its associated 95% confidence interval. The overall estimate of the intervention effect with its 95% confidence interval also is displayed, in this example at the bottom of the plot. The measures of heterogeneity are included in the figure and suggest that it is appropriate to calculate an overall effect estimate. The overall effect is incorporated into the forest plot as a diamond. The centre line of the diamond is the point estimate of the summary effect, with the horizontal points corresponding to the bounds of the confidence interval. The numerical values of the overall effect estimate derived from the fixed-effect or random-effects models are provided, as are the weights used.

The effect size from each study used in the calculation of the summary effect size should have the following properties: (1) consistency; that is, a measure that tends to be consistent across studies; (2) mathematical properties valid for meta-analysis; and (3) ease of interpretation. For the relative measures and dichotomous outcomes, the odds ratio has preferable statistical properties making meta-analysis easier to conduct, despite the fact that the risk ratio is easier to interpret for clinicians and end-users (O'Connor, 2013). As the risk difference is inconsistent across studies and affected by the baseline risk, it is not preferred for meta-analysis. In addition, as the number needed to treat (see Chapter 17) has no simple estimate of variance it is also not preferred for meta-analysis. A more

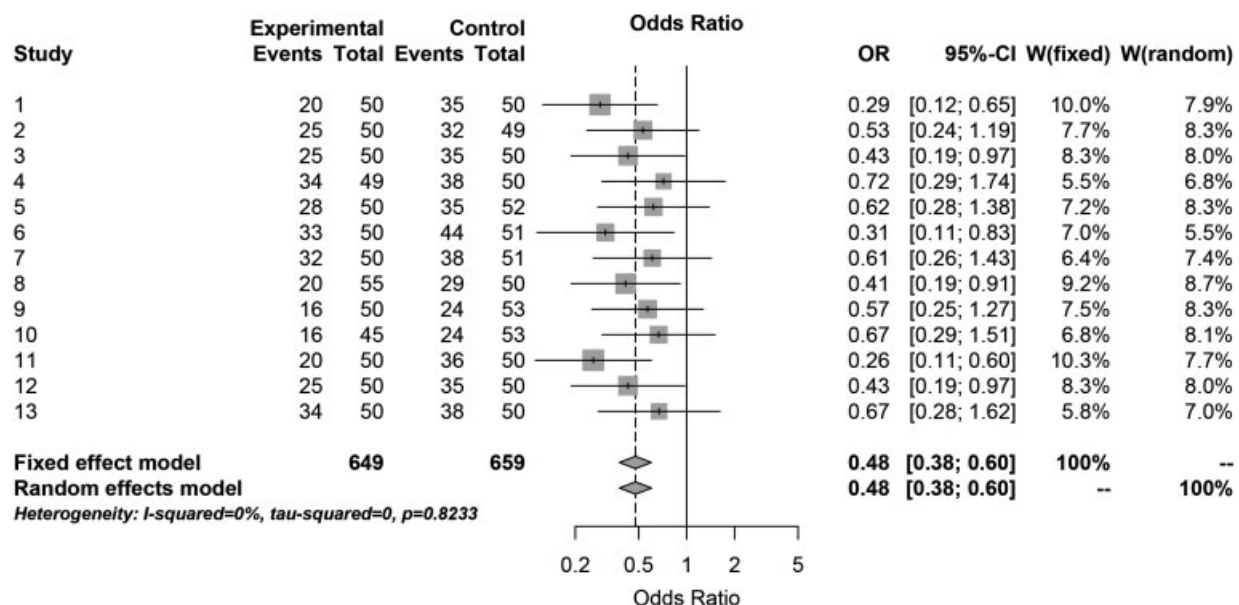


Fig. 19.2 Results and forest plots from an example of a pairwise meta-analysis, using hypothetical data. The meta-analysis includes data from 13 studies, uses the odd ratio (OR) from each study as the effect size, and calculates summary odds ratios using both a random-effects model and a fixed-effect model.

detailed discussion of the properties of various effect sizes for meta-analysis is available elsewhere (Deeks, 2002; Deeks *et al.*, 2011). For continuous outcomes, such as average daily gain or weaning weight, use of the mean difference is common. Where the studies do not exactly measure the same outcome, but a case can be made that each measure is a reasonable proxy for the other, the outcomes may be converted to a standardized mean difference. When using the standardized mean difference, it is important to verify that the direction of benefit is the same for all measures; that is, benefit should not be documented by a decrease in one measure and an increase in an alternative measure.

Another issue to consider prior to conducting meta-analysis is the assumption about the intervention effect to be adopted, which affects the choice of model; that is, a random-effects or fixed-effect model (see Chapter 17 and Cooper *et al.*, 2009). The combination of intervention effect size across studies might incorporate an assumption that the studies are not all estimating the same value of intervention effect, but estimating intervention effects that arise from the same intervention effect distribution. This is the basis of a random-effects model. Alternatively, if it is assumed that the effect of the intervention being investigated is common across studies, a fixed-effect model is used. Random-effects models generally produce a point estimate of the summary effect size that is similar to that obtained from a fixed-effect model, but which has a wider confidence interval than a fixed-effect model. The most common method of calculating the random-effects model in available software is the DerSimonian and Laird approach (DerSimonian and Laird, 1986); however, the Hartung–Knapp–Sidik–Jonkman method for random-effects meta-analysis is considered straightforward and preferable in many situations (IntHout *et al.*, 2014).

Regardless of the modelling approach adopted, the meta-analysis should also take into account that the precision of the estimates of intervention effect is not equal among studies. Meta-analyses therefore use a system of weighting study results based on the precision of their estimates to compute the summary-intervention effect. Common weighting methods are the **DerSimonian and Laird** (also known as the **inverse variance** method), **Mantel–Haenszel** and **Peto** methods (see Chapter 15 and Deeks *et al.*, 2011). The Mantel–Haenszel procedure might be better than the inverse variance approach if data are sparse. The Peto approach is applicable to studies in which odds ratios or time-to-event outcomes are being pooled. It is not recommended to weight using any subjective measure of study quality.

Step 7: Presenting the results

The presentation of the results of the review should include the following components:

- the results of search and study selection;
- summary information about the characteristics of the studies identified as relevant to the review (including those that could not be included in a meta-analysis);
- risk of bias assessment for the individual studies;
- outcomes reported by the relevant studies;
- meta-analysis results including sub-group analysis and/or meta-regression;
- risk of bias across the studies.

The results of the search and abstract screening are usually summarized in a figure, as proposed in the PRISMA statement (Liberati *et al.*, 2009). This figure includes the number of studies screened, the number of relevant studies and the number of studies that were included in any quantitative analysis. A list of reasons for exclusion should be included for studies that were excluded after full text evaluation.

For the individual studies included in the review, three aspects are usually reported: characteristics, outcomes and risk of bias. Differences in the characteristics of the population and intervention in each study are usually reported in a study characteristics table. Where it was not possible to extract important characteristics from the relevant studies, this should be indicated. This proactive reporting of missing data increases the transparency of the review outcome and clarifies that missing information was not the result of an omission by the review team.

The outcome reported in each relevant study also should be described. Again, if it was not possible to extract results from all the studies due to the reporting approach, this should be indicated. For a dichotomous outcome, a table may list all studies in the first column, with number of events in each treatment group in the next columns, and then the effect size and the 95% confidence interval observed in each study. If a meta-analysis was conducted, the final row of such a table may contain the overall summary effect size and corresponding 95% confidence interval.

For a continuous outcome, a table may list all studies in the first column, with group mean, standard deviation of the mean and number of animals in each treatment group in the next columns, and then the effect size and 95% confidence interval observed in each study. The final row of such a table usually contains the overall summary effect size and corresponding 95% confidence interval from the meta-analysis if conducted.

	1	2	3	4	5	6	7	
	+	+	⊖		⊖	⊖	⊖	Random sequence generation (selection bias)
	+	+	⊖	⊖		+	⊖	Allocation concealment (selection bias)
	⊖	⊖		⊖		⊖	⊖	Blinding of participants and personnel (performance bias)
	+	⊖	+	⊖	⊖	+	+	Blinding of outcome assessment (detection bias)
	⊖		⊖	+	+	⊖	⊖	Incomplete outcome data (attrition bias)
	+	+			⊖	+	⊖	Selective reporting (reporting bias)
	+			⊖		+	⊖	Other bias

Fig. 19.3 An example of a risk of bias summary table from *Cochrane Rev-Man* software (RevMan, 2014) presenting the reviewer's judgement of the individual risk of bias for each bias domain. Dark grey circles indicate a high risk of bias; grey circles indicate a low risk of bias; missing circles indicate an unclear risk of bias; numbers identify each study.

An alternative and useful output from a meta-analysis is a graphic representation of the results using a forest plot (see earlier).

The approach to presenting the risk of bias assessment can vary. The *Cochrane risk of bias tool* recommends a figure or table that lists the determination of each aspect of bias for each study. An example is provided in *Figure 19.3*. By reporting the risk of bias assessment at the individual study level, it is possible for end-users to evaluate if studies that failed to properly control for the potential for confounding were the same studies that failed to adjust for the potential for differential information bias due to absence of blinding the outcome assessor to the treatment allocation. The approach to reporting risk of bias in *Figure 19.4* is less preferred than *Figure 19.3*, because *Figure 19.4* does not allow for identification of joint bias issues.

A summary of the evidential value of the body of work should be presented in addition to the individual study risk of bias. One approach used by the Cochrane Collaboration is to provide an evidence profile (Higgins and Green, 2011). The evidence profile provides a structured means of summarizing the risk of bias assessment for each outcome included in the systematic review. The evidence profile provides information about the impact of inconsistency (Guyatt *et al.*, 2011d), risk of bias in the studies (Guyatt *et al.*, 2011h), indirectness, imprecision and other considerations (usually publication bias) for the entire body (i.e., all the studies included in the review) on the findings of the review. The final column in the evidence

table is an overall assessment of the quality of evidence based on a four-point scale from very low to high. In the example presented in *Table 19.6* the review authors had concerns about consistency and publication bias and these resulted in a downgrading of the quality of the evidence. Inclusion of footnotes in the evidence profile, which indicate the issues identified by the review authors (as shown in *Table 19.7*), increases the transparency of this judgement.

Frequently, the evidence profile is presented in conjunction with the summary of findings table. This contains the outcomes considered by the review, and then, for each outcome, the number of participants and studies, the assumed risk (baseline) and the corresponding risk (intervention group), and measures of relative and absolute effect of the intervention. In the example in *Table 19.8*, the intervention is associated with a risk ratio of 0.74 (95% CI = 0.65, 0.83). When using the baseline risk of disease in the study population of 69.5%, this translates into an absolute reduction of 180 cases (95% CI from 118 to 243 cases) per thousand. When using the baseline risk of disease in a hypothetical population of 10%, this translates into an absolute reduction of 26 cases (95% CI from 17 to 35 cases) per thousand.

More information about creating summary of findings tables and evidence profiles is provided in a very thorough series of publications from the GRADE working group (Guyatt *et al.*, 2008, 2011a–h; Balshem *et al.*, 2011). The GRADE working group provides software, *GRADEpro* (Version 3.2: Brozek *et al.*,

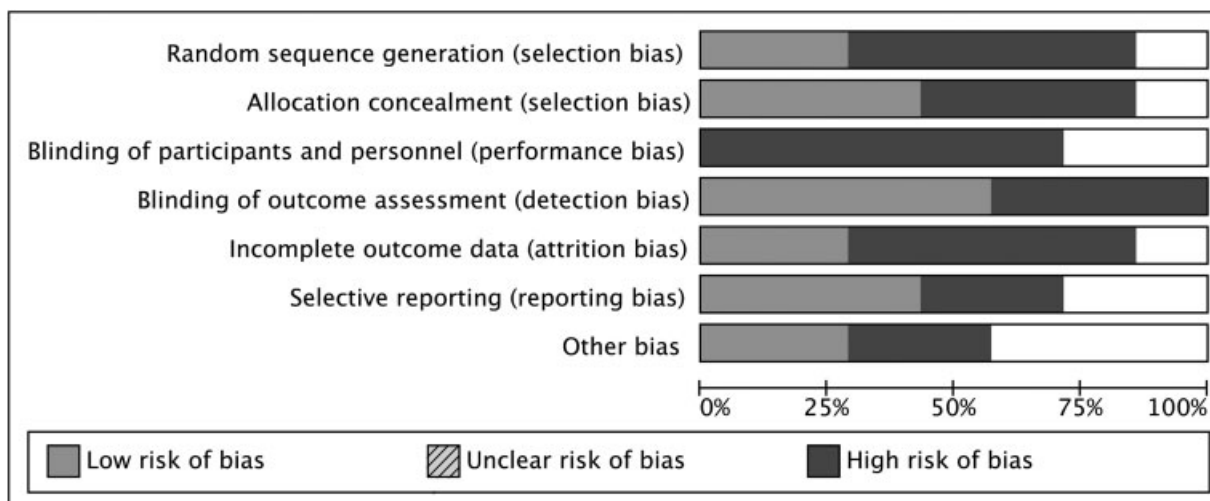


Fig. 19.4 An example of a risk of bias table from *Cochrane Rev-Man* software (RevMan, 2014) presenting the reviewer's judgement of the risk of bias for each bias domain over all studies. Dark grey bars indicate a high risk of bias; grey bars indicate a low risk of bias; and hatched bars indicate an unclear risk of bias (none identified by the reviewer in this example).

Table 19.6 Evidence profile for a hypothetical review of an intervention to prevent disease.

No. of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Quality of the evidence ¹
10	Six randomized and four non-randomized challenge studies	Not serious	Serious ²	Not serious	Not serious	Publication bias ³	Low

¹GRADE working group grades of evidence:

High quality: further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: we are very uncertain about the estimate.

²Significant heterogeneity was noted in the meta-analysis ($I^2 = 60\%$).

³The funnel plot suggested evidence that larger studies showed effects closer to the null.

Table 19.7 Example of the group-level data and study-level data and meta-analysis-level effect sizes. The group-level and study-level data are extracted from primary research studies.

Examples of type of outcome measured on individual factor	Group-level data	Study-level effect size	Meta-analysis summary effect size
Weight gain	Average group mean and standard deviation	Mean difference (group 1 mean – group 2 mean)	Summary mean difference and standard error (weighted average of mean difference)
Disease incidence (yes/no)	Risk (number of new events/number at risk)	Risk ratio (group 1 risk/group 2 risk)	Summary risk ratio (weighted average of risk ratio)
Disease incidence	Odds (number of new events/number of non-events)	Risk odds ratio (group 1 risk odds/group 2 risk odds)	Summary risk odds ratio (weighted average of odds ratio)
Disease prevalence	Prevalence (number of current events/number in population)	Prevalence ratio (group 1 prevalence/group 2 prevalence)	Summary prevalence ratio (weighted average of prevalence ratio)
Disease prevalence	Prevalence (number of current events/ number of current non-events)	Prevalence odds ratio (group 1 prevalence odds/group 2 prevalence odds)	Summary prevalence odds ratio (weighted average of prevalence odds ratio)

Table 19.8 Summary of findings table for hypothetical review of an intervention to prevent disease.

	<i>Illustrative comparative risks (95% CI)</i>		<i>Relative effect (95% CI)</i>	<i>Absolute effect (95% CI)</i>	<i>Number of participants (studies)</i>	<i>Quality of the evidence¹</i>
	<i>Assumed risk (no intervention)</i>	<i>Corresponding risk (intervention)</i>				
Study population	695 cases per 1000	515 (452 to 577) cases per 1000	Risk ratio 0.74 (0.65 to 0.83)	180 fewer (from 118 fewer to 243 fewer) cases per 1000	2547 (10 studies)	Low due to inconsistency and publication bias
Low-risk population	100 per 1000	74 (65 to 82) cases per 100		26 fewer (from 17 fewer to 35 fewer) cases per 1000		

¹GRADE working group grades of evidence:

High quality: further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: we are very uncertain about the estimate.

2008), that guides review teams through the steps of producing evidence tables and summary of findings tables.

Finally, the results of the quantitative synthesis (meta-analysis) are presented, if appropriate. This will include the results of heterogeneity assessment and publication bias assessment.

Step 8: Interpret the results and discussion

As with any research project, conclusions about the results of the review and a discussion of potential biases of the review should be discussed to enhance the end-user's understanding of the issues related to the review and the review author's interpretation of the meaning of the results. The PRIMSA guidelines for reporting a systematic review (Moher *et al.*, 2009) suggest the following aspects should be covered in the discussion and interpretation: summarize the evidence, discuss the limitations of the review, and provide conclusions. The discussion of the evidence

should include information on the magnitude of the summary effect size, precision based on the confidence interval and impact of bias. The summary effect size may suggest no effect, or may reflect a strong protective effect, a weak protective effect, a strong deleterious effect, or a weak deleterious effect. Based on the width of the confidence interval and quality of body of work, the certainty about this effect will differ. The discussion of the impact should include the expected direction of bias, rather than mere mention that such biases could occur.

The discussion of the limitations of the review should include two components: (1) the issues identified in the studies themselves; and (2) the approach to the review. Despite attempts to be transparent and comprehensive, all reviews have limited resources. For instance, translations may not have been conducted or perhaps assumptions were made in the approach to extracting measures of variation for continuous outcomes; these types of issues should be included in the discussion on a review's limitations.

Further reading

Alonso, S., Lindhal, J., Roesel, K., Traore, S.G., Yobouet, B.A. *et al.* (2016) Where literature is scarce: observations and lessons learnt from four systematic reviews of zoonoses in African countries. *Animal Health Research Reviews*, **17**, 28–38

Borenstein, M., Hedges, L.V., Higgins, J.P.T. and Rothstein, H.R. (2009) *Introduction to Meta-Analysis*. John Wiley and Sons, Chichester

Connor, A.M. and Sergeant, J.M. (2014) An introduction to systematic reviews in animal health, animal welfare, and food safety. *Animal Health Research Reviews*, **15**, 3–13

Cooper, H.M., Hedges, L.V. and Valentine, J.C. (2009) *The Handbook of Research Synthesis and Meta-analysis*. Russell Sage Foundation, New York

European Food Safety Authority (2010) Application of systematic review methodology to food and feed

- safety assessments to support decision making. *EFSA Journal*, **8**, 1–90
- Higgins, J. and Green, S. (Eds) (2008) *Cochrane Handbook for Systematic Reviews of Interventions*. The Cochrane Collaboration. John Wiley and Sons, Chichester
- Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gotzsche, P.C. *et al.* (2009) The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Journal of Clinical Epidemiology*, **62**, e1–34
- Morton, S.C., Adams, J.L., Suttorp, M.J. and Shekelle, P.G. (2004) *Meta-regression Approaches: What, Why, When, and How?* Technical Review 8. Agency for Healthcare Research and Quality, Rockville

20

Diagnostic testing

The range of diagnostic techniques that are currently available for the diagnosis of infectious and non-infectious diseases is wide, and includes clinical and pathological examination; microbiological, biochemical and immunological investigation; and diagnostic imaging (e.g., radiography and ultrasound). These techniques may be used either to diagnose disease in the individual animal, or to investigate disease in populations. This chapter focusses primarily on the assessment and performance of diagnostic tests when applied to populations¹. Diagnosis of infectious diseases using **serological** methods is addressed first. This is followed by a more general discussion of diagnostic testing.

Serological epidemiology

Serological epidemiology is the investigation of disease and infection in populations by the measurement of variables present in serum. A range of constituents of serum can be measured, including minerals, trace elements, enzymes and hormones. One of the main constituents that is frequently measured is the specific antibody activity of immunoglobulins, and it is investigation of antibodies that is commonly understood to comprise serology. Alternative terms for antibody measurement are 'titration' and 'assay'. Antibodies provide evidence of current and previous exposure to infectious agents; their assay is commonly employed in veterinary medicine as a relatively efficient and cheap means of detecting this exposure in both individual animals and populations.

The statistical methods employed to analyse antibody levels are equally applicable to other serological tests, such as those that detect enzymes and minerals, in which case, however, results can be compared with normal reference ranges. These commonly include: (1) the mean $\pm$ two standard deviations for Normally-

distributed data, selected from a normal (i.e., reference) population; and (2) the middle 95% of values (i.e., from the 2.5th to 97.5th percentile: see Chapter 12) from a reference population for data that are not Normally distributed (Farver, 2008). Although values for reference levels are available in published tables (e.g., Kaneko *et al.*, 2008), each laboratory should establish its own norms. If the values are Normally distributed, or can be transformed to Normality, then a one-sample *t*-test (see *Table 14.1*) can be applied to compare a sample's values with those of a reference population; otherwise one-sample non-parametric methods may be appropriate (see *Table 14.2*). Appropriate methods depend on not only statistical distribution but also the size of the reference population, which can be increased by integrating results from several laboratories. Farver (2008) and Friedrichs and others (2012) discuss determination of reference values in detail.

The serological diagnosis of disease based on the detection of circulating antibodies is one of the techniques available for the identification of current and previous exposure to infectious agents. This and other methods are listed in *Table 20.1*. A range of tests to detect antigen/antibody reactions has been developed over the last 100 years, and more are being added to the range. Detection of antigens provides the best proof of current infection (Engelkirk and Duben-Engelkirk, 2008).

Descriptions of these techniques are found in standard immunology texts (e.g., Hay and Westwood, 2002; Delves *et al.*, 2013; Tizard, 2013), and a basic knowledge of them is assumed.

Assaying antibodies

Methods of expressing amounts of antibody

The concentration of antibody is expressed as a **titre**. This is the highest dilution of serum that produces a test reaction. Thus, if the highest dilution that

¹ A detailed discussion of diagnostic testing applied to the individual in clinical practice is presented by Haynes and others (2006).

Table 20.1 Methods of diagnosing infectious disease.

Evidence of current infection
Isolation of agent
Identification of agent's genes (molecular epidemiology)
Clinical signs
Pathognomonic (characteristic) changes
Biochemical changes
Demonstration of an immune response: detection of antigens and antibodies (serological epidemiology)
Evidence of past infection
Clinical history
Pathognomonic changes
Demonstration of an immune response: detection of antibodies

produces a test reaction is 1 in 32, then the titre is 1/32. Alternatively, the reciprocal, 32, can be quoted, indicating that the undiluted serum contains 32 times the antibody for the reaction. Animals with detectable antibody titres are **seropositive**; animals with no detectable antibodies are **seronegative**. Animals previously seronegative and now seropositive have **seroconverted**. However, classification of an animal as seropositive often is based on the titre being above a certain **threshold level (cut-off point)** (see 'Evaluation and interpretation of diagnostic tests' later in this chapter). For example, animals with titres >1/32 may be classed as positive, whereas animals with titres ≤1/32 may be classified as negative.

Logarithmic transformation of titres

Serum is usually diluted in a geometric series, that is, with a constant ratio between successive dilutions. The commonest ratio is two. Thus, serum is diluted 1/2, 1/4, 1/8, 1/16, 1/32 and so on. This suggests that the titres should be measured on a logarithmic scale. There are two reasons for this measurement:

1. the frequency distribution of titres often is approximately lognormal (see *Figure 12.5*); statistical tests that assume Normality therefore may be applied;
2. geometric dilution series are equally spaced on a logarithmic scale; thus serum may be diluted geometrically 1/2, 1/4, 1/8, 1/16 and so on, corresponding to log transformation to base two, the respective logs to base two of the reciprocals of the dilutions being 1, 2, 3, 4 and so on; the dilution can be coded as the value of these logarithms to base two (*Table 20.2*).

Table 20.2 Antibody titres expressed as reciprocal dilutions (X) and coded titres ($\log_2 X$).

Reciprocal dilution (X)	Coded titre ($\log_2 X$)
1 (undiluted serum)	0
2	1
4	2
8	3
16	4
32	5
64	6

In some cases, high concentrations of serum that react non-specifically are avoided by initially diluting by $\log_{10}$, and then continuing in $\log_2$ dilutions, thus: 1/10, 1/20, 1/40, 1/80.

Mean titres

If several coded (i.e., $\log_2$ transformed) titres are recorded, their **arithmetic mean** can be calculated. This is simply the sum of the coded titres divided by the number of titres. For example, if five titres are 1/2, 1/4, 1/2, 1/8 and 1/4, then the coded titres are 1, 2, 1, 3 and 2, respectively. The arithmetic mean therefore is $(1 + 2 + 1 + 3 + 2)/5 = 1.8$.

The **geometric mean titre (GMT)** is the antilog₂ of the arithmetic mean. This can be obtained from a pocket calculator with an 'x' function key on it. For instance, if the arithmetic mean of several coded titres is 1.8, then $\log_2 \text{GMT} = 1.8$; thus $\text{GMT} = 2^{1.8} = 3.5$.

If an initial $\log_{10}$ dilution has been carried out, subsequently followed by $\log_2$ dilutions, values are divided by 10 before taking logarithms to base 2. For example, dilutions of 1/10, 1/20, 1/40, 1/80 would be coded as 0, 1, 2, 3 (1/10 is coded 0 because it is equivalent to undiluted serum), giving a mean of 1.5. Then: $\text{GMT}/10 = 2^{1.5} = 2.8$. Thus, $\text{GMT} = 28$.

The logarithm of zero cannot be expressed because it is 'minus infinity'. Therefore, when calculating means of coded titres, seronegative animals have to be excluded because their reciprocal titres are zero and therefore cannot be coded; *mean titres can be calculated only for seropositive animals*. Thus, when comparing coded antibody titres in populations, two parameters must be considered before inferences are made: the relative proportion of seropositive animals, irrespective of titre, and the GMTs of the seropositive populations. For instance, it might be found that in two dairy herds approximately 20% of cows in each herd were seropositive to *Leptospira interrogans* serovar Pomona, but that the GMT in one herd was

40 while in the other it was 640. Such circumstances might indicate a recent epidemic in the second herd and merely the persistence of antibodies in convalescent animals in the first. Conversely, a serological survey of workers in two different abattoirs might reveal similar *GMTs* of complement-fixing antibodies to *Coxiella burnetti* in each group of seropositive workers, but at one abattoir 30% of workers had titres, while at the other only 3% were seropositive. Such results would indicate a much greater probability of infection at the first abattoir, although the *GMTs* of the groups were similar.

Quantal assay

A quantal assay measures an 'all-or-none' response; for example, agglutination or no agglutination, infected or non-infected. Two systems frequently are used:

1. single serial dilution assay;
2. multiple serial dilution assay.

The first is the commoner. Both techniques utilize geometric (logarithmic) dilutions, the range of dilution depending on the sensitivity of the test. Sensitivity here refers to the ability of a system to detect amounts of antibody and antigen: the more sensitive the test, the smaller the amount of antibody or antigen (or other substances) it will detect. This sometimes is more fully termed **analytical sensitivity** (Saah and Hoover, 1997), to avoid confusion with sensitivity as a validity parameter of a diagnostic test – which is more fully termed **diagnostic sensitivity** (see later in this chapter). Synonyms for analytical sensitivity include 'limit of detection' and 'minimal detectable concentration'.

Single serial dilution assay

In a single serial dilution assay, each dilution is tested once. For instance, in a virus haemagglutination-inhibition test, the highest dilution that prevents agglutination of erythrocytes on a test plate is the antibody's haemagglutination-inhibition titre. This is a relatively 'weak' form of measurement. If the titre is 1/32 it implies that 1/33 would not produce the effect. However, since 1/64 is the next highest dilution that is tested, the actual titre could lie between 1/63 and 1/32. Thus, this type of titration, which tests only dilution intervals, actually divides the dilutions into blocks. The blocking is more marked when titres are expressed as 'less than' or 'greater than' (e.g., <1/8 or >1/256). The data therefore are essentially *ordinal* (see Chapter 10).

Multiple serial dilution assay

In a multiple serial dilution assay, each dilution is tested several (preferably at least five) times. The object is to achieve a 'strong' measure. The end-point is the dilution of a substance at which a specified number of members of a test group show a defined effect, such as death or disease. The most frequently used and statistically useful end-point is 50% (Gaddum, 1933). Thus, in pharmacology, the toxicity of a drug can be expressed as an LD_{50} (lethal dose₅₀): the amount of drug that will kill 50% of test animals. An amount of drug therefore can be expressed in terms of the number of LD_{50} s that it contains.

Fifty per cent end-point titrations also can be used to estimate antibody concentrations, in which case antibody titres are expressed in terms of the dilution of serum that *prevents* an effect in 50% of members of a test group, the effect being produced by the infectious agent responsible for induction of the antibodies that are being titrated. For example, the dilution of serum that prevents infection of 50% of cell culture monolayers with a standard concentration of virus can be estimated: an 'effective dose₅₀' (ED_{50}). Several methods of calculating 50% end-points are available, including the Reed–Muench and Spearman–Kärber methods, and moving averages. The Reed–Muench method is not recommended because precision cannot be assessed, there is no validity test, and the method is less efficient than some of the alternatives (Finney, 1978). The second method (Spearman, 1908; Kärber, 1931), which involves relatively simple calculations, is described here.

Example of a Spearman–Kärber titration The antibody titre to a virus is required. The defined measured response is a cytopathic effect (CPE) in cell culture monolayers. The test serum is diluted (usually in two-fold geometric increments). One-tenth of 1 ml of each dilution is inoculated into groups of five cell culture monolayers, each of which has been inoculated with a fixed, potentially lethal, dose of the virus. *Table 20.3* depicts the results. The 50% end-point is the dilution of serum that prevents a CPE in 50% of the monolayers in a group, that is, in two and a half monolayers (note that this is clearly a statistical estimation).

According to the Spearman–Kärber formula:

$$\log ED_{50} = L - d(\Sigma P - 0.5),$$

where:

L = log highest dilution at which all monolayers survive intact;

d = log of the dilution factor (i.e., the difference between the log dilution intervals);

Table 20.3 Example of a 50% end-point titration (Spearman–Kärber method).

Serum dilution	Log ₁₀ dilution	Monolayers showing cytopathic effect	Intact monolayers	Proportion 'positive' (intact) <i>P</i>	1 – <i>P</i>
1/1	0.0	0	5	1.00	0.00
1/2	–0.3	0	5	1.00	0.00
1/4	–0.6	0	5	1.00	0.00
1/8	–0.9	1	4	0.80	0.20
1/16	–1.2	1	4	0.80	0.20
1/32	–1.5	3	2	0.40	0.60
1/64	–1.8	4	1	0.20	0.80
1/128	–2.1	5	0	0.00	1.00

ΣP = sum of the proportion of 'positive' tests (i.e., intact monolayers), from the highest dilution showing a positive result to the highest dilution showing all results positive (i.e., $P = 1$).

From Table 20.3:

$$\begin{aligned}
 L &= -0.6, \\
 d &= \log_{10} 2 \\
 &= 0.3, \\
 \Sigma P &= 0.20 + 0.40 + 0.80 + 0.80 + 1.00 \\
 &= 3.2.
 \end{aligned}$$

Thus:

$$\begin{aligned}
 \log_{10} \text{ED}_{50} &= -0.6 - \{0.3(3.2 - 0.5)\} \\
 &= -0.6 - (0.3 \times 2.7) \\
 &= -0.6 - 0.8 \\
 &= -1.4.
 \end{aligned}$$

Therefore,

$$\begin{aligned}
 \text{ED}_{50} &= \text{antilog}(-1.4) \\
 &= 1/\text{antilog} 1.4 \\
 &= 1/25.1
 \end{aligned}$$

Thus 0.1 ml of serum contains 25.1 ED_{50} s, and 1 ml contains 251 ED_{50} s.

The estimated standard error (e.s.e.) is calculated using:

$$\text{e.s.e.}(\log_{10} \text{ED}_{50}) = d \sqrt{\Sigma\{P(1-P)\}/(n-1)},$$

where n = number of animals in each group.

Substituting the values from Table 20.3:

$\log_{10} \text{e.s.e.}$

$$\begin{aligned}
 &= 0.3 \sqrt{\{(0.2 \times 0.8) + (0.4 \times 0.6) + (0.8 \times 0.2) + (0.8 \times 0.2)\}/(5-1)} \\
 &= 0.3 \sqrt{(0.16 + 0.24 + 0.16 + 0.16)/4} \\
 &= 0.13.
 \end{aligned}$$

Virus multiple serial dilution assays were conducted originally using either test animals or embryonated hens' eggs as the assay medium, but have now been largely superseded by plaque assays (MacLachlan and Dubovi, 2011). Multiple serial dilution assays are now also generally less common than previously because they are more expensive and slower than single serial dilution assays, and titrations conducted on single dilutions – notably the enzyme-linked immunosorbent assay (ELISA). However, they still have a role in measuring vaccine production consistency, determining product shelf-life, and calibrating reference preparations (Lee *et al.*, 2015).

Serological estimations and comparisons in populations

Antibody prevalence

The presence of detectable antibody indicates that an animal or its dam has been exposed to the antigen that stimulates the antibody's production. In the absence of further challenge, the antibody level will decline. The rate of decline, usually measured in terms of the antibody's **half-life** (the mean time taken for its level to halve), varies between antibodies. Titres to some antibodies persist because the antibodies have a long half-life or there is persistent infection or repeated challenge. The possession of a long half-life explains why a single infection or vaccination course can produce lifelong immunity (e.g., the estimated half-life of human mumps antibodies is over 500 years, and, for measles antibodies, is over 3000 years: Amana *et al.*, 2007). The half-life of vaccinal antibodies therefore is an important aspect of vaccinal efficacy (see also Chapter 17) and of passively-acquired immunity in young animals.

If the amount of antibody in an animal population is to be estimated, without particular regard to the frequency distribution of antibody titres, animals are

categorized as either 'positive' or 'negative', and the prevalence of antibodies in the population (i.e., seroprevalence), with its associated confidence interval, can be calculated using the methods described in Chapter 13. A titre cut-off point, below which animals are considered to be negative, and above which animals are categorized as positive, is often defined (see later in this chapter).

The prevalence of detectable antibody depends on the rate of infection, the rate of antibody loss, and the time at which these rates have been effective. A high prevalence therefore may reflect not a high rate of infection but a low rate of antibody loss; recall that prevalence, P , is related to incidence, I , and duration, D (see Chapter 4):

$$P \propto I \times D.$$

It follows that not only the prevalence of detectable antibody in a population but also the titre in the individual is related to the half-life of the antibody.

If the frequency distribution of antibodies is required, then, if the scale of measurement is 'strong' (e.g., an ED_{50}) and the distribution is Normal, the mean and standard deviation can be quoted and confidence intervals can be calculated (see Chapter 12).

The much more common single serial dilution assays, which define a titre as the highest dilution producing a test reaction, produce ordinal data; this is particularly evident when a large proportion of the titres are expressed as 'less than' or 'greater than' a particular dilution. If there are not any 'less than' or 'greater than' titres, and there is a reasonable spread of titres, then the log titres can be regarded as crude approximations to Normally-distributed measurements, and the mean, standard deviation and confidence intervals can again be quoted. However, if these assumptions are not met, the median and semi-interquartile range should be quoted. Confidence intervals for the median can also be calculated (see Chapter 12).

Rate of seroconversion

If a population is susceptible to infection at birth, the duration of antibodies following infection is lifelong, and mortality due to infection is negligible, a simple mathematical model can be used to describe the age distribution of antibodies for various rates of seroconversion (Lilienfeld and Lilienfeld, 1980).

If p = probability of becoming infected in one year (i.e., rate of seroconversion); y = age in years; $(1 - p)^y$ = probability of not having become infected by age y (i.e., in y years); P_y = proportion of population that have become infected by age y (i.e., seroprevalence at age y), then:

$$P_y = 1 - (1 - p)^y.$$

It is also possible to estimate the rate of seroconversion from age-specific seroprevalence values by inversion of the formula:

$$\log(1 - p) = \{ \log(1 - P_y) \} / y.$$

Therefore:

$$(1 - p) = \text{antilog} \{ \{ \log(1 - P_y) \} / y \},$$

and:

$$p = 1 - \text{antilog} \{ \{ \log(1 - P_y) \} / y \}.$$

A series of age-specific seroprevalence values therefore can produce estimates of rates of seroconversion, and changes in these can provide information on the patterns and effects of infection in a herd. Table 20.4 lists the age-specific seroprevalence values for antibodies against bovine leukosis virus in a random sample of beef cattle. There is a slow increase in seroprevalence with age, up to and including 10 years. This infection produces chronic latent infections with persistent antibodies, and so the rate of seroconversion can be calculated.

Thus, for one-year-old animals ($y = 1$):

$$\begin{aligned} p &= 1 - \text{antilog} \{ \{ \log(1 - 0.15) \} / 1 \} \\ &= 1 - \text{antilog}(-0.0706/1) \\ &= 1 - 0.850 \\ &= 0.150; \end{aligned}$$

Table 20.4 Age-specific seroprevalence and annual mean seroconversion rates for bovine leukosis virus reactors in a sample of Louisiana beef cattle, 1982–1984. (Adapted from Hugh-Jones and Hubbert, 1988.)

Age (years)	Number of cattle tested	Seroprevalence (P_y)	Annual mean seroconversion rates (p)
1	67	0.15	0.150
2	191	0.21	0.111
3	105	0.23	0.083
4	143	0.39	0.116
5	167	0.40	0.097
6	137	0.47	0.100
7	98	0.53	0.102
8	92	0.55	0.095
9	32	0.63	0.105
10	53	0.60	0.088
>10*	19	0.37	0.036

* Average age used in calculating $p = 12.5$.

for two-year-old animals ($y = 2$):

$$\begin{aligned} p &= 1 - \text{antilog}[\{\log(1 - 0.21)\}/2] \\ &= 1 - \text{antilog}(-0.1024/2) \\ &= 1 - 0.889 \\ &= 0.111; \end{aligned}$$

and so on.

There is a steady estimated rate of seroconversion in animals up to 10 years of age, suggesting that the disease is having little impact on the herd: if diseased animals were being culled, a reduction in estimated seroconversion rates (because of the removal of seropositive animals) could be expected from about six years of age. The relatively high seroconversion rate in animals 12–23 months of age could (speculatively) be due to the curiosity of young heifers or persistent passive immunity. The low seroprevalence and seroconversion rate in animals older than 10 years suggests that preferential culling of affected animals is only taking place at that age.

Houe and Meyling (1991) and Houe and others (1995) further exemplify calculation of rate of seroconversion in relation to bovine virus diarrhoea virus infection, and Engel and her colleagues (1999) explore rate of seroconversion to Aujeszky's disease.

More complex models can be applied to infections in which antibodies decline during life, when

previously seropositive individuals therefore may become seronegative (i.e., 'serorevert') (Muench, 1959; Griffiths, 1974; Cunha *et al.*, 2014).

Comparison of antibody levels

Comparison of two different populations

If a comparison of two different populations in terms of presence and absence of antibody (i.e., 'positive' or 'negative' animals) is required, then the χ^2 test can be used; alternatively, confidence intervals for differences between two proportions for independent samples can be calculated (see Chapter 14).

If the frequency distributions of antibodies in two populations are to be compared, then, if the scale of measurement is 'strong', a parametric test can be used. Moreover, since antibodies are usually lognormally distributed, standard tests that assume Normality can be used.

An ED_{50} , calculated in multiple serial dilution assays, is a 'strong' measurement. The following example uses the data in Table 20.5 relating to vaccination titres in two groups of five dogs, one group vaccinated with killed rabies virus of porcine origin, and the other with vaccine of feline origin. The comparison is between the titres in each group, 60 days after vaccination. Log titres are used; this transformation allows the assumption of Normality. Student's t -test for

Table 20.5 Serum antibody titres (SN_{50} : serum neutralizing dose₅₀) of dogs, for two types of rabies vaccine, before and 60 days after vaccination. (From Merry and Kolar, 1984.)

Vaccine	Dog number	Pre-vaccination titre		Titre 60 days after vaccination	
		Reciprocal	$\log_{10}$	Reciprocal	$\log_{10}$
Killed vaccine feline cell origin	A653	3	0.48	214	2.33
	A616	3	0.48	182	2.26
	2C10	2	0.30	280	2.45
	2B39	2	0.30	267	2.43
	2B47	2	0.30	198	2.30
Mean		2.4	0.372	228	2.354
$\Sigma x_1 = 11.77$; $n_1 = 5$; $\Sigma x_1^2 = 27.7339$; $\bar{x}_1 = 2.354$; $s_1 = 0.083$.					
Killed vaccine porcine cell line origin	A603	3	0.48	10	1.00
	A654	2	0.30	51	1.71
	A618	2	0.30	9	0.95
	2C16	2	0.30	16	1.20
	2C3	2	0.30	38	1.58
Mean		2.2	0.366	25	1.288
$\Sigma x_2 = 6.44$; $n_2 = 5$; $\Sigma x_2^2 = 8.763$; $\bar{x}_2 = 1.288$; $s_2 = 0.342$.					

independent samples less than 30 can be used (see Chapter 14), assuming unknown variance. Using the same notation as that in Chapter 14:

$$n_1 = 5, \bar{x}_1 = 2.354, s_1 = 0.083,$$

$$n_2 = 5, \bar{x}_2 = 1.288, s_2 = 0.342.$$

The hypothesis to be tested is that there is no difference in 60-day antibody titres between the dogs vaccinated with killed rabies virus of porcine origin and dogs vaccinated with vaccine of feline origin.

Let μ_1 and μ_2 be the mean 60-day titres in the two groups, and let $\delta = \mu_1 - \mu_2$. The hypothesis then may be written as $\delta = 0$.

First it is necessary to check that s_1^2 and s_2^2 are estimates of a common population variance. This is done by calculating the ratio of the two variances, where the numerator is the greater of the two. This ratio is then compared with the appropriate percentage points of an F distribution (see Appendix XXII) with a pair of degrees of freedom, the first being one less than the sample size used in calculating the variance in the numerator, and the second being one less than the sample size used in calculating the variance in the denominator. In this particular case:

$$s_2^2/s_1^2 = 17.0,$$

with (4, 4) degrees of freedom.

The 1% point of the corresponding F distribution is 15.98. The sample value of 17.0 is greater than this value. The sample value therefore is significant at the 1% level and there is strong evidence to suggest that the variances of the $\log_{10}$ of 60-day antibody titres differ between groups.

The test statistic in this situation is now:

$$t = (\bar{x}_1 - \bar{x}_2 - \delta) / \sqrt{(s_1^2/n_1) + (s_2^2/n_2)},$$

with approximate degrees of freedom, ν , given by:

$$\nu = (\nu_1 + \nu_2)^2 / \{ \nu_1^2/(\nu_1 - 1) + \nu_2^2/(\nu_2 - 1) \},$$

where:

$$\nu_1 = s_1^2/n_1,$$

and:

$$\nu_2 = s_2^2/n_2,$$

to take account of unequal variances (Snedecor and Cochran, 1989).

For this example:

$$\begin{aligned} t &= (2.354 - 1.288 - 0) / \sqrt{(0.083^2/5) + (0.342^2/5)} \\ &= 1.066 / \sqrt{0.00138 + 0.02339} \\ &= 6.773. \end{aligned}$$

Thus:

$$\begin{aligned} \nu &= (0.00138 + 0.02341)^2 / (0.000000466 + 0.00037) \\ &= 4.47. \end{aligned}$$

Rounding down to the nearest whole number, when using the t -table (see Appendix V) there are only four degrees of freedom because the variances differ significantly. From Appendix V, the 5% value for four degrees of freedom is 2.776, which is less than 6.773. Therefore, the two groups of dogs have significantly different mean titres at the 5% level. Note that the result is also significant at the 2% level and the 1% level.

An alternative approach is estimation of confidence intervals for the difference between the means of two independent samples, noting that the variances (and therefore the standard deviations) differ (see Chapter 14).

Single serial dilution assays present a more difficult choice of statistical test because the titres are ordinal. Again, if there are not any 'less than' or 'greater than' titres, and there is a reasonable spread of titres, then the log titres can be regarded as crude approximations to Normally-distributed measurements, and a t -test for independent samples can be used; otherwise the non-parametric Wilcoxon–Mann–Whitney test should be applied; alternatively, confidence intervals can be calculated for the difference between two medians for independent samples (see Chapter 14).

Comparison of different estimates on the same population

If a population is sampled twice over a period of time, and animals are classified as positive or negative, then a suitable comparison can be made using McNemar's change test (see Chapter 14). If the frequency distribution of antibodies is to be compared, then a t -test for related samples should be applied – again using log titres to assume Normality (see Chapter 14). The test is described in standard statistical texts. The appropriate non-parametric equivalent is the Wilcoxon signed ranks test (see Chapter 14). Again, confidence intervals can be calculated for the difference between two means or two medians for related samples (see Chapter 14).

Interpreting serological tests

Refinement

Infectious agents have a variety of antigens on their surfaces and in their interiors. Additionally, non-structural antigens can be detected in the early stages

of virus replication. Some of the antigens are shared by several groups of isolates and are the basis of division into broad categories. Other antigens are unique to a particular group of isolates. For instance, influenza type A viruses are distinguished from types B and C by their core nucleoproteins and matrix proteins. Influenza A viruses are divided into subtypes on the basis of their surface haemagglutinin and neuraminidase antigens. Similarly, subtypes are divided further into strains according to more refined differences in the antigenic composition of the haemagglutinins and neuraminidases (*Table 20.6*). This refinement in antigenic definition also is called specificity. This is sometimes more fully termed **analytical specificity**, which refers to the ability of an assay to measure one particular organism or substance (Saah and Hoover, 1997). This avoids confusion with specificity as a validity parameter of a diagnostic test – more fully termed **diagnostic specificity** (see later in this chapter).

Table 20.6 The classification of some influenza A viruses. (Mainly from Murphy and Webster, 1990; Zambon, 1998; and Wright *et al.*, 2013.)

<i>Haemagglutinins (H) and neuraminidases (N)</i>	<i>Strains</i>
H1 N1	PR/8/34
H1 N1	Sw/Ia/15/30
H2 N2	Sing/1/57
H3 N2	HK/1/68
H3 N2	Sw/Taiwan/70
H3 N8	Eq/Miami/1/63
H3 N8	A/Eq-2/Suffolk/89
H3 N8	A/Eq-2/Newmarket2/93
H3 N8	A/Eq-2/Newmarket2/95
H4 N6	Dk/Cz/56
H5 N1	A/chicken/Scotland/59
H5 N1	A/grey heron/Hong Kong/861.1/2002
H5 N2	A/ostrich/South Africa/2004
H5 N3	Tern/South Africa/61
H5 N8	A/turkey/Ireland/1378/83
H6 N2	Ty/Mass/3740/65
H7 N3	A/chicken/Saskatchewan? HR-00011/2007
H7 N7	Eq/Prague/1/56
H7 N7	A/Bury/1239/94
H7 N7	A/chicken/Spain/6279-2/2009

The epidemiological value of a serological test, for example when tracing the spread or origin of a particular infection, increases in relation to the test's ability to detect more refined antigenic differences. The new molecular diagnostic techniques are particularly valuable in this respect (see Chapter 2).

Serological tests vary in their ability to detect subtle antigenic differences. *Table 20.7* illustrates varying refinement of serological tests for influenza A viruses. The complement fixation test (CFT), using virus extracted from chorioallantoic membranes as antigen, will detect antibody against virus nucleoprotein, and therefore is specific only to the level of virus type. However, the use of whole virus as antigen results in a CFT that is specific for subtypes because it will detect particular subtypes of haemagglutinins and neuraminidases. Identification of specific strains is possible if carefully-selected reference strains are used as antigens; the titre of antibodies to haemagglutinins is highest when the antibodies are directed against the

Table 20.7 Summary of tests for influenza serology. (From Stuart-Harris and Schild, 1976.)

<i>Test</i>	<i>Test antigens</i>	<i>Antibody detected</i>	<i>Recommended use*</i>	
			<i>Serosurvey</i>	<i>Serodiagnosis</i>
HI	Whole virus	HA [†]	++++	++++
NI	Whole virus	NA [†]	++++	
CF	CAM extract	NP		++
	Whole virus	HA, NA		+++
SRD	Whole virus [‡]	HA, NA	+++	++++
	Disrupted virus [‡]	NP, MP		+++
IDD	Disrupted virus [‡]	HA, NA	+	++
	Disrupted virus [‡]	NP, MP	+	++
N-IHA	NA	NA	++++	

HI, haemagglutination inhibition; NI, neuraminidase inhibition; CF, complement fixation; SRD, single radial immunodiffusion; IDD, immuno-double-diffusion; N-IHA, neuraminidase-indirect haemagglutination; HA, haemagglutinin; NA, neuraminidase; NP, nucleoprotein; MP, matrix protein; CAM, chorioallantoic membrane.

* The usefulness of the test for the indicated purpose is expressed on a scale of + (least useful) to ++++ (most useful).

[†] Serum containing high antibody titre to the second surface antigen (HA or NA) may at low dilutions and under certain conditions cause inhibition.

[‡] Test refinement (specificity) achieved by selecting viruses or recombinant viruses with the required antigenic composition.

strain-specific antigens (Katz *et al.*, 2011). It should be emphasized that one type of test is not always more refined than another (e.g., radial immunodiffusion vs complement fixation) for all antigen/antibody reactions; the refinement also depends on the nature of the antigen that is used in the test.

Accuracy

In common with other diagnostic tests, false-positives and false-negatives can occur (see Chapter 10). *Table 20.8* lists the reasons for positive and negative results in serological tests.

Positive results

A true-positive result derives from actual infection.

False-positive results occur for a variety of reasons. **Group cross-reactions** can occur between an infectious agent and antibodies to different organisms with similar antigens. For example, infection with *Yersinia enterocolitica*, serotype O:9, can produce antibodies that cross-react with *Brucella* spp. antigens (Godfroid *et al.*, 2010). Similarly, cases of paratuberculosis (Johne's disease) can produce positive reactions to mammalian and avian tuberculin (Körmendy, 1988).

Non-specific inhibitors present in serum may inhibit reactions that normally are associated with the action of intact antigens that are not specifically

bound to antibody. These inhibitors therefore mimic the effects of antibody in the latter's absence. An example is non-specific inhibitors in haemagglutination tests against influenza viruses (Boliar *et al.*, 2006). Agglutination of antigen by **non-specific agglutinins** similarly mimics the effect of antibodies that are agglutinins.

Negative results

A true-negative result indicates absence of infection.

Again, false-negative results can occur for several reasons. Some animals show **natural** or **induced tolerance** to antigens and therefore do not produce antibodies when challenged with the agent. Thus, exposure of the bovine fetus to bovine virus diarrhoea in the first half of gestation results in offspring that do not produce detectable antibodies when challenged with the same strain of virus (Coria and McClurkin, 1978).

Improper timing may result in a test's failure to detect infection. For instance, sampling of some cows before abortion, using the CFT, may not detect *Brucella abortus* because detectable complement-fixing antibodies may not appear until after abortion (e.g., Robertson, 1971).

Some tests may be **unsuitable** for detecting infection. Thus, infection by African swine fever virus cannot be detected using a serum neutralization test because infected pigs do not produce detectable levels of neutralizing antibodies (De Boer, 1967); an immunofluorescence test will, however, detect antibodies.

Some **non-specific inhibitors** will produce false-negative results by their mode of action (c.f. those above that produce false-positive results). Some sera, notably contaminated and haemolysed specimens, are anticomplementary; thus complement cannot be fixed in the CFT and the test is therefore assumed to be negative although antibodies may be present. This can occur with CFTs for *Br. abortus* infections (Worthington, 1982). Similarly, substances that are toxic to tissue culture monolayers may mimic the effects of un-neutralized virus, giving the impression that neutralizing antibodies are absent when they may be present.

Some antibodies are **incomplete** and so cannot take part in antigen/antibody test reactions. A common type of canine autoimmune haemolytic anaemia is characterized by incomplete antibodies on the surface of red blood cells, which can only be detected by an antiglobulin test (Halliwell, 1978). Occasionally **blocking antibodies** prevent antigen/antibody reactions occurring. This occurs sometimes when conducting CFTs for bovine *Br. abortus* infection (Plackett and

Table 20.8 Reasons for positive and negative results in serological tests. (From Stites *et al.*, 1982.)

Positive results		
Actual infection		true + ve
Group cross-reactions	}	false + ve
Non-specific inhibitors		
Non-specific agglutinins		
Negative results		
Absence of infection		true – ve
Natural or induced tolerance	}	false – ve
Improper timing		
Improper selection of test		
Non-specific inhibitors, e.g., anticomplementary serum; tissue culture toxic substances		
Antibiotic-induced immunoglobulin suppression		
Incomplete or blocking antibody		
Insensitive tests		

Table 20.9 Relative analytical sensitivity of assays for antigens and antibodies. (From Stites *et al.*, 1997.)

<i>Technique</i>	<i>Approximate sensitivity (per dl)</i>
Total serum proteins (by biuret or refractometry)	100 mg
Serum protein electrophoresis (zone electrophoresis)	100 mg
Analytical ultracentrifugation	100 mg
Immunoelectrophoresis	5–10 mg
Immunofixation	5–10 mg
Single radial diffusion	<1–2 mg
Double diffusion in agar (Ouchterlony)	<1 mg
Electroimmunodiffusion (rocket electrophoresis)	<0.5 mg
One-dimensional double electroimmunodiffusion (counterimmunoelectrophoresis)	<0.1 mg
Nephelometry	0.1 mg
Complement fixation	1 µg
Agglutination	1 µg
Enzyme immunoassay (ELISA)	<1 µg
Quantitative immunofluorescence	<1 pg
Radioimmunoassay	<1 pg

Alton, 1975), as a result of excess IgG₁ blocking IgG₂ (the latter being responsible for complement fixation) at low concentrations: this is the ‘prozone’ effect.

Finally, a serological test may be too **insensitive** to detect antibody. Sensitivity in this context again refers to the ability of a test to detect amounts of antibody or antigen (i.e., analytical sensitivity). *Table 20.9* lists some common serological tests and their approximate analytical sensitivities. Some molecular techniques (e.g., the polymerase chain reaction) are extremely analytically sensitive (Hoffmann *et al.*, 2009) and therefore compare favourably with serological tests in detecting infection (Kitchin *et al.*, 1990).

Evaluation and interpretation of diagnostic tests

The second part of this chapter describes the procedures for evaluating diagnostic tests in general, and relates test characteristics to the performance of various testing strategies.

Sensitivity and specificity

Diagnostic sensitivity and **diagnostic specificity** were introduced in Chapter 10 as indicators of the **validity** of diagnostic tests; and, for brevity, were just termed sensitivity and specificity; this convention will be followed in the remainder of this chapter. Although the discussion is largely in the context of serological investigations, it is equally relevant to other types of diagnostic test and their application (e.g., in genetic screening), as well as to the evaluation of questionnaires. To reiterate: the **sensitivity** of a diagnostic method is the proportion of true-positives that are detected by the method; the **specificity** of the method is the proportion of true-negatives that are detected².

Continuous and ordinal test variables

Sensitivity and specificity can be calculated for tests in which the variable that is measured is nominal and dichotomous, for example presence or absence of tapeworms (see *Table 10.4*). However, in many tests, the test variables are continuous (e.g., α -mannosidase levels: see Chapter 27), or are measured on the ordinal scale (e.g., single serial dilution antibody titres). Although methods for evaluating such tests are available (e.g., estimating the mean and standard deviation of the difference between the test measurement and a valid reference measurement: Bland and Altman, 1986; Krouwer, 2008; see also ‘Agreement between tests: Continuous data: the Bland–Altman plot’ later in this chapter), it is common practice to dichotomize measurements into ‘positive’ or ‘negative’. This requires definition of a **cut-off point**.

When a cut-off point is identified, there is then clearly an inverse relationship between sensitivity and specificity in a particular test. This is illustrated in *Figure 20.1* in which the upper graph represents the frequency distribution of a variable in a healthy population and the lower graph represents its frequency distribution in a diseased population. Individuals for whom the variable’s value is to the right of the cut-off point, *C*, are classified as test-positive; individuals for whom the variable’s value is to the left of the cut-off point are classified as test-negative. Animals with values to the right of the cut-off point in the upper graph are false-positives; animals with values to the left of the cut-off point in the lower graph are false-negatives. If the area under each curve represents 100%, then the marked area to the right of the cut-off point corresponds to the test’s sensitivity, whereas the marked area to the left of the cut-off point corresponds

² Recently, sensitivity also has been applied to the effectiveness of surveillance systems (see Chapter 21).

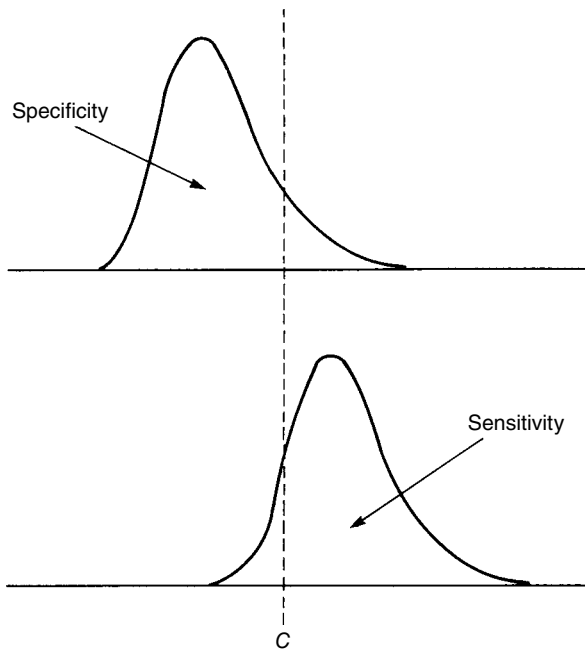


Fig. 20.1 The relationship between sensitivity and specificity for continuous test variables (see text for explanation). Upper graph shows the frequency distribution of a variable in a healthy population; lower graph shows the frequency distribution of the variable in a diseased population. *C*, cut-off point defining the boundary between healthy and diseased individuals.

to the test's specificity. If fewer false-positives are required, *C* is moved to the right: specificity increases and sensitivity decreases. However, if fewer false-negatives are required, *C* is moved to the left: sensitivity increases and specificity decreases.

Table 20.10 also illustrates the inverse relationship between sensitivity and specificity in the ELISA for antibody to *Br. abortus*. As the cut-off point (positive threshold) increases, sensitivity decreases and specificity increases.

Defining the cut-off point Cut-off points have been determined in several ways. They have been arbitrarily defined as two (e.g., Coker-Vann *et al.*, 1984) or three (e.g., Gottstein, 1984) standard deviations greater than the mean of the test values of the unaffected individuals. Alternatively, the value that minimizes the total number (Cummings and Richard, 1988) or total cost (Anderson, 1958) of misdiagnoses can be selected. The optimum cut-off point also depends on the frequency distribution of the test variable in the healthy and diseased populations, which may be complicated. This topic is discussed in detail by Weinstein and Fineberg (1980), Vizard and colleagues (1990), Pencina and others (2008, with commentaries), Felder

and Mayrhofer (2011) and López-Ratón and others (2014). Although these sophisticated methods are required for optimum selection of the ideal cut-off point, some relatively simple techniques can be used as guides to selection. Two of these – calculation of **likelihood ratios**, and construction of **ROC curves** – are introduced later in this chapter.

When a large cross-section of the population is initially tested to detect disease (e.g., in screening: see Chapter 2), interpretation of the test is generally directed towards increased sensitivity at the expense of specificity. This is because initial tests are not usually intended to provide definitive diagnoses, but are designed to detect as many cases as possible³. Therefore, a high proportion of false-positives (resulting from increased sensitivity) is not as critical as a high proportion of false-negatives (resulting from increased specificity), and the cut-off point is shifted accordingly.

Ascertaining true status

Calculation of sensitivity and specificity requires an independent, valid criterion – also termed a '**gold standard**' – by which to define an animal's true disease status (see later for estimation in the absence of a gold standard). Thus, when evaluating the centrifugation/flotation technique for diagnosing equine cestodiasis (see Table 10.4), post-mortem examination of the intestinal tract was the gold standard. Similarly, in the evaluation of the ELISA for brucellosis, bacterial culture was used to define true-positives, whereas true-negative animals were selected from herds known to be *Brucella*-free (Table 20.10). A battery of alternative tests also may be used as a gold standard, animals being defined as true-positives if they are simultaneously positive to several tests, and categorized as true-negatives if they are simultaneously negative to the tests (Mateu-de-Antonio *et al.*, 1993). Sometimes, several standards may be used. For example, the sensitivity of the caudal-fold tuberculin test has been estimated as being between 80.4% and 84.4%, using one or more of four standards: positive on bacteriological culture alone; positive on histological examination alone;

³ The theory and principles of screening were set out by the *World Health Organization* 50 years ago (Wilson and Junger, 1968). Essentially, screening sorts individuals into two groups: those at low risk of having or developing a disease, and those at high risk. The low-risk group will always contain some individuals with the condition, and the high-risk group will contain some without it. Evaluating a screening programme involves assessing how well it performs these sorting procedures (Snowdon and Stewart-Brown, 1997). Screening programmes therefore cannot be promoted fully until they have been evaluated and proved to be effective. This also involves assessing potential consequences – negative, as well as positive (Petticrew *et al.*, 2000).

Table 20.10 Effect of various positive thresholds on the serodiagnostic interpretation of the enzyme immunoassay (ELISA) for the detection of bovine antibody to *Brucella abortus*. (From Agriculture Canada, 1984.)

Positive ^a threshold	Sensitivity ^b	Specificity ^c		Prevalence ^e of disease (%)	Predictive value		
					+ve test		–ve test
		Non-vac	Vac ^d		Non-vac	Vac ^c	Either
≥0.220	0.960	0.990	0.852	10	0.92	0.42	1.00
				1	0.50	0.06	1.00
				0.1	0.09	0.01	1.00
≥0.260	0.943	0.995	0.930	10	0.95	0.60	0.99
				1	0.64	0.12	1.00
				0.1	0.15	0.01	1.00
≥0.300	0.937	0.998	0.948	10	0.98	0.67	0.99
				1	0.84	0.15	1.00
				0.1	0.34	0.02	1.00
≥0.340	0.920	0.999	0.969	10	0.99	0.77	0.99
				1	0.90	0.23	1.00
				0.1	0.48	0.03	1.00

^a Minimum reactivity (expressed as Absorbance⁴¹⁴) considered to be antibody positive.

^b Sensitivity based on 175 sera from *Brucella* culture-positive cattle.

^c Specificity (non-vaccinates, 'non-vac') based on 1128 sera from *Brucella*-free herds; specificity (vaccinates, 'vac') based on 1079 sera from field vaccinated cattle.

^d *Br. abortus* strain 19 vaccination status.

^e Theoretical disease prevalence.

simultaneously positive to bacterial culture and histological examination; and positive to either bacteriological culture or histological examination (Whipple *et al.*, 1995).

Gold standards may themselves be imperfect⁴, and this can distort the apparent performance of the test that is being evaluated (Limmathurotsakul *et al.*, 2012). A reduction in the sensitivity of a gold standard test causes a decrease in the apparent specificity of the test that is being appraised. Similarly, a decrease in the gold standard's specificity is associated with a decrease in the apparent sensitivity of the test that is being assessed. A further complication may arise if the results of both the test and gold standard depend on the subjective interpretation of individuals (Omutag and Fenton, 2012). This can result in disagreement not only between two or more assessors of a test result, but also between the same assessor who judges the same test result on different occasions (see 'Agreement

between tests' later in this chapter). The issue of imperfect tests is discussed further later in this chapter ('Validating diagnostic tests when there is no gold standard').

The diseased and healthy animals to which the gold standard is applied should be representative of the population in which the test is to be applied⁵. Thus, a screening test is conducted in the general population, and so the gold standard should be applied to a sample of diseased and healthy animals drawn from this population⁶.

In contrast, a clinical diagnostic test is run on animals for which there is usually already evidence of disease, and the test needs to distinguish between animals with the relevant condition (the 'diseased' animals) and those animals with diseases that are similar to the condition (these therefore constitute the 'healthy' animals). It follows that the sensitivity and specificity

⁴ True gold standards, which provide full certainty of health status, rarely exist. Some authorities (e.g., Knottnerus *et al.*, 2009) therefore recommend the term 'reference standard' to describe a test that approximates the gold standard as closely as possible. This should not be confused with reference values that are used to establish normal serological reference ranges, as discussed earlier.

⁵ Unrepresentative individuals can result in overestimation of the performance of diagnostic tests (Lijmer *et al.*, 1999).

⁶ If a gold standard is only applied to a sub-sample of individuals who were initially tested (e.g., those with equivocal test results) then this can result in **verification bias** (Begg and Greenes, 1983). Thus, if the presence of disease is not established in individuals who are diseased, but who had a negative test result, then the apparent sensitivity of the test will be inflated (Ransohoff and Feinstein, 1978).

of a test may be different when it is applied as a screening test than when it is used as a diagnostic test in a veterinary clinic.

Martin and Bonnett (1987) describe the derivation of sensitivity and specificity in a clinical setting.

Confidence intervals for sensitivity and specificity

Confidence intervals for sensitivity and specificity can be calculated using Wilson's method for interval estimation of a proportion (see Chapter 12). This involves calculating three values, A , B and C , where, for a 95% interval:

$$A = 2r + 1.96^2;$$

$$B = 1.96\sqrt{1.96^2 + 4r(1-P)};$$

$$C = 2(n + 1.96^2);$$

where:

r = number of individuals with the feature of interest;

n = number in the sample;

P = observed proportion.

The confidence interval is then $(A - B)/C$, $(A + B)/C$.

For example, if 130 known negative animals are tested, and 128 are test negative (d in Table 20.11), the point estimate of specificity = $d/b + d = 128/130 = 98.5\%$, and the 95% confidence interval is derived thus:

$$A = 2 \times 128 + 1.96^2$$

$$= 256 + 3.84$$

$$= 259.84;$$

$$B = 1.96\sqrt{1.96^2 + 4 \times 128 \times (1 - 0.985)}$$

$$= 1.96\sqrt{3.84 + (512 \times 0.015)}$$

$$= 1.96\sqrt{11.52}$$

$$= 6.65;$$

$$C = 2(130 + 1.96^2)$$

$$= 267.68.$$

Table 20.11 Possible results of a diagnostic test.

Test status	True status		Totals
	Diseased	Not diseased	
Diseased	a	b	$a + b$
Not diseased	c	d	$c + d$
Totals	$a + c$	$b + d$	$a + b + c + d$

Thus

$$(A - B)/C = (259.84 - 6.65)/267.68$$

$$= 0.946,$$

$$\text{and } (A + B)/C = (259.84 + 6.65)/267.68$$

$$= 0.996.$$

That is, the 95% confidence interval = 94.6%, 99.6%.

Appendix VII also may be used if the sample size is small.

If other confidence intervals are required, then 1.96 is replaced by the appropriate multiplier (see Appendix VI).

Another approach to estimation of the precision of sensitivity and specificity involves modelling the most likely mean, minimum and maximum values from a series of point estimates from several studies. A common method uses the beta-pert distribution⁷. An example, relating to diagnostic tests for bovine brucellosis, is given by Jones and others (2004a). Alternatively, the two extreme percentiles and the median may be modelled (Johnson, 1997). Estimates also may be based on several test results from the same individuals (Genders *et al.*, 2012). Estimation by modelling is reviewed by Branscum and others (2005).

Youden's index

Youden's index, J (Youden, 1950), combines sensitivity and specificity in a single value of test performance: J = sensitivity + specificity - 1; or, using the notation in Table 20.11:

$$J = \frac{a}{a + c} + \frac{d}{b + d} - 1.$$

It can take values between 1 (when sensitivity and specificity each equal 100%) and -1 (when sensitivity and specificity each equal 0%: an unlikely situation).

An approximate 95% confidence interval is given by:

$$J - 1.96\sqrt{\frac{ac}{(a + c)^3} + \frac{bd}{(b + d)^3}},$$

$$J + 1.96\sqrt{\frac{ac}{(a + c)^3} + \frac{bd}{(b + d)^3}}.$$

The index can be used to define a cut-off point for continuous and ordinal test variables by selecting the cut-off point that maximizes the value of J (Greiner *et al.*, 2000; Fluss *et al.*, 2005). However, this

⁷ The beta distribution is a basic distribution of statistics for variables bounded at both sides (Snell, 1987). 'Pert' (Pleguezuelo *et al.*, 2003) is an acronym for Project Evaluation and Review Techniques (originally developed in the context of the *Polaris* missile system), which are stochastic modelling techniques for estimating probability distributions (see Chapter 23).

assumes that sensitivity and specificity are of equal importance, which, as has already been noted, is not usually the case. Its use in judging the utility of a test at different stages of a disease-control programme therefore is limited.

Diagnostic odds ratio

The **diagnostic odds ratio** of a test, *DOR*, is another single indicator of test performance (Kraemer, 1992). It is the ratio of the odds (see Chapter 15) of positivity in diseased individuals relative to the odds of positivity in healthy individuals. Using the notation in Table 20.11:

$$\begin{aligned} DOR &= (a/c)/(b/d) \\ &= ad/bc. \end{aligned}$$

Alternatively, it can be read as the ratio of the odds of disease in test-positives relative to the odds of disease in test-negatives:

$$\begin{aligned} DOR &= (a/b)/(c/d) \\ &= ad/bc. \end{aligned}$$

In terms of sensitivity and specificity:

$$DOR = \frac{\text{sensitivity}/(1 - \text{sensitivity})}{(1 - \text{specificity})/\text{specificity}}.$$

The value of a *DOR* ranges from zero to infinity, with higher values indicating better discriminatory test performance. A value of one means that a test does not discriminate between diseased and healthy individuals. A value less than one points to improper test interpretation (i.e., there are more negative tests among diseased, than healthy, individuals).

Confidence intervals for the *DOR* can be calculated, noting that this parameter is lognormally distributed. An approximate 95% confidence interval is given by:

$$\log_e DOR \pm 1.96, \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}},$$

and then taking the natural antilogarithm of this expression.

Glas and colleagues (2003) discuss the relative merits of Youden's index and the *DOR*, and the latter's relationship to other diagnostic-test parameters. In common with Youden's index, the *DOR* is independent of prevalence and is a useful single measure of test performance when sensitivity and specificity are deemed to be of equal importance.

Predictive value

When using either serological or other screening tests to determine the presence of disease in a population, it

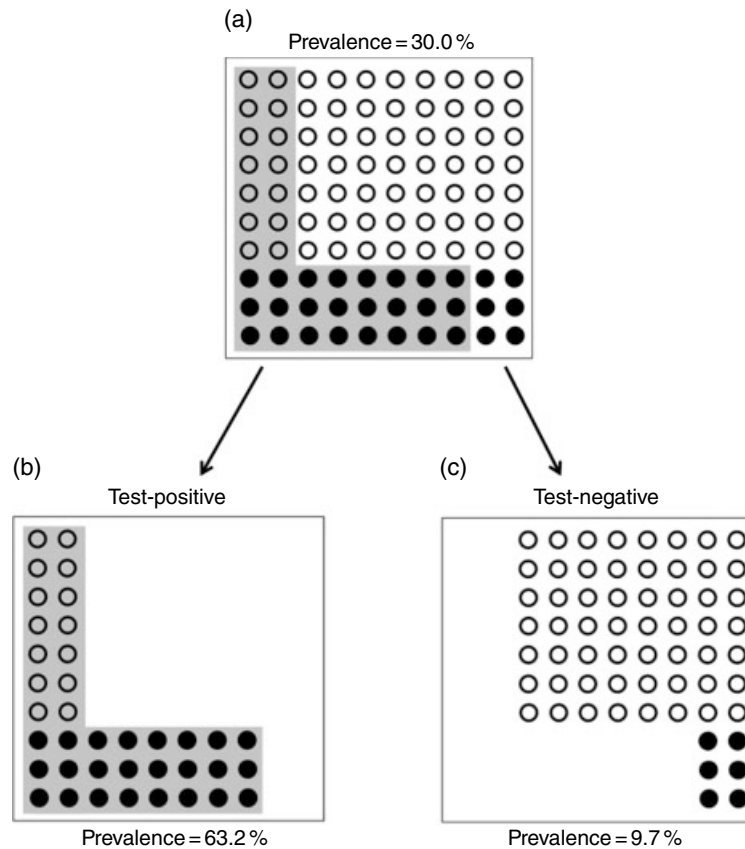
is important to know the probability that an animal, 'positive' according to the test, is actually positive; alternatively that a test-negative animal is a true-negative. These probabilities are the **predictive values** of the test. The parameter most often quoted as the predictive value of a test is the predictive value of a positive (as opposed to negative) test result, but both are important when interpreting diagnostic test results.

One way to interpret the role of diagnostic tests applied to a population is that they enable it to be divided into two sub-populations: a test-positive population and a test-negative population. A perfect test would assign all diseased animals to the test-positive population and all the healthy animals to the test-negative population; that is, the prevalence of disease would be 100% in the sub-population testing positive and 0% in the sub-population testing negative. However, when a diagnostic test is imperfect, this categorization also is imperfect and the predictive values then provide information on the prevalence of disease in the test-positive and test-negative sub-populations. In Figure 20.2a the overall true prevalence is 30%; the sensitivity of the diagnostic test is 24/30 = 80%; and the specificity is 56/70 = 80%. The population can be divided according to the results of the diagnostic tests (Figure 20.2b and c). Compared with the prevalence in the total population, the sub-population that tested positive has a higher prevalence: 24/38 = 63.2% (Figure 20.2b). In contrast, the sub-population that tested negative has a lower prevalence: 6/62 = 9.7%, and the percentage of test-negative animals that are healthy is therefore 100% - 9.7% = 90.3% (Figure 18.2c). In this example, therefore, the predictive value of a positive test result is 63.2% and the predictive value of a negative result is 90.3%.

The predictive value depends on specificity and sensitivity and prevalence. Sensitivity and specificity are innate characteristics of a test for a given reference population, and (for a defined cut-off point) are relatively stable⁸, but the prevalence of a disease in a population being tested will affect the proportion of test-positive animals, P^T , that are actually diseased.

⁸ Variations in sensitivity and specificity, however, can occur (Leefang *et al.*, 2013). For example, they may be related to differences in severity of lesions, and host characteristics such as body condition (Sergeant *et al.*, 2003). The underlying continuous traits on which tests are usually based have frequency distributions that vary between populations, and so the distribution of these traits, relative to the cut-off point, also varies. Since the distribution also determines prevalence, and misclassification of individuals is more likely when they have values close to the cut-off point, sensitivity and specificity can vary with prevalence (Brenner and Gefeller, 1997). The variation in sensitivity and specificity with the distribution of the traits is **spectrum bias** (Ransohoff and Feinstein, 1978).

Fig. 20.2 Schematic representation of the effect of using diagnostic test results to divide individuals within a population into test-positive and test-negative sub-populations. Solid circles indicate truly diseased individuals; empty circles indicates truly healthy individuals. The grey-shaded background identifies individuals that test positive; the white background indicates individuals that test negative. See text for further explanation. (Adapted from Loong, T.-W. (2003))



There are two components to P^T :

1. the true-positives;
2. the false-positives.

The proportion of animals, P^T , is then:

$$\{P \times \text{sensitivity}\} + \{(1-P) \times (1 - \text{specificity})\},$$

where P equals true prevalence.

For example, if $P = 0.01$ (1%), sensitivity = 0.99 (99%) and specificity = 0.99 (99%), then:

$$P^T = \{0.01 \times 0.99\} + \{(1 - 0.01) \times (1 - 0.99)\} = 0.02.$$

This represents an overestimation of 100% (the true prevalence is 0.01 and the estimated prevalence is 0.02). The smaller the prevalence, the larger the proportional overestimation; that is, the lower the predictive value (positive test result).

The predictive value (positive test result) is given by:

$$\frac{P \times \text{sensitivity}}{(P \times \text{sensitivity}) + \{(1-P) \times (1 - \text{specificity})\}},$$

and the predictive value (negative test result) is given by:

$$\frac{(1-P) \times \text{specificity}}{\{(1-P) \times \text{specificity}\} + \{P \times (1 - \text{sensitivity})\}}$$

(Galen, 1982). (If these parameters are quoted as percentages, one is replaced by 100 in the formulae.)

Alternatively, the calculation can be expressed more simply in terms of the values in *Table 20.11*:

- sensitivity = $a/(a + c)$;
- specificity = $d/(b + d)$;
- the predictive value (positive test result) = $a/(a + b)$;
- the predictive value (negative test result) = $d/(c + d)$.

Predictive values are proportions, and so confidence intervals can be calculated in similar fashion to those for sensitivity and specificity.

Five screening tests (or modifications of them) are available for brucellosis testing: the tube agglutination test (TAT), the CFT, the Brewer card test, the ELISA and the milk ring test. Sufficient data are available to estimate the sensitivity and specificity of the first four (MAF, 1977; Agriculture Canada, 1984). These are summarized in *Table 20.12*.

Assume that the TAT will be applied to 100 000 cattle in three different areas in which the prevalence of brucellosis is 3%, 0.1% and 0.01% respectively. From these data, and the figures in *Table 20.12*, the sensitivity, specificity and predictive value (of a positive test result) of the test can be calculated for the populations in each of the three areas.

Table 20.12 Sensitivity and specificity of four screening tests for bovine brucellosis.

	Sensitivity (%)	Specificity (%)
Tube agglutination test	62.0	99.5
Complement fixation test	97.5	99.0
Brewer card test	95.2	98.5
ELISA*	96.0	99.0

* Positive threshold ≥ 0.220 ; specificity in non-vaccinated cattle (see Table 20.10).

The results are given in Table 20.13a, b and c, respectively. As the prevalence of disease declines, so does the predictive value of the test, which could result in an increasing proportion of healthy animals being destroyed in a test and slaughter programme. This is also demonstrated in Table 20.10.

At low prevalence levels, even relatively 'good' tests (sensitivity = 99%; specificity = 99%) have a low predictive value (Figure 20.3). If a test with a sensitivity of 0.990 (99%) and a specificity of 0.999 (99.9%) were used in a disease-eradication campaign, then, if the prevalence were 0.1 (10%), a single test conducted on 10 million animals would record 990 000 true-positives and 9000 false-positives. The test therefore would be acceptable at the beginning of the campaign. However, as the campaign proceeded, the prevalence would fall. When the prevalence was reduced to 0.0001 (0.01%), the test would record 9900 true-positives and 9990 false-positives⁹. *The number of false-positives would be unchanged after the disease was eradicated.* Therefore acceptable levels of sensitivity and specificity depend on the stage of a control or eradication campaign. Ideally, towards the end of an eradication campaign, a more sensitive and specific test is required if the campaign depends only upon a single serological test. In practice, other techniques are used, such as serial testing (see later in this chapter), isolation of infected farms and maintenance of disease-free areas.

In some tests there are *no false-positives*, for example, when identifying blood parasites by microscopic examination of blood films. Estimation of true prevalence in this circumstance is discussed by Waltner-Toews and others (1986).

⁹ The situation where the prevalence of a condition in a population is lower than the false-positive rate results in false-positive tests being more probable than true-positive tests (as in Table 20.13b and c). Thus, when the test indicates that a condition exists, it is more likely not to exist. This is termed the **false-positive paradox**.

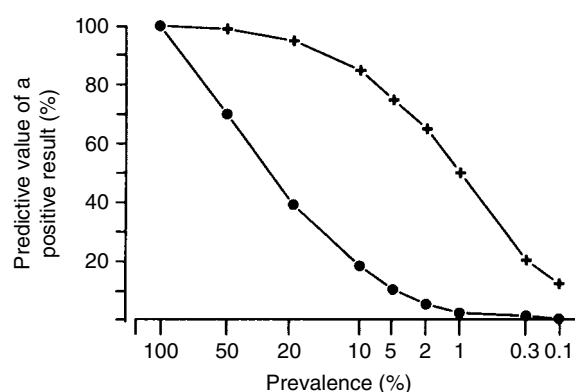


Fig. 20.3 The relationship between prevalence and predictive value of a positive test result. +: Sensitivity = 99%, specificity = 99%; •: sensitivity = 70%, specificity = 70%.

Further discussion of the predictive value of serological and other diagnostic tests is provided by Vecchio (1966), Galen and Gambino (1975) and Rogan and Gladen (1978).

Likelihood ratios

The dependence of predictive values on prevalence is a major disadvantage when a summary measure of a test's performance, when the test is applied in a population, is required. The **likelihood ratio** provides a suitable summary measure, which is independent of prevalence (Deeks and Altman, 2004). It compares the proportion of animals with and without disease, in relation to their test results.

The **likelihood ratio of a positive test result** ($LR+$) is the ratio of the proportion of affected individuals that test positive, and the proportion of healthy individuals that test positive. Using the notation and data of Table 20.13a:

$$\begin{aligned}
 LR+ &= \{a/(a+c)\} / \{b/(b+d)\} \\
 &= (1860/3000) / (485/97\,000) \\
 &= 0.620\,00 / 0.005\,00 \\
 &= 124.
 \end{aligned}$$

Thus, a positive result is 124 times as likely to come from an animal with brucellosis, as from an animal without the disease. The $LR+$ is therefore a quantitative indication of the *strength* of a positive result. The perfect diagnostic test would have an $LR+$ equal to infinity (detecting all true-positives, and generating no false-positives), and the best test for ruling in a disease is therefore the one with the highest $LR+$.

Table 20.13 Predictive value (positive test result) of the tube agglutination test for bovine brucellosis at three different prevalence levels (sensitivity = 62%; specificity = 99.5%).

Test status	True status		Totals
	Brucellosis present	Brucellosis absent	
(a) Prevalence of brucellosis: 3%			
Brucellosis present	1 860 (a)	485 (b)	2 345
Brucellosis absent	1 140 (c)	96 515 (d)	97 655
Totals	3 000	97 000	100 000
Predictive value (positive result) = $a/(a + b) = 79.3\%$			
(b) Prevalence of brucellosis: 0.1%			
Brucellosis present	62	500	562
Brucellosis absent	38	99 400	99 438
Totals	100	99 900	100 000
Predictive value (positive result) = 11.0%			
(c) Prevalence of brucellosis: 0.01%			
Brucellosis present	6	500	506
Brucellosis absent	4	99 490	99 494
Totals	10	99 990	100 000
Predictive value (positive result) = 1.2%			

Note that the above formula comprises $a/(a + c)$, that is, the sensitivity (the true-positive rate); and $b/(b + d)$, that is, $1 - \text{specificity}$ (the false-positive rate). It may therefore be expressed, alternatively, as:

$$LR+ = \text{sensitivity} / (1 - \text{specificity}).$$

The **likelihood ratio of a negative test result** ($LR-$) is the ratio of the proportion of affected individuals that test negative, and healthy individuals that test negative; that is:

$$\begin{aligned} LR- &= \{c/(a + c)\} / \{d/(b + d)\} \\ &= (1140/3000) / (96\,515/97\,000) \\ &= 0.380\,00 / 0.995\,00 \\ &= 0.382. \end{aligned}$$

Thus, a negative result is approximately only 0.4 times as likely to come from an animal with brucellosis, as from an animal without the disease. The perfect

diagnostic test would have an $LR-$ equal to zero (producing no false-negatives, but detecting all true-negatives), and the best test for ruling out a disease is therefore the one with the lowest $LR-$.

Note, again, that the above formula comprises $c/(a + c)$, that is, $1 - \text{sensitivity}$ (the false-negative rate) and $d/(b + d)$, that is, the specificity (the true-negative rate). It may therefore be expressed, alternatively, as:

$$LR- = (1 - \text{sensitivity}) / \text{specificity}.$$

The above formulae for the $LR+$ and $LR-$ indicate that these parameters are a function only of sensitivity and specificity. It is for this reason that they are relatively stable.

Note that algebraically the ratio of $LR+$ to $LR-$ equals the diagnostic odds ratio, DOR . Thus, using the data in Table 20.13a:

$$\begin{aligned} DOR &= LR+ / LR- \\ &= 124 / 0.382 \\ &= 324; \end{aligned}$$

also:

$$\begin{aligned} DOR &= (a/c) / (b/d) \\ DOR &= (1860/1140) / (485/96\,515) \\ &= 324. \end{aligned}$$

The likelihood ratio is the ratio of two proportions, and approximate confidence intervals can be calculated via a logarithmic transformation using the formulae for computing approximate confidence intervals for the relative risk, based on cumulative incidence (see Chapter 15). The calculation is not possible with zero values in either cells a or b . Altman and his colleagues (2000) recommend the addition of 0.5 to each of the four cells to facilitate the calculation in this circumstance. Nam (1995) and Bolboacă and Jäntschi (2005) describe more complex methods, which are reputed to perform better than the previous method. Simel and others (1991) discuss sample-size estimation.

Relationship between likelihood ratios and odds

Further characteristics of the likelihood ratio can be explored when the parameter is considered in the context of odds. Four new terms may now be introduced, exemplified using data in Table 20.13a.

First, the **pre-test probability** (Bayes' 'prior probability': see Chapter 3) of disease is the proportion of animals in a population that have a disease before a test is applied; this is simply the prevalence of disease (0.03).

Secondly, the **post-test probability** (Bayes' 'posterior probability') of disease is the proportion of test-positive animals that are diseased (i.e., the prevalence of disease in the sub-population of disease-positive animals. This has been introduced earlier as the predictive value of a positive test result (0.793)).

Thirdly, the **pre-test odds of disease** is the ratio of the pre-test probability of disease and the pre-test probability of not being diseased:

$$\begin{aligned} & (3000/100\,000)/(97\,000/100\,000) \\ &= 0.0300/0.9700 \\ &= 0.030\,93. \end{aligned}$$

Finally, the **post-test odds of disease** is the ratio of the post-test probability of disease and the post-test probability of not being diseased:

$$\begin{aligned} & (1860/2345)/(485/2345) \\ &= 0.7932/0.2068 \\ &= 3.8356. \end{aligned}$$

The $LR+$ is also the ratio of the post-test odds of disease to the pre-test odds of disease; that is, $3.8356/0.030\,93 = 124$.

The pre-test, and post-test, odds of disease are therefore related through the $LR+$:

$$\text{pre-test odds of disease} \times LR+ = \text{post-test odds of disease}.$$

Probability, however, is a more familiar quantity than odds, and so may be considered a more 'user-friendly' value with which to work. The two quantities are related thus:

$$\frac{\text{pre-test probability}}{1 - (\text{pre-test probability})} = \text{pre-test odds},$$

and:

$$\frac{\text{post-test odds}}{(\text{post-test odds}) + 1} = \text{post-test probability}.$$

For example, using the data in *Table 20.13a*:

$$\text{pre-test probability (i.e., prevalence)} = 0.03, LR+ = 124.$$

Therefore:

$$\text{pre-test odds} = 0.03/(1 - 0.03) = 0.030\,93,$$

$$\text{post-test odds} = 0.030\,93 \times 124 = 3.84,$$

$$\text{and post-test probability} = 3.84/(3.84 + 1) = 0.793$$

(i.e., the positive predictive value).

These calculations can be obviated by using a nomogram (*Figure 20.4*)¹⁰. For example, if the pre-test probability is 0.20 (20%), and the likelihood ratio associated with a positive test result, $LR+$, is 5, a ruler is placed through the 0.20 point on the left-hand vertical line and through the value 5 on the middle line. A post-test probability of 0.57 (57%) is then identified on the right-hand line.

An even simpler method, which avoids these calculations and a nomogram, uses the estimates given in *Table 20.14*. A finding with a LR of 2.0 increases the post-test probability of disease by about 0.15, and a finding with a LR of 0.3 decreases the probability of disease by about 0.25. Therefore, applying this table to the nomogram example immediately above, a $LR+$ of 5 increases the post-test probability of disease by approximately 0.30, giving a post-test probability of $0.20 + 0.30 = 0.50$ (50%). This value is just 7% lower than the value derived from the nomogram (57%). The estimates in *Table 20.14* are generally accurate to within 10% of the calculated values for all pre-test probabilities between 0.10 and 0.90 (10% and 90%). The values in the table are also easy to recall simply by remembering three specific LR values – 2, 5 and 10 – and the first three multiples of 15 (15, 30 and 45). A LR of 2 increases probability by 0.15 (15%); a value of 5 increases it by 0.30 (30%); and a value of 10 increases it by 0.45 (45%). For LR estimates between 0 and 1, the numbers 2, 5 and 10 are inverted (i.e., $1/2 = 0.5$; $1/5 = 0.2$; $1/10 = 0.1$): just as a LR of 2 increases probability of disease by 0.15%, its inverse, 0.5, decreases probability by 0.15%. These 'benchmark' values are relatively easy to remember in clinical practice.

Likelihood ratios in clinical practice

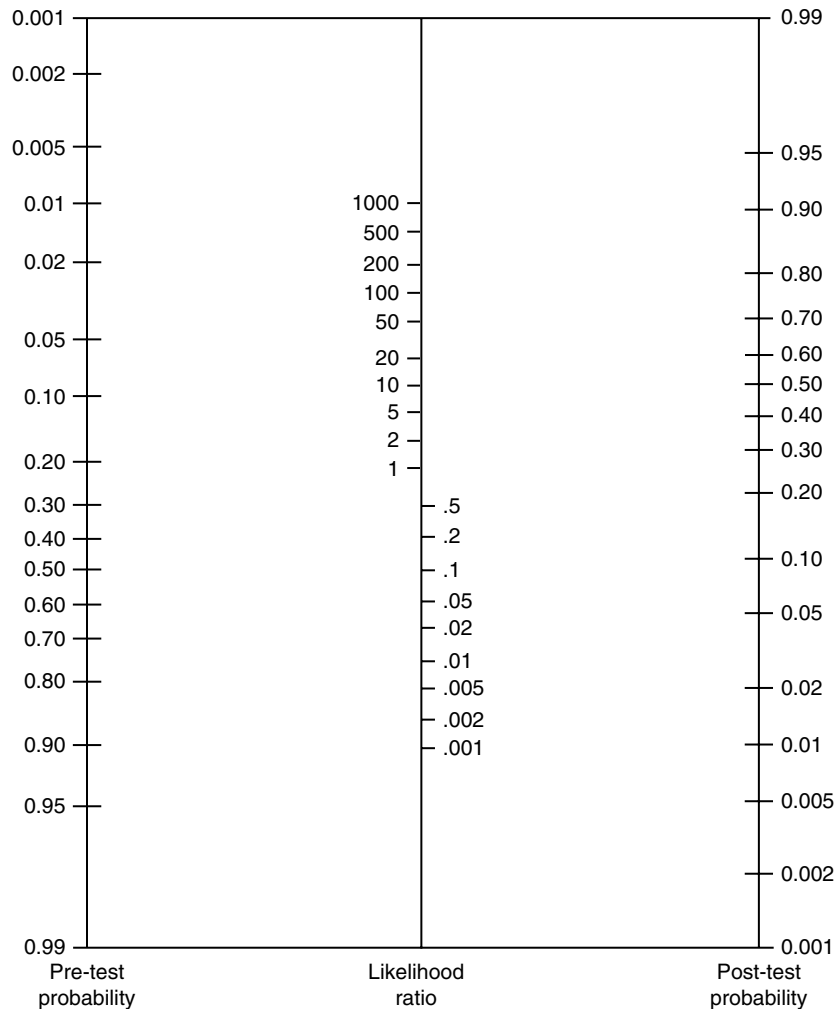
A major advantage of likelihood ratios over sensitivity and specificity is that they can be computed for different *ranges* of values of continuous or ordinal test variables. Additionally, in contrast to sensitivity and specificity, they can be used to compare quickly different diagnostic strategies. These advantages are of particular benefit in assisting diagnosis in the individual patient in the veterinary clinic¹¹.

Table 20.15 lists likelihood ratios of a positive test result for hip dysplasia in dogs. The diagnostic test involves radiographing dogs' hips and recording a dorsolateral subluxation score (DLS), based on the

¹⁰ A nomogram is a graphical representation of the relationship between more than two quantities.

¹¹ Variables used in the clinical environment may involve composite measurement scales (see Chapter 10), including, for example, aggregates of clinical signs and history (Cockcroft, 2002).

Fig. 20.4 Nomogram depicting the relationship between pre-test probability of disease, likelihood ratios and post-test probability of disease. (Adapted from Fagan, 1975.)



percentage of overlap between the femoral head and the acetabulum. A *low* score is more likely in affected dogs. The presence of dysplasia is confirmed at post-mortem examination.

The likelihood ratios are computed for two cut-off values (<45 and ≤ 55). Thus, for the ≤ 55 cut-off, true-positive rate = $(14 + 6)/24 = 0.8333$; false-positive rate = $(6 + 8)/82 = 0.1707$; $LR+ = 0.8333/0.1707 = 4.9$. They are also computed for the three 'bands' of DLS scores. Thus, for the 45–55 band, true-positive rate = $6/24 = 0.2500$; false-positive rate = $8/82 = 0.0976$; $LR+ = 0.2500/0.0976 = 2.6$. The use of a single cut-off value of ≤ 55 ($LR+ = 4.9$) does not describe the performance of the test over the full spectrum of results, which clearly varies (<45 : $LR+ = 8.0$; 45–55: $LR+ = 2.6$). Similarly, selection of <45 as the cut-off point (with affected animals with values ≥ 45 therefore being classified as healthy) disregards the strength of the positive result in the 45–55 band ($LR+ = 2.6$). It is therefore desirable to estimate likelihood ratios for various band intervals, rather than just for

cut-off points (Gardner and Greiner, 2006). Additionally, confidence intervals should always be quoted; this is particularly relevant when there is a limited number of test results in each band.

The values of likelihood ratios, over the full range of test results, can assist the clinician in deciding on a diagnosis. First, an indication of the pre-test probability of disease is required. This is quite different from the value obtained when a diagnostic test is applied to screen the general population, where most animals are assumed to be healthy (exemplified in *Table 20.13*), and where this probability is simply the prevalence of disease in the population. Animals presented at a veterinary clinic are a highly restricted sector of the population with clinical histories and signs that have driven the owners to present them for examination. The pre-test probability, in this circumstance, is the probability that they have a given condition, and is usually based on the judgement of the clinician. If the latter considers that the animal is just as likely to

have the condition as not to be affected, then the pre-test probability is 0.5. Thus, if hip dysplasia is the putative condition, and the animal has a DLS between 45 and 55, then the $LR+$ is 2.6 and the post-test probability, read from the nomogram, is approximately 0.75. (Approximating 2.6 to 3, *Table 20.14* gives an increase in probability of approximately 0.20; that is, an approximate post-test probability of $0.50 + 0.20 = 0.70$: close to the value judged from the nomogram.) In contrast, if the animal's DLS score is <45 , the $LR+$ is 8.0 and the post-test probability increases to approximately 0.90. (*Table 20.14* gives an increase in probability of roughly 0.40; that is, an approximate post-test probability of $0.50 + 0.40 = 0.90$: the same as that

obtained from the nomogram.) The exact values can be calculated using the formula, above, for converting probability to odds, and vice versa. Thus, knowledge of the likelihood ratios for the full range of intervals for ordinal or continuous test variables, coupled with measurement of the variable's value in a specific patient, is a powerful aid in narrowing down the diagnostic options in that patient.

Likelihood ratios also may be used to identify which clinical signs are the most useful indicators of disease. For example, the diagnosis of ovine hypocalcaemia is complicated by other diseases that are also associated with ataxia and/or recumbency (e.g., pregnancy toxæmia, hypomagnesaemia, mastitis, listeriosis and scrapie). *Table 20.16* lists several relevant clinical signs and their likelihood ratios ($LR+$ and $LR-$) associated with hypocalcaemia in recumbent/ataxic ewes. The most compelling argument for hypocalcaemia is the presence of constipation (with the highest $LR+$ of 6.90), increasing the absolute probability of disease by over 0.35 (*Table 20.14*). In contrast, the most compelling argument against hypocalcaemia is the absence of ruminal stasis (with the lowest $LR-$ of 0.10), which decreases the likelihood of disease by approximately 0.45 (*Table 20.14*). This demonstrates that clinical signs are not diagnostically equivalent, and that an informed diagnosis may depend on both their presence and their absence. (Note that, in this example, the likelihood ratio estimates are probably imprecise because they were calculated from a sample of only 13 ataxic/recumbent hypocalcaemic animals and 10 ataxic/recumbent animals without hypocalcaemia, and confidence intervals were not quoted. However, this does not undermine the underlying principles.)

Additionally, likelihood ratios provide the scientific framework for the interpretation of evidence in human forensic medicine (Nordgaard and Rasmusson, 2012) and have potential for a similar role in veterinary forensic medicine (Andersson *et al.*, 2013).

Table 20.14 The relationship between the likelihood ratio and approximate change in probability of disease. (From McGee, 2002.)

Likelihood ratio	Approximate absolute change in probability
Values between zero and one decrease the probability of disease	
0.1	-0.45
0.2	-0.30
0.3	-0.25
0.4	-0.20
0.5	-0.15
1	0
Values greater than one increase the probability of disease	
2	+0.15
3	+0.20
4	+0.25
5	+0.30
6	+0.35
7	
8	+0.40
9	
10	+0.45

Table 20.15 Likelihood ratios of a positive test result ($LR+$) for hip dysplasia in dogs, using a dorsolateral subluxation score (DLS). (Data from Lust *et al.*, 2001.)

DLS	Hip dysplasia present	Hip dysplasia absent	$LR+$ for different cut-offs		$LR+$ for different ranges	
			DLS cut-off	$LR+$	DLS range	$LR+$
<45	14	6	<45	8.0	<45	8.0
45–55	6	8	≤ 55	4.9	45–55	2.6
>55	4	68			>55	0.2
Totals	24	82				

Table 20.16 Likelihood ratios when a clinical sign is present ($LR+$) or absent ($LR-$) in 13 hypocalcaemic ewes with ataxia and/or recumbency. (Modified from Cockcroft and Whiteley, 1999.)

Clinical sign	Likelihood ratio: sign present ($LR+$)	Likelihood ratio: sign absent ($LR-$)
Constipation	6.90	0.34
Ruminal stasis	4.60	0.10
Salivation	3.85	0.29
Hyposensitive	3.07	0.11
Loss of anal reflex	2.70	0.58
Ruminal tympany	2.70	0.58
Recumbent but able to stand	1.55	0.86
Recumbent but unable to stand	1.53	0.77
Tremors	1.20	0.98
Tachypnoea (>40/min)	1.15	0.78
Tachycardia (>100/min)	1.10	0.77
Regurgitation	0.80	1.02
Ataxia	0.46	1.54
Dry muzzle	0.40	1.15
Pyrexia	0.40	1.15

ROC curves

The $LR+$ for various cut-off values for continuous or ordinal test variables can be presented graphically by drawing a **receiver-operating characteristic (ROC) curve** (Figure 20.5)¹². This depicts the relationship between pairs of true-positive rates (sensitivity), on the vertical axis, and false-positive rates ($1 - \text{specificity}$), on the horizontal axis, for a range of cut-off values. The perfect test, which discriminates perfectly between diseased and healthy animals, would generate a 'curve' that coincided with the left side and top of the plot. A nugatory test, in contrast, would produce a straight-line plot, from bottom left to top right. Thus, tests with the ROC curves furthest into the top left-hand corner are the better tests. The 'best' cut-off for a given test – in terms of minimizing the proportions of animals that are misclassified (i.e., producing the largest value of $\{\text{sensitivity} + \text{specificity}\}/2$) – is the point on the ROC closest to the top left-hand

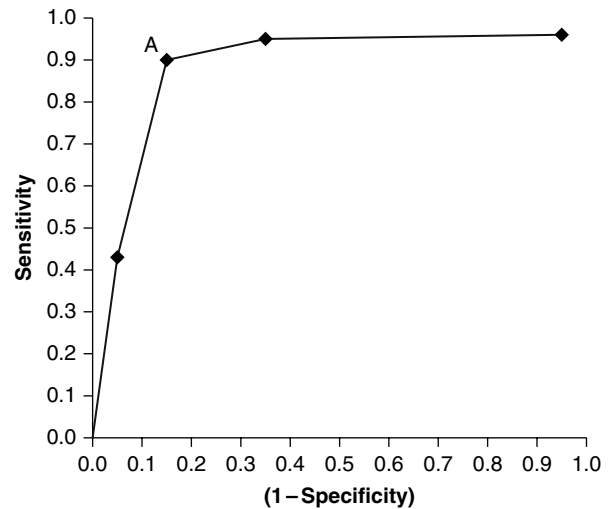


Fig. 20.5 A ROC curve of an enzyme-linked immunosorbent assay for *Neospora caninum*. (Data from Table 20.18.)

corner. Moreover, when prevalence is 50%, this results in the minimum number of animals that are misclassified.

The ROC curve depicted in Figure 20.5 is plotted from the results of an ELISA for *Neospora caninum*, a major infectious cause of bovine abortion in many countries (Table 20.17). The ELISA values are expressed as '% positivity' (PP)¹³, and five cut-off values have been tabulated. Thus, classifying animals with a PP >20% as affected:

$$\text{sensitivity} = (4 + 5 + 98)/114 = 0.9386,$$

$$\text{specificity} = (170 + 18 + 2)/199 = 0.9548;$$

and so on, for the other cut-off values.

Likelihood ratios can now be computed for each cut-off. Thus at the >20% PP point:

$$\begin{aligned} LR+ &= \text{sensitivity}/(1 - \text{specificity}) \\ &= 0.9386/(1 - 0.9548) \\ &= 21, \end{aligned}$$

and:

$$\begin{aligned} LR- &= (1 - \text{sensitivity})/\text{specificity} \\ &= (1 - 0.9386)/0.9548 \\ &= 0.06; \end{aligned}$$

and so on, for other cut-off values.

¹² ROC curves were developed in the 1950s to assess the detection of radar signals, and became established in medicine in the early 1980s (Hanley and McNeill, 1982).

¹³ In common with other ELISA tests, the test for *Neospora caninum* generates an ELISA value by comparison with positive reference serum; this can lead to PP values in excess of 100%.

Table 20.17 Estimates of sensitivity and specificity, and likelihood ratios, for different bands of percentage positivity values for an ELISA for *Neospora caninum* infection in dairy cattle. (Study reported in Davison *et al.* (1999); raw data supplied by Helen Davison, Veterinary Laboratories Agency, New Haw, UK.)

% positivity	Known positives	Known negatives	Cut-off	Sensitivity	Specificity	LR+	LR–
0–10	0	170	–	–	–	–	–
>10–15	3	18	>10	1.000	0.8543	6.9	0.00
>15–20	4	2	>15	0.9737	0.9447	18	0.03
>20–25	4	3	>20	0.9386	0.9548	21	0.06
>25–30	5	0	>25	0.9035	0.9648	30	0.10
>30–107	98	6	>30	0.8596	0.9648	29.9	0.15
Totals	114	199					

LR+ = likelihood ratio of a positive test result.

LR– = likelihood ratio of a negative test result.

Table 20.18 Calculation of the area under the ROC curve. (Data from Table 20.17.)

% positivity	Number of times known-positive values > known-negative values	Total scores: number known-positive values > known negative values (A)	Number of ties	Total scores: ties (number of ties × 0.5) (B)	Total score (A + B)
0–10	0 × 170	–	0	–	–
>10–15	3 × 170	510	3 × 18	27	537
>15–20	(4 × 170) + (4 × 18)	752	4 × 2	4	756
>20–25	(4 × 170) + (4 × 18) + (4 × 2)	760	4 × 3	6	766
>25–30	(5 × 170) + (5 × 18) + (5 × 2) + (5 × 3)	965	0	–	965
>30–107	(98 × 170) + (98 × 18) + (98 × 2) + (98 × 3)	18 914	98 × 6	294	19 208
Totals	21 901			331	22 232

The curve is close to the top left-hand corner, suggesting that the test is a good one. Moreover, the PP cut-off point that maximizes sensitivity and specificity is >15 ($\{sensitivity + specificity\}/2 = 0.9592$ – the largest value obtainable from Table 20.17).

Altman and others (2000) describe computation of confidence intervals for the ROC curve, and Pepe (2003) discusses construction of ROC curves for ordinal test results.

Area under the curve

The **area under the curve (AUC)** – also termed **diagnostic accuracy** – is a global assessment of a test's performance. This area equals the probability that a random individual with disease has a higher value of the test variable than a random healthy individual (if the variable is elevated in sick individuals). A perfect test thus yields an AUC of one, whereas an uninformative test gives a value of 0.5.

Calculation of the AUC is related to the Mann–Whitney statistic (see Chapter 14), and is based on every comparison of individuals in the known-positive and known-negative groups. A score is attached to each comparison pair: the value, one, is awarded when the known-positive value is greater than the known-negative value; 0.5 is scored when the two values are equal ('ties'); and zero is allotted when the known-positive value is less than the known-negative value¹⁴. The scores are then summed, and divided by the number of comparisons.

This calculation is exemplified in Table 20.18, using the data in Table 20.17. Thus, in the >10–15 PP class, three known-positive animals have values greater than 170 known-negative animals (those with PP values

¹⁴ Since the method uses scores, test results need not necessarily be accurate, as long as they can be ranked. Moreover, it is not restrained by distributional assumptions.

0–10), and so there are 3×170 such comparisons, each with a score of one, resulting in a total score value of 510. Additionally, in this class, there are three known-positive animals and 18 known-negatives, constituting 3×18 ties, each with a score value of 0.5, that is, a sum of $(3 \times 18) \times 0.5 = 27$. Thus, the total score attributed to this class is $510 + 27 = 537$, and so on for the other classes. (Score values of zero need not be tabulated because they do not contribute to the score total.) The final score total is therefore 22 332, and there are 22 686 comparisons (114 known-positive animals multiplied by 199 known-negative animals). The AUC is thus $22\,332/22\,686 = 0.984$. This value is high (the perfect test having a value of one), again providing evidence that the ELISA test is a good one.

Confidence intervals can be calculated for the AUC (Altman *et al.*, 2000), and Hanley and McNeil (1982) discuss sample-size calculation.

Comparing ROC curves

Two or more tests can be compared visually by graphing their ROC plots together (Zweig and Campbell, 1993). The relative positions of the plots indicate qualitatively the relative accuracies of the tests. A plot lying above and to the left of another plot indicates greater observed accuracy.

More rigorous quantitative comparison requires application of formal statistical procedures (Zweig and Campbell, 1993). Hanley and McNeil (1982) describe a method for comparing curves when tests are independent; that is, when each test has been applied to different subjects. A different procedure is required if the tests are applied to the same subjects (Hanley and McNeil, 1983; DeLong *et al.*, 1988; Hanley and Hajian-Tilaki, 1997; Li *et al.*, 2008; Zou *et al.*, 2008).

Greiner and others (2000), Lasco and others (2005), Zou and others (2007) and Kryzanowski and Hand (2009) present a fuller discussion of ROC curves. McGough and Faraone (2009) discuss generalization of ROC curves to assess the clinical relevance of treatment effects.

Aggregate-level testing

Tests also may be applied to **aggregates of animals** (e.g., pens and herds) with the object of classifying the aggregate, rather than its individual members, as either diseased or healthy. If there is one biological sample (e.g., a bulk milk sample or a pooled faeces sample) from each aggregate, the formulae for sensitivity, specificity and predictive value, described earlier, may be applied. Assessment of the aggregate's status is also uncomplicated if the true status of test-

positive animals can be ascertained quickly using a gold standard. However, if only a *sample* of animals is tested in each aggregate, sensitivity and specificity at the aggregate level are affected not only by the sensitivity and specificity at the individual level but also by the sample size. The overall effect is that, for a defined sensitivity and specificity at the individual level, aggregate-level sensitivity increases and aggregate-level specificity decreases as the sample size increases.

Aggregate sensitivity, Se_{agg} (the proportion of affected herds that test positive), depends on individual-animal sensitivity, specificity and prevalence, and the number of animals sampled:

$$Se_{agg} = 1 - (1 - P^T)^n,$$

where:

P^T = test prevalence;

n = number of animals sampled.

For example, if the true prevalence, P , is 0.25 in each of several herds, sensitivity = 0.97, specificity = 0.98, and four animals are selected from each herd, then:

$$\begin{aligned} P^T &= \{P \times \text{sensitivity}\} + \{(1 - P) \times (1 - \text{specificity})\} \\ &= \{0.25 \times 0.97\} + \{(1 - 0.25) \times (1 - 0.98)\} \\ &= 0.2575. \end{aligned}$$

Thus:

$$\begin{aligned} Se_{agg} &= 1 - (1 - 0.2575)^4 \\ &= 1 - 0.7425^4 \\ &= 1 - 0.3039 \\ &= 0.70. \end{aligned}$$

That is, 70% of all affected herds have one or more test-positive animals in the sample of four animals that are tested in each herd; alternatively, 30% of affected herds will not be detected.

Aggregate specificity, Sp_{agg} (the proportion of unaffected herds that test negative), depends on individual-animal specificity and the number of animals sampled:

$$Sp_{agg} = (\text{specificity})^n.$$

For example, if a test of specificity 0.98 is applied to samples of four animals from several herds, then:

$$\begin{aligned} Sp_{agg} &= 0.98^4 \\ &= 0.92. \end{aligned}$$

Therefore, 92% of all unaffected herds will have no test-positive animals in the samples of four animals

that are tested in each herd; alternatively, 8% of disease-free herds will be scored as affected.

Note that the formula for Se_{agg} and Sp_{agg} above, should only be applied when the sampling fraction in each aggregate is less than approximately 5%.

Martin (1988), Jordan and McEwen (1998) and Faes and others (2011) discuss this topic in detail.

Multiple testing

Multiple testing involves the use of more than one test, and is commonly encountered in clinical diagnosis and herd testing. Two main approaches can be adopted (Fletcher *et al.*, 2014): **parallel testing** and **serial testing**.

Parallel testing

Parallel testing involves conducting two or more tests on animals *at the same time*, and animals are considered to be affected if they are positive to *any* of the tests. For example, during brucellosis eradication in the UK, cows that aborted were tested routinely for brucellosis by means of bacterial culture from a vaginal swab, the Rose Bengal test on serum and the milk ring test on milk, and animals were defined as affected if they were positive to any test. Parallel testing is often undertaken when animals are admitted to clinics, and an assessment is required quickly. In comparison with each individual test, parallel testing increases sensitivity and therefore the predictive value of a negative test result, but reduces specificity and positive predictive value. A disease is therefore less likely to be missed; however, false-positive diagnoses are more likely. Parallel testing effectively asks the animal to 'prove' that it is healthy.

The sensitivity of two tests, A and B, applied in parallel, is calculated thus:

$$1 - [(1 - Se_A) \times (1 - Se_B)],$$

where:

Se_A = sensitivity of test A;
and Se_B = sensitivity of test B.

The parallel specificity is derived thus:

$$Sp_A \times Sp_B,$$

where:

Sp_A = specificity of test A;
and Sp_B = specificity of test B.

The derivation of parallel values may be better understood by a numerical example. Consider two

Table 20.19 The effect of parallel and serial testing on sensitivity, specificity and predictive value for two tests (A and B). (Adapted from Fletcher *et al.*, 1988.)

Test	Sensitivity (%)	Specificity (%)	Predictive value (positive result)(%)*	Predictive value (negative result)(%)*
A	80	60	33	92
B	90	90	69	97
A and B (parallel)	98	54	35	99
A and B (serial)	72	96	82	93

* For 20% prevalence.

tests, A and B (Table 20.19), applied to a population in which disease prevalence is 0.20 (20%).

Test A detects 80%, leaving 20% undetected (i.e., sensitivity = 0.80).

Test B detects 90% of the remaining 20% = 18% (i.e., sensitivity = 0.90).

Therefore, *parallel* sensitivity (proportion positive to *either* test)

$$\begin{aligned} &= 80\% + 18\% \\ &= 98\%. \end{aligned}$$

Applying the appropriate formula:

$$\begin{aligned} \text{parallel sensitivity} &= 1 - [(1 - Se_A) \times (1 - Se_B)] \\ &= 1 - [(1 - 0.80) \times (1 - 0.90)] \\ &= 1 - (0.20 \times 0.10) \\ &= 0.98 (98\%). \end{aligned}$$

Test A categorizes 60% of true-negatives as negative (i.e., specificity = 0.60).

Test B categorizes only 90% of these as negative (i.e., specificity = 0.90).

Therefore, *parallel* specificity (proportion negative to *both* tests)

$$\begin{aligned} &= 90\% \times 60\% \\ &= 54\%. \end{aligned}$$

Applying the appropriate formula:

$$\begin{aligned} \text{parallel specificity} &= Sp_A \times Sp_B \\ &= 0.90 \times 0.60 \\ &= 0.54 (54\%). \end{aligned}$$

Table 20.20 Relationships (test status and true status) under parallel testing, derived from *Table 20.19*.

	True + (%)	True – (%)	Totals (%)
Test + (either) (%)	19.6	36.8	56.4
Test – (both) (%)	0.4	43.2	43.6
Totals (%)	20	80	100

These parallel values can now be used to complete a contingency table, and compute predictive values (*Table 20.20*).

$$\begin{aligned}\text{Positive predictive value} &= 19.6/56.4 \\ &= 35\%.\end{aligned}$$

$$\begin{aligned}\text{Negative predictive value} &= 43.2/43.6 \\ &= 99\%.\end{aligned}$$

Serial testing

In serial testing, tests are conducted *sequentially* (i.e., consecutively), based on the results of a previous test. Conventionally, only those animals that are *positive* to an initial test are tested again; therefore only animals that are positive to *all* tests are considered to be affected. Thus, *Brachyspira hyodysenteriae* infection (see Chapter 5) is initially diagnosed by a fluorescent antibody test performed on faecal or gut mucosal smears; the status of positive animals is then confirmed by bacterial culture. Serial testing maximizes specificity and the predictive value of a positive test result, but lowers sensitivity and negative predictive value. More credence therefore can be attached to positive test results, but there is an increased risk that disease will be missed. Serial testing effectively asks the animal to 'prove' that it is affected by the condition that is being investigated. The test with the highest specificity should be used first to decrease the number of animals that are tested again.

The sensitivity and specificity of two tests, A and B, applied in series, are calculated thus:

$$\begin{aligned}\text{Sensitivity } Se_A \times Se_B, \\ \text{Specificity } 1 - [(1 - Sp_A) \times (1 - Sp_B)],\end{aligned}$$

using the same notation as above.

Employing the values in *Table 20.19*:

Test A detects 80%.

Test B defines 90% of these as positive.

Therefore, *serial* sensitivity (proportion positive to *both* tests)

Table 20.21 Relationships (test status and true status) under serial testing, derived from *Table 20.19*.

	True + (%)	True – (%)	Totals (%)
Test + (both) (%)	14.4	3.2	17.6
Test – (either) (%)	5.6	76.8	82.4
Totals (%)	30	80	100

$$\begin{aligned}&= 80\% \times 90\% \\ &= 72\%.\end{aligned}$$

that is, $Se_A \times Se_B = 0.80 \times 0.90 = 0.72$ (72%).

Test A correctly categorizes 60% of true-negatives, leaving 40%.

Test B categorizes 90% of these as negative = $90\% \times 40\% = 36\%$.

Therefore, *serial* specificity (proportion negative to *either* test)

$$\begin{aligned}&= 60\% + 36\% \\ &= 96\%.\end{aligned}$$

that is, $1 - [(1 - Sp_A) \times (1 - Sp_B)] = 1 - [(1 - 0.60) \times ((1 - 0.90))] = 1 - 0.40 \times 0.10 = 0.96$ (96%).

These serial values can again be used to complete a contingency table, and predictive values computed (*Table 20.21*).

$$\begin{aligned}\text{Positive predictive value} &= 14.4/17.6 \\ &= 82\%.\end{aligned}$$

$$\begin{aligned}\text{Negative predictive value} &= 76.8/82.4 \\ &= 93\%.\end{aligned}$$

Serial testing is an important part of disease-eradication campaigns in which positive animals are culled from herds (see Chapter 27). Animals defined as diseased by an initial screening test¹⁵ are subjected to further tests to confirm their status, so that false-positives are not unnecessarily removed.

The formulae for calculating parallel and serial sensitivity and specificity assume that the tests are independent (i.e., uncorrelated); that is, they measure different manifestations of disease (e.g., antibodies or presence of microbes identified microscopically). If tests are not independent (e.g., two variations of an antibody-detection test), then the dependence (correlation) between their sensitivities and specificities affects their sensitivity and specificity when used in combination. Positive dependence in test sensitivity

¹⁵ Ideally, the first test should detect all cases.

Table 20.22 Characteristics of multiple test strategies. (Modified from Smith, 2006.)

Consideration	Test strategy		
	Parallel	Serial	Negative-herd retesting
Effect of strategy	Increase sensitivity	Increase specificity	Increase sensitivity at aggregate level
Greatest predictive value	Negative test result	Positive test result	Negative test result at aggregate level
Purpose	Rule out a disease	Rule in a disease	Rule out a disease
Application and setting	Rapid assessment of individual patients; emergencies	Diagnosis when time is not crucial; test and removal programmes	Test and removal programmes
Comments	Useful when there is an important penalty for missing a disease (i.e., false-negative results)	Useful when there is an important penalty for false-positive results	Useful when there is an important penalty for missing a disease (i.e., false-negative results)

reduces the sensitivity of parallel test interpretation, and positive dependence in test specificity reduces specificity under serial interpretation. Gardner and colleagues (2000) address this issue and its consequences in detail. Kraemer (1992) argues that two tests for the same disease are frequently not independent and that empirical evaluation is required by conducting both tests on samples.

Negative-herd retesting

Testing can also be conducted only on animals that are negative to an initial test. This is usually applied at *herd* level, and involves periodically retesting animals in previously test-negative herds with the *same* test.

Negative-herd retesting is an important component of eradication campaigns. It improves aggregate-level sensitivity; that is, it increases the likelihood of detecting on a premises an infectious agent that eluded detection earlier (e.g., because antibodies had not yet been produced, or the infection was subsequently reintroduced). It therefore asks a herd to 'prove' that it is free from the condition that is being investigated. For instance, in tuberculosis eradication in the UK, using the comparative intradermal test, reactors are traditionally culled, and inconclusive reactors retested after 42–60 days, with usually up to two retests allowed. If visible lesions of tuberculosis are found at post-mortem examination, or laboratory testing of pooled lymph nodes gives a positive result, the whole herd is subjected to two 'short-interval' tests 60 days apart. If post-mortem examination fails to find lesions, and pooled lymph nodes yield negative results, only one 60-day test is required. If the short-interval tests are clear, movement restrictions are lifted, and a further test is carried out in six months; if still clear, a further

check is conducted in another 12 months' time. If the latter whole-herd test is clear, the herd is returned to a less stringent four-year testing cycle, or to a routine testing interval for the area¹⁶.

The important characteristics of multiple test strategies are listed in *Table 20.22*. Multiple testing is discussed in more detail by Smith (2006).

Diagnostic tests in import risk assessment

Knowledge of a test's sensitivity and specificity enables predictions to be made about the risk of importing infected animals or products with various importation rules. The probability of missing an infected individual using a given diagnostic test is $(1 - \text{sensitivity})$; this probability would therefore be 0.05 if a test with a sensitivity of 0.95 (95%) were being applied. Recall, however, that the predictive value of a diagnostic test also depends on disease prevalence. The probability of an animal that is negative to a test being actually infected, p_n , is:

$$\frac{P(1 - Se)}{P(1 - Se) + (1 - P) \times Sp},$$

where P = the true prevalence, Se = sensitivity, and Sp = specificity (Marchevsky *et al.*, 1989).

If animals were being quarantined, exclusion of false-positive animals is of little concern, and so specificity can be assumed to be one. *Table 20.23* lists the values of p_n for various values of P when a test with a

¹⁶ In areas where tuberculosis is a major problem, an alternative approach, seeking a balance between sensitivity and specificity according to likely prevalence within a herd, is justified (van Dijk, 2013).

Table 20.23 The probability that a test-negative animal is actually infected, when sensitivity = 0.95 and specificity = 1. (From MacDiarmid, 1991.)

Prevalence	Probability (p_n)
0.01	5.05×10^{-4}
0.05	2.63×10^{-3}
0.10	5.52×10^{-3}
0.20	1.23×10^{-2}

sensitivity of 0.95 is used. The probability of any test-negative animal being infected increases when the prevalence in the source population increases.

Moreover, at a given prevalence, the probability of including even one test-negative infected animal in a group of imported animals, p_c , increases as the number of animals in the group increases (Marchevsky *et al.*, 1989):

$$p_c = 1 - \left\{ \frac{(1-P) \times Sp}{(1-P) \times Sp + P(1-Se)} \right\}^n,$$

where n is the group size.

If a policy dictates that a positive test result only disqualifies the *individual* animal that reacts positively, then the risks associated with such a policy are p_n (Table 20.23) and p_c (Table 20.24). Alternatively, it may be decided that a positive test result in any one animal will disqualify the entire group, in which circumstance the probability of disqualifying an infected group increases as the prevalence and/or group size increases. The probability of a test failing to detect at least one test-positive animal in an infected group, β , thus identifying the group as infected, can be calculated (MacDiarmid, 1987):

$$\beta = \{1 - (t \times Se)/n\}^{pn},$$

where t = the number of animals that are tested in the group, n = group size, and p = group prevalence.

Thus, the difference in risk between these two policies can be compared (Table 20.25). The risk of an infected animal being imported is considerably reduced when a single reactor disqualifies the entire group, rather than only the test-positive individual.

Jones and others (2004a) develop this approach for serial testing strategies in the context of the risk of importing brucellosis-infected cattle from Ireland into the UK, where surveillance for infection in the exporting countries involves an initial screening test using either the serum agglutination test (Northern Ireland) or the micro-agglutination test (Republic of Ireland), followed by confirmation of test-positives by the complement-fixation test.

Table 20.24 The probability that a test-negative, infected animal will be included in a group destined for import when only reactor animals are excluded (prevalence = 0.01, sensitivity = 0.95 and specificity = 1). (From MacDiarmid, 1991.)

Group size	Probability (p_c)
10	5.04×10^{-3}
20	1.00×10^{-2}
30	1.50×10^{-2}
50	2.49×10^{-2}
100	4.92×10^{-2}
500	2.23×10^{-1}

Table 20.25 The probability that a test-negative, infected animal will be included in a group destined for import (prevalence = 0.01, sensitivity = 0.95 and specificity = 1; entire group tested.) (Adapted from OIE Scientific and Technical Review, 12 (4), December 1993.)

Group size	Probability (if reactor animal only excluded) (p_c)	Probability (if a single reactor disqualifies group): probability of no test-positives (β)
100	4.92×10^{-2}	5.00×10^{-2}
200	9.61×10^{-2}	2.50×10^{-3}
300	1.41×10^{-1}	1.25×10^{-4}
400	1.83×10^{-1}	6.25×10^{-6}
500	2.23×10^{-1}	3.13×10^{-7}

Import risk assessment is addressed comprehensively by Murray (2002) and Murray and others (2004c, 2010b).

Guidelines for validating diagnostic tests

The components of a comprehensive protocol for validating a diagnostic test are summarized in Table 20.26, and also are discussed by Irwig and others (2009). Some authorities recommend that at least 300 true-positives and 1000 true-negatives are used to determine sensitivity and specificity, respectively (Jacobson, 2000)¹⁷. Such values will dictate the

¹⁷ If validation is conducted on a random sample of the target population, sample size depends not only on precision, anticipated sensitivity/specificity and level of confidence but also on disease prevalence in the population (Buderer, 1996): the required sample size to estimate sensitivity increases as prevalence decreases; whereas the required sample size to estimate specificity increases as prevalence increases.

Table 20.26 Guidelines for validation of diagnostic tests. (Adapted from Greiner and Gardner, 2000. Reproduced with permission from Elsevier.)**General**

The test purpose and the analytical unit are described
 The test protocol is sufficiently described

Reference test (gold standard)

The choice of the reference method is justified (a necessary condition is that it should be more accurate than the test that is being evaluated); and the method is fully described or referenced

Selection of reference populations

The reference population is sufficiently described (time, location and animal characteristics such as breed, age and gender)
 The reference population should reflect the target population (see Chapter 13) and include an appropriate spectrum of disease and spectrum of other conditions
 The sampling frame should be an unbiased representation of the reference population (see Chapter 13)
 Selection criteria must be stated and should reflect the testing situation

Sampling of the reference population

The sampling procedure is described in detail
 Exclusion or inclusion criteria (if any) are described (see Chapter 17)
 Sample sizes must be stated and should reflect the degree of the required statistical precision
 Random and systematic sampling are the preferred option (see Chapter 13)

Performance of test and reference test

The testing protocols are sufficiently described (including definition of negative and positive results)
 Results of test and reference test are evaluated independently (blinded) (see Chapter 17)

Presentation of results

Methods of estimating parameters are explained by formulae; estimates are presented together with sample sizes and confidence intervals (exact confidence intervals are preferred to approximate); sensitivity and specificity are always required, additional parameters may be presented as necessary; the 2×2 table used in generating estimates should be displayed
 ROC analysis should be presented for test outcomes measured on ordinal or continuous scales
 The number of intermediate results and results that cannot be interpreted (if any) and reasons for missing data are given

Discussion of results

The test performance parameters should be discussed in relation to the study design and the intended or current use of the test; if the gold standard is imperfect, this should be discussed in relation to the effect on the study results

precision with which the two parameters are estimated. The precision can be varied and predetermined by using the appropriate formula for estimation of sample size for a simple proportion (see Chapter 13: 'Estimation of disease prevalence: simple random sampling'). Reviews of sample-size determination for various diagnostic test parameters are presented by Arkin and Wachtel (1990), Obuchowski (1998) and Hajian-Tilaki (2014)¹⁸.

¹⁸ Carley and others (2005) reproduce a simple nomogram to calculate sample size in diagnostic studies. If any three of four parameters – prevalence, confidence interval, sensitivity/specificity and sample size – are known, the fourth can be estimated. Therefore, this also may be used to explore sample size retrospectively from published research.

Bossuyt and others (2003) and Bossuyt and Smidt (2009) provide guidelines for reporting studies of diagnostic test characteristics, and Gardner (2010) addresses methodological issues. Guidelines for conducting systematic reviews of studies evaluating diagnostic tests are given by Buntinx and others (2009).

Validating diagnostic tests when there is no gold standard

In many circumstances, results are available for a test under evaluation (the index test), but test data from a gold standard (or from a reference standard that best approximates the gold standard) are not available, either because the gold standard/reference standard was not applied to some individuals or because a true

gold standard does not exist. However, if a gold standard is not available, it may still be possible to assess an index test's validity (e.g., sensitivity and specificity). Rutjes and others (2007) describe four main approaches:

1. imputing or adjusting for missing data on the reference standard;
2. correcting for imperfections in the reference standard;
3. constructing a reference standard;
4. validating index test results.

Imputing or adjusting for missing data on the reference standard

This approach is relevant when a reference standard is available but is not applied to all individuals (e.g., for ethical or financial reasons: Kosinski *et al.*, 2009). Methods then either **impute** (i.e., infer) or **adjust** (i.e., correct) for the missing information in the subset of individuals from which it is absent. This is therefore part of the wider problem of missing values in epidemiological data (see Chapter 11). Values may be assumed to be missing at random, in which circumstance the frequency of missing values is similar to that in individuals for which reference-standard results are available. Alternatively, the missing values may result from a non-random process (e.g., if individuals to which tests are applied are subjectively chosen by clinicians); this poses a greater difficulty than if omission was random.

Single missing values may be selected (single imputation). Alternatively, a range of missing values may be selected and analysed (multiple imputation). Results are then combined to produce point and interval estimates that properly reflect the uncertainty stemming from the missing values (Little and Rubin, 2002).

In adjustment methods, it is assumed that estimates of test validity are likely to be biased only if individuals who received verification with the reference standard (i.e., for which there are complete results for both the index test and reference standard) are analysed. A mathematical correction of the estimates and their confidence intervals is then undertaken (Begg and Greenes, 1983; Zhou, 1998). The strengths and weaknesses of adjustment and imputation are detailed by Harrell and others (1996) and Harel and Zhou (2006).

Correcting for imperfections in the reference standard

In the absence of a gold standard, imperfections in a preferred reference standard will lead to bias ('reference standard bias': Whiting *et al.*, 2004). However, this can be **corrected** if the error rates are known

(e.g., from previous studies) by the application of appropriate formulae (Staquet *et al.*, 1981; Hadgu *et al.*, 2005). This assumes that all individuals have received the index test and reference standard (i.e., there are no missing data) and that both tests are independent. If this is not the case, different formulae are required (Brenner, 1996).

If the sensitivity and specificity of the reference test are unknown, it may be possible to assign a plausible range of values to them, and then to undertake a sensitivity analysis (see Chapter 23) to produce a range of estimates for the index test. Alternatively, the uncertainty relating to the sensitivity and specificity of the reference test can be described explicitly within the framework of Bayesian latent class analysis (see following). Latent class analysis also can be used to address another weakness associated with an imperfect reference standard: dependence between the index test and reference test.

Constructing a reference standard

This approach combines multiple sets of information to construct a reference standard. First, instead of ignoring, imputing or correcting for incomplete results, a **second** reference standard can be used to achieve complete verification. This tends to be applied selectively (i.e., non-randomly) and can lead to more favourable estimates of index-test validity than if a single reference test were applied (Lijmer *et al.*, 1999).

Secondly, a **combination** of reference tests can be used sequentially. All individuals are initially tested with an imperfect reference test and the index test, and those cases in which the two tests disagree (i.e., in which there are discrepant results) are subjected to an additional reference standard to resolve the discrepancy. Such discrepant analysis is dependent on the results of the index test, and does not provide information on individuals that are not retested (which often is the majority), and therefore can lead to major bias, with sensitivity and specificity being overestimated (Hadgu, 1996; Miller, 1998).

Thirdly, a **composite** reference standard can be created by combining the results of several imperfect tests, the composite standard being considered to perform better than each of its component tests (Alonzo and Pepe, 1999). Unlike discrepant analysis, the application of further reference standards is independent of the index test results. Criteria are required for defining the presence of disease, the commonest being that any positive component test registers disease. A weakness of this technique is the possibility that the different tests may be targeting subtly different conditions (Pepe, 2003).

Fourthly, disease status can be determined by the consensus of a **panel of experts** (commonly a Delphi conference: see Chapter 23). An uneven number of experts is recommended in the event of majority voting (Gagnon *et al.*, 2005)¹⁹. This approach is very flexible, and is likely to focus on clinically-relevant aspects of conditions (e.g., their differing degrees of severity). However, there may be poor agreement between experts (see 'Agreement between tests' following).

Validating index test results

The broad approaches described above focus on either correcting imperfect reference standards or developing improved reference standards in terms of traditional diagnostic test validity parameters such as sensitivity and specificity. Sole consideration of these parameters can be eschewed by focussing, instead, on the index test's ability to measure various key aspects of the condition at which it is targeted; that is, its **construct validity** (see Chapters 10 and 17). The condition can be defined in terms of future clinical events such as disease outcome (e.g., 'poor' vs 'favourable'), which may be assessed either by comparing the events in test-positive and test-negative individuals or by randomizing comparisons between individuals that are tested and those that are not tested (Bossuyt *et al.*, 2000; Lijmer and Bossuyt, 2009).

This approach addresses many of the wider purposes to which diagnostic tests may be put: notably, guiding treatment decisions, and monitoring treatment and outcome. The traditional validity paradigm, with its requirement for a gold standard (often based on pathological or biochemical findings), and its focus on unequivocal diagnosis of disease (Ledley and Lusted, 1959), although of considerable value, cannot address these wider aspects of disease and its consequences (Rutjes *et al.*, 2007).

Latent class analysis

Latent class analysis is a statistical modelling procedure that is used to group observed variables into distinct clusters, based on the assumption that there are one or more unobserved (i.e., underlying, **latent**) variables to explain the relationships among the observed variables. In the context of diagnostic tests, the observed variables are test results, usually dichotomized into a binary classification (positive and negative)²⁰. The latent variable is true disease status. The

observed data are usually presented in terms of a cross-tabulation of the observed variables. Thus, if three dichotomous diagnostic tests, each of whose sensitivity and specificity are not known, are applied to an individual, they provide eight (i.e., 2^3) possible outcomes of test result, ranging from all testing negative to all testing positive. Latent class analysis then can be used to estimate parameters relating to each of the three tests (e.g., sensitivity, specificity) and prevalence, using the pattern of the tests' outcomes, without knowledge of true disease status (Hui and Walter, 1980). Statistical approaches may be 'frequentist' (Vermunt and Magidson, 2004) or Bayesian (Joseph *et al.*, 1995).

Latent class analysis is used extensively in veterinary medicine. Examples include testing for canine neosporosis (Frössling *et al.*, 2003), foot-and-mouth disease (Bronsvoot *et al.*, 2006), brucellosis (Muma *et al.*, 2007), maedi-visna (Toft *et al.*, 2007) and canine echinococcosis (Hartnack *et al.*, 2013), and identification of lesions at meat inspection (Bonde *et al.*, 2010).

Latent class analysis requires specialized software. Suitable software includes *TAGS* (Pouillot *et al.*, 2002), *Latent GOLD* (Vermunt and Magidson, 2005) and packages within the *R* software environment (Haughton *et al.*, 2009). Bayesian latent class modelling can be performed using *WinBUGS* (Lunn *et al.*, 2000) and *OpenBUGS* (Thomas *et al.*, 2006).

The strengths and weaknesses of latent class analysis are outlined by Rutjes and others (2007).

Agreement between tests

Nominal data: the *kappa* statistic

A further approach to comparing tests is to assess the **agreement** between different tests, without assuming that one test is the best²¹. The logic of using this approach as a form of test evaluation, argued by some authorities, is that agreement between tests is evidence of validity, whereas disagreement suggests that the tests are untrustworthy (although it is possible that tests could agree by being consistently wrong). Alternatively, the specific goal of the comparison simply may be to measure the agreement between tests, rather than validation of the tests.

Table 20.27 presents the results of an examination of pigs' heads to identify atrophic rhinitis using two techniques: cross-sectional and longitudinal examination.

The observed proportion agreement between the two tests, $OP = (a + d)/n$, where $n = (a + b + c + d)$. Substituting the values in *Table 20.27*:

¹⁹ The consensus approach can improve on individual expert diagnoses, and performs at least as well as decisions based on majority or unanimous voting (Gabel *et al.*, 2010).

²⁰ It also is possible to incorporate continuous and ordinal results, and counts (Magidson and Vermunt, 2004; Grilli and Rampichini, 2012).

²¹ The proportion of all test results on which two or more different tests agree is sometimes termed **concordance** (Smith, 2006).

Table 20.27 Number of pigs' heads showing turbinate atrophy by cross-sectional and longitudinal examination of 248 heads. (Data derived from Visser *et al.*, 1988.)

Longitudinal examination	Cross-sectional examination	
	Atrophy present	Atrophy absent
Atrophy present	8 (a)	1 (b)
Atrophy absent	16 (c)	223 (d)

$$OP = (8 + 223)/248$$

$$= 0.932.$$

This comparison, however, does not consider the agreement between the two tests that could arise just by *chance*. A more rigorous comparison can be made by calculating a statistic, **kappa** (see Table 14.2), which takes account of chance agreement. First, the expected proportion of agreement by chance, *EP*, is calculated. This is simply the sum of the expected proportion of agreement for the positive and negative results.

Expected proportion agreement by chance (both positive):

$$EP+ = \{(a + b)/n\} \times \{(a + c)/n\}$$

$$= \{(8 + 1)/248\} \times \{(8 + 16)/248\}$$

$$= 0.0363 \times 0.0968$$

$$= 0.00351.$$

Expected proportion agreement by chance (both negative):

$$EP- = \{(c + d)/n\} \times \{(b + d)/n\}$$

$$= \{(16 + 223)/248\} \times \{(1 + 223)/248\}$$

$$= 0.964 \times 0.903$$

$$= 0.87049.$$

Thus:

$$EP = (EP+) + (EP-)$$

$$= 0.00351 + 0.87049$$

$$= 0.874.$$

Observed agreement beyond chance:

$$OA = OP - EP$$

$$= 0.932 \times 0.874$$

$$= 0.058.$$

Maximum possible agreement beyond chance:

$$MA = 1 - EP$$

$$= 1 - 0.874$$

$$= 0.126.$$

Kappa is the ratio of the observed agreement beyond chance to the maximum possible agreement beyond chance, that is:

$$kappa = OA/MA$$

$$= 0.058/0.126$$

$$= 0.46.$$

Kappa ranges from one (complete agreement beyond chance) to zero (agreement is equal to that expected by chance), whereas negative values indicate agreement less than is expected by chance. Arbitrary 'benchmarks' for evaluating observed *kappa* values have been recommended. Fleiss and others (2003) suggest ≥ 0.75 indicates excellent agreement, whereas ≤ 0.40 indicates poor agreement. Everitt (1989) suggests > 0.81 : almost perfect agreement; $0.61-0.80$: substantial agreement; $0.41-0.60$: moderate agreement; $0.21-0.40$: fair agreement; $0-0.20$: slight agreement; 0 : poor agreement. Altman (1991a) suggests > 0.80 : very good agreement; $0.61-0.80$: good agreement; $0.41-0.60$: moderate agreement; $0.21-0.40$: fair agreement; and ≤ 0.2 : poor agreement. Thus, the point estimate of *kappa*, 0.46, suggests moderate agreement between the two methods of examination of pigs' heads.

The same approach can be used to assess clinical agreement; *kappa* values between 0.5 and 0.6 being expected when comparing the results of a diagnosis made on the same animals by different clinicians.

Confidence intervals can be calculated for the observed proportion of agreement (Samsa, 1996) and for *kappa* (Everitt, 1989), in which circumstance the lower confidence limit should lie above 0.40 before at least moderate agreement can be inferred (Basu and Basu, 1995). Sim and Wright (2005) discuss sample size for determination of *kappa*.

The *kappa* statistic also can be generalized to studies involving dichotomous nominal data with several ratings of subjects, to nominal data with several categories (Fleiss *et al.*, 2003), and to ordinal data (Cicchetti and Allison, 1971).

Kappa values need to be interpreted with caution. For instance, a low *kappa* value could indicate that only one test is good, or that both tests are bad, or that both tests are good but negatively correlated (which can occur with some antigen and antibody tests). Moreover, its value depends on the *prevalence* of the attribute of concern (the effect of prevalence on *kappa* is greater for large values of *kappa* than for small values) and the degree of *bias* (i.e., the extent to which raters disagree on the proportion of positive, or negative, cases, reflected in the difference between cells *b* and *c* in Table 20.27, and which exerts a greater effect

when $kappa$ is small than when it is large) (Feinstein and Cicchetti, 1990). Byrt and others (1993), Lantz and Nebenzahl (1996) and Sim and Wright (2005) discuss methods of adjusting for prevalence and bias.

Note that McNemar's change test (see Chapter 14) is not suitable for assessing agreement because it only tests the null hypothesis that the proportion of the individuals scored positive by one rater is the same as the proportion scored positive by the other, and only utilizes values from discordant pairs of observations.

Continuous data: the Bland–Altman plot

A widely-used graphical method for assessing agreement between two sets of continuous data has been developed by Bland and Altman (1986). It focusses on estimating by how much one test or measurement procedure differs from another.

Table 20.28 lists the weight of 11 horses, estimated by two different measurement methods: using a weigh tape to determine weight based on (1) abdominal and (2) thoracic measurement (Marante *et al.*, 2007). Examination of the rows in the table reveals that each weigh tape generally gives different readings in the same horses. A simple plot of the results of one method against the other would not readily assist in visualizing the actual differences in the two methods, but a plot of the differences between the methods against their mean, a **Bland–Altman plot**, is more informative (Figure 20.6). For example, for Horse 1, weigh method 1 versus weigh method 2, the difference is $419 - 402$ kg = 17 kg; and the mean is $(419 + 402)/2 = 410.5$ kg.

Table 20.28 Weight of 11 horses measured by two weigh tape methods. (Raw data supplied by Rachel Jago, Bryony Lancaster and Bruce McGorum, Royal (Dick) School of Veterinary Studies, University of Edinburgh.)

Horse	Abdominal weigh tape method (1) kg	Thoracic weigh tape method (2) kg	Difference (1 – 2) kg
1	419	402	17
2	341	511	–170
3	435	532	–97
4	585	593	–8
5	192	240	–48
6	329	329	0
7	272	307	–35
8	428	461	–33
9	577	624	–47
10	489	532	–43
11	441	489	–48

The figure shows considerable lack of agreement, with discrepancies of up to 170 kg (Horse 2 in Table 20.28). Additionally, the plot facilitates investigation of the relationship between the measurement error and the true value. Although the latter is not known, the mean of the two values provides the best estimate.

The lack of agreement can be summarized by calculating the **bias**, estimated by the mean of the differences between the two methods undertaken on each individual, $\bar{d}$, and the standard deviation (see Chapter 12) of these differences, s . If one method is sometimes higher, and the other method also is sometimes higher, the mean of the differences will be close to zero. If the mean of the differences is not close to zero, it indicates that the two methods are systematically producing different results.

For the data in Table 20.28, $\bar{d} = -46.5$ and $s = 50.8$. Most differences can be expected to fall between $\bar{d} - 2s$ and $\bar{d} + 2s$ (Figure 20.6). If the differences are Normally distributed²², 95% of the differences will lie within these limits (precisely, between $\bar{d} - 1.96s$ and $\bar{d} + 1.96s$; see Figure 12.2). These limits are termed the **limits of agreement**. That is, for any future sample, the difference between measurements using these two methods should lie within the limits of agreement 95% of the time. If differences within these limits are not biologically or clinically²³ important, the two measurement methods may be used interchangeably. If the differences within these limits are biologically or clinically important, then the methods may not be used interchangeably. In general, if the bias is small and the limits of agreement are narrow then the two methods are equivalent.

For the data in Table 20.28:

$$\bar{d} - 1.96s = -46.5 - (1.96 \times 50.8) = -146.1 \text{ kg}$$

$$\bar{d} + 1.96s = -46.5 + (1.96 \times 50.8) = 53.1 \text{ kg.}$$

Thus, the thoracic weigh tape method could estimate weight 146 kg above or 53 kg below the abdominal weigh tape method. These values represent major discrepancies between the two methods, suggesting that one method should not be used as an alternative to the other.

Confidence intervals can be calculated for the bias and the limits of agreement, using the formulae that follow. With respect to the bias:

$$\text{estimated standard error of } \bar{d} = \sqrt{s^2/n},$$

²² This can be simply checked by drawing a histogram. (The individual values do not need to be Normally distributed.)

²³ The relevance of error to clinical outcome can be judged by **error grid analysis** (Parkes *et al.*, 2000; Johnson *et al.*, 2009).

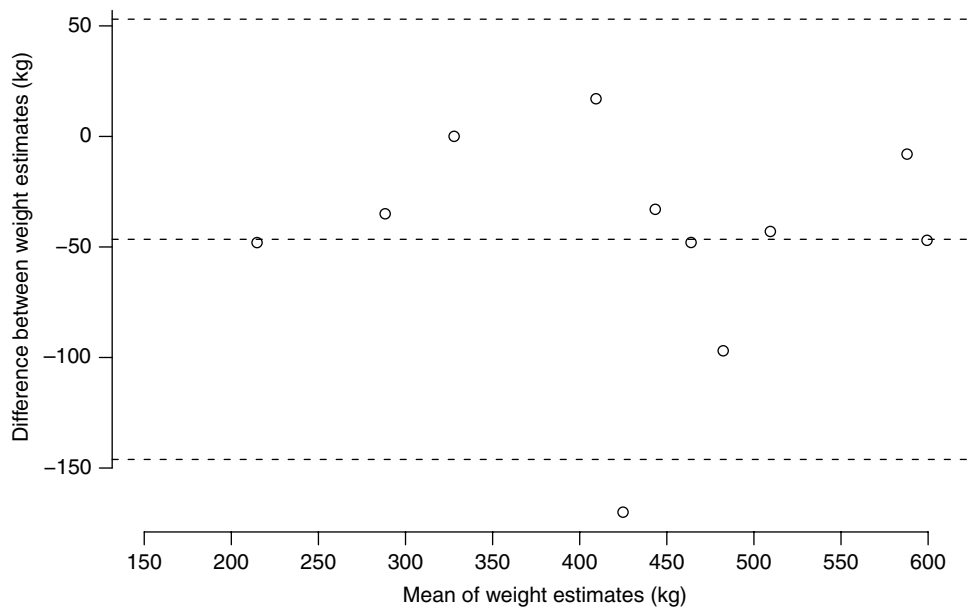


Fig 20.6 Bland–Altman plot of the weights of 11 horses, estimated by two weigh tape methods. (Data from Table 20.28.) Central horizontal dashed line: mean of the differences between the two estimates for each horse; lower horizontal dashed line: lower limit of agreement; upper horizontal dashed line: upper limit of agreement.

where n is the sample size.

Thus, for the data in Table 20.28:

$$\begin{aligned}\sqrt{(s^2/n)} &= \sqrt{(50.8^2/11)} \\ &= 15.3 \text{ kg.}\end{aligned}$$

To construct a 95% confidence interval, select the appropriate multiplier from the table of Student's t -distribution (Appendix V) from the column headed '0.05'. There are 10 degrees of freedom ($11 - 1$), and so the appropriate multiplier is 2.228.

Thus the 95% confidence interval for the bias is:

$$\begin{aligned}&-46.5 - (2.228 \times 15.3), -46.5 + (2.228 \times 15.3) \\ &= -80.6, -12.4 \text{ kg.}\end{aligned}$$

With respect to the limits of agreement, the standard error of $\bar{d} - 1.96s$ and $\bar{d} + 1.96s$ is approximately:

$$\begin{aligned}\sqrt{(3s^2/n)} &= \sqrt{(3 \times 50.8^2/11)} \\ &= 26.5 \text{ kg.}\end{aligned}$$

The 95% confidence interval for the lower limit of agreement is therefore:

$$\begin{aligned}&-146.1 - (2.228 \times 26.5), -146.1 + (2.228 \times 26.5) \\ &= -205.1, -87.1 \text{ kg.}\end{aligned}$$

The 95% confidence interval for the upper limit of agreement is therefore:

$$\begin{aligned}&53.1 - (2.228 \times 26.5), 53.1 + (2.228 \times 26.5) \\ &= -5.9, 112.1 \text{ kg.}\end{aligned}$$

Thus, even the most sanguine interpretation (the upper confidence limit of the lower limit of agreement of -87.1 kg; the lower confidence limit of the upper limit of agreement of -5.9 kg; and the upper confidence limit of the bias of -12.4 kg) concludes that there still could be major discrepancies between the two methods, and therefore that they cannot be used interchangeably (e.g., when comparing the weights of horses, some of which have been estimated by one method, and some by the other).

Two further aspects also may require consideration: (1) is there a trend (i.e., does the difference between the methods either increase or decrease as the mean increases); and (2) is the variability consistent across the graph (i.e., does the scatter around the bias line get larger as the mean increases) (Bland and Altman, 1999)?

Johnson and others (2009) give a Bland–Altman plot to compare devices for measuring blood glucose concentrations in dogs, and Sargent and others (2015) apply the plot to quantification of blood flow using cardiac magnetic resonance imaging in relation to canine mitral regurgitation.

The Bland–Altman approach can be applied when there are multiple observations per individual (Bland and Altman, 2007; Myles and Cui, 2007). Agreement between continuous measurements is discussed further by Bland and Altman (1999), Christley and Reid (2003) and Bland (2015), and further discussion of the Bland–Altman technique is provided by Krouwer (2008) and Giavarina (2015).

Note that the correlation coefficient (see Chapter 14) is not a valid indicator of agreement between two tests. Perfect correlation will exist between two tests if the points lie along any straight line; whereas perfect agreement is obtainable only if the points lie along the line of equality. Moreover, a significance test conducted on the data may indicate that the two tests are related, but this would be unsurprising because they are measuring the same quantity. Testing significance is irrelevant to the issue of agreement.

Reliability

The value of a diagnostic test is also judged by its **reliability** (see Figure 10.6); that is, the extent to which its results are *stable*. This can be explored by running the test two or more times on the same samples in the same laboratory under the same conditions, and assessing the **repeatability** of the results (see Chapter 10). Tests that are used in several laboratories (e.g., those that are recognized as international standards) also require their **reproducibility** (see Chapter 10) to be determined.

The statistical procedures for assessing repeatability and reproducibility are based on agreement between results. Thus, calculation of a *kappa* value is appropriate for nominal and ordinal data. An individual clinician's diagnoses made on the same animals on different occasions, for example, are likely to produce *kappa* values between 0.6 and 0.8. Bartlett and Frost (2008) discuss appropriate methods for continuous measurements.

The individual repeatability of two different diagnostic tests limits the amount of agreement that is possible between them. If one test has poor repeatability, then agreement between the two tests is bound to be poor, too. Similarly, if an established test has poor repeatability, then a new test, even if perfect, will not agree with it. If both tests have poor repeatability, the problem is compounded.

Guidelines for reporting studies of reliability and agreement are given by Kottner and others (2011).

Serological tests The repeatability of serological tests depends on a variety of factors including the degree of standardization of test reagents and the expertise of the tester; similarly, reproducibility might be affected when the same serum sample is analysed by different laboratory technicians in different laboratories. Thus, a twofold difference in antibody titre between samples from the same animal, taken at different times, may reflect either a true change in titre or similar titres associated with low repeatability or reproducibility.

Table 20.29 Typical titres of sera from 100 individuals tested twice. (From Paul and White, 1973.)

First reading (reciprocal titre)	Second reading (reciprocal titre)	Frequency
<8	<8	24
<8	8	2
8	16	2
16	8	2
16	32	3
32	16	3
32	32	8
32	64	6
64	32	4
64	64	16
64	128	1
128	64	3
128	128	6
128	256	2
128	512	2
256	128	4
256	256	6
256	1024	2
512	256	2
512	512	2

Generally, a geometric fourfold change in antibody titre (e.g., from 1/16 to 1/64) is assumed to reflect a real change; a twofold change is not considered to be significant (e.g., Wood *et al.*, 2012). Table 20.29 illustrates the reasoning behind this decision. The table shows typical results when sera from 100 individuals are tested twice. Each of the two readings should be identical for a single serum sample. However, the table shows that this is true only for 62 samples. Of the remaining 38 samples, 34 show a twofold shift and four show a fourfold shift. The shift is caused by the value of the test's repeatability not being 100%. Note also that the fourfold shift in titres occurs with high dilutions. If these titres are typical of the test, then, when future samples are drawn from the study population, 4% of the animals might be expected to show a fourfold shift in titre, which does not represent a true change. Since this shift can be caused by the second sample showing either a higher or a lower titre than the first, then, when evidence of a rising titre is required from two samples taken from the same animal at different times, 2% of the animals can be expected to show spurious fourfold increases in titre. This degree of error is generally acceptable.

Table 20.30 Recommended testing strategies for various diseases of cattle under different circumstances. (Extracted from Caldwell *et al.*, 2001.)

Circumstances	Disease	Strategy
Screening herds	Bovine virus diarrhoea	Active infection identified by sampling a small group (5–10 animals); assuming that seroprevalence will be high if a persistently infected animal is present, providing (1) maternal immunity has waned (animals tested are older than nine months) and (2) the group has been together for a lengthy period
	Paratuberculosis	All the herd needs to be tested because <i>Mycobacterium johnei</i> infection spreads slowly. Moreover, ELISA test sensitivity is no more than 50%. Thus, the level of prevalence to be detected would need to be set very low (say, 0.01), and aggregate testing is unsuitable
Testing animals added to herds The imperfect nature of diagnostic tests, and the possibility of carrier animals testing negative during some periods, means that quarantine and testing will never obviate the risk of introduction of infection. Ideally, therefore, animals should be purchased from herds where certified programmes, testing several animals, demonstrate absence of infection. If certified animals are not available, imported animals should be tested on arrival to quarantine, then again three weeks later Testing added animals is generally not required if infection is endemic	Bovine virus diarrhoea	Screen for antigen. If seroconversion occurs, retain the affected animal in quarantine for a further month, and vaccinate in-contact seronegative animals
	Bovine herpes virus 1	Antibody-positive animals remain potentially infectious for life, and therefore should be excluded from 'clean' herds
	<i>Leptospira hardjo</i>	Agglutination titres may have fallen below detectable levels; accordingly, an ELISA test is preferred. If the initial test titres are inconclusive, repeat the test in one month. Treat with antibiotics if there is residual doubt
	Paratuberculosis	Low test sensitivity and slow spread suggest that animals under two years of age will test negative. Faecal culture is preferred, but can take up to four months to reach a negative result. Quarantine screening is therefore impracticable. Annual testing by ELISA and faecal culture offer the best prospects for detection
	Neosporosis	Test heifers on entry to the herd, and at calving (infected animals are most likely to test positive at calving)
	Salmonellosis	Serology not routinely used. Culture faeces on three occasions, at 14-day intervals
	Tuberculosis	Tuberculin testing should be undertaken if animals are obtained from areas in which the infection is known to be a problem. However, animals will not react up to six weeks after infection, and sometimes will not react around parturition
	Venereal <i>Campylobacter</i> infection	Serological tests are of no value, and bacteriological culture has poor sensitivity. Accordingly, detection is difficult. If a bull is imported into the herd (e.g., as an emergency replacement), the prepuce should be irrigated with antibiotics
Monitoring progress		
If bulk milk can be screened for antibodies (e.g., bovine virus diarrhoea), conduct three-monthly tests. Alternatively, monitor sentinel groups of seronegative animals. Animals showing specific clinical signs should be tested (e.g., animals with diarrhoea and weight loss, for <i>M. johnei</i>).		

Practical application of diagnostic tests

Diagnostic tests are applied to specific populations of animals (notably, herds and flocks) in the context of specific diseases. Thus, the characteristics of each disease should be known before either a testing strategy is developed or the results can be meaningfully interpreted. For example, lifelong infection with *Neospora caninum* is associated with bouts of waxing and waning antibody titres (Dannat, 1997). Therefore, infected animals may test positive on one occasion, and nega-

tive on another. Consequently, it may be necessary to test an animal more than once to determine its infection status. Similarly, antibodies are produced late in infection with paratuberculosis (Johne's disease), therefore limiting the value of screening tests at quarantine.

Table 20.30 lists some diseases, the circumstances in which they may be investigated, and appropriate testing strategies. It serves to emphasize that a thorough knowledge of the relevant diseases is required before tests can be applied and interpreted with confidence.

Further reading

- Alonzo, T.A. and Pepe, M.S. (1999) Using a combination of reference tests to assess the accuracy of a new diagnostic test. *Statistics in Medicine*, **18**, 2987–3003
- Banoo, S., Bell, D., Bossuyt, P., Herring, A., Mabey, D. *et al.* (2010) Evaluation of diagnostic tests for infectious diseases: general principles. *Nature Reviews, Microbiology*, **8**, Suppl. 12, S17–S29. (Includes discussion of sample-size calculations for estimating sensitivity and specificity)
- Bossuyt, P.M., Reitsma, J.B., Bruns, D.E., Gatsonis, C.A., Glasziou, P.P. *et al.* (2003) The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. *Clinical Chemistry*, **49**, 7–18
- Choudhary, P.K. and Nagaraja, H.N. (2017) *Measuring Agreement: Models, Methods, and Applications*. John Wiley and Sons, Hoboken
- Crowther, J.R., Unger, H. and Viljoen, G.J. (2006) Aspects of kit validation for tests used for the diagnosis and surveillance of livestock diseases: producer and end-user responsibilities. *Revue Scientifique et Technique, Office International des Epizooties*, **25**, 913–935. (A discussion of diagnostic test validation with particular reference to the enzyme-linked immunosorbent assay and polymerase chain reaction)
- Gardner, I.A. and Greiner, M. (Eds) (2000) Validation and application of diagnostic tests used in veterinary epidemiologic studies. *Preventive Veterinary Medicine*, **45**, 1–162
- Jensen, A.L. and Kjelgaard-Hansen, M. (2006) Method comparison in the clinical laboratory. *Veterinary Clinical Pathology*, **35**, 276–286. (A review of methods for comparing two laboratory diagnostic tests)
- Knottnerus, J.A. and Buntix, F. (Eds) (2009) *The Evidence Base of Clinical Diagnosis: Theory and Methods of Diagnostic Research*, 2nd edn. Wiley-Blackwell
- Chichester. (A comprehensive discussion of the evaluation and reporting of clinical diagnostic tests)
- Kraemer, H.C. (1992) *Evaluating Medical Tests*. Sage Publications, Newbury Park. (A comprehensive guide to test evaluation, particularly in a clinical setting)
- Linnet, K. (1988) A review on the methodology for assessing diagnostic tests. *Clinical Chemistry*, **34**, 1379–1388
- Martin, S.W. and Bonnett, B. (1987) Clinical epidemiology. *Canadian Veterinary Journal*, **28**, 318–325. (A discussion of the application of diagnostic tests in clinical practice)
- Meier, K.L. (2002) Reporting results from studies evaluating diagnostic tests. *Clinical Microbiology Newsletter*, **24**, 60–63
- OIE (2012) *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, 7th edn. Office International des Epizooties, Paris
- Paul, J.R. and White, C. (Eds) (1973) *Serological Epidemiology*. Academic Press, New York and London
- Pepe, M.S. (2004) *The Statistical Evaluation of Medical Tests for Classification and Prediction*. Oxford University Press, Oxford
- Rutjes, A.W.S., Reitsma, J.B., Coomarasamy, A., Khan, K. S. and Bossuyt, P.M.M. (2007) Evaluation of diagnostic tests when there is no gold standard. A review of methods. *Health Technology Assessment*, **11** (50), iii, ix–72
- Smith, R.D. and Slenning, B.D. (2000) Decision analysis: dealing with uncertainty in diagnostic testing. *Preventive Veterinary Medicine*, **45**, 139–162
- Worthington, R.W. (1982) Serology as an aid to diagnosis: uses and abuses. *New Zealand Veterinary Journal*, **30**, 93–97

21

Surveillance

An essential part of disease control is the ability to document the occurrence of disease with the goal of developing effective control and eradication strategies; this is **surveillance**¹. This chapter outlines basic principles of surveillance, whereas some of its components, including data collection, the design of appropriate information systems, survey-sampling theory, and the application of diagnostic tests are discussed in Chapters 11, 13 and 20. Outbreaks of major epidemic diseases (e.g., classical swine fever and foot-and-mouth disease) require rapid surveillance protocols, linked to prompt control procedures, to ensure that epidemics are brought under control quickly; this topic is addressed in Chapter 27 (under 'Outbreak investigation').

Some basic definitions and principles

Definition of surveillance

Surveillance was originally applied to individuals; primarily to contacts of serious communicable diseases (e.g., pneumonic plague), who were closely watched for the development of the first signs of illness (Langmuir, 1971). Gradually, it was broadened to include diseases and related factors, such that there is no standard definition (*Table 21.1*)². Some

definitions describe it in terms of monitoring³. Some authorities have used 'monitoring' and 'surveillance' synonymously and interchangeably (Acheson *et al.*, 1976). However, there is a contemporary consensus that, although these two terms are closely interdependent (Weatherall and Haskey, 1976), they now have separate and distinct meanings.

Monitoring is the routine collection of information on disease, productivity and other characteristics possibly related to them in a population, without a predefined plan to mitigate disease risk. For example, in the UK, isolation of *Mycoplasma* spp. from ruminant specimens submitted to regional veterinary diagnostic laboratories regularly documented *M. bovis* over the 10-year period, 1990–2000 (Ayling *et al.* 2004). Thus, monitoring is firmly grounded in the recording of vital statistics initiated by William Farr (see Chapter 1 and Langmuir, 1976).

Surveillance, in contrast, is a more intensive form of data recording than monitoring, with a predefined plan to mitigate disease risk. It therefore has three distinct elements:

1. **gathering, recording and analysis** of data;
2. **dissemination** of information to interested parties, so that:
3. **action** can be taken to **control disease**.

It therefore has been likened to a nerve cell, with an afferent arm that receives data; a cell body that

¹ The procedure is not new, having its origins in the reporting of disease outbreaks (see Chapter 1). The Committee of Inquiry following epidemics of rinderpest in the UK in the 18th century (see *Table 1.1*) recommended compulsory notification of disease (Spinage, 2003), and in France in the 19th century the *Conseil de Salubrité* advised that the authorities were immediately notified of outbreaks of foot-and-mouth disease (Reynal, 1873). Surveillance of non-notifiable diseases in the UK was expanded in the early 20th century (MAFF, 1965), and international interest in surveillance increased with the establishment of the *Office International des Epizooties* in 1924 (see Chapter 11).

² 'Surveillance' is derived from the French, *surveiller*, 'to watch or guard a person' (*Oxford English Dictionary*, 1971). It came into

English use in this context at the beginning of the 19th century, and may have entered English from the *Reign of Terror* in France, when 'surveillance committees' were formed in French municipalities in March 1793 to monitor the actions and movements of suspect persons, outsiders and dissidents.

³ 'To monitor' is 'to keep under observation, especially so as to regulate, record, or control' (Latin: *monere* = 'to warn, advise, admonish': *Oxford English Dictionary*, 1971). It was introduced in the field of regulating recorded sound early in the 20th century, and is now in general use to indicate regular measurements of any continuous process.

Table 21.1 Some definitions of surveillance.

Definition	Source
Surveillance <i>of individuals</i> : close observation to detect early signs of infection without restricting [<i>their</i>] freedom of movement.	Langmuir (1963)
Surveillance <i>of disease</i> : the continued watchfulness over the distribution and trends of incidence through the systematic collection, consolidation and evaluation of morbidity and mortality reports and other relevant data.	Langmuir (1963)
... a more active system [<i>than monitoring</i>] and implies that some form of directed action will be taken if the data indicate a disease level above a certain threshold.	Martin <i>et al.</i> (1987)
Continuous analysis, interpretation, and feedback of systematically collected data, generally using methods distinguished by their practicality, uniformity, and rapidity rather than by accuracy or completeness.	Eylenbosch and Noah (1988)
The collection, collation, analysis and dissemination of data; a type of observational study that involves continuous monitoring of disease occurrence within a population.	Stedman (1989)
The maintenance of an ongoing watch over the status of a group or a community.	Abramson and Abramson (1999)
Ongoing systematic and continuous collection, analysis and interpretation of health data (often designed to detect the appearance of specific diseases), allowing epidemiologists to follow in time and space the health status and some risk factors associated with diseases for a given population, for use in the planning, implementation, and evaluation of disease control measures.	Toma <i>et al.</i> (1999)
The ongoing systematic collection, collation, analysis and interpretation of accurate information about a defined animal population with respect to disease and/or infection, closely integrated with timely dissemination of that information to those responsible for control and prevention measures.	Ministry of Agriculture, Fisheries and Food, UK: Meah and Lewis (2000)
... refers to a specific extension of monitoring where obtained information is utilized and measures are taken if certain threshold values related to disease status have been passed. It therefore is part of disease control programmes.	Noordhuizen and Dufour (2001)
The ongoing systematic collection, analysis, and interpretation of outcome-specific data essential to the planning, implementation, and evaluation of public health practice, closely integrated with the timely dissemination of these data to those who need to know.	Centers for Disease Control and Prevention, US: quoted by Thacker and Birkhead (2008)
The systematic (continuous or repeated) measurement, collection, collation, analysis, interpretation, and timely dissemination of animal-health and [<i>animal</i>]-welfare data from defined populations. These data are essential for describing health-hazard occurrence [<i>i.e., prevalence or incidence</i>] and to contribute to the planning, implementation, and evaluation of risk-mitigation actions.	Hoinville <i>et al.</i> (2013)
1. Systematic and continuous collection, analysis and interpretation of data, closely integrated with the timely and coherent dissemination of the results and assessment to those who have the right to know so that action can be taken.	Porta (2014)
2. Continuous analysis, interpretation, and feedback of systematically collected data, generally using methods distinguished by their practicality, uniformity, and rapidity, rather than by accuracy or completeness.	
... the systematic ongoing collection, collation, and analysis of information related to animal health and the timely dissemination of information so that action can be taken.	Office International des Epizooties: OIE (2015a)

analyses data; and an efferent arm that takes appropriate action (Thacker and Birkhead, 2008).

Goals of surveillance

The broad aims of veterinary surveillance follow the goals of veterinary medicine in general: namely, maintenance of high standards of animal health and welfare,

and the protection of public health (by the control of zoonoses and foodborne infections). Several specific objectives can be identified:

- rapid detection of disease outbreaks;
- early identification of disease problems (endemic and non-endemic);
- assessment of the health status of a defined population;

- definition of priorities for disease control and prevention;
- identification of new and emerging diseases;
- evaluation of disease-control programmes;
- provision of information to plan and conduct research;
- confirmation of absence of a specific disease.

Types of surveillance

There are several types of surveillance, defined by function and method. The terminology has undergone several changes since types were initially described (Hoinville *et al.*, 2013), with consequent overlap of types, and potential for confusion⁴.

Disease surveillance

Disease surveillance addresses aspects of the occurrence and spread of disease that are pertinent to disease control. Thus, during outbreaks of foot-and-mouth disease, sources of infection must be traced, isolated and removed. Disease surveillance is therefore more focussed than surveillance in general, which, for instance, may include recording of the distribution of agents and vectors, and serological ‘imprints’ of past infection.

Epidemiological surveillance

Epidemiological surveillance is a fuller description of surveillance, as generally and variously defined in Table 21.1. Some authors (e.g., Toma *et al.*, 1999) relate it closely to monitoring.

Sentinel surveillance

Surveillance can include the entire national herd (e.g., testing for bovine tuberculosis). Alternatively, a few farms, abattoirs, veterinary practices or laboratories may be selected; these are then referred to as **sentinel** units, because they are designed to ‘keep watch’ on a disease⁵. Thus, sentinel equine premises can be used to investigate persistence of vesicular stomatitis virus, using previous history of the disease as the selection criterion (McCluskey *et al.*, 2002). The *National Arbovirus Monitoring Program* (NAMP, 2015) utilizes sentinel cattle herds to plot the distribution of insect-borne viruses in Australia, and, in the same country, chicken flocks are used as sentinels of Murray Valley encephalitis virus, seroconversion in flocks alerting public-health authorities to the human risk (Bennett,

2008). Cattle herds are sampled in parts of Switzerland that are considered to be at high risk of incursion of bluetongue (Racloz *et al.*, 2008). Non-specific declines in milk production and reproduction, detected in routinely-collected dairy-cattle data, also may point to the early stages of disease outbreaks (Veldhuis *et al.*, 2016).

Alternatively, attention may be focussed on a species in general. Horses can be used as sentinels for Venezuelan equine encephalitis virus infection (Navarro *et al.*, 2005), stray dogs as sentinels for canine parvovirus infection (Gordon and Angrick, 1985) and West Nile virus (Resnick *et al.*, 2008), and the elkhorn coral, *Acropora palmata*, as a sentinel for the health of coral reefs (Woodley *et al.*, 2015).

Other species of animal that also are susceptible to an infectious agent can be used as sentinels for the infection in the main population of concern. For example, wild canids may serve as sentinel species for emerging vector-borne infections relating to translocation, climate change and habitat fragmentation (Aguirre, 2009; see also Chapter 7). Sea otters act as sentinels for transmission of *Toxoplasma gondii* in marine ecosystems (Conrad *et al.*, 2005). Wild birds can be used to monitor the activity of St Louis encephalitis virus, providing early information on the activity of the virus at a time when avian infection rates are still too low to pose an immediate threat to humans (Lord *et al.*, 1974). Eastern equine encephalitis (a mosquito-borne virus disease) infects horses and other vertebrates, including humans, but has a reservoir in birds. Surveillance of this infection therefore includes regular serological testing of sentinel flocks of chickens or pheasants kept outdoors, in association with the results of virus culture on captured mosquitoes, and veterinary surveillance of Eastern equine encephalitis-like illness in horses (Thacker and Birkhead, 2008). Similarly, in the US, an integrated approach to surveillance of West Nile virus, which emerged in the western hemisphere in 1999 (Nash *et al.*, 2001), involves surveillance of humans, horses, dogs, cats, wild birds (particularly dead crows), the vector mosquitoes, and sentinel chicken units (USGS, 2015).

Domestic animals also can be used as sentinels of human environmental health hazards such as carcinogens and insecticides (see Chapter 9 and ‘Sentinel health events’ following). Animals also offer prospects as sentinels of chemical terrorism agents (Rabinowitz *et al.*, 2008).

Humans also may act as sentinels of events in animals. Thus, Ascione and others (2006) interestingly report domestic violence as a ‘sentinel’ indicator of physical abuse of pets.

‘Sentinel’ therefore can refer to either a specific unit of observation or a species of animal.

⁴ A complete list of definitions of types of surveillance is given by Hoinville (2013).

⁵ The origin is Italian: *sentinella* = ‘sentry; guard’.

The linking of animal health and human health sentinel data is discussed by Rabinowitz and others (2009), Scotch and his colleagues (2009) and Pearce and Douwes (2013). A framework for evaluating animals as sentinels for infectious-disease surveillance is outlined by Halliday and her colleagues (2007).

Sentinel health events The occurrence of a preventable disease, condition or untimely death that indicates that the quality of preventive or therapeutic care may need to be improved is termed a **sentinel health event** (Rutstein *et al.*, 1983). Thus, deaths of canaries were used to indicate risk of carbon monoxide poisoning in coal mines in the UK until the late 20th century (see also Chapter 9). Die-offs in birds in the mid-20th century alerted the American public to the detrimental effect of pesticides and the associated risk to animal and human health (Carson, 1963), resulting in the ban on the use of dichlorodiphenyltrichloroethane (DDT) in agriculture. Mercury poisoning in cats that consumed fish contaminated with discharged industrial mercury presaged the disease in humans in Japan (Harada, 1995). Informative disease or mortality events thus can occur prior to, or concurrent with, signs of disease events in humans, justifying improved reporting of such incidents and closer integration with human disease-reporting systems (Bisson *et al.*, 2015).

Serological surveillance

Serological surveillance (serosurveillance) is the identification of patterns of current and past infection using serological tests (see Chapter 20). For instance, following epidemics of foot-and-mouth disease in the European Union (EU), all small-ruminant holdings within a 3-km radius of infected premises must be serologically sampled according to a precise statistical sampling protocol (see Chapter 13) before the area can be declared free from infection, and movement restrictions lifted. Sometimes more extensive sampling may be undertaken; this occurred in the UK following the 2001 epidemic, when serological surveillance extended to a 10-km radius (see *Figure 11.11*).

Passive and active surveillance

Passive and active surveillance each have been given different meanings. First, passive surveillance has been defined as the examination of only clinically affected cases of specified diseases; this contrasts with active surveillance, which involves the sampling (including post-mortem examination) of clinically-normal animals in the population, therefore being important in the surveillance of diseases in which subclinical cases and carriers predominate (Blood and Studdert, 2002).

Secondly – and most commonly in veterinary medicine – passive surveillance has been described as the continuous monitoring of the existing disease status of the populations that are studied, using routinely-collected data to produce outputs that can feed into policy decisions (Scudamore, 2000). Examples include reports of laboratory diagnoses, routine meat inspection findings and statutory notification of disease. Passive surveillance is therefore essentially monitoring with the intention of acting on its findings. Active surveillance, in contrast, involves the committed effort of veterinary authorities to collect information, commonly by undertaking investigations of specific diseases.

The term **enhanced passive surveillance** has been coined to describe the provision of data with the active involvement of investigators (e.g., by encouraging producers to report certain types of disease by active follow-up of suspect disease reports) (Hoinville *et al.*, 2013). Thus, farmer-awareness campaigns are encouraged to increase reporting of clinical signs suggestive of bluetongue, especially if circulating serotypes or strains only produce mild clinical signs (European Commission, 2006).

Passive and active surveillance each have strengths and weaknesses (Meah and Lewis, 2000; Scudamore, 2000). Passive surveillance uses data that may be biased (e.g., voluntary submissions to diagnostic laboratories), frequently lacking denominator values (see Chapter 4), and so cannot give unbiased estimates of disease frequency. In contrast, active surveillance, based on well-designed surveys, can produce such estimates. Passive surveillance also may underestimate the frequency of disease; for example, in Kenya, only 12 rabid dogs per 100 000 dogs were identified by passive surveillance, in contrast to 860 per 100 000 by active surveillance (Kitala *et al.*, 2000). However, passive surveillance is the first stage in identifying new and emerging diseases, which active surveillance cannot target (because the target is not yet identified); examples have included the initial identification of bovine spongiform encephalopathy and porcine reproductive and respiratory syndrome (see *Tables 1.6* and *1.7*). Additionally, passive surveillance can be efficient at detecting diseases of moderate prevalence, whereas active surveillance is more likely to capture diseases of high prevalence (Ouagal *et al.*, 2010). Moreover, in passive surveillance, submission of samples to laboratories, with feedback of results to farmers and veterinarians, establishes good relationships, facilitating 'informal' intelligence on animal health. Passive surveillance also usually involves a lesser cost than active surveillance. Therefore, both passive and active surveillance programmes are necessary components of national surveillance systems: the US's *National*

Animal Health Monitoring System is a good example (see Chapter 11).

Targeted, scanning and risk-based surveillance

The term 'passive' has the further disadvantage of failing to describe adequately the functions of surveillance, outlined earlier. Moreover, the term can give the impression of being 'unscientific', sometimes dependent on chance, and might imply that action is not taken (i.e., it may be categorized as monitoring *per se*). Additionally, active surveillance now includes activities in addition to regular surveys (e.g., outbreak investigations). Therefore, a new vocabulary has evolved to complement 'active' and 'passive'.

Targeted surveillance collects specific information about a defined disease so that its level in a defined population can be measured and its absence monitored. It is often planned using appropriate statistical sampling theory, and commonly focusses on populations that are at increased risk of being affected, thereby increasing the efficiency of detection. Examples are the targeting of fallen stock for surveillance of bovine spongiform encephalopathy (Doherr *et al.*, 2000) and equine colic cases for nosocomial *Salmonella* spp. infection (Kim *et al.*, 2001).

Profiles that increase the risk of detection can be broad. Thus, surveillance for the mosquito-transmitted Usutu virus of birds in the UK involves post-mortem examination of birds that die during periods of peak vector abundance, and are known to be susceptible to the virus, including residents and summer migrants (Horton *et al.*, 2013). Additionally, birds of other species with either splenomegaly, hepatomegaly or traumatic injuries (due to the possibility of neurological disease precipitating trauma) also are examined. Such broad profiles therefore logically may include sentinel species.

Scanning (global) surveillance maintains a continuous watch over endemic disease, so that unexpected changes can be recognized. Thus, questionnaires distributed to veterinarians in Scotland provided a regional picture of calf pneumonia and enteritis (Gunn and Stott, 1996). If scanning surveillance identifies an unusually high frequency of similar, undiagnosed cases (e.g., of respiratory disease), it may trigger a more detailed investigation to explore the likelihood of a new disease occurring; this is **syndromic surveillance**⁶ (Dorea *et al.*, 2011). Syndromic surveillance has rapidly detected outbreaks of bluetongue in The Netherlands (Elbers *et al.*, 2008) as well as identifying new syndromes such as

Pseudamphistomum triuncatum infection in otters, and 'bleeding calf syndrome' (DEFRA, 2011a)⁷.

Hoinville and her colleagues (2013) have suggested that 'scanning surveillance' should be replaced by '**early-warning surveillance**' to describe surveillance aimed at the early detection of threats.

Risk-based surveillance attempts to optimize surveillance by targeting categories of animal in which there is an increased likelihood of detecting disease (Stärk *et al.*, 2006), and frequently uses methods that are utilized in risk analysis (see Chapter 24). For example, the estimated prevalence of *Trichinella* spp. in Danish pigs is estimated at less than one case per million (essentially negligible), and so surveillance targeted at outdoor-reared pigs, sows and boars is justified because they are judged to have a higher risk of acquiring the parasite (Alban *et al.*, 2008). Similarly, monitoring of tetracycline residues in calves slaughtered in Switzerland increases efficiency of detection if focussed on animals from larger farms (Presi *et al.*, 2008). Table 21.2 lists some diseases and the risk factors that determine the efficiency of disease detection.

Targeted surveillance and risk-based surveillance represent overlapping approaches because the former frequently targets categories of animal identified by the latter. Consequently, some authorities suggest that the term 'risk-based surveillance' should replace 'targeted surveillance' (Hoinville *et al.*, 2013).

Some general considerations

Nature of data

Data from some sources may be unsuitable because they are **inaccurate**. Moreover, accuracy may vary over time (e.g., as a result of the changing ability to diagnose disease), producing spurious changes in disease trends (see Chapter 8).

Data also may be of the **wrong type**. For example, a record that just notes 'lameness' would be useful in a general estimation of the prevalence of bovine foot problems, but would be of little value to a detailed study of the various lesions and their causes that produce lameness. Hierarchic coding systems allow data of varying degrees of refinement ('granularity') to be recorded (see Chapter 10) and therefore may assist in the recording of disease profiles according to the available diagnostic detail (e.g., Stärk *et al.*, 1996).

Additionally, sources of veterinary data may be **biased** (see Chapter 10); a common type of bias being selection bias. The bias in the sources of data listed below is indicated when they are described.

⁶ The term derives from 'syndrome': the aggregate of signs associated with a disease that provides a description of it (Greek: *syn* = 'together'; *dromos* = 'running').

⁷ The value of syndromic surveillance in the context of bioterrorist attacks is discussed by Reingold (2003).

Table 21.2 Examples of risk-based surveillance used in animals and man. (Adapted from Oidtmann *et al.*, 2013.)

<i>Pathogen/agent/disease</i>	<i>Species</i>	<i>Risk factors</i>
Poliovirus	Man	Region; age; population immunity
Bovine spongiform encephalopathy prions	Ox	Age; feeding practices (use of meat-and-bone meal); intensive feeding
Enzootic bovine leucosis; infectious bovine rhinotracheitis	Ox	Importation
Avian influenza	Poultry; wild bird	Contact with wild birds; husbandry system
<i>Trichinella</i> spp.	Pig, wild boar	Husbandry system (outdoor access, contact with wildlife); feeding practice (swill)
<i>Salmonella</i> spp.	Pig	Spatial risk factors
Rabies	Racoon	Environmental factors (habitat, topography)
Several	Ox; poultry	Animal movements; movement networks

Cooperation

Lack of cooperation can pose a problem to surveillance and other epidemiological investigations. There are several reasons why people may not be willing to supply data.

The **reasons** for collecting information may not be clear to potential suppliers of data, and so they may be discouraged. This emphasizes that the **objectives** of data gathering should be explained to all who are involved in it. Farmers and government officials also may be **reluctant to report** disease if reporting is likely to lead to economic losses within their community (Kaufman, 2008).

Cooperation is more likely if data collection is part of a planned disease-control programme than if it is undertaken in isolation. For example, Sudanese farmers cooperated in a survey of mortality due to schistosomiasis because this survey was part of an investigation of the financial viability of a new vaccine against *Schistosoma bovis* (McCauley *et al.*, 1983a,b). However, surveys in Haiti, conducted without a concomitant animal health programme, eventually met with resistance from local animal owners (Perry and McCauley, 1984).

Motivation may be difficult to maintain in long-term data collection. This is of relevance not only to surveillance but also to prospective epidemiological studies (e.g., cohort studies: see Chapter 15). For example, during a five-year study of urinary incontinence in bitches, only 7% of veterinary practitioners returned completed questionnaires, despite initial enthusiasm, agreement to participate and regular contact by the investigators (Thrusfield *et al.*, 1998). Problems also may appear to be insufficient to maintain enthusiasm. For example, in Indonesia, local official support for avian influenza surveillance waned

when human cases did not reach the impending epidemic proportions that were initially reported in the press, when there were also more exigent concerns such as the risk of earthquakes, an increase in dengue cases, and sharp rises in the price of fuel and rice (Padmawati and Nichter, 2008). Additionally, motivation may be dampened by a loss of trust in surveillance activities. This occurred in Uruguay after an outbreak of foot-and-mouth disease in 2001 near its border with Argentina, which resulted in loss of its foot-and-mouth disease-free status without vaccination, with a consequent major impact on trade (Otte *et al.*, 2012).

The collection of information may risk a breach of **confidentiality**; for example, if practitioners' records that contain details of financial transactions of practices are examined. This, too, may prevent cooperation. Regulatory surveillance therefore frequently is accompanied by guidelines to guarantee confidentiality. For instance, in the reporting of data on the sales and use of veterinary antimicrobial agents, the European Medicines Agency ensures that it will not disclose confidential information on specific products, and only publishes aggregated data (EMA, 2011).

Cooperation is unlikely if data collection is **laborious** or **time-consuming**, for instance completing a complex or cumbersome questionnaire (see Chapter 11). The method of data collection therefore should be as simple as possible, within the constraints of the requirements of the data-collection programme.

The cost of data collection

The collection of data always incurs a cost, and this is particularly relevant to active surveillance. Costs include laboratory examination charges and postal

charges when the data are collected by postal questionnaire (see Chapter 11). The value of data therefore has to be judged in the context of the cost of collecting them. In most countries, the collection of information on diseases of national importance is supported by government funds⁸. Similarly, in many countries funds are available for research on diseases of economically-important livestock. However, investigation of companion animals' diseases, especially if they are not demonstrably of public-health significance, relies on the limited financial support available from welfare societies and charities. Lack of funds therefore can restrict companion-animal data collection.

Traceability

Traceability (Latin: *tractus* = 'track', 'course') is a neologism referring to the ability to trace the history, application or location of an item by recorded identification (EPA, 1998) and, in relation to the tracing of animals and animal products, can be measured in three ways (Golan *et al.*, 2004):

1. **breadth**: the amount of information that is collected through the production process;
2. **depth**: how far forward or backward in a production process the animal product can be traced;
3. **precision**: the degree of assurance with which the system can pinpoint its movement.

Increased attention has been paid to the traceability of animals and animal products over the past three decades, fostered, in Europe, by crises such as bovine spongiform encephalopathy, foot-and-mouth disease and dioxin contamination of feed (Vallat, 2001)⁹, bovine spongiform encephalopathy in the US (Boehler, 2013), and by the development of livestock product assurance and accreditation schemes (see Chapter 26 and Liddell and Bailey, 2001). Tracing systems¹⁰ thus have become a priority for national and international veterinary services.

⁸ The total cost of animal health surveillance in Great Britain in 2011 was an estimated £47.3 million (mainly spent on bovine tuberculosis), with only approximately 11% of declared funding coming from the private sector (Drewe *et al.*, 2014).

⁹ The idea is not new: the marking of animals is documented in the *Code of Hammurabi*, sixth king of the Amorite Dynasty of Old Babylon (18th century BC), and written certification of the origin of animals was introduced by Friedrich William, King of Prussia, in 1716, as a component of the control of rinderpest (Tornor, 1927). A detailed history of the traceability of animals and their products is presented by Blancou (2001).

¹⁰ Caporale and others (2001) draw a nice distinction between tracing systems and traceability systems. The former are implemented specifically to assist in disease control (e.g., tracing the origin of tuberculosis reactors), whereas the latter aim to document the history of a product along the whole production chain, 'from

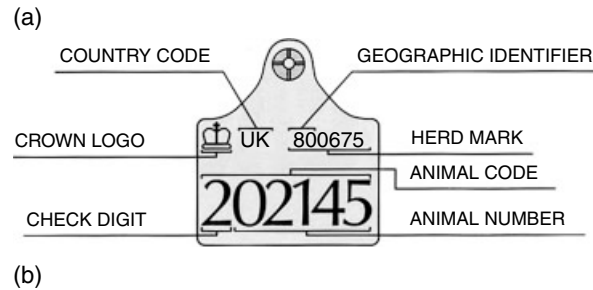


Fig. 21.1 An example of animal identification: ear tagging of cattle in the UK: (a) ear-tag design (the geographical identifier is illustrative); (b) location of tagged animals according to the first two digits of a tag's geographical identifier.

The starting point for a tracing system is animal identification (commonly by ear tag; e.g., *Figure 21.1*¹¹), which has increased in sophistication with

paddock to plate' (see Chapter 1 and Meisinger *et al.*, 2008). The term 'tracking' also is used to describe the ability to follow animals down the food chain from producer to slaughter or retail outlet, in contrast to 'tracing', which refers to the ability to follow a meat product up the supply chain by means of the records that have been kept at each stage in the chain (Bass *et al.*, 2008).

¹¹ Gonzales-Barron and others (2009) describe problems associated with ear tags.

the development of electronic 'microchip' implants, both in livestock and companion animals (see Chapter 4), ruminal boluses (Trenkle, 2006; Carné *et al.*, 2009), electronic ear tags (Fallon, 2001; Ribó *et al.*, 2001; Shanahan *et al.*, 2009; Ruiz-Garcia and Lunadei, 2011) and genetic markers (Cunningham and Meghen, 2001; Wilkinson *et al.*, 2011)¹². Individual-animal documentation (e.g., animal 'passports': see Chapter 4), linked to details of diagnostic tests, movements and livestock holdings, then forms the basis of a system for documenting an animal's history and current location. For example, the control of chlamydiosis in Californian parakeets was facilitated by a state law requiring all parakeets to be banded with details of their year of birth and breeding establishment (Schachter *et al.*, 1978). In contrast, the UK's *Vetnet Tracing and Verification System* proved insufficient to trace efficiently all potentially infected sheep during the 2001 foot-and-mouth disease epidemic because they could only be traced in batches and, when broken up, consequently became progressively more difficult to track (Gibbens *et al.*, 2001b). Similarly, in Canada, lack of animal identification hampered final efforts to eradicate bovine tuberculosis and to trace sheep potentially infected with scrapie (Greenwood, 2004).

Examples of tracing systems include a tracing system for cattle in Northern Ireland (Houston, 2001) and Canada (Stanford *et al.*, 2001) and systems for various livestock species in the US, Canada and Mexico (Murphy *et al.*, 2008). The US's *National Animal Identification System* (USDA, 2007) is designed to facilitate tracing of animals within 48 hours of a disease outbreak (Scanga *et al.*, 2007). Electronic identification of dogs, with registration in computerized databases accessible via the Internet (Mockford, 2004), offers a solution for tracing of companion animals (Dalla Villa *et al.*, 2013).

Increased world trade also means that there is a requirement for minimum standards in animal health, including the need to track animals and their products between countries (Wilson and Beers, 2001). This is because one needs to know the origin of animals and the places in which they have stayed because, for trade, health status is linked to location (e.g., some types of farm in some countries are known to be disease-free and so animals on such farms need not be proved to be individually disease-free). Thus, in the EU, *ANIMO* (*ANImal MOvement*) is a computerized network linking veterinary authorities (EUROPA,

2015a). It exchanges information about intra-Community trade, importation or transit in the EU of live animals, semen and embryos, as well as trade in mammalian animal waste, and importation of certain kinds of animal products. A development is the integration of *ANIMO* with *SHIFT* (a computerized network for veterinary import procedures) to produce *TRACES* (*TRAdE Control and Expert System*), which has several goals, including recording and tracing the origin, destination, and all stages in the movement of animals, animal products, by-products, semen and embryos (EUROPA, 2015b).

Sources of data

The organizations and groups described below represent a comprehensive list of possible sources for surveillance and other types of epidemiological investigation. The membership of the list will vary between countries. Some countries, particularly the more-economically-developed ones, have veterinary infrastructures that facilitate potential or actual data collection from the majority of these sources. The less-economically-developed countries may be able to utilize only a few of them. Some organizations record and store data routinely and therefore provide structured collections of data (databases) and information systems to which reference can be made for surveillance activities and when mounting other epidemiological studies (see Chapter 11).

Government veterinary organizations

Most countries have organized government veterinary services. These services investigate diseases of national importance, particularly those infectious ones for which legislation enforces reporting (the 'notifiable diseases'). Many governments also operate **diagnostic laboratories**. Reports are sometimes prepared and published routinely; for example, records of diagnoses made at diagnostic laboratories in the UK (see Chapter 11: 'VIDA'). Diagnostic laboratories are potential sources of syndromic-surveillance data (Dórea *et al.*, 2013, 2014) and have regionally or initially identified pathogens; for example, *Microsporium canis* (Ayling *et al.*, 2004) and *Neospora caninum* as a cause of bovine abortion (O'Toole, 2010). Submissions to diagnostic laboratories from practitioners are often voluntary and may therefore reflect personal interest and motivation, causing selection bias. Reports of notifiable diseases, similarly, depend upon the conscientiousness of observers.

¹² Other recognition technologies, such as retinal scans (Allen *et al.*, 2008) and muzzle patterns (Barry *et al.*, 2007), also have been explored.

Publications that collate information from a variety of government sources, including field and laboratory investigations, are also available routinely (e.g., the UK's *Animal Health & Plant Health Agency's* surveillance reports: APHA, 2015). In some cases routine reports are prepared but are confidential and therefore not readily available. An international database, the *World Animal Health Information Database* (WAHID), covering most countries of the world, is maintained by the *Office International des Epizooties/World Organisation for Animal Health* (OIE, 2015b). The database provides a statement of disease prevalence and control measures, based on regular reports of member countries of the *World Organisation for Animal Health* (see Chapter 11).

Sometimes surveys have been undertaken that use data collated from several sources. An early good example is the survey of livestock diseases in Northern Ireland that was undertaken in 1954 and 1955 (Gracey, 1960). This included demographic and disease data relating to a wide range of infectious and non-infectious diseases in cattle, sheep and pigs. It included data collected from 70% of farms in the country by field officers, and abattoir data.

Veterinary practices

In countries with private veterinary practices (notably the more-economically-developed countries), practitioners have contact with farm and companion animals, although the extent of contact varies. Farm-animal veterinarians have the greatest contact with dairy cattle, less contact with pigs and beef cattle, and the least with sheep. Problems in ruminants tend to be seasonal and related to parturition.

Owners of companion animals usually attend private clinics. Thus, the practitioner is a major potential source of small-animal and equine data that relate to a reasonably representative cross-section of animals.

In companion-animal practices, the cost of therapy may prevent animals being presented for treatment, and so diseases of animals owned by the poor may be under-represented, therefore inducing selection bias. Additionally, there is the more general issue of owners of sick animals not seeking veterinary care¹³.

In large-animal practices mild diseases and hopeless cases are not usually brought to the practitioner's attention by the farmer and so may be under-represented.

Even when information is freely available, it usually exists as separate accumulations of records in the individual practices and therefore may be difficult to collect and collate. Questionnaires can be used (see Chapter 11), but are time consuming. A few continuous monitoring programmes using veterinary practice data were instigated towards the end of the last century, for example, those that collected data from several veterinary practices in Minnesota in the US (Diesch, 1979; see also *Table 11.4*).

Computerized practice records Modern computer technology is increasing the accessibility of data (see Chapter 11), and now offers the possibility of using routinely-collected veterinary practice data for surveillance and epidemiological research. There are several computerized livestock health and productivity recording schemes operated by veterinary practices, bureaux and universities (see *Table 11.4* and Chapter 26), which have facilitated the recording of general morbidity in intensive production systems (Whitaker *et al.*, 2004), and specific epidemiological studies and surveys; for example, of bovine lameness (Russell *et al.*, 1982; Whitaker *et al.*, 1983) and fertility (Esslemont, 1993a); however, the data are of variable quality (Velasova *et al.*, 2015). There have been a few early attempts to pool small-animal data from computerized practice records (e.g., Thrusfield, 1991). However, the Internet is an expanding resource, and is now being used to collect small-animal surveillance data (see 'Companion-animal surveillance' later in this chapter, and Chapter 11 and Gobar *et al.*, 1998).

The use of pre-existing data is attractive because these data can be extensive, covering a representative cross-section of the population, and are less expensive than data that are collected for a particular purpose. However, there are problems associated with routinely-collected computerized data. First, some of the data may be of insufficient refinement for a proposed study. Secondly, data from various recording systems may be difficult to unify and compare because the systems contain varying categories of data of varying degree of refinement. For example, one computerized system may record lesions of the bovine foot in considerable detail (e.g., Russell *et al.*, 1982), whereas another simply may record that lameness is present (Smith *et al.*, 1983). Thirdly, the researcher has little control of the collection, and

¹³ This phenomenon, termed the 'symptom iceberg', in which most symptoms are managed in the community without people seeking professional health care, was first identified in human medical practice (Last, 1963). A veterinary example, relating to limber tail in Labrador retrievers, is given by Pugh and her colleagues (2016).

therefore the quality, of the data (Willeberg, 1986), which can be poor (e.g., Pollari *et al.*, 1996); this may result in inaccurate records (e.g., caused by 'finger trouble': see Chapter 10), incomplete data, selection bias, misclassification and confounding¹⁴. Fourthly, conversion of the records into a form that is suitable for analysis can be complex (Mulder *et al.*, 1994).

Abattoirs

Red-meat abattoirs process large numbers of animals for human consumption and identify some diseases during meat inspection. Only clinically-healthy animals usually are presented for slaughter, therefore the majority of diseases that are diagnosed at meat inspection are subclinical. Most reports relate to helminth diseases and internal lesions such as hepatic abscesses.

The objective of meat inspection is to safeguard the health of the human population. Traditionally this is practised by preventing the sale of meat and offal that are *obviously* unfit for human consumption. Therefore, most conditions are diagnosed only by macroscopic post-mortem examination; experience has shown that this approach is adequate.

A secondary objective is to record details of abnormalities that are found, because these findings may be of epidemiological value; for example, in associating outbreaks of disease in humans with infections in animals (Watson, 1982). Thus, an increase in the prevalence of tuberculosis lesions in cattle at slaughter was the first indication of a human tuberculosis epidemic in Barbados (Wilson and Howes, 1980).

Abattoirs therefore can be primary sources of data for disease surveillance for conditions for which other diagnostic methods are not appropriate. In Europe, for example, clinical signs are not reliable indicators of contagious bovine pleuropneumonia, and the probably low prevalence of antibodies makes serological screening inefficient. Therefore, in Switzerland, examination of lungs at slaughter was proposed as the basis of a surveillance system for this disease (Stärk *et al.*, 1994). Additionally, abattoirs may be targeted for specific surveys (e.g., of *Escherichia coli* O157: Chapman, 2000).

Epidemiological investigations are essentially a subordinate goal of meat inspection at abattoirs, although it is possible to conduct auxiliary investigations of blood, sputum, lymph nodes and other tissues for specific surveys and studies. Thus, in Ireland, bovine

kidneys condemned at meat inspection by the usual macroscopic techniques have been subjected to electron-microscopic examination to determine the nature of the lesions (Monaghan and Hannan, 1983). This is an example of using auxiliary aids to increase refinement of a diagnosis. Similarly, a survey of *Cysticercus tenuicollis* infection of sheep in Britain has been undertaken (Stallbaumer, 1983). This was necessary because the condition was not routinely recorded separately but was included in the 'coarse-grained' category: 'other conditions'.

Animals examined at meat inspection do not originate from the abattoir; they have travelled there. Trace-back therefore is desirable if the diseases that are identified are to be associated with their farm or area of origin. This is relatively easy in countries that have a simple marketing system. In Denmark, for example, pigs are shipped directly from producers to cooperative slaughterhouses from which data are collectively pooled, thereby allowing rapid trace-back and surveillance of disease (Willeberg, 1980a; Willeberg *et al.*, 1984).

Even when trace-back from the abattoir is possible, improper identification of viscera can be a further problem in some countries. Carcasses may be identified by ear tag or tattoo, but viscera containing lesions may not be labelled adequately and it therefore may be impossible to associate them with the carcasses from which they were removed.

Some countries publish routine meat inspection findings. These include Australia, Cyprus, Denmark, India, Luxembourg, New Zealand, Nigeria, Norway, the UK and the US. The published data may originate from the majority of abattoirs (e.g., in Denmark); from a sample (e.g., in Britain: Blamire *et al.*, 1970, 1980); or from only a single abattoir (e.g., in India: Prabhakaran *et al.*, 1980). These sources have been used for epidemiological surveys; for instance, surveys of reasons for condemnation of sheep at Scottish abattoirs (Cuthbertson, 1983) and of numerous diseases identified in animals in English abattoirs (Blamire *et al.*, 1970, 1980).

Abattoir investigations also can be used to detect deficiencies in farm-animal welfare and husbandry (Harley *et al.*, 2012). A survey of hoof and pedal horn lesions in sows at a Budapest abattoir has assisted in the identification of defects in floor construction in pig houses (Kovacs and Beer, 1979).

There are also formal abattoir-surveillance programmes. For example, the *Enhanced Abattoir Surveillance Program* records lesions identified in sheep in South Australian abattoirs, and notifies participating farmers of the percentage of their sheep affected with the conditions or diseases, accompanied by fact-sheets of the relevant conditions (PIRSA, 2015). No

¹⁴ For accounts of assessment of the quality of data, see also Canner and others (1983), Neaton and others (1990), Roos and others (1989) and Willeberg (1986).

producers are identified if the data are used for research or reports.

Abattoir surveillance has been a key component of surveillance for bovine tuberculosis for many years. It dates back to the early 20th century in the US (Frye, 1995), and has increased in sophistication (Kaneene *et al.*, 2006). It usually complements surveillance by herd testing (e.g., in the UK: Abernethy *et al.*, 2013), which also may be refined by a risk-based approach (Bessell *et al.*, 2013).

Poultry slaughterhouses

In several countries, poultry are slaughtered separately from the larger food animals in poultry slaughterhouses. Post-mortem examination results from these premises constitute another source of information. Most clinically-diseased and dead birds are excluded before slaughter. Conventional inspection of carcasses appears to compare favourably with risk-based approaches (Huneau *et al.* 2012).

Knacker yards

In some countries, animals that are ill or have died, and are therefore unfit for human consumption, are sent for slaughter to premises other than abattoirs. These premises are called 'knacker yards'. The carcasses may be fed to animals. In Europe, dogs kennelled for hunting are often supplied with flesh from these sources. Knacker yards also handle horses in countries in which horse flesh is not routinely eaten by humans.

Data from knacker yards are biased, but in quite a different way from those obtained from abattoirs: animals sent to knacker yards usually are either dead or ill – not alive and healthy. Data from this source are difficult and costly to acquire; they are distributed over many premises and would require professional veterinary inspection of lesions to ensure accuracy.

Registries

A registry is a reference list (more commonly, the word describes the building in which the list is kept). In human medicine, registries of diseases (notably of tumours) are maintained using hospital and death-certificate data as numerators and census data as denominators in morbidity and mortality rates. There are some veterinary tumour registries but these usually lack census data and so the denominators tend to be biased by non-response. For instance, when using estimates of population size based on the enumeration of licensed or vaccinated dogs (e.g., Cohen *et al.*, 1959), the denominator will tend to be underestimated because of a low public response to licensing and vaccination.

Some registries reduce the non-response bias by utilizing demographic surveys in specified areas. A Californian tumour registry, which ran between 1963 and 1966, defined a 'veterinarian using' reference population, counted by household survey, as the denominator (Dorn, 1966). The Tulsa (US) *Registry of Canine and Feline Neoplasms* (MacVean *et al.*, 1978) defined a 'veterinarian using' reference population, enumerated by a census of all veterinary practices in the Tulsa area, which included not only sick animals but also healthy animals presented for routine examination, vaccination and elective surgery; the numerator comprised diagnoses made by registry pathologists from specimens submitted by practitioners. The *Animal Tumor Registry*, a research unit at the National Institute for Research on Cancer of Genoa, Italy, documented histopathological diagnoses of canine tumour specimens submitted by veterinarians between 1985 and 2002, and related these to the canine population registry of the Local Health Authority (Merlo *et al.*, 2008). A similar project was instigated in the Veneto Region of north-eastern Italy in 2005, with denominator data gathered by a telephone survey (Vascellari *et al.*, 2009). A *Norwegian Canine Cancer Registry*, running between 1990 and 1998, related histologically-confirmed tumour diagnoses in a defined area to registrations recorded by the Norwegian Kennel Club (Arnesen *et al.*, 2001). A *Swiss Canine Cancer Registry* gathered data between 1955 and 2008 (Grüntzig *et al.*, 2015) and a *Danish Veterinary Cancer Registry* accumulated data between 2005 and 2008 (Brønden *et al.*, 2007, 2010). A companion-animal *National Veterinary Cancer Registry* was launched in the US in 2013, with an intended focus on clinical trials (NVCR, 2015).

Although these registries can reduce the selection bias inherent in specialized populations (e.g., of clinical cases), the true population at risk may not be estimated. Animals may not attend veterinary practices and therefore would be undetected, and some animals may attend more than one practice, one animal therefore counting twice in the denominator. Similarly, the completeness of demographic registries is uncertain. The extent of these inaccuracies is difficult to assess.

Companion-animal cancer registries extant in 2007, and previous (often ephemeral) ones, are reviewed by Brønden and others (2007). The varied methods of data collection make comparisons between them difficult (e.g., denominators may facilitate estimation of incidence or prevalence; or may be based on restricted clinic data, in which circumstance only standardized morbidity ratios may be calculated: see Chapter 4).

Insurance companies

Insurance companies supply insurance to animal owners (notably, for companion animals: see Chapter 4). Limited information on disease is recorded, but has facilitated estimation of incidence; for example, of lymphoma (Edwards *et al.*, 2003) and neoplasia (Dobson *et al.*, 2002) in the UK, and disease patterns in Sweden (Bonnett and Egenvall, 2010; Heske *et al.*, 2014; Pelander *et al.*, 2015).

Sales of pharmaceuticals

The sales records of pharmaceutical companies provide an indirect means of assessing the amount of disease, as well as monitoring drug use. The sale of antibiotics, for example, may be used as a rough guide to the prevalence of bacterial diseases, although estimates made from this information may be inaccurate. Antibiotics may be used without isolating specific bacteria – indeed, without even positively incriminating a bacterium – and so may be used when a bacterial infection is not present. They also may be used prophylactically; for example, following routine surgery. Similarly, they may be used for a purpose other than that for which they were intended: some small-animal practitioners, for instance, sometimes have used bovine intramammary antibiotics to treat otitis externa. The extent of unjustifiable and unusual use of some drugs is difficult to estimate (Murphy *et al.*, 2012).

Pharmaceutical sales are recorded in some countries: the *European Surveillance of Veterinary Antimicrobial Consumption* project, established in response to a request to strengthen surveillance systems of consumption of antimicrobial agents and of antimicrobial resistance (CEC, 2008), collates sales from several countries (Grave *et al.*, 2012). Pharmaceutical sales also can be linked with other sources of data. In Denmark, for example, where pharmacies supply 95% of the weight of antimicrobial compounds, the *VETSTAT* surveillance system integrates data from pharmacies, veterinarians and feed mills to provide a picture of the use of drugs at farm level, to promote prudent use of drugs in the context of resistance to antimicrobial compounds (Stege *et al.*, 2003; Dupont and Stege, 2013). Additionally, on-farm records can be used to monitor the use of antimicrobials (Chauvin *et al.*, 2005; Merle *et al.*, 2012), and the electronic medical records of veterinary practices also offer potential for monitoring such use (Regula, *et al.*, 2009; Anholt *et al.*, 2014). The *World Organisation for Animal Health* also has issued criteria for the development of national antimicrobial-resistance surveillance and monitoring programmes, and for the harmonisation of existing programmes (OIE, 2015d).

Zoological gardens

Most zoos maintain detailed records of animals and their diseases (e.g., Griner, 1980; Pugsley, 1981), and may be required to monitor specific diseases (DEFRA, 2012a). There is a general awareness of the need for recording systems for the surveillance of diseases in captive species (Cook *et al.*, 1993; Munson and Cook, 1993). The *MedARKS* (*Medical Animal Record Keeping System*) software (Fidget *et al.*, 2008), distributed by the International Species Information System (Flesness, 2003; ISIS, 2015), facilitates recording of clinical, pathological and laboratory data by participating zoos, and is now being incorporated in the *Zoological Information Management System* (ZIMS, 2015).

Some computerized recording systems focus on particular species; for example, bustards (Bailey *et al.*, 2003) and cheetahs (Evermann *et al.*, 1993). Other systems target specific aspects of health (e.g., reproductive health: Moresco and Agnew, 2013). Additionally, veterinary facilities attached to zoos can provide information on wildlife that is submitted to them. Six zoos in Australia, for example, feed data into the country's wildlife health surveillance system (Cox-Witton *et al.*, 2014). McNamara (2007) argues in favour of an integrated surveillance system, incorporating information from public-health, livestock, wildlife and zoo surveillance, partly using the initial identification of West Nile virus in crows in a zoo as justification.

Agricultural organizations

There are many agricultural bodies associated with the livestock industry that record information on animal production, such as liveweight gain, food conversion and milk yield. Although these data are not related directly to disease, they can provide information on the composition and distribution of populations that can be of value in defining populations that can be studied. An early example was the use of the *British National Milk Records File* to locate Friesian herds for a survey of reasons for culling and wastage in dairy cows (Young *et al.*, 1983). Currently, in The Netherlands, routinely-collected cattle census data form the basis of a cattle-health surveillance system (Santman-Berends *et al.*, 2016).

Commercial livestock enterprises

The intensification of animal industries mentioned in Chapter 1 has resulted in the establishment of commercial enterprises, particularly in the pig and poultry sector, where large units are common (see *Table 1.11*). Many of these enterprises have their own recording systems, although again some data may be confidential. These sources have been utilized in some surveys;

for example, mortality in broiler chickens (Reece and Beddome, 1983; Yassin *et al.*, 2009), losses in commercially-reared rabbits (Rosell and de la Fuente, 2009, 2016), unthriftiness in weaned pigs (Jackson and Baker, 1981) and lesions associated with the movement of weaners (Walters, 1978).

Farm records

Many farmers, particularly those keeping dairy cattle and pigs, routinely record production data. Some record information on disease. The recorded data may be computerized, and computerized schemes operated by bureaux, universities and veterinary practices have already been discussed earlier in this chapter (see 'Veterinary practices').

Veterinary schools

Veterinary schools have clinics that record the results of consultations. Many have established databases, often using computerized techniques that allow rapid access to records. Early examples include those at the Florida (Burridge and McCarthy, 1979), Edinburgh (Stone and Thrusfield, 1989), Liverpool (Williams and Ward, 1989b) and Glasgow (Knox *et al.*, 1996) schools. Some veterinary schools also pool records to form composite databases (e.g., the *Veterinary Medical Databases* in the US, and the *National Animal Disease Information Service* in the UK: see Chapter 11). The study population is frequently biased, especially when clinicians have specialist interests resulting in a high proportion of referred cases (Bartlett *et al.*, 2010).

Wild-animal organizations

Wildlife and animal conservation organizations and pest-control centres record data on wild animals, particularly relating to the size of populations. Wildlife can be important sources of infection to domestic animals and humans; for example, of rabies (in skunks in the US and foxes in Europe) and may be potential sources of infection (e.g., if hares were infected with *Brucella suis* in the UK). Routine monitoring of disease in these animals would be expensive, although they are increasingly the focus of sentinel surveillance (see earlier in this chapter). However, *ad hoc* surveys can be undertaken. Demographic data also are valuable when investigating the actual and potential spread of infection (see, for example, the discussion of potential fox rabies in Britain in Chapter 23). Wildlife surveillance is considered briefly later in this chapter.

Research laboratories

Research laboratories record data on primates, lagomorphs, rodents and caviés that are used in

experiments. These sources are very specialized, closed communities, and therefore are obviously biased.

Pet-food manufacturers

Manufacturers of pet food sometimes collect animal demographic data as part of their market research (see Chapter 4 and Anderson, 1983), although commercial interests may prevent release of all of these data.

Certification schemes

Schemes certifying freedom from disease involve compulsory and often regular examination of animals belonging to participating owners, and can therefore provide morbidity figures for a range of diseases. The *British Veterinary Association* and *Kennel Club*, for example, jointly run four schemes (BVA, 2015). The main purposes of the Hip Scheme are the examination of radiographs of hips of dogs for hip dysplasia and the issue of examination certificates, and breed-specific median hip scores are published. The main purposes of the Elbow Scheme are the examination of radiographs for elbow dysplasia, and breed-specific statistics again are produced. The Eye Scheme, run in conjunction with the *International Sheep Dog Society*, issues certificates attesting to freedom from 11 inherited ocular conditions. The goal of the Chiari Malformation/Syringomyelia Scheme is the elimination of this genetically-transmitted disease complex, predominantly from Cavalier King Charles spaniels, based on neurological examination. Data from these Schemes have been used to estimate disease morbidity and changes in disease trends (e.g., Curtis and Barnett, 1989; Lewis *et al.*, 2010; Parker *et al.*, 2011).

Pet shops

Pet shops are potential sources of information on conditions of young animals, such as congenital lesions (Ruble and Hird, 1993), but trace-back (e.g., for genetic studies) is difficult or impossible. They also can provide information on the zoonotic risk associated with a range of pets (e.g., Ferreira-Paim *et al.*, 2010; Roble *et al.*, 2012).

Breed societies

Companion-animal and pedigree-livestock breed societies have information on breed numbers and distribution. The extent of cooperation can vary. If a survey could highlight a certain problem in a particular breed, then that breed's society may be unwilling to help. This fear is real, although it is based on the false notion that epidemiological investigations just produce incriminating morbidity figures. The main goal of these investigations, however, is to detect causes, with a view to developing beneficial preventive strategies.

Serum banks

A serum bank is 'a planned catalogued collection of serum forming a random sample that is as representative as possible of a population and that is stored to preserve its immunological and biochemical characteristics' (Moorhouse and Hugh-Jones, 1981, after Timbs, 1980). Serum samples may be collected routinely during mandatory control and eradication campaigns in which serological tests are used to diagnose infection (e.g., brucellosis eradication where, in the UK, samples also were screened for warble fly and enzootic bovine leucosis). They also may be collected during specific surveys. For example, in a serological survey of warble-fly infestation of cattle in England and Wales (Sinclair *et al.*, 1989), over 74 000 specimens, from over 3000 farms, were collected. Much of the serum is not used, particularly now that diagnostic techniques using very small volumes of serum are available, and so the unused serum usually is discarded.

Applications of serum banks Serum samples can provide useful epidemiological information on infectious diseases, notably:

- identification of major health problems;
- establishment of vaccination priorities;
- demarcation of the distribution of diseases;
- investigation of newly discovered diseases;
- determination of epidemic periodicity;
- an increase in the knowledge of disease aetiology;
- evaluation of vaccination campaigns;
- assessment of economic losses due to disease.

Sources of serum Table 21.3 lists the potential sources of serum and some of their characteristics. This indicates that each source has its own advantages and disadvantages. The validity of results obtained from a serum bank depends on:

- the sensitivity and specificity (see Chapters 10 and 20) of the tests performed on the serum;
- the quality of the survey design used in collecting the serum;
- the degree of degradation that has occurred in the serum during storage.

The first component is an inherent characteristic of the tests that are employed. The second factor hinges on an appropriate sampling strategy (see Chapter 13). The third component is related directly to the means of storage.

Collection and storage of serum Samples should be taken aseptically. Blood should be allowed to clot for one to two hours at room temperature, stored horizontally overnight at 4°C, and then the serum should be separated by centrifugation at 2000–3000 rpm for 10–15 minutes. Blood also can be collected on filter paper discs; the serum can then be eluted later, although the amount of serum that is stored is small and may only allow semi-quantitative investigations.

Three storage techniques are available: **deep freezing**, **dry storing** on paper discs, and **lyophilization (freeze-drying)**. Four options are available for the first:

1. liquid phase of liquid nitrogen: –196°C;
2. vapour phase of liquid nitrogen: –110°C;
3. ultra-low deep freeze: –70°C to –90°C;
4. standard deep freeze: –20°C to –40°C.

Prolonged storage at –20°C may allow deterioration of antibodies. Refrigeration at 4°C is satisfactory for short-term storage. Lyophilization is a better technique than deep freezing, but is relatively expensive and technically complex. The following facts should be remembered in relation to freezing:

- the degree of deterioration at –20°C depends on the type and quantity of immunoglobulins; sera with

Table 21.3 Potential sources of samples for serum banks and some of their characteristics. (From Moorhouse and Hugh-Jones, 1981.)

Source	Potential no. of samples	Selection bias in study population	Cost to serum bank	Ease of collection	Standard of documentation
<i>Ad hoc</i> field visits	High	Low	High	Low	High
Other field visits	High	Low–high	Low	High	Moderate–high
Slaughter houses	High	High	Low	High	Low
Livestock marketing chain	High	High	Low–moderate	Moderate	Low
Veterinary diagnostic laboratories	Low	High	Low	High	Moderate–high
Private individuals	Low	High	Low	Moderate	Moderate–high
Existing collection	Low–high	Low–high	Low	High	Low–high

high IgM levels may be expected to lose specific activity more rapidly than those with high IgG levels, due to fragmentation (Moorhouse and Hugh-Jones, 1983); this is particularly salient to chronic infections in which levels of IgM generally decline relative to IgG levels (Racine and Winslow, 2009);

- repeated thawing and refreezing can be deleterious; this is apparently not due to the thawing/freezing process *per se* (Cecchini *et al.*, 1992), but may result from the bacterial and enzymatic contents of non-sterile samples; samples should therefore be refrozen as soon as possible;
- the use of cryoprotectants and/or enzyme inhibitors will reduce or eliminate deterioration;
- uninterrupted storage at -20°C appears to be a satisfactory procedure with very little loss of specific activity for at least two years; however, precise indications of the longevity of whole serum stored under serum bank conditions are not available;
- rapid freezing of samples is required;
- sterility is important;
- samples should be tested immediately after being thawed;
- delays will lead to increased rates of proteolysis.

Dry-stored samples probably provide satisfactory results for up to about five years (OIE, 2012).

Despite a recommendation, over 50 years ago, for the establishment of veterinary and medical serum banks (WHO, 1959), this recommendation has not been heeded widely, particularly in veterinary medicine. Veterinary banks include one in Canada, using serum collected during the bovine brucellosis eradication campaign (Kellar, 1983), and others in New Zealand (Timbs, 1980) and in Louisiana, US (Moorhouse and Hugh-Jones, 1981; Hugh-Jones, 1986). The latter included serum samples salvaged from previous studies, which provided information on diseases in domesticated and wild animals (leptospirosis in horses and leprosy in armadillos).

Several countries maintain national medical serum banks; for example, for influenza surveillance in the UK (Zambon and Joseph, 2001) and, in Scandinavia, for recording nutritional, biochemical, immunological or other changes that might be indicative of cancer development at early stages (Jellum *et al.*, 1995; Tuohimaa *et al.*, 2004). Serum banks also have a broader general role in cancer research (Evans and Mueller, 1992) and recently have been applied to assessing the allergenicity of genetically modified organisms (Barber *et al.*, 2008). Use of serum bank samples has the advantage of not usually requiring informed consent (Broberg *et al.*, 2011). However, despite their value, *World Health Organization* (WHO) funding

for some international medical serum banks was withdrawn in the late 1980s (Lederberg *et al.*, 1992).

The general principles of serum banks were established in two WHO Technical Reports (WHO, 1959, 1970), and are discussed by Paul (1965), Moorhouse and Hugh-Jones (1981) and Evans (1984). Additionally, Moorhouse and Hugh-Jones (1981) describe a computerized database for storing serum bank information.

Internet searches

The Internet is increasingly exploited to access health-care information (Dutton *et al.*, 2013), resulting in its use to explore determinants of health (so-called 'infodemiology': Eysenbach, 2009). Investigation may involve the use of Internet search engines such as Google™ (Brownstein *et al.*, 2009). A valuable tool is Google Trends (Choi and Varian, 2012), which is a public, free, Web facility showing how often a particular search term is entered relative to the total search volume across various regions of the world, aggregated into time series (see Chapter 8). Examples of its use include identification of the seasonality of chicken pox (Bakker *et al.*, 2016) and Lyme disease (Seifter *et al.*, 2010).

Google Trends data have been used to estimate the distribution and frequency of canine leishmaniasis in several European countries, when complemented by data from online questionnaires and veterinary practices (Mattin *et al.*, 2014) and to predict tick paralysis in cats and dogs in Australia (Guernier *et al.*, 2016). Although there is currently no standardized method for their use in veterinary medicine, such 'big data' techniques (see also Chapter 27) are likely to expand considerably, with an initial focus on detection of events that may justify further investigation, rather than acting as primary surveillance mechanisms (Hartley *et al.*, 2013), noting that predictions can be inaccurate because of 'big data hubris' (the assumption that 'big data' replace, rather than complement, traditional sources of data: Lazer *et al.*, 2014).

Mechanisms of surveillance

There are six main mechanisms of surveillance:

1. voluntary notification;
2. mandatory notification;
3. outbreak investigation;
4. sentinel surveillance;
5. structured surveys;
6. censuses.

These, and their characteristics, with examples from the UK, are presented in *Table 21.4*. One or more mechanisms may be used in a surveillance strategy.

Table 21.4 Mechanisms of surveillance. (Adapted from DEFRA, 2002c.)

<i>Mechanism</i>	<i>Description</i>	<i>Advantages</i>	<i>Disadvantages</i>	<i>Comment</i>	<i>Relative cost</i>
Voluntary notification	Observation of event of interest (e.g., welfare infringement, unusual clinical syndrome) is reported to federal/state veterinary services either directly or through routinely-reviewed publications or other sources	Provides a route for new syndromes to be identified Can be effective if the sources of data are motivated (e.g., university veterinary schools) Useful for recording unusual events of limited consequence (e.g., <i>Babesia canis</i> in dogs in the UK)	Will miss cases due to under-reporting Hard to define or measure the population at risk (i.e., the denominator in measures of morbidity) and so trends and changes cannot be measured	Reports are received when the observed event is recognized as being of interest and the observer is aware of the route by which a report can be made Can be improved by increasing awareness (e.g., by specific campaigns) and by offering financial incentives, but these will increase cost	Low
Mandatory notification	Legal obligation for observer to report events of interest (e.g., outbreaks of foot-and-mouth disease, salmonellosis in certain species) to defined government official or department	Good for syndromes with easily recognized clinical signs, particularly if awareness is raised (e.g., bovine spongiform encephalopathy) Requirement to notify is universal across the country Notification facilitates the swift implementation of investigation and control measures Clinical notification enables action to be taken in respect of diseases that are not rapidly or routinely confirmed in a laboratory Location data are supplied with notifications, which may enable population-based morbidity values to be calculated, and comparisons over time and geographical area to be made	Under-reporting is likely, unless training in awareness of clinical signs and route of reporting is maintained among the appropriate people The level of under-reporting of a particular disease is often biased; there are many reasons why a report may not be made, and these can be different for different areas or types of livestock owner Reduced sensitivity for syndromes with similar presentation to endemic syndromes (e.g., classical swine fever and post-weaning multisystemic wasting syndrome) Events with non-specific signs (e.g., Aujeszky's disease) may be missed Adverse consequences to the owner may act as disincentives and can increase under-reporting (e.g., a scrapie case on a holding in the previous two years prevents export) It may not be possible to estimate the size of the population to which the disease report relates, and so incidence or prevalence cannot be calculated	Reports are received when the observed event is recognized or suspected of being 'notifiable', and the observer is aware of route by which a report should be made Can be improved with incentives such as compensation	Low when prevalence is low
Outbreak investigation (see Chapter 27)	Veterinarians with appropriate expertise are recruited to investigate outbreaks of unusual disease syndromes; investigation of apparent association of human disease with exposures to animals	'Syndromic surveillance' provides a route for novel conditions to be identified	Depends on clear, agreed definition of an 'outbreak' (see Chapter 4) and agreed 'intervention levels' (e.g., bovine abortion rate >1% per year) Depends upon existing relationship between primary veterinarian and owner and therefore may omit farms that lack this relationship. Such farms may be 'high risk' (i.e., more likely to have disease problems) because there	Variety of observation points may trigger investigations (e.g., mortality statistics from carcass-disposal system; information from the public health authorities; lesions observed at meat inspection)	Can be high if intervention levels not appropriate

Sentinel surveillance (primary and secondary data sources*)	Key observers are recruited to provide routine returns listing the type, number and other specified details of specified events if observed. Events of interest are defined and may differ in different time periods Sentinels can be either primary data sources (e.g., as farmers) or secondary sources (e.g., veterinary practitioners)	Can be trained for the purpose Specialized and trained sentinels (e.g., veterinary practices) can provide estimates of level of defined events in the population surveyed Flexible: once the network is recruited and established, events surveyed and data collected can be varied in response to changing needs Can provide data on common conditions that are not notifiable, and for which laboratory diagnosis is not routine An estimate of the population size under surveillance may be available and so population-based morbidity values can be calculated If sentinel sites are representative, estimates can be generalized to a wider population Additional information that enables interpretation of trends can be collected Can monitor the reason for, and outcome of, particular laboratory tests or other health event, thus improving interpretation of other surveillance data	is less veterinary involvement, and they are less likely to report or investigate problems Not useful for rare events because (due to cost) sentinels usually form a very small proportion of the population surveyed Can be hard to recruit sentinels that are representative of the population of interest, and so results may be biased	Observers are recruited appropriate to the event(s) to be detected and recorded Diagnostic specificity can be improved by linking with diagnostic laboratories To be effective, contact must be maintained and regular feedback provided, so as to retain the commitment of the data source	Moderate or high
Sentinel surveillance (tertiary data sources)	Contributing data sources (e.g., diagnostic laboratories) report the number of diagnoses made of particular diseases, with a variable amount of supporting information (e.g., species affected, date of diagnosis, geographical location, specimen type). These are collated to give national statistics, which can indicate long-term trends and the effects of interventions	Efficient surveillance method for indicators that cannot be confirmed by primary or secondary data sources (e.g., diseases with non-specific clinical signs) Can be universal, involving all sources, with a broad range of indicators reported, and so it is sensitive enough to detect rare but important changes at an early stage (e.g., emergence of new diseases) Can provide information on relative morbidity due to particular indicators (e.g., causes of abortion) and so guide the setting of priorities	Under-reporting is likely (because one is dependent on both primary and secondary data sources for material) and so it is impossible to calculate the total number of cases of disease May not be universal. Distribution of contributing laboratories may not be comprehensive, leading to geographical 'blind spots' Cases that are easily diagnosed at primary and secondary level will not contribute to the data set because there is no need to refer cases or diagnostic material The frequency of diagnosis is biased by the rate at which cases or specimens		Variable

(Continued)

Table 21.4 (Continued)

<i>Mechanism</i>	<i>Description</i>	<i>Advantages</i>	<i>Disadvantages</i>	<i>Comment</i>	<i>Relative cost</i>
Structured surveys (see Chapter 13)	A selected sample of the population of interest is surveyed for the presence of the indicator of interest	Highly specific if accredited data sources used, so links between cases reported from different areas can be recognized Provides a mechanism for identifying cases which can contribute to further study of priority diseases about which more information is required (e.g., first case-series study of bovine spongiform encephalopathy) Data on some indicators are not available routinely by any other means The population of interest and the information needed can be defined Provided a sampling frame exists, the data can, and must, be collected from a representative sample of the population, ensuring unbiased estimates of morbidity The survey can be repeated over time A range of surveys can be carried out targeted at different populations to define the overall level and distribution of disease (e.g., fallen stock and casualty slaughter surveys for bovine spongiform encephalopathy are targeted at animals at particular risk with non-specific clinical signs) This contributes to defining the level of disease in that population, which may otherwise be missed Cost can be controlled by restricting the precision of the estimate	are referred; however, if the same factors affect all groups (e.g., economic hardship), relative morbidities will still be valid There is no tertiary diagnostic support (e.g., suitable laboratory test) for some diseases Targeted, and so can only measure that which is defined; cannot respond to new information or measure diseases that are new or unexpected Differences in diagnostic methods, or changes over time, can limit the comparability of surveys that set out to measure the same thing Can have problems of non-participation Denominator data sets may be incomplete or unavailable Very expensive if large sample sizes are prescribed	Surveys with small sample size can give useful results if the indicator is not very rare Surveys of animals at slaughter can measure the level of subclinical disease in animals of different ages Cost depends on disease characteristics and precision required	Variable
Censuses	Measurement of an indicator in all members of a defined population (e.g., tuberculosis testing in all cattle of a defined age, or meat inspection of all animals slaughtered for human consumption)	All members of the defined population contribute information and so true prevalence or incidence values (rather than sample estimates) can be measured	Requires a mechanism for identifying all members of the population to be counted Expensive		High

* The particular definition of primary, secondary and tertiary data sources in this table refers to the 'closeness' of relationship between the affected animals and the source of data relating to them. For example, farmers have regular and direct contact with livestock and so are primary sources; veterinarians may only have contact with selected clinical cases and so are secondary sources; and diagnostic laboratories have only indirect contact with animals through laboratory submissions. It is therefore subdividing primary data, as generally defined.

For example, surveillance of porcine brucellosis in the US involves mandatory reporting, active and passive routine surveillance, outbreak surveillance and sentinel surveillance (NCAHP, 2004).

Surveillance networks

The institutional infrastructure, personnel and agencies assembled to conduct surveillance of one or more diseases in a given region is termed a **surveillance network** (Dufour and Audigé, 1997; Dufour and Hendrikx, 2009)¹⁵. Such networks are usually centralized (i.e., data are managed at a specific centre). However, a network implies that information circulates in all directions, both from the field to the data-collection centre and vice versa, and between all personnel and agencies that are involved.

The objectives, remit and mechanisms of surveillance that are applied permit a classification of surveillance networks (outlined by Dufour and Audigé, 1997; Dufour and Hendrikx, 2009) that assists understanding of the scope of contemporary veterinary surveillance:

- management of the network: integrated (i.e., coupled to another activity such as disease control or prevention) versus autonomous (i.e., developed independently);
- scope of surveillance: local, national, regional or international;
- type of surveillance: targeted (one of more well-defined diseases) or global (all diseases or syndromes);
- epidemiological situation: exotic or emerging disease, or endemic disease;
- population under surveillance: sample or exhaustive population;
- data-collection method: passive or active.

Thus, the Canadian *RAIZO (Alert and Information Network on Animal Health in Quebec)*: RAIZO, 2015) is local; the French *REPAMO (National Network for Surveillance and Monitoring of Mollusc Health)*: Garcia *et al.*, 2011) is national; *MECIDS (Middle East Consortium on Infectious Disease Surveillance)*: MECIDS, 2015) is regional; and *WAHIS (World Animal Health Information System)*: OIE, 2015b) is international.

¹⁵ A distinction is sometimes drawn between networks and systems (see Chapter 11) on the grounds that the latter have broader-based objectives (e.g., servicing epidemiological studies, as well as surveillance), and therefore may comprise several networks. Hitchcock and others (2007) present a notional scheme for the global integration of networks and systems for the detection and containment of infectious-disease outbreaks, including those caused by zoonoses.

Networks may be components of veterinary information systems (see Chapter 11). Additionally, surveillance and monitoring may be spread over several different systems. For example (Stärk and Nevel, 2009), information about the health of pigs in England is garnered from several sources, including *VIDA* and *NADIS* (see Chapter 11) and the British Pig Health Scheme (see Chapter 26). Examples of French surveillance networks are given in *Table 21.5*.

The national and international aspects of veterinary surveillance are reviewed by Blajan (1979), Davies (1980, 1993), Ellis (1980), Blajan and Welte (1988), Bernardo and others (1994), Dufour and Audigé (1997) and Dufour and Hendrikx (2009).

Surveillance in less-economically-developed countries: participatory epidemiology

A major constraint to veterinary surveillance and disease control in less-economically-developed countries has been the lack of information on disease morbidity. Data collection may be thwarted by poor laboratory diagnostic support, insufficient manpower and difficult terrain (Broadbent, 1979). There also may be a general failure of conventional veterinary services (Swift *et al.*, 1990). This has given impetus to the elaboration of alternative methods of data collection as part of the wider goal of improving the delivery of veterinary services in the context of closer participation in disease control by rural communities¹⁶.

The need for methods of data collection and analysis that are powerful, quick, careful and cheap led to a range of **rapid rural appraisal** techniques, using multidisciplinary teams, both in the agricultural sector generally (McCracken *et al.*, 1988) and specifically in relation to animal health (Ghirotti, 1992). Involvement of communities in data gathering resulted in the term **participatory appraisal** being applied to this mainly qualitative approach (Pretty, 1995). This also is termed **participatory rural appraisal** when it is set in a rural

¹⁶ Appreciation of the need for community participation in the wider agricultural theatre originated in the US during the 1950s and 1960s, when Roosevelt's Country Life Commission encouraged self-reliance among deprived communities (Christenson and Robinson, 1980). This concept spread to developing countries after decolonization (Rifkin *et al.*, 1998). Hitherto, underdevelopment had been associated with a lack of technology, which could be remedied merely by passive adoption of Western technologies (Cohen and Uphof, 1980).

Table 21.5 Animal epidemiological surveillance networks operating in France in 1997. (Adapted and reduced from Dufour and La Vieille, 2000.)

<i>Name of network</i>	<i>Aims</i>	<i>Type of surveillance</i>	<i>Scope of surveillance</i>	<i>Sample or all animals</i>	<i>Data collection</i>	<i>Operation</i>
Rabies	To detect cases	Targeted	National	All	Passive	Integrated
Tuberculosis	To monitor disease control	Targeted	National	All	Active	Integrated
Brucellosis	To monitor disease control	Targeted	National	All	Active	Integrated
Bovine leukosis	To monitor disease control	Targeted	National	All	Active	Integrated
BSE (bovine spongiform encephalopathy)	To detect cases	Targeted	National	All	Passive	Integrated
Scrapie	To evaluate prevalence	Targeted	National	All	Passive	Integrated
<i>SAGIR</i> (wildlife surveillance network)	To estimate causes of death in game	Scanning	National	Sample (by hunters, on a voluntary basis)	Passive	Autonomous
<i>RENESA</i> (national poultry epidemiological surveillance network)	To monitor contamination of poultry flocks with <i>Salmonella</i> spp.	Targeted (<i>Salmonella</i> spp. and mycoplasma)	National	Sample (by voluntary farmers, on a voluntary basis)	Active	Integrated
<i>RNOEA</i> (national network for monitoring poultry production)	To monitor diseases in poultry flocks	Scanning	National	Sample (by veterinarians, on a voluntary basis)	Active	Integrated
<i>RESAN</i> (beekeeping epidemiological surveillance network)	To monitor bee diseases in apiaries	Targeted (five diseases)	National	Representative sample	Passive	Autonomous
<i>REPFI</i> (phytosanitary surveillance network for shellfish)	To monitor toxicology contamination in shellfish	Targeted	National	Representative sample	Active	Autonomous
<i>REMI</i> (microbiological surveillance network for shellfish)	To monitor bacteriological contamination in shellfish	Targeted	National	Representative sample	Active	Autonomous
<i>RESABO</i> (network for monitoring antibiotic resistance of the principal bacterial pathogens of cattle)	To monitor the development of antibiotic resistance in the main bacterial pathogens affecting cattle	Targeted	National	Sample (by laboratories, on a voluntary basis)	Passive	Integrated
<i>CESAM</i> (epidemiological surveillance network for contagious equine metritis)	To monitor changes in contagious equine metritis	Targeted	National	Sample (by breeders, on a voluntary basis)	Passive	Integrated
<i>Salmonella</i> network	To monitor changes in <i>Salmonella</i> serotypes	Targeted	National	Sample (by laboratories, on a voluntary basis)	Passive	Partly integrated and partly autonomous
<i>RESSAB</i> (epidemiological surveillance network for suspected cases of bovine salmonellosis)	To monitor suspected cases of salmonellosis in adult cattle	Targeted	National	Sample (by veterinarians, on a voluntary basis)	Passive	Partly integrated and partly autonomous
<i>OPA</i> (network for monitoring bovine pasteurellosis)	To monitor bovine pasteurellosis	Targeted	National	Sample (by veterinarians, on a voluntary basis)	Passive	Autonomous
<i>VEGA*</i>	To monitor the main diseases of cattle and sheep	Scanning	Regional	Sample (by veterinarians, on a voluntary basis)	Active	Partly integrated and partly autonomous
<i>VIALINE*</i>	To monitor changes in infectious diseases of cattle (infectious bovine rhinotracheitis, paratuberculosis, listeriosis)	Scanning	Regional	Sample (by livestock farmers, on a voluntary basis)	Passive	Integrated

* Name of regional network.

context (Mukherjee, 1993). More recently, **participatory epidemiology** has been coined to describe community participation in data collection and disease control (Mariner, 1996; Catley and Mariner, 2001; Catley *et al.*, 2012)¹⁷.

An important, but sometimes overlooked, distinction exists between rapid rural appraisal and participatory rural appraisal. This relates to whether researchers see themselves as being engaged in the collection of objective data (typical of rapid rural appraisal, which tends to be short and conducted by external investigators), or whether they see themselves as being part of the system under study, in which case they are part of a multidisciplinary team that includes community members, where the predominant mode is participatory with the aim of facilitating community outcomes (Webber and Ison, 1995). The intention of participatory rural appraisal therefore is to '*stimulate community awareness*', rather than to identify or formulate problems (Chambers, 1992). Hence, there may be a contrast between activities usually associated with veterinary epidemiology and those of participatory epidemiology. Typically, the former attempt to identify and solve problems identified by scientific (and other) experts for the benefit of 'local people', and may be seen as extracting information by outsiders (Chambers, 1992). In contrast, participatory epidemiology includes the essential involvement of the community in defining and prioritizing problems, and in the development of solutions to service delivery, disease control and surveillance: the use of the term 'participatory epidemiology' to describe projects that do not involve the community in this way therefore is misplaced (Catley *et al.*, 2012). Thus, it is important to note that these techniques are not merely data-gathering procedures, but are an integral part of community-focussed animal disease intervention strategies, of which surveillance is only a part¹⁸.

Although these participatory methods have been developed for use in less-economically-developed countries, and it has been stated that they only apply to work in such countries (Toribio and Rushton, 2012), there also is potential for the methods to be of value in more-economically-developed countries (Catley *et al.*, 2012).

¹⁷ Explicitly in the context of surveillance, this approach also has been termed '**participatory surveillance**' (Hoinville *et al.*, 2013).

¹⁸ The active inclusion of local community-based animal health workers in disease control (e.g., Allport *et al.*, 2005), which began in the Horn of Africa in the 1980s (Leyland *et al.*, 2014), represents the maturing of a process that had begun in colonial times, when, for example, herders were used as 'vetscouts' and vaccinators (Jack, 1961).

Principles of participatory epidemiology

Participatory epidemiology relies heavily on indigenous knowledge and terminology, and respect for this knowledge therefore is of paramount importance. Indigenous knowledge is known to be extensive (albeit sometimes coloured by traditional notions of the cause and treatment of disease: Beinart and Brown, 2013), and, in general, farmers and pastoralists have greater capabilities than has been customarily supposed (Chambers, 2010). Thus, the Maasai were the first to suggest that wildebeest were associated with bovine malignant catarrhal fever¹⁹ (see Chapter 2), and nomadic cattle owners could competently diagnose rinderpest (Plowright, 1998). Moreover, local diagnosis often compares favourably with conventional scientific investigation (Baumann, 1990; Edelsten, 1995; Mariner and Roeder, 2003; Catley *et al.*, 2004; Catley, 2009; Robyn *et al.*, 2012). It therefore has a useful role to play in the timely identification of disease in the field, and to confirm the absence of clinical disease.

Participatory epidemiology differs from traditional epidemiology in key respects, Catley and Mariner (2002) highlighting five broad principles:

1. attitude and behaviour;
2. combined methods of triangulation;
3. use of key informants;
4. action orientation;
5. methodological flexibility, adaptation and development.

Attitudes and behaviour

Researchers should assess their own professional and cultural biases, and have respect for local knowledge and culture. This includes recognizing that their point of view is not necessarily better than that of the farmer or other participant. They therefore must be willing to learn from local people and to patiently and actively listen, rather than to lecture.

Combined methods and triangulation

Most participatory epidemiology involves a range of methods such as interviewing, scoring and ranking, and visualization techniques (see later). These methods enable individuals to express their own knowledge and to understand other people's knowledge. Importantly, they enable knowledge to be expressed in different ways (verbal, numerical and visual) because this may help to expose subtle differences in understanding and to highlight areas of misunderstanding

¹⁹ The Maasai use the same word for 'wildebeest' and 'malignant catarrhal fever' (Barnard *et al.*, 1994).

between the researchers and local people. These methods may be combined with one another, with more traditional epidemiological approaches, and with other forms of information (e.g., disease records, and scientific and 'grey literature'²⁰) in a process called **triangulation**, in which information is gained from, and compared with and to, several intentionally different perspectives.

Use of key informants

Although pastoral communities generally are recognized as being knowledgeable about animal health, certain people have special knowledge of livestock systems and health, and may possess particular skills. Such local experts are important key informants. Five types of non-academic partners have been identified (Cargo and Mercer, 2008). These include:

1. the **end-users**, who represent the group in which impact or change can be measured (e.g., farmers);
2. the **support network** of the end-users (e.g., family members and friends);
3. the **general public**, who know about or support the relevant issues;
4. people who **directly interact** with the end-users or their networks (e.g., community animal health workers and veterinarians);
5. people who operate at an **administrative** or **political** level, who typically interact with service providers rather than with end-users or their networks (e.g., policy makers and administrators).

Selection of participants raises important issues because, although individuals may seek to represent the views of the wider community, they will only be able to present their own perceptions of the views of others (Webber and Ison, 1995). It is therefore important to ensure that all relevant groups (i.e., points of view) are included in the optimal mix of participants.

Action orientation

The focus of participatory epidemiology is on the generation of locally verified information that is used to determine agreed action. This does not mean that a particular study should immediately result in action because further investigation, either by the community or through more traditional scientific approaches, may be warranted before action can be agreed. Therefore, care should be taken to ensure that expectations

are not raised unrealistically, with the risk of disappointment.

Methodological flexibility, adaptation and development

Key features of the methods of participatory epidemiology are their adaptability to particular requirements and their ability to be combined with other methods (including standard epidemiological techniques). Participatory epidemiological appraisals are not rigidly pre-planned and executed without deviation; techniques and questions may be changed during the investigation. As a relatively new discipline, participatory epidemiology is still developing, both in terms of methodology and application.

These principles presuppose that the community can be readily identified, whilst a community also can be defined in numerous ways: a collection of people in the same geographical location (Webber and Ison, 1995); a subset of people who share similar interests; or people engaged in a network of communication (Maturana and Varela, 1998; Gochenour, 2006). Thus, in a participatory epidemiological study in India, Wright and Thrusfield (2016) defined the community as the Van Gujjar: an ethnically-distinct, forest-dwelling, vegetarian, pastoral, transhumant population, predominantly following Islam, whose culture and livelihood are intrinsically linked to their herds of buffalo (Gooch, 2004).

Chambers (1992) dichotomizes a community into **insiders** and **outsiders**. This highlights another central principle of participatory epidemiology: **reversal of learning**. The concept of 'reversal of learning' is that outsiders (the researchers, policy makers, administrators, and so on) should learn from the insiders (the local community) face-to-face (Chambers, 1994). Differentiating individuals within a community on the basis of particular characteristics, such as interests and gender, may help to ensure that the poor, women and other groups that are frequently marginalized in such processes are included and are able to participate fully in the **focus groups** that are enlisted for participatory exercises (Chambers, 1994).

Techniques of data collection

The main means of collecting information are by **semi-structured interviews**, **scoring and ranking**, and **visualization**. The methods also can be classified according to the types of information they gather, and hence may be **space-related** (useful for exploring spatial dimensions of people's reality, largely by mapping), **time-related** (useful for investigating the temporal dimensions of people's reality) or **relational** (designed

²⁰ 'Grey literature' is generally defined as academic literature that is not formally published and controlled by commercial publishing, and which therefore may be difficult to trace via conventional channels such as published journals. Examples include technical reports and consultants' reports.

to explore relationships between various items or various aspects of the same item; e.g., the various clinical signs associated with different diseases) (Kumar, 2002)²¹. A key feature of these methods is that they do not require participants to be literate. Typically, they also involve group participation, through either discussion or activities that require the informants to work together. Only a brief description of the more common methods is provided here²².

Semi-structured interviews

Interviewing is a major participatory method, being used in circumstances that render questionnaires impracticable (e.g., because of illiteracy) or inappropriate (e.g., because there is insufficient knowledge to construct a valid questionnaire). In contrast to the structured design of interviews and questionnaires often used in traditional epidemiological studies (see Chapter 11), interviews used in participatory epidemiology are *informal* and should have only a basic structure, leaving the interviewer to improvise, depending on the responses and interests of the informant (Slim and Thomson, 1994). The interviewer also should be willing to make culturally-appropriate gestures of understanding, sympathy or encouragement.

Probing Asking detailed questions on a specific subject raised by the respondents is termed 'probing' (Mariner, 2000). This is both a data-gathering and data-quality-control technique. Probing can be used to verify the consistency of information or simply to gather more detailed information on a particular subject. Probing often is used to expand the description of a particular disease volunteered by respondents. Thus, respondents might describe a disease that causes sudden death in livestock without rigor mortis. The appraisal team could then inquire if the disease can affect people and, if so, what does the disease look like in humans. A positive response with a characteristic description of anthrax abscesses will confirm this description as anthrax.

Scoring and ranking methods

Two methods are commonly used: **matrix scoring** and **proportional piling**²³. Matrix scoring involves the creation of a matrix with, for example, different

Signs	Diseases				
	<i>Liei</i>	<i>Dat</i>	<i>Maguar</i>	<i>Doop</i>	<i>Macueny</i>
Chronic weight loss	●●●●●	●	●●	●	●
Animal seeks shade		●●●●●			
Diarrhoea	●●		●●●●●		●●
Reduced milk yield	●●	●●●●●	●●	●	
Coughing				●●●●●	
Reduced appetite		●●●●●		●●	
Loss of tail hair	●●●●●				
Tearing	●●●	●●	●●		●●
Salivation	●●	●●●●●	●●	●	

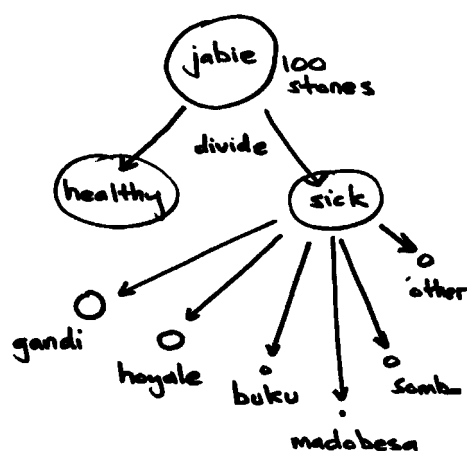
Fig. 21.2 Matrix scoring of disease signs for diseases of adult cattle in Nyal, southern Sudan. (Nuer names in italics.) (Adapted from Catley, 2004.)

diseases on the horizontal axis, and characteristics associated with the diseases (e.g., clinical signs) on the vertical axis. Piles of counters are then used to complete the cells of the matrix, indicating the relative magnitude of the relationships. An example, relating to diseases in the Upper Nile region of southern Sudan occupied by the Nuer, is given in *Figure 21.2*. Three Nuer conditions, *liei*, *maguar* and *macueny*, are associated with chronic wasting. Two other conditions, *dat* and *doop*, had already been identified as foot-and-mouth disease and contagious bovine pleuropneumonia, respectively. Matrix scoring of the signs of *dat* and *doop* (e.g., salivation and coughing for the two respective diseases) suggested that the pastoralists could effectively monitor the occurrence of these specific diseases. The three other conditions posed a more thorny diagnostic issue. Further matrix scoring of these conditions against putative causal factors (e.g., liver flukes, exposure to snails or biting flies), supported by conventional laboratory investigations, suggested that *macueny* and *maguar* involved helminths, with fascioliasis predominating in the former, and gastrointestinal parasites in the latter. *Liei* was more complex, including fascioliasis, gastrointestinal parasites, trypanosomiasis and schistosomiasis. Thus, the local monitoring of *liei*, *macueny* and *maguar* indicates only a broad range of conventional potential diseases,

²¹ There also are other systems of classification (Mikkelsen, 2005).

²² A related approach – the 'Knowledge, Attitude and Practice (KAP)' survey (WHO, 2008) – has been applied recently in veterinary medicine (Grace *et al.*, 2009).

²³ Proportional piling is a visualization method, but is included here because results are recorded numerically and used for scoring or ranking.



jabie: calves
 gandi: trypanosomiasis
 hoyale: foot-and-mouth disease
 buku: acute trypanosomiasis due to *Trypanosoma vivax*
 madobesa: rinderpest
 somba: contagious bovine pleuropneumonia

Fig. 21.3 Proportional piling: method for incidence estimates for diseases of calves in Orma communities, Kenya. (Number of stones in each pile indicated by sizes of circles.) (Figure provided by Andrew Catley, African Union/Interafrican Bureau for Animal Resources, Nairobi, Kenya.)

rather than specific ones. However, for a syndrome with non-specific clinical signs, herders were able to sub-classify three 'local diseases', based on seasonality and causal factors, better than veterinarians, who tended to diagnose *liei* cases as trypanosomiasis *per se*.

Matrix scoring also is a useful means of confirming verbal descriptions given in interviews (i.e., triangulation) and can be used as the basis of further interviews.

Proportional piling uses counters to indicate the relative frequencies of diseases or disease-associated characteristics. *Figure 21.3*, for example, depicts the relative annual incidence of various diseases in calves in Orma communities in Kenya. A range of objects, such as stones, seeds, bottle tops or other locally available materials, can be used as counters. However, care should be taken to ensure that the choice is acceptable locally. For example, in some areas of northern Ethiopia it is highly inappropriate to be wasteful of, or play with, food and so seeds would be a very poor choice.

Confidence in scoring and ranking methods is increased if there is a high degree of agreement (see Chapters 10 and 20) between different groups of informants²⁴. However, disagreement between groups

may indicate real differences that should be investigated further.

Visualization methods

A variety of methods may be used to present events pictorially. These include maps, time lines and seasonal calendars. **Participatory mapping** is an example of a space-related method that is used to explore local perceptions of the spatial distribution of disease and risk factors (or other issues). *Figure 21.4* is a map of a village in the Tana River District of Kenya, inhabited by Orma pastoralists. The map was originally drawn on the ground using branches, leaves and stones. Key features, such as rivers, the school, the mosque, grazing, cultivation and water resources are depicted. This allowed villagers to indicate proximity of cattle to tsetse-infested areas, and the information was triangulated against matrix scoring to substantiate villagers' perception of high-risk areas with respect to transmission of trypanosomiasis (*gandi*).

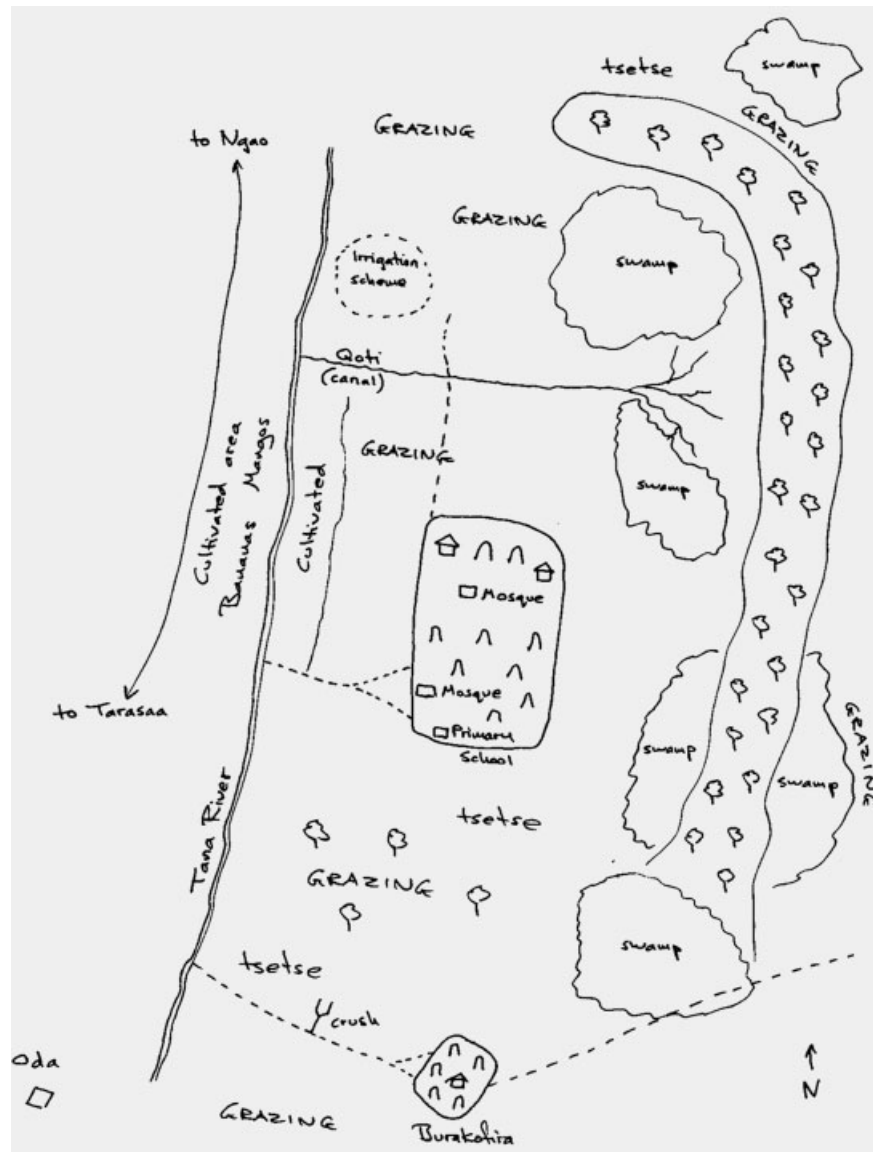
A **time line** (e.g., *Figure 12.12*) is a useful way of recording what a community considers to be important past events, with their chronology. This can be used to develop discussion about changes over time relevant to the issue of interest, such as past animal health interventions and changing livestock demographics or production methods.

A **seasonal calendar** is a time-related method that uses counters to indicate the relative occurrence of diseases. *Figure 21.5* is an example relating to several diseases and disease-related factors, also in the Tana River District. Thus, *gandi* was common in the hot and wet seasons, *Hageiya* and *Bona hageiya* (Gregorian-calendar months October–March), and is therefore consistent with the observations of contact with tsetse flies. This information was triangulated with two other participatory methods: (1) matrix scoring of disease signs indicated that herders characterized *gandi* using similar clinical signs and causal factors to those described by veterinarians; (2) mapping showed that herders identified tsetse habitats and considered these areas to be high-risk areas for *gandi*.

A **transect walk** involves use of direct observation, informal interview and visualization to describe and show the location and distribution of resources, features, landscape and main land uses along a given narrow, straight section (transect) of an area (Ameri *et al.*, 2009). A key informant accompanies, and is interviewed by, the researcher. Other community members who are encountered may be informally interviewed. A diagram showing land use and livestock can then be constructed. Transect walks can be used to identify

²⁴ In the studies from which the examples in this section are drawn, agreement was quantified using the Kendal coefficient of concordance (see *Table 14.2*) and was found to be generally good.

Fig. 21.4 Participatory map of Kipao village, Tana River District, Kenya. (Source: Catley *et al.*, 2004. Reproduced with permission of Elsevier.)



animal-husbandry systems and other production activities; identify major problems; learn about local technology and practices; probe the information that has already been mentioned by the community; and triangulate data collected through other tools such as mapping.

All of these methods that aim to produce a tangible output (i.e., proportional piling, matrices, mapping, calendars), however, generate a final physical product that only provides some of the available data. Valuable information also can be obtained from the discussion that arises during the exercise, or by the participants presenting the results to the individual who facilitates the exercise. The facilitator also may use the results as the basis of an informal interview (called 'interviewing the matrix' or 'interviewing the map', and so on), in

which the facilitator asks participants to discuss and explain features of the output.

Table 21.6 lists some topics that can be investigated by participatory epidemiology, with appropriate methods.

Strengths and weaknesses of participatory epidemiology

Conventional survey methods (especially questionnaires) are subject to several particular types of bias when used in less-economically-developed countries (Chambers, 1983):

- **spatial bias:** investigators may be more likely to travel on better roads; therefore more remote areas

	Orma seasons												
	Hageiya			Bona hageiya			Gana			Shur-icha	Bona adolesia		
	O	N	D	J	F	M	A	M	J	J		A	S
	Gregorian calendar												
Rainfall	•••						•••••			••			
Trypanosomiasis	•••••			•••••			•			•		••	
Foot-and-mouth disease	••			•••••			••			••		••	
Haemorrhagic form <i>T.vivax</i> (<i>buku</i>)	•••••			••••• ••••• •••••						•		••	
Contagious bovine pleuropneumonia (<i>somba</i>)				•			••			•••		••••• •••••	
Contact with tsetse (<i>gandi</i>)	•••••			•••••			••			•••		••	
Contact with Tabanids (<i>kobabe</i>)	••••• ••••• •••••			••			••						
Contact with ticks (<i>shilmi</i>)	•••			••••• •••••			•			•		••	
Contact with buffalo (<i>gadarsi</i>)	••			••••• •••••								•• ••	

Fig. 21.5 Seasonal calendar for livestock diseases and disease-related factors in Tana River District, Kenya. (Adapted from Catley *et al.*, 2002b.)

may be under-represented (i.e., sampling is by convenience: see Chapter 13);

- **project bias:** researchers may be channelled to areas considered to be important, where work is ongoing, where staff are located and so on;
- **person bias:** only influential, rich people may be interviewed, and they may be ignorant of the circumstances of the poor, and therefore of their animals;
- **gender bias:** women usually are not included as participants, or do not participate fully in mixed-gender groups²⁵;

²⁵ Men and women often have different knowledge, different access to resources, including health-related resources, and different responsibilities (e.g., women often manage small livestock, particularly poultry). Gender-sensitive methods therefore have been developed (Bagnol, 2008; Ahlers *et al.*, 2009).

- **dry-season bias:** malnutrition and high levels of morbidity and mortality are more common in the dry season; surveys conducted at other times may therefore underestimate problems;
- **'diplomatic' bias:** 'politeness' and 'diplomacy' may try to hide poverty and its attendant problems.

Participatory approaches can help to overcome some of these biases; for example, by reaching places away from main roads, meeting all kinds of people (rich and poor), visiting areas beyond project areas, and understanding seasonality (e.g., by using seasonal calendars). They also may be the only alternative when sample surveys are precluded (Carruthers and Chambers, 1981); notably if the target population is scattered over a wide and remote area, if there is no base for a sampling frame, and if a study aims to look at many variables, rendering choice of which variable on which to base sample-size calculations difficult.

Table 21.6 Some topics investigated by participatory epidemiology. (Adapted from Conroy, 2005.)

Topic	Participatory methods*
Contact between adjacent livestock populations	Participatory mapping
Contact with disease vectors or high-risk disease areas	Participatory mapping
History of livestock diseases in the community	Time lines
Prioritizing livestock diseases	Ranking
Differential diagnosis of diseases	Matrix scoring
Seasonal variations in diseases and exposure to vectors	Seasonal calendars
Age-specific disease incidence and mortality	Proportional piling
Economic and social losses due to disease	Matrix scoring; proportional piling
Analysis of control options	Matrix scoring; ranking
Analysis of service providers	Matrix scoring; ranking
Assessing effect of treatment or control	'Before and after' scoring

* Semi-structured interviews can provide information on all topics.

Participatory epidemiology, however, also has weaknesses (Catley, 2000; Mariner, 2000):

- there are negative perceptions of qualitative approaches among some veterinary epidemiologists, who focus on numerical methods;
- there is a need for good training to ensure best practice and to correct misconceptions (e.g., that questionnaires are participatory methods);
- success depends on indigenous knowledge; this is often good amongst pastoralists, but may be less developed in communities that are less dependent on livestock.

Participatory approaches and methods are described in detail by Catley (1999, 2005), Mariner (2000), Catley and Mariner (2001), Catley and others (2002a, 2013), Kumar (2002), Conroy (2005), Bagnol (2008), Ameri and others (2009), VSO (2009) and Tchangai and others (2010).

Some examples of participatory epidemiology

Some examples of participatory studies and the techniques that they used are listed *Table 21.7*. Initiatives also have included general improvement in animal health (Mugunieri *et al.*, 2004), and delivery of rinderpest vaccination to remote areas of Ethiopia, Sudan

and Uganda (Catley and Leyland, 2001) as part of the global rinderpest eradication campaign. Jost and others (2007) list some national participatory disease-surveillance programmes. Grace and others (2008) describe **participatory risk assessment** for assessing and managing food safety in less-economically-developed countries.

Companion-animal surveillance

Surveillance of companion animals has been traditionally hampered by the scattered nature of companion-animal practices and limitations to data-recording and extraction techniques (Robotham and Green, 2004; Ward and Kelman, 2011). Surveillance therefore has been limited generally to data in established databases that record details of companion-animal conditions, such as the US's *Veterinary Medical Databases* (see Chapter 11), registries and insurance-company data, or to passive surveillance of particular diseases, notably rabies (e.g., Blanton *et al.*, 2006; Tenzin *et al.*, 2011) and leishmaniasis (Palatnik-de-Sousa and Day, 2011). Research on companion-animal surveillance has tended to focus on systems to detect adverse drug reactions; for example, Moore and others (2006) describe the recognition of adverse vaccinal reactions in dogs attending over 400 veterinary practices enlisted in a system utilizing standardized electronic medical records in the US. Although individual surveys and observational studies provide valuable information on conditions in companion animals, they lack a key characteristic of surveillance: *ongoing* data collection (see *Table 21.1*).

Some companion-animal health schemes provide continuous aspects of conditions. In the UK, these include the *British Veterinary Association/Kennel Club* schemes (see earlier in this chapter); *CICADA* (*Computer-based Investigation of Companion Animal Disease Awareness*), which allows reporting of several diseases of pets; *Acarus*, the University of Bristol's laboratory for the detection of arthropod-borne infections; *DACTARI* (the Department for Environment, Food and Rural Affairs' *Dog and Cat Travel and Risk Information* scheme), a voluntary scheme for reporting cases of exotic disease in dogs and cats; and *SARSS* (*Suspected Adverse Reaction Surveillance Scheme*), which monitors reports of, and trends in, adverse drug reactions (Carruthers, 2009). Other companion-animal surveillance systems are exemplified in Chapter 11.

More extensive systems are being developed. For example, a national surveillance project, *Disease Watchdog*, was launched in Australia in 2010 to capture

Table 21.7 Some examples of veterinary participatory studies.

Country	Topic	Participatory methods	Source
Cambodia	Impact of foot-and-mouth disease	Semi-structured interviews; ranking; matrix scoring; proportional piling	Bellet <i>et al.</i> (2012)
Corsica	Evaluation of surveillance systems	Proportional piling	Calba <i>et al.</i> (2015a)
Ethiopia	Impact of epizootic lymphangitis	Focus groups; ranking; matrix scoring	Scantlebury <i>et al.</i> (2015)
India	Perceptions of animal and zoonotic diseases by the transhumant Van Gujjar community	Semi-structured interviews; ranking; proportional piling; transect walks	Wright and Thrusfield (2016)
Indonesia	Surveillance for highly-pathogenic avian influenza	Semi-structured interviews; proportional piling; participatory mapping	Azhar <i>et al.</i> (2010); Mariner <i>et al.</i> (2014)
Japan	Post-traumatic stress disorder associated with foot-and-mouth disease control	Semi-structured interviews	Hibi <i>et al.</i> (2015)
Kenya	Bovine trypanosomiasis	Matrix scoring; proportional piling; seasonal calendars	Catley <i>et al.</i> (2002b)
	Diseases of camels	Semi-structured interviews; matrix scoring; proportional piling; seasonal calendars; time lines	Mochabo <i>et al.</i> (2005)
	Pastoralist knowledge of peste des petits ruminants	Semi-structured interviews; matrix scoring; proportional piling; participatory mapping	Kihu <i>et al.</i> (2012)
	Incidence and impact of livestock diseases on nomadic pastoralists	Focus groups; semi-structured interviews; matrix scoring; proportional piling; seasonal calendars; participatory mapping	Bett <i>et al.</i> (2009)
Kenya/ Somalia	Identification of rinderpest resulting from transhumance	Semi-structured interviews; time lines; participatory mapping	Mariner and Roeder (2003), OIE (2003)
Madagascar	Spectrum and significance of livestock diseases	Semi-structured interviews; ranking; matrix scoring	Bardsley and Thrusfield (2014)
Nigeria	Characteristics of foot-and-mouth disease	Semi-structured interviews	Olabode <i>et al.</i> (2014)
	Ethnoveterinary practices among Fulani pastoralists	Semi-structured interviews; transect walks; probing; triangulation	Alhaji and Babalobi (2015)
	Importance of diseases in livestock among Fulani pastoralists	Focus groups; semi-structured interviews; proportional piling; seasonal calendars; transect walks; triangulation	Bolajoko <i>et al.</i> (2011); Elelu <i>et al.</i> (2016)
	Surveillance for highly-pathogenic avian influenza	Matrix scoring; proportional piling; seasonal calendars; time lines; transect walks; participatory mapping	Kwaghe <i>et al.</i> (2011)
Tanzania	Association between heat intolerance syndrome and foot-and-mouth disease	Matrix scoring; proportional piling	Catley (2004)
Tanzania/ Sudan	Spectrum and significance of livestock diseases	Semi-structured interviews; matrix scoring; proportional piling; time lines	Mariner <i>et al.</i> (2002)
Uganda	Management and importance of tick-borne diseases in transhumant cattle	Semi-structured interviews; ranking; matrix scoring; proportional piling; participatory mapping	Byaruhanga <i>et al.</i> (2015)
Zimbabwe	Constraints to livestock production on smallholder farms	Focus groups; semi-structured interviews; proportional piling; transect walks; triangulation	Chatikobo <i>et al.</i> (2013)

data on diseases of dogs and cats (Ward and Kelman, 2011). Registration is voluntary but, within its first year, an estimated 31% of veterinary practices in Australia joined the project. A major benefit is the project's focus on geographical analysis of data, facilitating detection of disease clusters.

The increasing use of electronic practice management systems (utilized, for example, by over 90% of veterinary practices in the UK: Brodbelt *et al.*, 2011), linked to standardized disease-coding systems (see Chapter 11), and integrated electronic reporting systems (Day *et al.*, 2012), should expand opportunities for companion-animal surveillance in the future.

O'Neill and others (2014) review the strengths and weaknesses of data sources for canine health surveillance.

Wildlife surveillance

Several purposes of wildlife disease surveillance can be identified (Willette *et al.*, 2012):

- **detection and monitoring of emerging infectious diseases** (including zoonoses), many of which are sylvatic and related to ecosystem dynamics (see Chapter 7);
- **detection of health threats** to wildlife, domestic animals and humans (e.g., risk of exposure to toxic levels of lead in game meat: Hunt *et al.*, 2006);
- most recently, **monitoring of potential bioterrorism threats**, including plague, tularaemia, Ebola and anthrax, all of which can be found in wildlife (CDC, 2010).

Surveillance in wildlife captures information on disease in four main ways (Wobeser, 2006²⁶):

1. **searching for sick or dead animals** (e.g., rabbits for infection with rabbit haemorrhagic disease: Vilafuerte *et al.*, 1994);
2. **searching for the causal agent** (e.g., lungworms in deer: Lankester, 2001);
3. **searching for physiological/immunological responses to the causal agent** (e.g., antibodies to tuberculosis in waterfowl: Cromie *et al.*, 2000);
4. **searching for evidence of the disease in species other than the primary species** (e.g., plague in predators rather than rodents: Gage and Monteneri, 1994).

²⁶ See this author for details of the problems and bias associated with these methods.

The need for a global wildlife disease surveillance system, with the general goals of detecting, monitoring and controlling disease in wildlife, was recognized at the beginning of this century (Reaser *et al.*, 2002). However, the depth to which wildlife surveillance has been undertaken is patchy (e.g., Figure 21.6). Some diseases are particular focusses of attention; for example, influenza (Fouchier *et al.*, 2003), with wild birds increasingly being targeted as sentinel species (Hoye *et al.*, 2010). Several organizations that undertake wildlife surveillance also may combine, share and distribute findings in formal networks. For example, in the UK, the *GB Wildlife Disease Surveillance Partnership* includes the *Animal and Plant Health Agency*, *Scottish Agricultural College*, *Institute of Zoology*, *Food and Environmental Research Agency*, *Centre for Environment, Fisheries and Aquaculture Science*, *Wildfowl Wetlands Trust*, *Natural England* and *Forestry Commission England*, and incorporates both scanning and targeted surveillance in a quarterly report (GBWDSP, 2015).

Further discussions of surveillance in wildlife are presented by Mörner and co-workers (2002), Nusser and others (2008), OIE (2010, 2015c) and Grogan and others (2014).

Aquatic-animal surveillance

The goals of surveillance of diseases of aquatic animals (in both wild and cultured species) are similar to those for terrestrial-animal diseases. However, notable differences in diseases between these two categories include, in the former, the absence of reliable methods of ridding carrier populations of directly-transmitted pathogens; the lack of scientific knowledge on the susceptibility of most non-cultured species; and the high variability in prevalence of infection, ranging from one infected individual to an entire population. It therefore may be necessary to establish baseline health status at the beginning of a surveillance programme (e.g., for coral reefs: Woodley *et al.*, 2015). Definition of zones delineating infected and uninfected populations, and therefore determining areas over which surveillance should be conducted, may be similarly difficult. In freshwater aquaculture enterprises, if farms obtain their water from unshared river systems then zones can be based on individual facilities. However, if facilities are connected to common river systems, a zone may comprise an entire river system, and may even be multinational (e.g., the Mekong Delta or Great Lakes). Zones also may extend into the sea if farms either discharge

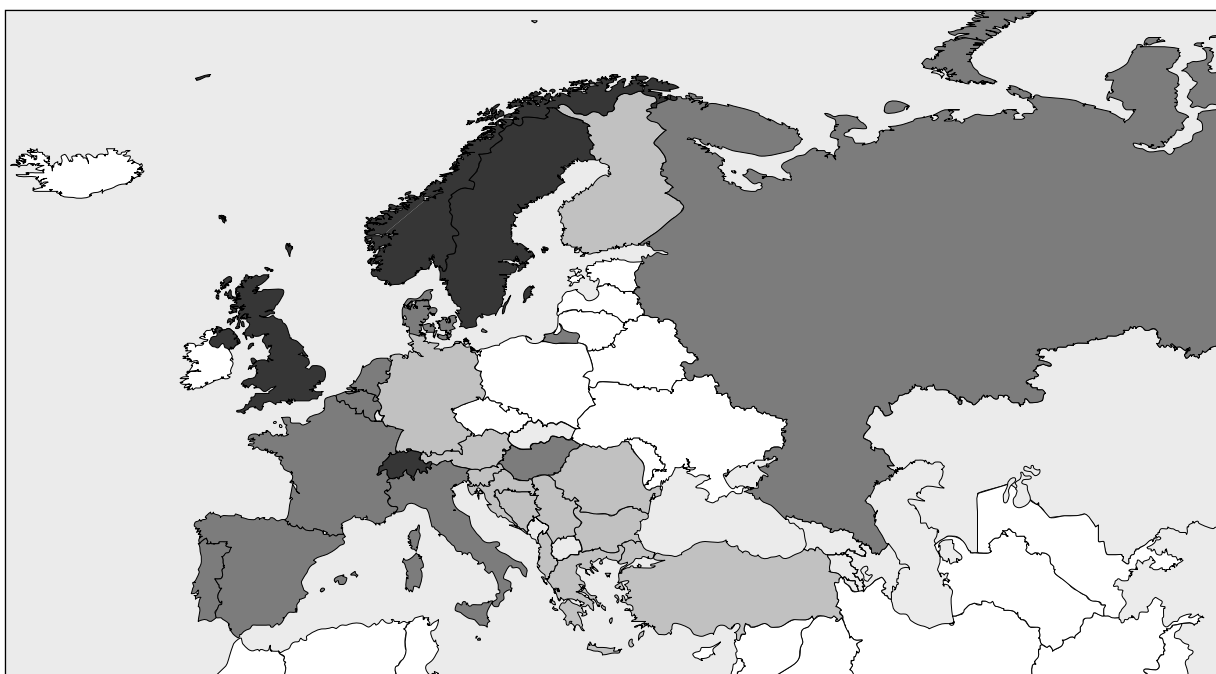


Fig. 21.6 Map of Europe depicting the level of wildlife health surveillance according to self-evaluation of participating countries. Dark grey: comprehensive general wildlife surveillance; medium grey: partial general wildlife surveillance (wide range of programmes, but restricted in various ways); light grey: no general wildlife health surveillance, but some degree of targeted surveillance for a few specified diseases. Categories based on Leighton (1995). (Source: Kuiken, *et al.*, 2011. Reproduced with permission of the World Organisation for Animal Health (OIE).)

effluent, or translocate juvenile animals, into it (e.g., abalone farms: Jones and Fletcher, 2012). Zoning in marine and estuarine areas also is complicated, depending on oceanographic characteristics, distribution of hosts and vectors, and unrelated human activities (e.g., recreational pursuits and shipping).

A range of surveillance programmes address aquatic-animal diseases in cultured and wild species, notably fish. For example, Nordic countries target virus pathogens (e.g., viral haemorrhagic septicaemia virus of salmonids), bacterial conditions (e.g., furunculosis) and parasitic diseases (e.g., infection with *Gyrodactylus salaris*), and Denmark, Norway and Scotland run a surveillance programme for virus infections in wild marine populations in European Coastal waters (Håstein *et al.*, 2001). Australia's surveillance system requires domestic reporting of diseases, including those on Australia's National List of Reportable Diseases of Aquatic Animals, and provides field and laboratory diagnostic resources and surveillance guidelines (AGDA, 2015).

A detailed discussion of surveillance for aquatic-animal diseases is presented by Subasinghe and others (2004).

Assessing the performance of surveillance systems

Evaluation of veterinary surveillance systems has been the focus of substantial recent attention, although medical systems have been assessed for some years (Klaucke *et al.*, 1988; WHO, 2006). Many criteria have been cited; Hendrikx and others (2011), for instance, list 78 assessment criteria for systems in animal health and food safety. *Table 21.8* presents some key attributes for evaluating surveillance activities (a full list is reported by Hoinville, 2013).

Improving the performance of surveillance: risk-based surveillance

The statistical basis of surveys to estimate disease prevalence and to detect disease have been presented in Chapter 13. These methods can be refined for particular surveillance approaches.

The surveillance sensitivity of detecting the presence of disease (*Table 21.8*), SSe_d , in a large population is given by:

$$SSe_d = 1 - (1 - P)^n,$$

Table 21.8 Attributes that can be used for evaluating surveillance activities. (Adapted from Hoinville *et al.*, 2013.)

<i>Evaluation attribute</i>	<i>Definition</i>
Economic efficiency	Whether the surveillance system produces the desired effect without wasting resources. Three levels of economic efficiency are defined: 1. <i>optimization</i>: maximizing the net benefit to society achieved by the allocation of scarce resources to animal health surveillance and intervention to avoid losses resulting from animal diseases; 2. <i>acceptability</i>: ensuring that the benefits generated by a mitigation policy at least covers its costs; 3. <i>cost minimization</i>: ensuring that a technical target for disease mitigation (e.g., time to detection) is achieved at minimum cost without quantifying the benefit in monetary terms
False-alarm rate	The false-alarm rate is the proportion of negative events (e.g., periods without outbreaks) incorrectly classified as events (outbreaks); this is equivalent to $1 - \text{specificity}$ (see Chapter 20); very low specificity would result in a surveillance system indicating many 'false' outbreaks
Sensitivity	The sensitivity of a surveillance system can be considered on three levels: 1. <i>surveillance sensitivity (of case detection)</i>: refers to the proportion of individual animals ('sensitivity' in Chapter 20) or herds ('aggregate sensitivity' in Chapter 20) in the population of interest that have the health-related condition of interest and that the surveillance system is able to detect; 2. <i>surveillance sensitivity (of outbreak detection)</i>: refers to the probability that the surveillance system will detect a significant increase (outbreak) of disease; this requires a clear definition of what constitutes an outbreak (see Chapter 4); 3. <i>surveillance sensitivity (presence)</i>: refers to the probability that disease will be detected if present at a certain level (see Chapter 13)
Timeliness	Timeliness is usually defined as <i>the time between any two defined steps in a surveillance system</i> . The time points that are chosen are likely to vary depending on the purpose of the surveillance activity. For outbreak detection, this can be defined using various time points (e.g., the time between exposure to the infectious agent and the initiation of the risk-mitigation measures; or the time between when disease could have been detected and reported, and the time when it actually was reported) For planning purposes, timeliness also can be defined as <i>whether surveillance detects changes in time for risk-mitigation actions to reduce the likelihood of further spread</i> The precise definition of timeliness that is chosen should be stated as part of the evaluation process

where P = anticipated overall prevalence, and n = number of animals sampled.

Thus, if $P = 0.05$ and $n = 25$:

$$\begin{aligned}
 SSe_d &= 1 - (1 - 0.05)^{25} \\
 &= 1 - 0.277 \\
 &= 0.723.
 \end{aligned}$$

This value also can be obtained indirectly from Appendix XIII. Consulting row 5 (prevalence = 5%), column 4 (25 animals sampled), the probability of *failing* to detect diseased animals is 0.277; therefore the probability of detecting disease (SSe_d) = $1 - 0.277 = 0.723$.

If the test that is being used does not have perfect sensitivity, the formula is developed thus:

$$SSe_d = 1 - \{1 - (P \times Se)\}^n,$$

where Se = test sensitivity.

Therefore, if $Se = 0.90$, $P = 0.05$ and $n = 25$:

$$\begin{aligned}
 SSe_d &= 1 - \{1 - (0.05 \times 0.90)\}^{25} \\
 &= 1 - 0.955^{25} \\
 &= 1 - 0.316 \\
 &= 0.684.
 \end{aligned}$$

Thus, if an imperfect test is used, the value of SSe_d falls relative to the value when a perfect test is used.

Additionally, prevalence of disease sometimes is unevenly distributed in a population. For example, disease may be more common in young, than old, animals. It is then possible to improve the performance of a surveillance strategy by adopting a risk-based approach, which targets animals that are at increased risk of disease.

For example, suppose that a disease is three times more likely to affect young animals than older animals; that is, there is a threefold increase in risk in the

high-risk group (young animals) relative to the low-risk group (older animals). Also suppose that 20% of the population comprises young animals.

First, the risk in the low-risk group needs to be adjusted according to the proportion of the population that is at low risk (Cameron *et al.*, 2014):

$$AR_L = \frac{1}{(R_H \times Pr_H) + Pr_L},$$

where:

AR_L = adjusted risk in low-risk group;

R_H = risk in high-risk group relative to risk in low-risk group;

Pr_H = proportion of the population that are high risk;

Pr_L = proportion of the population that are low risk.

Therefore:

$$\begin{aligned} AR_L &= \frac{1}{(3 \times 0.2) + 0.8} \\ &= 1/1.4 \\ &= 0.714. \end{aligned}$$

Then, the adjusted risk in the high-risk group,

$$\begin{aligned} AR_H &= AR_L \times R_H \\ &= 0.714 \times 3 \\ &= 2.142. \end{aligned}$$

Thus, if the anticipated overall prevalence is 0.05, then the prevalence in the low-risk group (older ani-

mals) = $0.05 \times 0.714 = 0.036$, and the prevalence in the high-risk group (young animals) = $0.05 \times 2.142 = 0.107$.

It is therefore logical to target surveillance at young animals, and so, also sampling 25 animals, with a test with a sensitivity of 0.90:

$$\begin{aligned} SS_{ed} &= 1 - \{1 - (P \times Se)\}^n \\ &= 1 - \{1 - (0.107 \times 0.90)\}^{25} \\ &= 1 - 0.080 \\ &= 0.920. \end{aligned}$$

Thus, by focussing surveillance on the group at higher risk, there has been an absolute increase in the surveillance sensitivity of detecting disease of approximately 24% (from 68.4% to 92.0%), with the same number of sampled animals.

General principles of risk-based surveillance and its statistical background in relation to demonstration of freedom from disease are addressed comprehensively by Cameron and others (2014). Evaluation of surveillance systems is discussed further by Kleinman and Abrams (2006), Hadorn and Stärk (2008), Amezcua and others (2010), Drewe and others (2012), Hoinville and others (2013) and Calba and others (2015b). An evaluation framework, *SERVAL* (*SuRveillance EVALuation framework*), is described by Drewe and others (2015), and a design and evaluation framework for risk-based surveillance, *RISKSUR*, is detailed by Comin and others (2016).

Further reading

- Babo Martins, S., Rushton, J. and Stärk, K.D. (2016) Economic assessment of zoonoses surveillance in a 'One Health' context: a conceptual framework. *Zoonoses and Public Health*, **63**, 386–395
- Beatty, A., Scott, K. and Tsai, P. (Rapporteurs) (2008) *Achieving Sustainable Global Capacity for Surveillance and Response to Emerging Diseases of Zoonotic Origin*. National Academies Press, Washington DC
- Blancou, J. (2003) *History of the Surveillance and Control of Transmissible Animal Diseases*. Office International des Épidémiologies, Paris
- Brückner, G., Knopf, L., MacDiarmid, S.C. and Münstermann, S. (Eds) (2014) *Guide to Terrestrial Animal Health Surveillance*. World Organisation for Animal Health (OIE), Paris
- Calb, C., Goutard, F.L., Hoinville, L., Hendrikx, P., Lindberg, A. *et al.* (2015) Surveillance systems evaluation: a systematic review of existing approaches. *BMC Public Health*, **15**, 448; doi 10.1186/s12889-015-1791-5
- Cameron, A. (2012) *Manual of Basic Animal Disease Surveillance*. African Union, Interafrican Bureau for Animal Resources, Nairobi
- Cameron, A., Njeumi, F., Chibeu, D. and Martin, T. (2014) *Risk-Based Disease Surveillance*. FAO Animal Production and Health Manual No. 17. Food and Agriculture Organization of the United Nations, Rome
- Carruthers, H. (2009) Disease surveillance in small animal practice. *In Practice*, **31**, 356–358
- Catley, A., Alders, R.G. and Wood, J.L.N. (2012) Participatory epidemiology: approaches, methods, experiences. *The Veterinary Journal*, **191**, 151–160
- Chenais, E., Sternberg-Lewerin, S., Boqvist, S., Emanuelson, U., Aliro, T. *et al.* (2015) African swine

- fever in Uganda: qualitative evaluation of three surveillance methods with implications for other resource-poor settings. *Frontiers in Veterinary Science*, **2**, 51; doi 10.3389/fvets.2015.00051
- Conroy, C. (2005) *Participatory Livestock Research: A Guide*. ITDG Publishing, Rugby
- Corsin, F., Georgidis, M., Hammell, K.L. and Hill, B. (Eds) (2009) *Guide for Aquatic Animal Health Surveillance*. Office International des Epizooties, Paris
- Curtis, C.R. and Farrar, J.A. (Eds) (1990) The National Animal Health Monitoring System in the United States. *Preventive Veterinary Medicine*, **8**, 87–225
- DEFRA (2002) *Partnership, Priorities and Professionalism. A Proposed Strategy for Enhancing Veterinary Surveillance in the UK*. Department for Environment, Food and Rural Affairs, London
- DEFRA (2003) *A Strategy for Enhancing Veterinary Surveillance in the UK*. Department for Environment, Food and Rural Affairs, London
- DEFRA (2011) *A Review of the Implementation of the Veterinary Surveillance Strategy (VSS)*. Department for Environment, Food and Rural Affairs, London
- Doherr, M.G. (Ed.) (2012) First International Conference on Animal Health Surveillance (ICAHS). *Preventive Veterinary Medicine*, **105**, 165–252
- Doherr, M.G. and Audigé, L. (2001) Monitoring and surveillance for rare health-related events: a review from the veterinary perspective. *Philosophical Transactions of the Royal Society of London, Series B*, **356**, 1097–1106
- Dórea, F.C., Sanchez, J. and Revie, C.W. (2011) Veterinary syndromic surveillance: current initiatives and potential. *Preventive Veterinary Medicine*, **101**, 1–17
- Drewe, J.A., Hoinville, L.J., Cook, A.J.C., Floyd, T. and Stärk, K.D.C. (2012) Evaluation of animal and public health surveillance systems: a systematic review. *Epidemiology and Infection*, **140**, 575–590
- Dufour, B. and Hendrickx, P. (2009) *Epidemiological Surveillance in Animal Health*, 2nd edn. Food and Agriculture Organization of the United Nations, Rome
- Dufour, B., Pouillot, R. and Toma, B. (2001) Proposed criteria to determine whether a territory is free of a given animal disease. *Veterinary Research*, **32**, 545–563
- East, I.J., Wicks, R.M., Martin, P.A.J., Sergeant, E.S.G., Randall, L.A. et al. (2013) Use of a multi-criteria analysis framework to inform the design of risk based general surveillance systems for animal health in Australia. *Preventive Veterinary Medicine*, **112**, 230–247. (An exploration of the spatial distribution of surveillance efforts in relation to likelihood of disease incursion)
- FAO (1999) *Manual on Livestock Disease Surveillance and Information Systems*. FAO Animal Health Manual No. 8. Food and Agriculture Organization of the United Nations, Rome
- FAO (2011) *Challenges of Animal Health Information Systems and Surveillance for Animal Diseases and Zoonoses*. Proceedings of the International Workshop Organized by FAO, 23–26 November 2010, Rome, Italy. FAO Animal Production and Health Proceedings No. 14. Food and Agriculture Organization of the United Nations, Rome
- Geering, W.A. (1992) Some epidemiological principles in disease surveillance and reporting. *Office International des Epizooties Quarterly Report (Asian and Pacific Region)*, **1**, 31–35. (A brief discussion of information requirements for disease surveillance systems)
- Greiner, M. and Dekker, A. (2005) On the surveillance for animal diseases in small herds. *Preventive Veterinary Medicine*, **70**, 223–234. (A discussion of disease detection in small herds)
- Hinman, A.R. (1977) Analysis, interpretation, use and dissemination of surveillance information. *Bulletin of the Pan American Health Organization*, **11** (4), 338–343
- Höhle, M., Paul, M. and Held, L. (2009) Statistical approaches to monitoring and surveillance for infectious diseases for veterinary public health. *Preventive Veterinary Medicine*, **91**, 2–10
- Hutton, N.E. and Halvorson, L.C. (1974) *A Nationwide System for Animal Health Surveillance*. National Academy of Sciences, Washington DC
- IOM (Institute of Medicine) and NRC (National Research Council) (2009) *Sustaining Global Surveillance and Response to Emerging Zoonotic Diseases*. National Academies Press, Washington DC
- Jebara, K.B. (2004) Surveillance, detection and response: managing emerging diseases at national and international levels. *Revue Scientifique et Technique, Office International des Epizooties*, **23**, 709–715
- Kellar, J.A. (2005) Portrait of the national veterinary service as a surveillance continuum. *Preventive Veterinary Medicine*, **67**, 109–115
- Keusch, G.T., Papaioanou, M., González, M.C., Scott, K. A. and Tsai, P. (Eds) (2009) *Sustaining Global Surveillance and Response to Emerging Zoonotic Diseases*. National Academies Press, Washington DC
- Konigshofer, H.O. (1972) *The Organisation of Surveillance of Animal Diseases*. World Health Organization inter-regional seminar on methods of epidemiological surveillance of communicable diseases including zoonoses and food-borne diseases, Nairobi, 9–20 October, 1972. Working Paper No. 8
- Kumar, S. (2002) *Methods for Community Participation: A Complete Guide for Practitioners*. Vistaar Publications/ITDG Publishing, New Delhi and Rugby
- Lemon, S.M., Hamburg, M.A., Sparling, P.F., Choffnes, E. R. and Mack, A. (Rapporteurs) (2007) *Global Infectious*

- Disease Surveillance and Detection: Assessing the Challenges – Finding the Solutions*. National Academies Press, Washington DC
- Lindberg, A., Berezowski, J. and Ward, M. (Eds) (2015) Second International Conference on Animal Health Surveillance (ICAHS). *Preventive Veterinary Medicine*, **120**, 1–130
- Lysons, R.E., Gibbens, J.C. and Smith, L.H. (2007) Progress with enhancing veterinary surveillance in the United Kingdom. *Veterinary Record*, **160**, 105–112
- MacDaniel, H.A. and Sheridan, M.K. (Coordinators) (2001) Traceability of animals and animal products. *Revue Scientifique et Technique, Office International des Epizooties*, **20**, 359–661
- Mariner, J.C. and Paskin, R. (2000) *Manual on Participatory Epidemiology – Method for the Collection of Action-Oriented Epidemiological Intelligence*. Food and Agriculture Organization of the United Nations, Rome
- Mariner, J.C., Hendrickx, S., Pfeiffer, D.U., Costard, S., Knopf, L. *et al.* (2011) Integration of participatory approaches into surveillance systems. *Revue Scientifique et Technique, Office International des Epizooties*, **30**, 653–659
- Mariner, J.C., Jeggo, M.H., van't Klooster, G.G.M., Geiger, R. and Roeder, P.L. (2003) Rinderpest surveillance performance monitoring using quantifiable indicators. *Revue Scientifique et Technique, Office International des Epizooties*, **22**, 837–847. (A description of performance monitoring of surveillance in less-economically-developed countries, with rinderpest as the example)
- Martinez, T.A., Kaneene, J.B., Lloyd, J.W. and Brown, M.I. (1992) Potential users and user requirements of an animal health monitoring system in a developing country: case study of Honduras. *Preventive Veterinary Medicine*, **13**, 1–11
- Meah, M.N. and Lewis, G.A. (2000) *Veterinary Surveillance in England and Wales – A Review*. Ministry of Agriculture, Fisheries and Food Publications, London
- Mörner, T., Obendorf, D.L., Artois, M. and Woodford, M. H. (2002) Surveillance and monitoring of wildlife diseases. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 67–76
- Moorhouse, P.D. and Hugh-Jones, M.E. (1981) Serum banks. *Veterinary Bulletin*, **51**, 277–290
- Oidtman, B., Peeler, E., Lyngstad, T., Brun, E., Jensen, B. *et al.* (2013) Risk-based methods for fish and terrestrial animal disease surveillance. *Preventive Veterinary Medicine*, **112**, 13–26
- OIE (2010) Proceedings of the First OIE Global Conference on Animal Identification and Traceability 'From Farm to Fork', 23–25 March 2009, Buenos Aires (Argentina). World Organisation for Animal Health (OIE), Paris
- PAHO/WHO (1999) Third Meeting of the Task Force on Surveillance for Emerging and Reemerging Infectious Diseases, Mexico City, Mexico, 16–17 November 1998. Final Report. Pan American Health Organization/World Health Organization Communicable Diseases Program, Division of Disease Prevention and Control. PAHO/HCP/HCT/141/99. Pan American Health Organization, Washington DC
- Palmer, S., Sully, M. and Fozdar, F. ((2009) Farmers, animal disease reporting and the effect of trust: a study of West Australian sheep and cattle farmers. *Rural Society*, **19**, 32–48
- Pini, A., Parodi, P., Demeneghi, D., Belemu, U., Kabilika, H. *et al.* (Eds) (1994) *Basic Procedures for Diagnostic Veterinary Laboratories in Developing Countries*. World Health Organization/Food and Agriculture Organization of the United Nations, Rome. (A guide to the selection and handling of appropriate tissue samples to identify animal diseases in less-economically-developed countries)
- Poppensiek, G.C., Budd, D.E. and Scholtens, R.G. (1966) *A Historical Survey of Animal-Disease Morbidity and Mortality Reporting*. Publication No. 1346. National Academy of Sciences, Washington DC
- Queenan, K., Häsler, B. and Rushton, J. (2016) A One Health approach to antimicrobial resistance surveillance: is there a business case for it? *International Journal of Antimicrobial Agents*, **48**, 422–427
- Radunz, B. (2006) Surveillance and risk management during the later stages of eradication. Experiences from Australia. *Veterinary Microbiology*, **112**, 283–290
- Saatkamp, H.W., Huirne, R.B.M., Dijkhuizen, A.A., Geers, R., Noordhuizen, J.P.T.M. *et al.* (1994) The potential value of identification and recording systems in contagious disease control. In: Proceedings of the Seventh International Symposium on Veterinary Epidemiology and Economics, Nairobi, 15–19 August 1994. Eds Rowlands, G.J., Kyule, M.N. and Perry, B.D. *The Kenya Veterinarian*, **18** (2), 391–393
- Salman, M.D. (Ed.) (2003) *Animal Disease Surveillance and Survey Systems: Methods and Applications*. Iowa State Press, Ames
- Salman, M.D. (2013) Surveillance tools and strategies for animal diseases in a shifting climate context. *Animal Health Research Reviews*, **14**, 147–150
- Salman, M.D., Stark, K.D.C. and Zepada, C. (2003) Quality assurance applied to animal disease surveillance systems. *Revue Scientifique et Technique, Office International des Epizooties*, **22**, 689–696
- Sargeant, J.M. (Ed.) (2012) Calvin W. Schwabe Symposium 2010: Animal Health Surveillance: From

- Science to Application. *Preventive Veterinary Medicine*, **105**, 253–330
- Slorach, S.A. (2013) Coordinating surveillance policies in animal health and food safety 'from farm to fork'. *Revue Scientifique et Technique, Office International des Epizooties*, **32**, 299–593
- Subasinghe, R.P., McGladerry, S.E. and Hill, B.J. (Eds) (2004) *Surveillance and Zoning for Aquatic Animal Diseases*. FAO Fisheries Technical Paper No. 451. Food and Agriculture Organization of the United Nations, Rome
- Thurmond, M.C. (2003) Conceptual foundations for infectious disease surveillance. *Journal of Veterinary Diagnostic Investigation*, **15**, 501–514
- USDA (1982) *International Directory of Animal Health and Disease Data Banks*. Miscellaneous Publication No. 1423. National Agriculture Library, United States Department of Agriculture, Washington DC
- Vergne, T., Del Rio Vilas, V.J., Cameron, A., Dufour, B. and Grosbois, V. (2015) Capture-recapture approaches and the surveillance of livestock diseases: a review. *Preventive Veterinary Medicine*, **120**, 253–264
- Vrbova, L., Stephen, C., Kasman, N., Boehnke, R., Doyle-Waters, M. *et al.* (2010) Systematic review of surveillance systems for emerging zoonoses. *Transboundary and Emerging Diseases*, **57**, 154–161
- Wagner, M.M., Moore, A.W. and Aryel, R.M. (Eds) (2006) *Handbook of Biosurveillance*. Academic Press, Amsterdam and Boston. (Includes a description of the types of data required for surveillance)
- Wendt, A., Kreienbrock, L. and Campe, A. (2015) Zoonotic disease surveillance – inventory of systems integrating human and animal disease information. *Zoonoses and Public Health*, **62**, 61–74
- WHO (undated) *STEPS Epi Info Training Guide*. World Health Organization, Geneva. (Training manual for chronic disease surveillance using Epi Info: see Appendix III)

22

Statistical modelling

Statistical testing, as a means of detecting associations, was introduced in Chapter 14. In that chapter, statistical methods were used to assess the evidence, provided by observed data, that two or more groups of observations had been drawn from a single underlying population. However, the goal of epidemiological research is frequently to evaluate a *theoretical model* of how the natural world behaves. For example, a researcher may develop a theoretical model of the causes of lamb mortality. Data are then collected in order to evaluate this theory through the use of a statistical model that captures the essential elements of the theoretical model. Statistical modelling is widely used in veterinary epidemiology, but it is unclear the extent to which it is used in linking a theoretical model of a natural phenomenon to observed data.

Statistical modelling of epidemiological data should not simply be the end-point of an epidemiological study, but should be integrated into the process of epidemiological research. Hence, modelling should be guided by, and guide, identification of the research problem, data collection and, ultimately, the need to make inference from the results of the study. Hence, a statistical model, when combined with data, enables inference regarding the problem:

Problem → Data → Model → Inference
→ Solution to problem.

Identification of the problem and, particularly, collection of appropriate data were dealt with in preceding chapters (see Chapters 13 and 15–18). However, formulation of a research question, and identification of the data to be collected, involves (formal or tacit) construction of a model linking the outcome of interest with the input variable(s) of interest. Hence, plans for modelling should be incorporated into all stages of the research process. Detailed awareness of the research question and other processes involved in data collection are important for modelling and subsequent inference.

The aim of statistical modelling is to extract appropriate information from data that is relevant to answering the research question. A useful metaphor is that of a radio transmission, in which the received data are composed of useful information (the signal) and unwanted noise:

$$\text{DATA} = \text{SIGNAL} + \text{NOISE}.$$

In statistical modelling, the signal is specified using a combination of fixed and random variables, often referred to as fixed and random effects, whereas the noise is always composed of one or more random variables.

This chapter provides a brief introduction to the role of statistical modelling in epidemiology, focussing on the use of generalized linear regression models. Several examples presented in this chapter are based on **example data** from a cohort of 189 lambs born to 113 ewes on a single farm. Each ewe had between one and three lambs. Outcomes of interest in the study were the weight of the lambs at eight weeks of age (continuous variable, measured in kilograms) and whether or not the lambs survived to 12 weeks of age (binary variable: 0 if survived, 1 if died). Possible explanatory variables included lamb birth weight (in kilograms) and litter size (single, $n = 47$ ewes/lambs; twin, $n = 56$ ewes and 112 lambs; or, triplet, $n = 10$ ewes and 30 lambs). In addition, the date of birth was recorded and the number of days since the first lamb was born was also available as an input variable. These data are modified from the data for one farm from Christley and others (2003).

Simple linear regression models

Simple linear regression models represent the relationship between a continuous output (dependent/response) variable and a single input (independent/explanatory) variable; that is, using the analogy with radio waves, between the *data* and *signal*. Where more

than one explanatory variable is included, it is called multivariable (or multiple) linear regression (see later). A simple linear relationship can be expressed using the *mathematical* model:

$$y = \alpha + \beta x,$$

where x is the input variable, y is the output variable, α represents the 'intercept' (that is, the value of y when $x = 0$) and β the **slope** of the line (that is, for unit increase in x , y increases by β). This model allows the relationship between x and y to take one of three forms:

1. there is, in fact, no relationship between x and y , in which case $\beta = 0$;
2. as x increases y also increases, in which case β is positive;
3. as x increases y decreases, in which case β is negative.

However, epidemiological data are unlikely ever exactly to follow this model, which implies that all data lie exactly on the straight line described by α and β . In reality, observations would be expected to deviate from this line due to numerous factors, including measurement errors and stochastic variation (see Chapter 23); that is, observations are subject to **noise**. For example, *Figure 22.1* displays the relationship between the birth weight of lambs and the weight of the same lambs at eight weeks of age. It is clear from the scatter plot that there is a tendency for the weight at eight weeks to be greater for lambs that were heavier

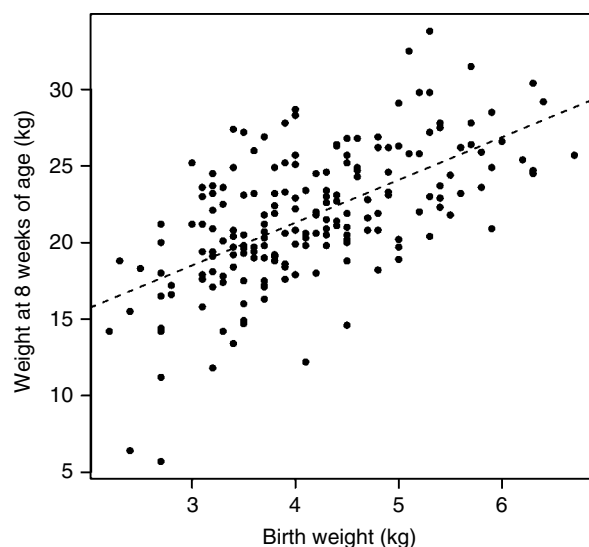


Fig. 22.1 The relationship between birth weight and weight at eight weeks among 189 lambs.

at birth, and a line of best fit (see later) representing this relationship has been included on the plot. However, it is also evident that data points vary around the line of best fit.

To allow for this variability, the mathematical model shown above can be extended to a statistical model:

$$Y_i = \alpha + \beta x_i + Z_i : i = 1, \dots, n,$$

which is depicted schematically in *Figure 22.2*. The mathematical and statistical versions of a simple linear regression model differ in several important respects. First, in the statistical representation, the term Z_i that has been included in the right side of the equation measures the amount that each observation (x_i, Y_i) deviates from the straight line $Y = \alpha + \beta x$. The Z_i are called the **residuals** and represent the noise in the system: by rearrangement, $Z_i = Y_i - (\alpha + \beta x_i)$. Notationally, the statistical model uses upper case letters to represent stochastic variables (i.e., ones that are subject to random variation) in order to distinguish these from deterministic variables (see Chapter 23), which are indicated by lower case letters. Although a seemingly subtle change, this highlights the distinction between the signal and the observed data, in that Y_i represents the predicted value of the outcome for the i th observation, given the model, whereas y_i is the observed value of the outcome for the same observation. The difference between Y_i and y_i is Z_i . The number of data points ($i = 1, \dots, n$) is also explicitly stated in the statistical model in order to highlight that estimation of α and β depends on the sample size.

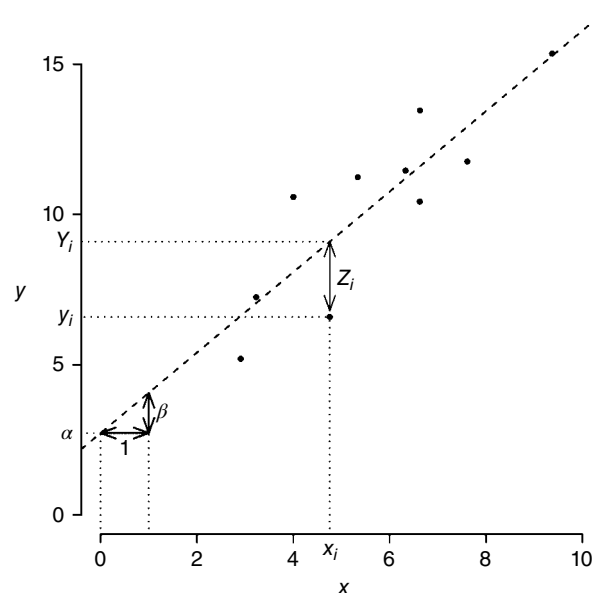


Fig. 22.2 Schematic representation of a simple linear regression model using statistical notation, $Y_i = \alpha + \beta x_i + Z_i$.

Note that the same physical quantity can be either a stochastic or a deterministic variable according to context. Suppose, for example, we wanted to study the growth of lambs. A possible study design would involve weighing each lamb at weekly intervals. In this context, a lamb's age in weeks would be a deterministic variable. In contrast, if we wanted to study premature mortality, the age at which a lamb dies would be a stochastic variable.

As already noted, the dashed lines in *Figures 22.1* and *22.2* represent the **regression lines** (or **lines of best fit**) for each dataset, described by the parameters α and β . Clearly, it is possible to draw many lines that could be considered reasonable descriptions of the relationship between the input and output variables. What is needed is a reproducible method to measure, and minimize, the distance that each observation lies from the line $y = \alpha + \beta x$.

The method used to measure the distance of each observation from the regression line is the *vertical* distance between the two. The vertical (rather than, for example, the perpendicular distance between) is used because the linear regression model describes how the outcome variable, y , would *vary* over independently repeated measurements of any *fixed* value of x . One consequence of this is that if the roles of x and y are interchanged, the so-called line of best fit also changes.

Expressed more formally, the line of best fit is the one that minimizes the sum of the squared vertical distances:

$$LS(\alpha, \beta) = \sum (Z_i)^2 = \sum (y_i - \alpha - \beta x_i)^2.$$

Here, LS represents the 'least (sum of) squared (residuals)', and the method of choosing α and β to minimize $LS(\alpha, \beta)$, is called **least squares** estimation. One (inefficient) way to do this would be to vary α and β repeatedly by trial and error, calculate the sum of squared residuals each time, and select the values for α and β that minimize this value. However, statistical software programs provide these values automatically.

Under the additional assumption that the noise terms, Z_i , are Normally distributed, the least squares estimates of α and β are **maximum likelihood** estimates. Maximum likelihood is a general method of estimation that is known to have good statistical properties (Diggle and Chetwynd, 2011).

The regression line depicted in *Figure 22.1* has, as parameter values, the least squares estimates, $\alpha = 10.1$ and $\beta = 2.8$. In this example, the value of the intercept has no biological relevance, as it implies that a lamb with a birth weight of zero is estimated to have a weight 10.1 kg at eight weeks of age; extrapolation

of a statistical model beyond the range of the data is at best a risky exercise, and at worst nonsensical¹. The slope is of more interest, as it characterizes the relationship between birth weight and weight at eight weeks of age: two lambs that differ by 1 kg at birth (at least within the range observed) are expected to differ by 2.8 kg at eight weeks of age.

Before drawing conclusions regarding the relationship between the input and output variables, it is important to determine whether or not the data are consistent with the null hypothesis, which is that there is no relationship (i.e., $H_0: \beta = 0$). To do this, the standard errors of α and β are estimated and used to calculate the t values and confidence intervals (see Chapter 14). Formulae for the calculation of the standard errors are available (see Crawley, 2007) but most statistical software programs provide standard errors alongside the estimates. *Table 22.1* displays typical output from a regression analysis of the data presented in *Figure 22.1*, following centring of birth weight data to the mean. Again, the estimated weight at eight weeks for a lamb of average birth weight is 21.6 kg, and the 95% confidence interval around this is from 21.1 to 22.1 kg, indicating that, at the conventional 0.05 level, this value is significantly different to zero – not a surprising result. The estimate of the slope parameter is $\beta = 2.79$, with 95% confidence intervals from 2.27 to 3.32, indicating the precision of the estimate.

The P value reported in the second line of the table (<0.001) is obtained from the t statistic and indicates that the data observed are very unusual under the assumption of the null hypothesis ($H_0: \beta = 0$). Similarly, the P value in the first row of the table indicates that the data observed are very unusual under the assumption of the null hypothesis ($H_0: \alpha = 0$), should this be of any interest. Here, as is usually the case, the parameter estimates and their associated

¹ It is possible to adjust the analysis in order to give the intercept a natural biological interpretation. For example, using a process called **centring**, a predetermined reference value for the input variable is subtracted from each observed value of that variable prior to estimation of α and β . In this way, the value of α estimates the value of Y for a subject with a value of x equal to the reference value; the estimate of β remains unchanged. Frequently, the reference value selected is the average value of x in the data, although other values can be used. For example, reconfiguring the regression model to:

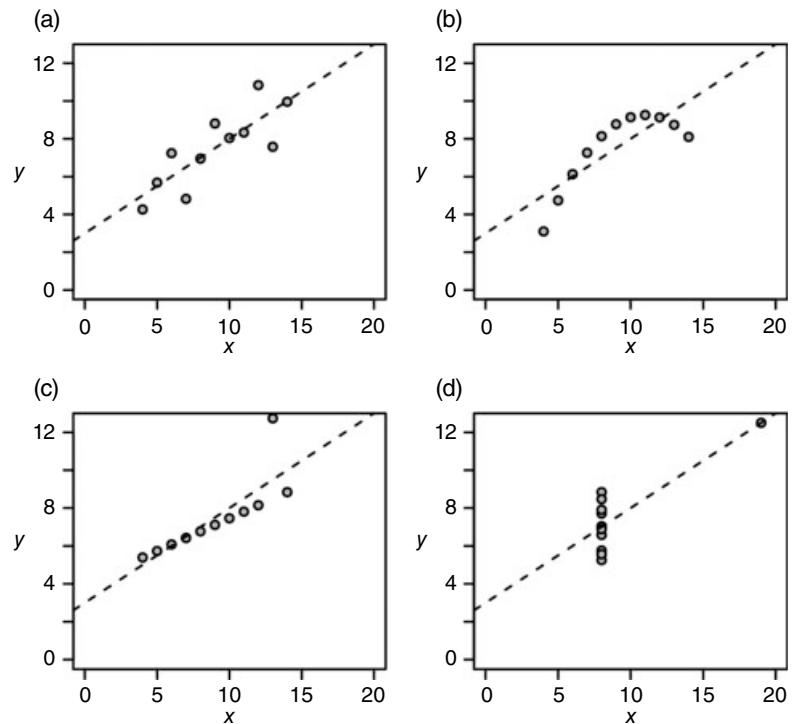
$$Y_i = \alpha + \beta(x_i - 4.1) + Z_i : i = 1, \dots, n,$$

leads to estimation of a regression line with parameter values $\alpha = 21.6$ and $\beta = 2.8$. The value of the slope remains unchanged, but the intercept, α , is now interpreted as the predicted weight at eight weeks of age of a lamb of mean birth weight (4.1 kg).

Table 22.1 Output from a linear regression analysis of the relationship between (centred) birth weight and weight at eight weeks of age among 189 lambs.

	<i>Estimate</i>	<i>Standard error</i>	<i>t statistic</i>	<i>P value</i>	<i>95% confidence interval</i>
Intercept	21.61	0.25	85.40	<0.001	21.11 to 22.10
Centred birth weight (kg)	2.79	0.27	10.45	<0.001	2.27 to 3.32

Fig. 22.3 Anscombe's Quartet. See text for further explanation. (Source: Anscombe, 1973. Reproduced with permission of the American Statistical Association.)



confidence intervals are more useful than the tests of statistical significance.

However, it is insufficient simply to rely on the estimated values of α and β when interpreting the fit of a data set to a linear regression model. This statement is clearly illustrated by considering a set of artificial data constructed by Anscombe (1973). 'Anscombe's Quartet' includes four sets of data, each consisting of the values of an input (x) and an output (y) variable. Linear regression analysis of each set provides the same estimates and standard errors of the intercept ($\alpha = 3.0$, standard error 1.1) and the slope ($\beta = 0.5$, standard error 0.1). However, plots of the Quartet (Figure 22.3) illustrate that for only one of the data sets is the linear regression model likely to be appropriate (i.e., Figure 22.3a). These plots highlight the importance of careful examination of the data to be modelled and of testing the data to ensure that the assumptions of the adopted model are not demonstrably false.

Key assumptions of linear regression models

The process of linear regression modelling entails a number of assumptions. The standard assumptions are that the random variables Z_i are an independent random sample from a Normal distribution with a mean of zero and standard deviation σ , written $Z_i = N(0, \sigma^2)$. That is, the Z_i are simply noise and contain no signal (or pattern). In decreasing order of importance, these assumptions can be set out as follows (adapted from Diggle and Chetwynd, 2011).

- Assumption 1:** Each Z_i has a mean of zero. This represents the essential assumption of linear regression, as it implies that the relationship between the input variable x and the outcome variable Y is linear.

- Assumption 2: The Z_i are independent with a standard deviation of σ regardless of the value of x_i . This assumption is important as it implies that the standard errors (e.g., for α and β) calculated using standard methods are reliable.
- Assumption 3: Each (Z_i) is Normally distributed. This is only really important if the behaviour of the deviations from the relationship between x and the mean of Y are of interest; it does not impact materially on conclusions regarding the relationship itself unless the evidence against the null hypothesis of no relationship is marginal.

It is important to check these assumptions when undertaking regression modelling in order to be confident that the model obtained is compatible with the observed data. Consideration of assumption 2 requires information regarding the study design. In veterinary epidemiology, observations are often not independent as, for example, data collection often involves collection of clustered observations, such as animals within farms. In the example data, observations on lambs are clustered within ewes, as many lambs are from multiple birth litters. As noted earlier, estimates of standard errors may then be unreliable. Analysis of clustered data is covered in more detail later in this chapter. For now, analysis of the example data will proceed as if the observations were independent. Similarly, unequal variances of the Z_i corresponding to different x_i leads to unreliable standard errors.

Evaluation of the assumptions through examination of the data is called **diagnostic checking**. In the basic form of the model,

$$Y_i = \alpha + \beta x_i + Z_i : i = 1, \dots, n,$$

but with the unknown values of the parameters α and β replaced by their estimated values, the quantity $\alpha + \beta x_i$ is called the **fitted value**, whilst the derived quantity $Z_i = Y_i - (\alpha + \beta x_i)$ is called the **residual**. Earlier in this chapter, we used the term residual also to refer to the true, but unknown, values of the original Z_i . Strictly, we should distinguish between **theoretical** residuals, which are the values of the residuals corresponding to the true, but unknown, values of the parameters α and β , and **empirical** residuals, which correspond to estimated values of α and β . In a well-fitting model, the statistical properties of theoretical

and empirical residuals should be similar; that is, they should behave as if they were an independent random sample from a Normal distribution with mean zero. However, this is only approximately true, to an extent that depends on the sample size; to take an extreme case, in a sample of size two, the best-fitting line simply connects the two data points and the empirical residuals are automatically zero. From now on, we use the term residual to mean an empirical residual unless stated otherwise.

Many approaches to diagnostic checking involve creating and examining plots of the residuals. These plots may use (Kleinbaum *et al.*, 1998):

- **ordinary residuals**, i.e., $\hat{z}_i$;
- **standardized residuals**, which are calculated by dividing the ordinary residuals by their estimated standard error (s):

$$\hat{z}_{si} = \frac{\hat{z}_i}{s};$$

- **(internally) studentized residuals**, which scale the standardized residuals by dividing them by $\sqrt{1-h_i}$, where h_i is the *leverage* of the i th observation and $0 \leq h_i \leq 1$, hence:

$$\hat{z}_{ri} = \frac{\hat{z}_{si}}{\sqrt{1-h_i}}.$$

Leverage is a measure of the importance of that observation in determining the model fit. If the i th observation has little influence on model fit, h_i is close to zero, $\sqrt{1-h_i}$ approaches one and the studentized residual will be similar to the standardized residual. In contrast, if the i th observation has a lot of influence on model fit, h_i is close to 1, $\sqrt{1-h_i}$ approaches zero and the studentized residual will be considerably greater than the standardized residual. Hence, studentized residuals are scaled according to the importance of each observation on the model fit;

- **jackknife residuals** (also called **externally studentized residuals**), which scale the residuals by a function of the leverage and by the standard deviation based on the remaining $n - 1$ residuals, so that the jackknife residual for the i th observation is:

$$\hat{z}_{t-i} = \frac{\hat{z}_i}{s_{(-i)}\sqrt{1-h_i}},$$

where $s_{(-i)}$ is the residual standard deviation calculated with the i th observation deleted.

If the regression assumptions hold, and approximately the same number of observations are made at

each of the values of the input variables, then the patterns observed in the ordinary, standardized, studentized and jackknife residuals are similar (Kleinbaum *et al.*, 1998). However, if there are deviations from these assumptions the studentized and, particularly, the jackknife residuals may make these more obvious, and they are particularly useful for detecting outliers (Armitage and Berry, 1994). For the purposes of illustration here, only the ordinary residuals, Z_i , will be considered.

Numerous graphical representations of the residuals have been suggested as aids to diagnostic checking (Atkinson, 1985; Armitage and Berry, 1994). Several potentially useful diagnostic plots include the following four types, which are illustrated in Figure 22.4 for the data presented in Figure 22.1:

1. Plotting the residuals against the fitted values from the model (the $\hat{\mu}_i$, Figure 22.4a). The residuals

should appear as an apparently random pattern. If the data are compatible with assumption 2, the variance (i.e., the spread of residuals around the mean) should be approximately constant over the range of the fitted values. If the data are compatible with assumption 1, the data should lie in an approximately horizontal band that does not curve. In the example, the variance is reasonably consistent across the fitted values, and the data generally lie in a horizontal pattern, although there may be some departure from this at lower fitted values; that is, in this example, lower birth weights. The fitted values from the model (the $\hat{\mu}_i$) may also be plotted against the observed outcome values (y_i) (Figure 22.4b), although this provides no additional value to plotting the residuals against the fitted values. In the example (Figure 22.4b), the pattern is equivalent to that in Figure 22.1a, but this is not the case for more complex, multivariable models.

Fig. 22.4 Diagnostic plots of the residuals for the linear regression model illustrated in Figure 22.1. See text for further explanation.

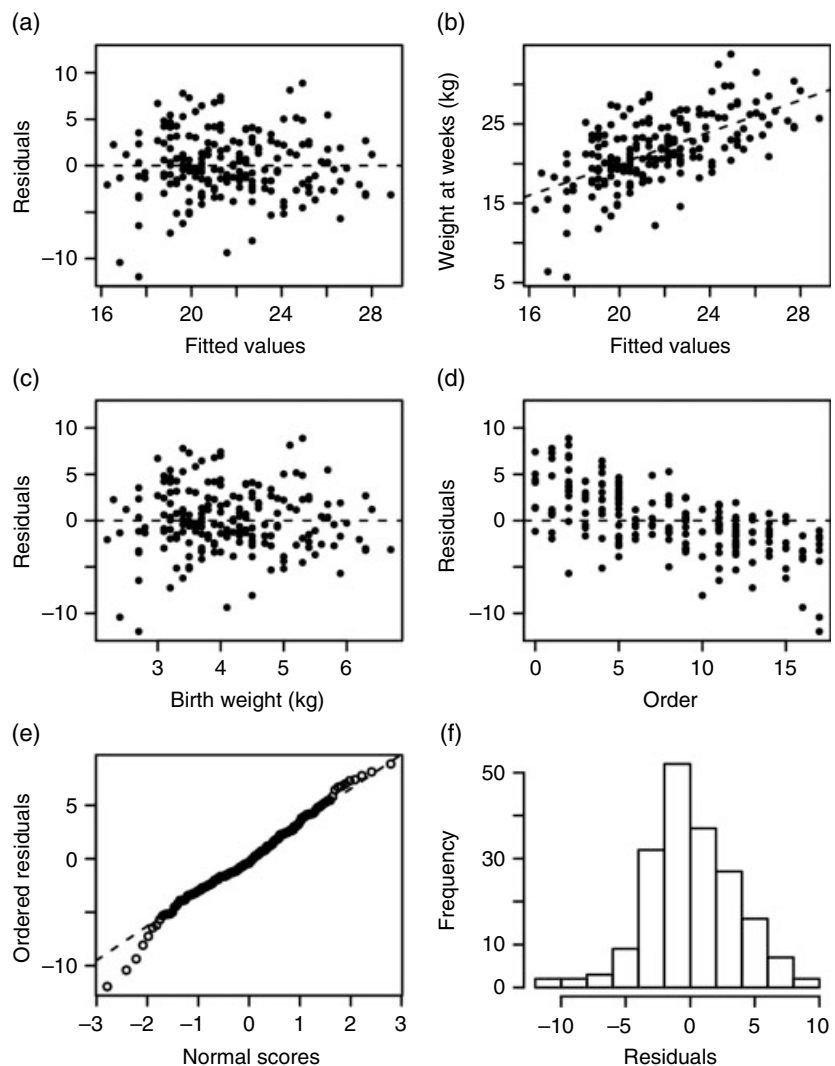


Table 22.2 Interpretation of patterns in QQ plots.

<i>Pattern</i>	<i>Possible interpretation</i>
Generally on a line, but a few points off the line	Outliers
Generally on the line, but left end below line, right end above line	Heavy (long) tails at both ends of the distribution. That is, the data lie further from the median than expected for a Normal distribution
Generally on the line, but left end above line, right end below line	Light (short) tails at both ends of the distribution. That is, the data lie closer to the median than expected for a Normal distribution
Left and right ends above the line, middle below the line (concave up). Points cross the line at two places	Right skewed data
Left and right ends below the line, middle above the line (concave down). Points cross the line at two places	Left skewed data
Points follow an S-shape, crossing the line at three places	Narrowly 'humped' data
Points follow an inverted S-shape, crossing the line at three places	Flatly distributed data
Stepped pattern	Discrete, clustered or rounded data

- Plotting the residuals against input variables included in the model (*Figure 22.4c*). In this example, the pattern is identical to that observed in *Figure 22.4a*, as we are using a simple (rather than a multivariable) model. Non-linear patterns suggest that the relationship between the plotted input variable and the outcome variable is not adequately expressed by a linear term.
- Plotting the residuals against measured input variables *not* included in the model. For example, if samples were collected over time, plotting the residuals in the sequence of data collection may indicate temporal patterns (*Figure 22.4d*). In this example, there is a clear systematic pattern in the plot, which suggests that the model should be extended to a multivariable model that includes day of birth (see later). This highlights the importance of carefully considering that variables that may need to be included in a model, and ensuring these are (accurately) obtained.
- Plotting the ordered residuals against standard normal deviates (also called a quantile-quantile, or QQ, plot). If the data are compatible with assumption 3, the residuals should lie approximately on the diagonal. Judgement is required to determine what constitutes an important deviation from normality. A QQ plot may be supplemented with a histogram of the residuals. The QQ plot for the example model (*Figure 22.4e*) lies on the diagonal, apart from at the lower scores, where the points curve below the diagonal. This suggests some departure from Normality, with a heavier (longer) left tail than predicted by a Normal distribution, as is evident in the histogram (*Figure 22.4f*). The interpretation of

departures from linearity in QQ plots is summarized in *Table 22.2*, and described in more detail by Chambers and others (1983) and Fowlkes (1987).

Detecting outliers and influential data

Outliers are data points that appear out of place with regard to the other data, leading to suspicion that they have been incorrectly recorded or that they originate from a different population to the bulk of the data (Porta, 2014). **Influential data** are those that have a particularly strong influence on the estimation of the regression coefficients. Sometimes outliers and/or influential data can be detected from summary statistics, scatter plots or from the diagnostic plots described earlier. However, this is not always the case and additional steps can help in their identification.

Outliers can be detected using jackknife (externally studentized) residuals. In the example data (*Figure 22.1*), the observations with the most extreme jackknife residual values ($\hat{z}_i$) are shown in *Table 22.3*. The most extreme value is $\hat{z}_i = -3.579$. This can be assessed notionally as a t statistic with degrees of freedom equal to $(n - p - 1)$, where n is the number of observations and p is the number of terms in the model, hence the degrees of freedom is $(189 - 1 - 1) = 187$ and, from Appendix V, $P < 0.001$ or, from computer software, $P = 0.0008$. However, the P value is spurious because it does not take into account that this observation was selected because it was the most extreme. A conservative bound on the significance is given by a **Bonferroni correction**, such that the corrected P value is $1 - (1 - 0.0008)^{189} = 0.14$, representing insufficient evidence that this observation is an outlier.

Table 22.3 Observations with the most extreme residual values from the example data shown in Figure 22.1.

Observation (i)	Birth weight (x)	Weight at eight weeks (y)	Predicted weight at eight weeks (Y)	Residual ($\hat{z}$)	Standardized residual ($\hat{z}_s$)	Studentized residual ($\hat{z}_t$)	Jackknife residual ($\hat{z}_j$)	Leverage (h)	Cook's distance (d)
185	2.7	5.7	17.7	-12.0	-3.5	-3.5	-3.6	0.017	0.11
84	2.4	6.4	16.8	-10.4	-3.0	-3.1	-3.1	0.023	0.11
178	4.1	12.2	21.6	-9.4	-2.7	-2.7	-2.8	0.005	0.02
24	5.3	33.8	24.9	8.9	2.6	2.6	2.6	0.013	0.04
23	5.1	32.5	24.4	8.1	2.4	2.4	2.4	0.011	0.03
119	4.5	14.6	22.7	-8.1	-2.3	-2.3	-2.4	0.006	0.02

An influential datum may, or may not, be an outlier. **Cook's distance** (Cook, 1977) provides a standardized measure (d_i) of the influence of each observation on the regression coefficients. Cook's distance is calculated as:

$$d_i = \frac{\hat{z}_{ri}^2 h_i}{(p+1)(1-h_i)}.$$

Cook's distance is always greater than zero, but with no upper limit; as a simplifying rule of thumb, observations with values of $d > 1$ should be considered as being potentially influential and more closely examined. The largest Cook's distance value for observations in the example (Figure 22.1) was 0.11 (for observations 185 and 84; Table 22.3), suggesting that no observations have excessive influence.

Table 22.3 also displays the leverage values (h). The mean leverage value is $(p+1)/n$; for the example this is $(1+1)/189 = 0.011$. Observations with leverage values greater than twice the mean value (i.e., $h_i > 2(p+1)/n$) should be more closely examined. As noted above, such rules of thumb should be used with caution. In the example, observation 84 has the highest leverage value, and this is greater than 0.022. However, neither the Cook's distance nor the jackknife residual for these observations suggested that they were not unduly influential or distant from the other data.

Context is an important factor in interpreting outlying and/or influential observations. Sometimes, a statistical outlier can subsequently be detected as a recording error, in which case it should either be corrected or removed from the analysis. If a statistical outlier is both scientifically plausible and not known to have been recorded incorrectly, its removal should only be considered if it is also influential, and even then only after careful consideration. Automatic removal of 'inconvenient' data values leads to spurious precision and should be avoided.

Modelling more than one input variable

A **multivariable (or multiple) linear regression**² model, also known as the **general linear model**, involves extending the simple linear model to include two or more input variables, hence:

$$Y_i = \alpha + \beta_1 x_{1i} + \beta_2 x_{2i} + \dots + \beta_p x_{pi} + Z_i \quad : \\ i = 1, \dots, n.$$

Direct visualization of such models is not possible, but the principles are the same as for simple linear regression. The value for β_1 provides the best estimate of the change in Y for each unit change in x_1 , irrespective of the values assigned to variables $x_2 \dots x_p$. This is beneficial for controlling for confounding (see Chapters 15 and 18), but can raise difficulties of interpretation if two variables in the model are highly correlated. In the latter circumstance, the two highly-correlated variables are essentially measuring the same thing, and the interpretation of their respective regression parameters β becomes ambiguous. Furthermore, epidemiological studies frequently collect data on many variables. Inclusion of all of these may increase the risk of inclusion of correlated variables, and makes the results difficult to interpret through inclusion of potentially extraneous information. As a general rule, the best explanation/understanding of the scientific

² The terms multivariable regression and multiple regression are preferred to the widely used **multivariate** regression in this situation, as the latter term more correctly describes a range of approaches used to analyse data containing several outcome (response) variables, whose joint probability distribution is of interest (see also Chapter 14). Multivariate methods include principal component analysis, factor analysis and cluster analysis, among others. See Everitt (2005) and Manly (2005) for further details.

Table 22.4 Output from a multivariable linear regression analysis of the relationship between input variables (centred) birth weight and day of birth, and the output variable weight at eight weeks of age, among 189 lambs.

	<i>Estimate</i>	<i>Standard error</i>	<i>t statistic</i>	<i>P value</i>	<i>95% confidence interval</i>
Intercept	25.31	0.37	67.63	<0.001	24.57 to 26.05
Centred birth weight (kg)	3.05	0.21	14.74	<0.001	2.64 to 3.46
Day of birth	-0.46	0.04	-11.38	<0.001	-0.54 to 0.38

question is obtained from a model that includes as few variables and is as simple as possible, whilst still providing an acceptable fit to the data (see later).

In the example data presented in *Figure 22.1* there was evidence (*Figure 22.4d*) that the data order (or more specifically, the day of birth of the lambs) should be included in the model. As expected, this variable is highly significant in the model (*Table 22.4*) and the coefficient suggests that the predicted weight at eight weeks of age decreases by approximately 0.5 kg per day for each day after the start of lambing, after controlling for the effect of birth weight. Controlling for day of birth results in a small increase in the estimate of the effect of birth weight. If this were considered the best model (that is, there were no other terms to be included in the model), the next step would be to undertake diagnostic checking in order to test the assumptions of the model.

Handling categorical input variables

Epidemiological analyses frequently involve consideration of categorical input variables (often called **factors**). These may include information on truly qualitative data, for example sex and breed, or categorized quantitative data, such as age or weight categories. These can be readily handled within a general linear model. The simplest way to see how categorical variables may be included within a general linear model is to consider a binary input variable.

In addition to measuring input variables birth weight and day of birth, the example data also measured whether each lamb was a singleton, twin or triplet. The variable *litter* recorded the litter size (1, 2 or 3) and the variable *multiple* recorded whether or not the lamb was a singleton ($x = 0$ if singleton and $x = 1$ if twin or triplet). Using the binary variable *multiple* it is possible to estimate the mean weight at eight weeks of age for singleton ($\mu_s = 24.94$ kg) and multiple-birth ($\mu_m = 20.85$ kg) lambs. If the mean weight of singleton lambs is defined as $\mu_s = \alpha$, the mean weight of multiple-birth lambs can be expressed as $\mu_m = \alpha + \delta$,

where δ is the population mean difference between singleton and multiple-birth lambs (-4.09 kg). It therefore follows that the mean weights at eight weeks of both singletons and multiple-birth lambs can be expressed as $\mu_i = \alpha + \delta x_i$, as this simplifies to $(\alpha + \delta \times 0) = \alpha$ for singleton lambs and $(\alpha + \delta \times 1) = (\alpha + \delta)$ for multiple-birth lambs. Therefore, the output variable, weight at eight weeks of age (Y_i), can be modelled as:

$$Y_i = \alpha + \delta x_i + Z_i : i = 1, \dots, n,$$

and this is equivalent to:

$$Y_i = \begin{cases} \alpha + Z_i : i = 1, \dots, n, & \text{for singleton lambs,} \\ \alpha + \delta + Z_i : i = 1, \dots, n, & \text{for multiple birth lambs.} \end{cases}$$

Using the least squares method, computer software produces parameter estimates for this model as shown in *Table 22.5a*. In this table, the intercept row indicates that the estimated value of the parameter α is 24.94 kg (95% confidence interval 23.65, 26.24). The row labelled multiple provides the estimated value of the parameter δ , -4.09 kg (95% confidence interval -5.54, -2.64). These estimates are the same as were calculated above. As the confidence interval for δ does not include zero, it is reasonable to reject the null hypothesis that the difference in weight at eight weeks of age between singleton and multiple-birth lambs is zero (i.e., $H_0: \delta = 0$).

It is important to note that this relatively straightforward interpretation of the output from the computer software program only applies if the input variable is binary and takes the values (0,1). When it is necessary to model categorical variables with more than two levels (i.e., $k > 2$), the method described earlier can be generalized by the inclusion of $k - 1$ **dummy variables** (also called **indicator variables**). When used in regression modelling, dummy variables usually only take the values (0,1), as in the case of the binary variable *multiple*, above. Dummy variables are created from categorical variables in order to indicate membership of one (and only one) of the levels of the categorical variable. For example, one possible set of

Table 22.5 Output from linear regression analysis of the effect of multiple births on weight at eight weeks of age: (a) the factor *multiple* takes the value 0 for singleton births and 1 for twins or triplets; (b) the dummy variable x_1 takes the value 1 if a twin, 0 otherwise, while x_2 takes the value 1 if a triplet, 0 otherwise; (c) the dummy variable x_1 takes the value 1 if a twin or a triplet, 0 otherwise, while x_2 takes the value 1 if a triplet, 0 otherwise.

	Estimate	Standard error	t statistic	P value	95% confidence interval
(a)					
Intercept	24.94	0.66	37.94	<0.001	23.65 to 26.24
Multiple	-4.09	0.74	-5.56	<0.001	-5.54 to -2.64
(b)					
Intercept	24.94	0.63	39.39	<0.001	23.69 to 26.19
x_1 (twin)	-3.37	0.73	-4.60	<0.001	-4.81 to -1.92
x_2 (triplet)	-6.25	0.90	-6.98	<0.001	-8.02 to -4.49
(c)					
Intercept	24.94	0.63	39.39	<0.001	23.69 to 26.19
x_1 (twin) compared to intercept	-3.37	0.73	-4.60	<0.001	-4.81 to -1.92
x_2 (triplet) compared to x_1	-2.89	0.73	-3.94	<0.001	-4.33 to -1.44

dummy variables for the input variable *litter* in the example is:

Group	x_1	x_2
1	0	0
2	1	0
3	0	1

The first dummy, x_1 , takes the value 1 for twins and 0 otherwise. The second dummy, x_2 , takes the value 1 for triplets and 0 otherwise. Together, the values of these two dummy variables identify the group membership of each observation for the variable *litter*: singletons are identified by (0,0); twins by (1,0); and triplets by (0,1). Thus, the effect of the variable *litter* on the output variable, Y_i , can be modelled as:

$$Y_i = \alpha + \delta_1 x_{1i} + \delta_2 x_{2i} + Z_i : i = 1, \dots, n,$$

which is equivalent to:

$$Y_i = \begin{cases} \alpha + Z_i & : i = 1, \dots, n, \text{ for singleton lambs,} \\ \alpha + \delta_1 + Z_i & : i = 1, \dots, n, \text{ for twin lambs,} \\ \alpha + \delta_2 + Z_i & : i = 1, \dots, n, \text{ for triplet lambs.} \end{cases}$$

Using the least squares method, computer software produces parameter estimates for this model as shown in *Table 22.5b*. The row labelled intercept indicates that estimated value of the parameter α (the mean weight at eight weeks of singleton lambs) is 24.94 kg (95% confidence interval 23.69, 26.19). The

information for x_1 indicates that the mean weight for twin lambs is 3.37 kg less than that of singletons (95% confidence interval -4.81, -1.92) and the information for x_2 indicates that the mean weight for triplet lambs is 6.25 kg less than that of singletons (95% confidence interval -8.02, -4.49). These results do not explicitly indicate whether or not the mean weights at eight weeks of age of twins and triplets are significantly different. If this is of particular interest, the dummy variables can be re-specified as:

Group	x_1	x_2
1	0	0
2	1	0
3	1	1

and the model re-estimated (*Table 22.5c*). Now, the assumed mean weights at eight weeks are α for group 1, $\alpha + \delta_1$ for group 2, and $\alpha + \delta_1 + \delta_2$ for group 3; hence δ_2 represent the difference in mean weight at eight weeks between twins and triplets. Given that the 95% confidence intervals for x_1 and x_2 in *Table 22.5b* barely overlap, it is not surprising that doing this indicates that these groups do, indeed, significantly differ in mean weight at eight weeks of age (difference = -2.89; 95% confidence interval -4.33, -1.44; $P < 0.001$).

Most statistical software allows the user to bypass the process of calculating dummy variables explicitly. For example, if y is an output variable and x an input variable coded as 1, 2 or 3, then the *R* software system interprets the command `lm(y ~ x)` as an instruction to fit a simple linear regression model with y as output

and x as a numerical input (in our example, litter size), whereas specifying x as a factor, using the command `lm(y ~ as.factor(x))`, instructs the software to fit a multiple linear regression model with y as output and x converted to dummy variables; that is, treating the 1, 2 and 3 simply as codes for three arbitrarily different values.

Non-linear modelling of quantitative input variables

The most important assumption of linear regression is that the residuals (Z_i) have a mean of zero, implying that the relationship between the input and output variables is indeed linear (assumption 1, earlier). It is essential that this key assumption is tested and, if necessary, additional steps taken in order that this assumption is met adequately.

Examination of plots of the data and of residuals may suggest that the relationship is not linear. Note that however many input variables are included in the model, residuals can be plotted against fitted values in a standard, two-dimensional scatter plot. This scatter plot is the single most widely-used method for diagnostic checking, but other methods can and should also be used. *Figure 22.5a* illustrates the relationship between an (artificial) input variable x and an (artificial) output variable Y . Although it is clear that Y increases as x increases, examination of the plot, including comparison with the line of best fit (dashed line), suggests that the relationship is not linear over the full range of values of x . This is confirmed by examination of a plot of the fitted values against the residuals derived from fitting a simple linear regression model, which show a distinctly curved pattern (*Figure 22.5b*). For completeness, the QQ plot (*Figure 22.5c*) is also shown. Superficially, this suggests that the residuals are approximately Normally distributed, but this is irrelevant if the assumed linear relationship between x and Y is not tenable. Examination of *Figure 22.5a* and *b* is sufficient to identify that the assumption of a linear relationship is dubious. However, this may not always be so and it may be beneficial to explore further the form of the relationship between the input and output variables. Several approaches to testing for, and dealing with, non-linear relationships are outlined here. The method(s) chosen may depend on both knowledge of the biological processes being modelled and practical considerations, including how the conclusions from the models can best be communicated. These methods include:

- additive models;
- categorization of the input variable;

- transformation of the input and/or output variable;
- piece-wise regression.

Additive models

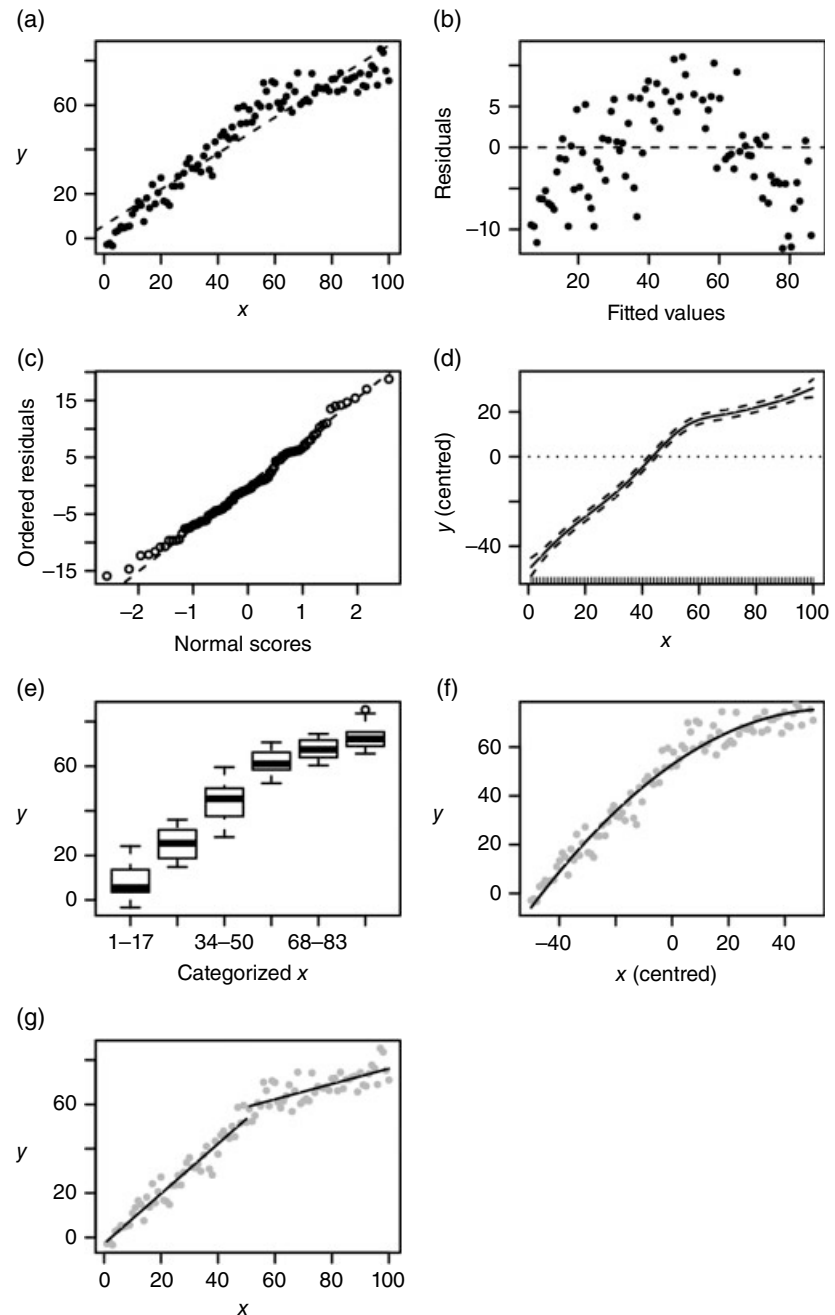
Additive models are a special case of generalized additive models (GAMs: Hastie and Tibshirani, 1986), which are described later. An additive model differs from a linear model in that the form of the relationship between the input variables and the output variable is not restricted to the linear form $\beta_1 x_{1i} + \beta_2 x_{2i} + \dots + \beta_p x_{pi}$, but instead is modelled as a sum of smooth functions $s_1(x_{1i}) + s_2(x_{2i}) + \dots + s_p(x_{pi})$, where the $s_i(\cdot)$ represent unspecified functions that are estimated using a non-parametric scatter plot smoother. Additive models are useful for exploring the form of the relationship between input and output variables. With care, they also can be used for confirmatory analysis when combinations of linear and non-linear relationships are indicated. The need for care arises for two reasons. First, the inferences produced by the fitting algorithms are only approximations. Secondly, and more importantly, all non-parametric scatter plot smoothers rely on the choice of a smoothing constant whose value materially affects the resulting estimates of the smooth functions. Several methods are available for guiding this choice but, in contrast to linear model fitting, there is no universal agreement on which of these is best. Software implementations typically offer defaults, but this hides rather than solves the problem.

Additive models can be fitted in a range of statistical software programs, although the methods used to fit the smooth functions differ between packages. For example, *Figure 22.5d* shows a plot of the estimated smooth function for the relationship between x and centred Y , derived using the default settings for the *gam* function in the R package *mgcv*. The estimate is plotted as a solid line, whilst the dashed lines represent the corresponding 95% point-wise confidence intervals. In this example, the confidence intervals are sufficiently narrow, and the function sufficiently non-linear, to suggest that the relationship is indeed non-linear. It is evident in this example that the functional form of the relationship illustrated in *Figure 22.5d* is consistent with scatter plot *Figure 22.5a*; however, this is not always so obvious, especially when considering models with more than one input variable.

Categorization of the input variable

Another method to assess the form of the relationship between the input (x) and output (Y) variable is to categorize x into an appropriate number of

Fig. 22.5 Modelling non-linear relationships: (a) plot of the relationship between an input variable, x , and output variable, y ; (b) plot of the residuals from the linear regression model, $Y_i = \mu(x_i) + Z_{ii}$; (c) QQ plot of the residuals; (d) plot of an additive model of the relationship between x and y ; (e) box plot of y following categorization of x ; (f) quadratic polynomial regression model fit to the data; and (g) piece-wise regression model fit to the data, based on a single break point at $x = 50$.



categories, estimate the mean of Y within each of the categories, and plot the result. For exploratory purposes the number of categories should be at least four or five and can be defined so as to contain approximately equal numbers of data points or to span equal ranges of x ; as with additive models, there is no objectively 'best' way to do this. In Figure 22.5e, x has been categorized into six equally-spaced categories and box plots drawn for Y within each category. As before, this plot suggests that Y increases more steeply with

increasing x over the lower values of x compared with the higher values of x . Categorization of linear input variables provides a possible solution to the problem of modelling variables that appear to have a curvilinear relationship with the outcome. However, this comes at the expense of increased model complexity due to the increased number of model parameters, as well as some loss of interpretability because the discontinuities between fitted values in adjacent categories are artificial.

Transformation of the input and/or output variable

In some instances, it is natural to consider a variable on a scale other than a simple linear scale. Many biological processes operate multiplicatively rather than additively, in which case a linear modelling assumption becomes more plausible for log-transformed outputs. For example, bacterial growth and milk somatic cell counts are often reported on a logarithmic scale. The formal mathematical justification for this is that if the underlying biological process involves a power law, $Y = ax^b$, then transforming to $Y^* = \log(Y)$ and $x^* = \log(x)$ gives the linear relationship:

$$Y^* = \alpha + \beta x^*,$$

where $\alpha = \log(a)$ and $\beta = b$.

Even when there is no specific biological reason to transform the input or output variables, doing so may be a useful way to make a linear model a better empirical approximation (Diggle and Chetwynd, 2011). *Figure 22.5f* again illustrates a scatter plot of the example data and, although x has been centred, the pattern is identical to that in *Figure 22.5a*. However, rather than model the relationship between x and Y as linear ($Y_i = \alpha + \beta x_i + Z_i$), a quadratic (squared) term has been added to the model: $Y_i = \alpha + \beta_1 x_{1i} + \beta_2 x_{1i}^2 + Z_i$. Inclusion of a quadratic polynomial term permits modelling of a curvilinear relationship; inclusion of higher-order polynomials (e.g., cubic and quartic polynomials) enables more flexible curves to be accommodated. The general principle of polynomial regression modelling requires inclusion of successive powers of individual predictor variables. Thus, extending the polynomial regression model above, if p powers of x were to be included, the model would be defined as:

$$Y_i = \alpha + \beta_1 x_{1i} + \beta_2 x_{1i}^2 + \dots + \beta_p x_{1i}^p + Z_i.$$

The aim should be to use the simplest appropriate model, so as low an order as possible should be specified. Hence, unless the more complex model explains more of the variation in the data, a linear model is preferred to a quadratic model, which is preferred to a cubic model, and so on (Armitage and Berry, 1994; see 'Model building' later in this chapter). Inclusion of polynomial terms in a model may cause problems with collinearity, but this can be easily overcome by using a centred variable or, more generally, orthogonal polynomials (Armitage and Berry, 1994). A more fundamental limitation of polynomial regressions of high degree is that they are often difficult to interpret, with artificial features including unrealistic extrapolations beyond the range of values of x included in the data. In most cases, polynomial regression models should be viewed as empirical curves whose individual parameters have no direct scientific interpretation. For example, it may not be immediately obvious to many readers how to interpret the relationship between x and Y from the model results in *Table 22.6a*. Graphical presentations of polynomial regression models, such as those shown in *Figure 22.5d* or *f*, are generally preferable.

Alternative methods based on polynomials include fractional polynomials (Royston and Altman, 1994) and splines, which are simply piece-wise polynomials (see 'Piece-wise regression' following) but constrained so that the pieces join smoothly.

Piece-wise regression

Piece-wise (or segmented) regression involves modelling the relationship between an input variable and output variable using different functions over distinct ranges of the input variable. Typically, linear functions are used for each segment, but other functional forms

Table 22.6 Output of (a) a quadratic polynomial regression analysis and (b) a piece-wise regression analysis of the artificial example data presented in *Figure 22.5*.

	Estimate	Standard error	t statistic	P value	95% confidence interval
(a)					
Intercept	52.75	0.75	70.73	<0.001	52.27 to 54.23
(Centred x) ²	-0.0072	0.00067	-10.80	<0.001	-0.0085 to -0.0059
Centred x	0.81	0.02	47.05	<0.001	0.78 to 0.84
(b)					
Intercept ≤ 50	-2.84	1.24	-2.29	0.03	-5.33 to -0.35
Intercept > 50	41.52	3.55	11.71	<0.001	34.39 to 48.65
x (≤ 50)	1.13	0.04	26.63	<0.001	1.04 to 1.21
x (> 50)	0.35	0.05	7.5	<0.001	0.25 to 0.44

may be used. As a specific and widely used example, the **regression spline** method fits a cubic polynomial within each segment, but adds algebraic constraints to make the pieces join up smoothly. Segmented relationships often make biological sense, at least as an approximation. For example, exposure to low levels of a toxin may result in no pathological response up to a certain level, beyond which increased exposure is linearly associated with increased pathology. The data in the example illustrated in *Figure 22.5a* are consistent with a piece-wise relationship described by two linear functions, as illustrated in *Figure 22.5g*. Fitting of piece-wise functions is relatively straightforward in many statistical software programs, but in any event is easily carried out using standard linear modelling methods. For example, to fit a piece-wise linear regression of Y on x with changes in slope at two points, say $x = a$ and $x = b$, new variables x_a and x_b can be defined such that x_a is equal to zero if x is less than a and equal to $x - a$ otherwise and, similarly, x_b is equal to zero if x is less than b and equal to $x - b$ otherwise. Then, the piece-wise model is fitted as a *linear* model:

$$Y_i = \alpha + \beta x_i + \gamma x_{ai} + \delta x_{bi} + Z_i.$$

Unless underlying biological theory suggests the appropriate number of segments and the locations of break-points for the segments, these decisions are often based on visual inspection of scatter plots or plots of additive model fits. Alternatively, multiple models could be considered with the preferred model identified by comparing measures of model fit. For example, the best two-segment model can be identified as the one that minimizes the residual standard error (Crawley, 2007). AIC (Akaike's information criterion; see later in this chapter) may also be used to compare models with different numbers of segments.

In the example, the single break-point is estimated to be at $x = 50$. The parameter estimates for this piece-wise regression model include separate intercept and slope values for each line (*Table 22.6b*). These results suggest that Y increases by approximately 1.13 units for each unit of x up to $x = 50$, but by only 0.35 units per unit of x subsequently.

The results presented in *Figure 22.5d–g* all suggest that the relationship between Y and x is non-linear. All of the methods presented here can be used both for data exploration and, with caution, for confirmatory analysis. The need for caution arises because, to a greater or lesser extent, they all involve making choices that may well deliver a good fit to the data, but usually lack a biological interpretation. This may not matter if the input variable in question is not

of direct interest. In general, the preferred method should balance the sometimes competing virtues of biological plausibility, parsimony and ease of communication.

Modelling interactions

Statistical interaction (introduced in Chapters 5, 15 and 18) occurs when the effect of one input variable depends on the value of another input variable (or several other input variables). Hence, interactions may be referred to as two-way interactions (between two variables), three-way interactions (between three variables) and so on. Interaction terms can be introduced into regression models by adding the cross-products terms (e.g., a two-way interaction between variables x_1 and x_2 is calculated as: $x_1 \times x_2$).

Interaction terms should be included in a model where their inclusion improves the model fit. Interpretation of interaction terms can be problematic. Where the interaction is between two binary variables, interpretation is relatively straightforward, with the coefficient for each variable indicating the effect of each variable alone and the coefficient for the interaction term indicating the additional effect of both variables being present, over and above the effect of each individually. When modelling interactions between categorical variables, an alternative approach is to create a new categorical variable explicitly identifying for each observation the level of its component variables. For example, in order to model the combined effects (interaction) of the variables sex (male = 0, female = 1) and breed (breed A = 0, breed B = 1), a new categorical variable could be created to indicate the four possible combined categories:

Sex	Breed	'Sex and breed'
0	0	0
0	1	1
1	0	2
1	1	3

The corresponding linear model has three regression parameters if the interaction is excluded, four if it is included. Hence, in this example, a single number can describe the interaction effect.

The coefficients for interactions that include continuous variables can be interpreted as follows. Consider the regression equation (omitting the residual term):

$$Y = \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2.$$

Table 22.7 Output of a linear regression model of the effects of (centred) birth weight and day of birth on weight at eight weeks of age in 189 lambs. The model includes the interaction between (centred) birth weight and day of birth.

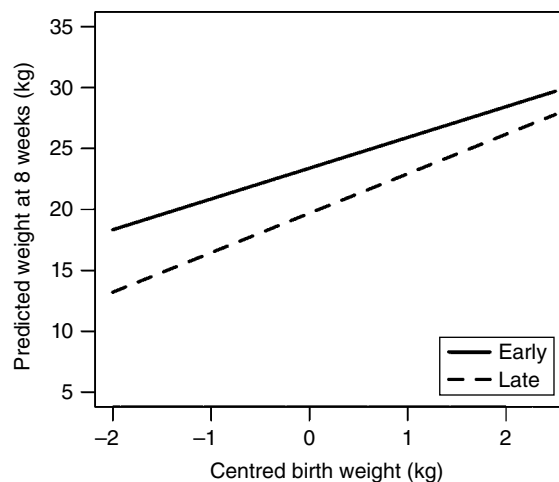
	Estimate	Standard error	t statistic	P value	95% confidence interval
Intercept	25.29	0.37	668.29	<0.001	24.55 to 26.02
Centred birth weight (kg)	2.27	0.49	5.68	<0.001	1.48 to 3.06
Day of birth	-0.46	0.04	-11.57	<0.001	-0.54 to 0.38
Centred birth weight × Day of birth (interaction)	0.09	0.04	2.28	0.02	0.01 to 0.17

Rewrite this as:

$$Y = \alpha + \beta_1 x_1 + (\beta_2 + \beta_3 x_1) x_2.$$

This allows us to interpret the model as saying that the effect of x_2 on Y depends on the value of x_1 . By the same token, we can interpret the model as saying that the effect of x_1 on Y depends on the value of x_2 . Illustrative plots can also be helpful. Extension of the model presented in Table 22.4 to include an interaction term between the two input variables, (*centred*) birth weight and day of birth, provides evidence that the effect of each of these interacts with the other, as the interaction term between these variables is significant in the model (Table 22.7). Interpretation of these parameters may be facilitated by plotting birth weight against the predicted values for different levels of day of birth. Figure 22.6 shows the fitted relationship between birth weight and eight-week weight for early lambs (those born on day 4, the lower quartile) and late lambs (those born on day 12, the upper quartile). This plot suggests, as we have already seen, that, as birth weight increases, the mean weight at eight weeks of age increases, but also that this relationship is somewhat different for early, compared with late, lambs, with the effect of birth weight being greater in late lambs (steeper slope). In addition, it is evident that late lambs tend to reach lower weights at eight weeks of age, compared with early lambs, for any given birth weight. Interactions between one categorical variable and one continuous variable are interpreted in a similar way: the relationship between the outcome variable and the continuous input variable varies depending on the level of the categorical variable (i.e., the two fitted lines are not parallel), perhaps even to the extent that the slope of the relationship between the continuous input variable and the outcome may be positive for some levels of the categorical input variable, and negative for others.

The key consideration when interpreting interaction terms is that the effect of one of the composite variables cannot be assessed without considering the effect of the other variable(s).

**Fig. 22.6** Predicted values of (centred) lamb weight (kg) at eight weeks of age, for early (born on day 4) and late (born on day 12) lambs. The predictions are based on a model that included the interaction between (centred) birth weight and day of birth.

Model selection

Selection of the 'best' model depends on the problem being addressed. The aim is usually to select the simplest ('most parsimonious') model that is not demonstrably incompatible with the data and adequately explains the system of interest. Often, a number of variables may be considered likely to influence the outcome of interest – this is natural in epidemiology, as most outcomes are believed to have multiple causes (see Chapter 3). Furthermore, causes can be thought of as being proximal, if they *directly* result in the outcome, and distal, if they affect other, more proximal, causes. Classification of causes in this way is useful but not definitive, as usually an even more proximal causal factor often can be identified for what may be thought of as a proximal cause in a particular causal-web model.

Regardless, it is common in statistical modelling of epidemiological data to be confronted with multiple possible explanatory variables, sometimes very many indeed. The process of using all or a selected sub-set

of these variables in a statistical model to represent our understanding of a process is called **model selection** or **model building**.

Model selection is not a simple task and, importantly, it is not a purely statistical task; it requires knowledge about the system being investigated. There is no single approach to selecting the best models, but most approaches involve selection of variables based on:

- *a priori* knowledge of the system and the question to be addressed. This may utilize construction of causal webs (see Chapter 3) or causal diagrams (see Chapter 18);
- a data-driven selection process, including single variable comparisons and model-building procedures such as forward selection, backward elimination or stepwise approaches.

A purely data-driven approach to variable selection in regression modelling is useful when the number of potential input variables is large and the primary goal of the model is empirical prediction. However, when scientific explanation is the goal, as it often is in epidemiological research, the interpretation of the parameters in a regression model fitted to observational data is not straightforward and the use of contextually-informed causal diagrams can help to avoid misinterpretation. This section first considers the subtleties that arise with a causal formulation, using the specific setting of Figure 18.3b as an example, before discussing data-driven variable selection methods.

***A priori* knowledge** should be used to develop a causal model before statistical modelling begins and, ideally, before data is collected. As noted in the introduction to this chapter, it is only through careful and detailed consideration of the problem to be addressed that appropriate data needs can be identified and a rational theoretical model identified. This process requires expert knowledge about the topic and close association between the knowledge expert and the statistician: epidemiologists often have an important part to play in this process.

To explain the statistical issues that arise, we consider the setting of Figure 18.3b. This illustrates a simple causal diagram of an outcome of interest (porcine respiratory disease, R), the explanatory variable of interest (the use of fans, F), and a potential confounder (herd size, H). The diagram shows that H is believed to be associated with the explanatory variable of interest, F , and with the outcome, R . A linear regression model for these three variables would take the form:

$$R = \alpha + \beta_1 F + \beta_2 H + Z_i,$$

where Z_i are the residuals. This is not a complete model for the data, but only for one component of it, namely the conditional distribution of R given F and H . Furthermore, it treats the variables F and H symmetrically. Hence, the regression parameters β_1 and β_2 have essentially the same interpretation: β_1 is the change in the mean value of R per unit change in F with H held fixed at any arbitrary value, and similarly for β_2 with the roles of F and H reversed. In a designed experiment where we are at liberty to fix the values of F and H independently, an exclusive focus on the conditional distribution of R given F and H is reasonable (and, incidentally, explains why explanatory variables in a regression model are sometimes called **independent variables**). In an observational study, the situation is less straightforward. In particular, Figure 18.3b does not treat F and H symmetrically, because H has two outward arrows whilst F has one inward and one outward, representing the belief that H is a confounder of the relationship between F and R .

Westreich and Greenland (2013) discuss in detail issues with interpretation of the effect estimates derived from multivariable regression models and the distinction between what they call **total** and **direct effects**. More detailed explanation of the use of causal diagrams is provided by Dohoo and others (2009). Where only a small number of variables are included in a causal model, the modeller may elect to present all variables identified *a priori*, regardless of whether or not their inclusion makes statistically significant improvements to the model fit. Interactions should also be considered, at least as much for their scientific interpretation as for their statistical significance.

In a **data-driven variable selection** process, standard practice is to reduce the number of variables included in the model to only those that are relevant to the research question at hand and whose inclusion gives a statistically significant improvement in the model fit. Whatever approach is taken, it is important that the key assumptions of regression are adequately fulfilled. Hence, it is common to start by exploring the relationships between the outcome variable and each input variable to check that these are approximately linear and that no two input variables are highly correlated; the definition of 'high' depends on context, but a rough rule of thumb is that two input variables with correlation greater than 0.9 should not both be included in the model. Perhaps the simplest such process is to screen variables to be included in a model on the basis of P values obtained by single variable regression of each possible input variable against the outcome variable. Cut-off values of 0.2, 0.25 and 0.3 are often used, and those variables with P values greater than the cut-off are not considered further in the model. This approach

should be used with caution, partly because of the arbitrariness of the chosen cut-off value but also because the role of a variable as a confounder of another relationship may only be evident in a multivariable model. Mechanical application of such approaches should therefore be avoided, but screening may nevertheless provide useful information, particularly when underpinning causal knowledge is lacking. Where variables are initially excluded in this way, it is common to include them, one at a time, into the final model as a check on the robustness of conclusions of scientific interest to marginal decisions on inclusion or exclusion of variables whose effects are not of direct interest. Most epidemiological studies collect data on input variables for two different purposes: either because their effects on the outcome variable are of direct scientific interest or because, whilst not of interest in themselves, their indirect effects on the outcome variable may distort or obscure the effects of scientific interest. Adjusting for the effects of variables in the second category can be thought of as analogous to blocking on the values of these variables in a randomized experiment. However, in contrast to the 'clean' analysis that results from a well-designed, randomized experiment, the epidemiologist is faced with the messier task of coping retrospectively with multiple sources of extraneous variation and, consequently, a multitude of possible analyses. A cautious strategy of including all of the variables in the second category risks unnecessary loss of precision through the need to estimate a large number of meaningless regression parameters. In contrast, a bolder strategy of selecting only a few 'important' variables from the second category also risks loss of precision because variability that could have been explained by the omitted variables is ascribed to residual variation.

Where categorical explanatory variables are present, it is worth checking how many data points are present in each category, as estimates associated with categories with very few data points will be very imprecise. When categories are ordered, it is useful to plot their effects against nominal category values; a clearly interpretable pattern in a plot of this kind may then suggest ways of simplifying the model, for example, by combining adjacent categories to represent a threshold effect (cf. piece-wise regression).

More complex model building may involve backward elimination, forward selection or stepwise approaches.

Backward elimination begins by specifying the **saturated model**³. The saturated model includes all variables, terms necessary to model non-linear relationships with the outcome (e.g., quadratic or piece-wise

terms) and some interaction terms. It may be impractical to include all second-order interactions terms, let alone higher-order terms. Hence, it is common to only include those that have some level of biological plausibility. Clearly this requires subjective judgement and *a priori* knowledge. Similarly, if two input variables are highly correlated, subjective judgement and *a priori* knowledge may be used to remove one of the two at the outset. The backward elimination process then proceeds by successive elimination of terms from the saturated model. Automated approaches are available, but these should be avoided in favour of a manual approach that can take context into account. At each stage a variable is considered for removal from the model if doing so does not significantly worsen the fit of the model as judged by the likelihood ratio test. However, terms whose removal substantially alters the estimate of one or more other terms should be retained, even if they are not themselves significant, if they are considered to be confounders of the relationship of interest.

Backward elimination first considers the removal of interaction terms, beginning with the highest-order interactions. If an interaction is retained, the component variables and lower-order interaction terms of these variables should also remain in the model. Hence, if the third-order interaction term ABC is retained, the second-order interactions AB, AC and BC, as well as the variables A, B and C, should also remain in the model, irrespective of whether they individually meet the criterion for retention.

Subsequently, non-significant polynomials should be removed from the model, again beginning with the highest-order terms. Hence, where A^3 , A^2 and A are present, the importance of A^3 should be assessed. If this term is retained, all three terms should remain in the model. If A^3 is deleted, attention shifts to A^2 and so on.

Individual variables not included in either interaction terms or polynomial sets can then be considered for removal one at a time.

The primary statistical criterion for deciding whether to retain a variable is the statistical significance, as judged by a likelihood ratio test. This consists of comparing the maximized log-likelihoods L_1 and L_0 corresponding to models with and without the variable in question, respectively. The test statistic is then $D = 2(L_1 - L_0)$ and its associated *P* value is the upper tail probability of a chi-squared distribution on *r* degrees of freedom, where $r = 1$ if the variable in question is continuous, $r = k - 1$ if it is a categorical variable with *k* categories. The likelihood ratio test is equally applicable to generalized linear models (see later).

³ Also called a **maximal** model.

For linear models, an equivalent test is the F -test. The degrees of freedom of the F -test are $k - j$ and $n - k - j - 1$, where n is the number of observations, whilst k and j are the numbers of terms in the model with and without the variable in question, respectively. For example, the F -test statistic for the comparison of the model in Table 22.7 with that in Table 22.4 in which the interaction term between *birth weight* and *day of birth* has been deleted, is $F_{1,186} = 5.2$. From Appendix XXII, $0.01 < P \text{ value} < 0.05$, and from a statistical software package $P = 0.02$, indicating that the model including the interaction term provides a significantly better prediction of the outcome, *weight at eight weeks of age*.

Alternative approaches for assessing the significance of the deletion of one or more variables include AIC and Schwarz's BIC (Bayesian information criterion; see later in this chapter).

Forward selection begins with inclusion of a single explanatory variable (typically the variable of interest, or perhaps the 'most significant' variable identified in single variable analyses). Each other variable is then added in turn, to select the 'best' two-variable model; that is, the one that most improves the fit, and this two-variable model then becomes the basis for adding additional variables from the remaining variables in turn, to make a three-variable model. This process then continues until adding more variables does not improve the model fit (i.e., no more variables are significant). The researcher may then test for the important interactions amongst the variables in the model. Automatic application of the forward approach is not recommended, as it may poorly control for confounding and interaction.

Stepwise regression combines elements of both forward and backwards selection approaches. For example, if model building commences with a backward elimination approach, excluded variables are re-entered into the model at each stage, using a forward selection process (i.e., one at a time), to check if they improve the fit of the model.

Hence, the process of model selection can be summarized in two stages.

1. Initial steps:

- identify variables to be considered in the model-building process and construct a causal diagram;
- examine and summarize each variable to be considered in the model;
- compare each potential explanatory variable to the outcome variable using:
 - plots (scatter and/or additive model plots for continuous variables, box plots for categorical variables);

- single variable regression analysis of the outcome against each explanatory variable, initially individually and, if indicated, against quadratic and cubic terms;
- explore the relationships between explanatory variables by:
 - plotting the continuous explanatory variables against each other and checking correlation coefficients;
 - tabulating pairs of categorical explanatory variables.

2. Model building:

- for example, a backward selection procedure could entail the following steps:
 - define the saturated model, including plausible interaction terms;
 - consider removal of interaction terms;
 - consider removal of polynomial terms;
 - consider removal of other explanatory terms;
 - possibly recheck previously excluded variables (stepwise regression);
 - consider grouping of category levels.

Diagnostic checking of the final model is always critical, and also may be used informally throughout the model selection process. Finally, and most importantly, *context matters*.

Modelling binary outcomes

General linear models can provide a useful method of analysis for models in which the outcome is on a ratio or interval scale. However, in epidemiological studies the outcome of interest is often binary, such as dead/alive, diseased/well or seropositive/seronegative. For example, in addition to the data presented in Figure 22.1, information was also collected on whether or not each lamb was alive at 12 weeks of age. This was recorded as a variable called *died* which took the value 0 if the lamb survived, and 1 if the animal died. The researchers wished to identify risk factors for death before 12 weeks of age, and one measured potential risk factor was birth weight. A scatter plot of *birth weight* against *died* (Figure 22.7a) provided some information and there is a suggestion that the lambs that died tended to have lower birth weights than those that survived. Nevertheless, it is clear that the relationship between *birth weight* and *died* would not be well explained by a straight line – the key assumption of linear regression. If, rather than using the binary outcome, the observed proportion of deaths at each unique value of birth weight is plotted, this again suggests that there is an increased risk of dying among

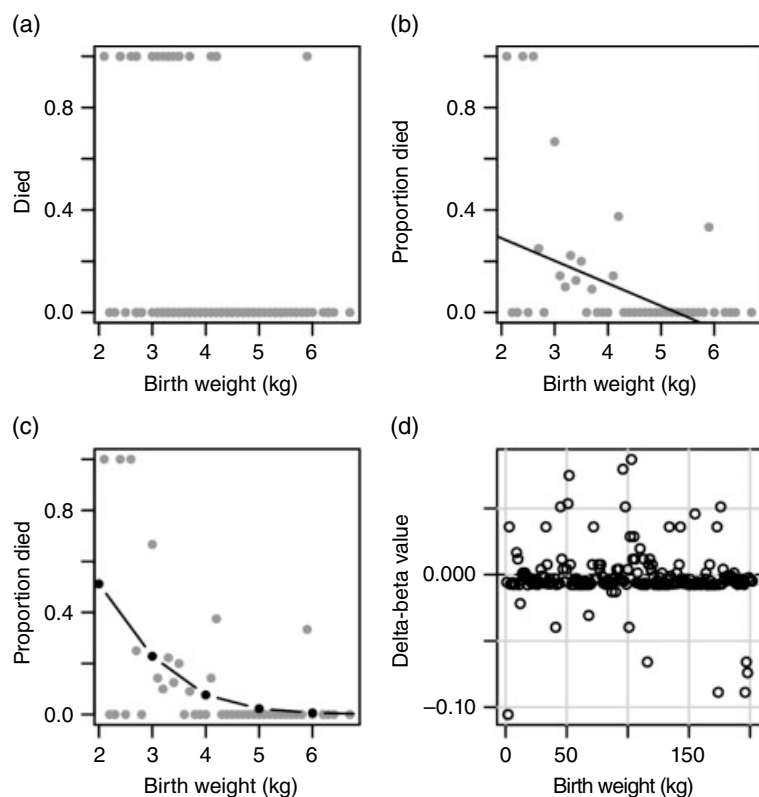


Fig. 22.7 The relationship between birth weight of lambs and the binary outcome *died*, which takes the value 0 if the lamb was alive 12 weeks after birth, and 1 if it was not. (a) Scatter plot of *birth weight* against *died*; (b) plot of the probability of dying at each observed birth weight; (c) plot of the predicted probability of mortality associated with different birth weights (see text for a description of the model); and (d) plot of the delta-beta values for the example logistic regression model.

lower birth weight lambs (Figure 22.7b). However, this still does not appear to be a linear relationship; moreover it is clear from the plot that a linear model fitted to these data would predict probabilities of dying outside the range (0,1) for plausible values of birth weight (e.g., 6 kg). This already gives a compelling reason to dismiss the linear model. But even without this, another objection to the linear model is that if Y is a binary variable with p denoting the probability that $Y = 1$, then the variance of Y is $p(1 - p)$ (see Chapter 12), so is 0 when $p = 0$ or $p = 1$, and maximal when $p = 0.5$. Hence, variance is not constant, which violates another assumption of linear regression.

There are several possible solutions to modelling binary outcome data, which involve transformation of the outcome. The most widely used in epidemiology is the **logistic** or **logit** transformation⁴. If p denotes the probability that $Y = 1$, then the quantity

$$\log\left(\frac{p}{1-p}\right)$$

is called the **logit** of p , or the **log odds** for $Y = 1$. As p varies over its permissible range from 0 to 1, the log

odds vary from minus to plus infinity. Hence, working on the log odds scale removes the first objection to the use of the linear model for binary data; this does not, of course, guarantee that a linear model for log odds will fit any given set of data. The logistic transformation does not address the remaining objections to using the linear model for binary data. To do so, a further step is needed, as follows. A generally accepted principle in statistical modelling is that inference should be based on the likelihood function. This is just the probability of the observed data, but considered as a function of the unknown model parameters. In the case of a binary outcome, suppose that the data consist of n independent pairs of values (x_i, Y_i) , where x_i is an input and Y_i the corresponding output, and let p_i be the probability that $Y_i = 1$. The **logistic regression model** assumes that $\text{logit}(p_i) = \alpha + \beta x_i$, or equivalently that:

$$p_i = \frac{e^{\alpha + \beta x_i}}{1 + e^{\alpha + \beta x_i}}.$$

The likelihood function is then the product of terms p_i for each $Y_i = 1$ and $(1 - p_i)$ for each $Y_i = 0$. The values of α and β that maximize the likelihood are called the **maximum likelihood estimates**, and the corresponding value of the likelihood forms the basis of **likelihood ratio** tests to compare different models (see later; also Hosmer and others, 2013). In particular, this

⁴ Other transformations that have been suggested are the **probit** transformation (Finney, 1971) and the **complementary log-log** transformation (Collett, 2003).

allows us to use the same model selection methods as discussed earlier for linear models. Note that 'log', here, refers to natural logarithms ($\log_e$ or $\ln$).

Generalized linear models

The logistic regression model is a special case of the generalized linear model (GLM), whose introduction by Nelder and Wedderburn (1972) had a transformational effect on statistical practice. These models generalize the linear model in three ways. First, instead of assuming that the mean of the outcome variable is linearly related to input variables, it assumes that some specified function of the mean, called the **link function**, is so related. Hence, in the logistic regression model discussed above, the link function is:

$$g(p) = \ln \left[\frac{p}{1-p} \right],$$

where p is the probability of the outcome at any value of x . As noted earlier, $g(p)$ takes the range $-\infty$ to ∞ . Secondly, instead of assuming a constant variance, a GLM assumes that the variance of Y is a specified function, called the **variance function**, of the mean of Y ; in the binary case the variance function is $V(p) = p(1-p)$. Finally, the Normal distribution is replaced by one of a number of other distributions, which are typically chosen to reflect the nature of the outcome variable. For a binary outcome Y , the only option is the so-called Bernoulli distribution, for which Y takes the value 1 with probability p , and 0 with probability $1-p$. When several independent binary outcomes are observed at each value of the input variable, a natural choice for the sum of the individual outcomes is the binomial distribution, but this is no longer the only possibility (see 'Modelling clustered data' later in this chapter). Poisson or negative binomial distributions are often used

to model open-ended count outcomes, whilst exponential, gamma or Weibull distributions are amongst the possible choices for time-to-event outcomes. Generalized linear models can also be used to analyse **multinomial** and **ordinal** outcome data. Discussion of these is beyond the scope of this book. For more information see Dohoo and others (2009).

The multiple logistic regression model

The multivariable version of the logistic regression model has the form:

$$g(p_i) = \ln \left[\frac{p_i}{1-p_i} \right] = \alpha + \beta_1 x_{1i} + \beta_2 x_{2i} \dots + \beta_m x_{mi} : \\ i = 1, \dots, n.$$

Once the value of $g(p_i)$ has been estimated, the corresponding value for the probability p_i is:

$$\frac{e^{g(\hat{p}_i)}}{1 + e^{g(\hat{p}_i)}} = \frac{e^{\hat{\alpha} + \hat{\beta}_1 x_{1i} + \hat{\beta}_2 x_{2i} \dots + \hat{\beta}_m x_{mi}}}{1 + e^{\hat{\alpha} + \hat{\beta}_1 x_{1i} + \hat{\beta}_2 x_{2i} \dots + \hat{\beta}_m x_{mi}}} : \\ i = 1, \dots, n.$$

Using a logistic regression model, estimates of the parameters for the example in *Figure 22.7a* are shown in *Table 22.8a*. Using these parameters, the probability of dying for a lamb with a birth weight of, say, 3 kg is:

$$p(x) = \frac{e^{2.58 + (-1.27 \times 3)}}{1 + e^{2.58 + (-1.27 \times 3)}} = 0.23.$$

Hence, the model predicts that, on this farm, almost a quarter of lambs with birth weight 3 kg will die before 12 weeks of age. Plotting such predictions illustrates that the model fits the observed data reasonably well (*Figure 22.7c*). Hence, these results suggest that, on this farm, the probability of dying before 12 weeks is almost negligible for lambs with a birth weight >5 kg,

Table 22.8 Output of the logistic regression model for: (a) the data presented in *Figure 22.7a* and (b) the model extended to include a dummy variable for multiple births (0 if singleton, 1 if twin or triplet).

	Estimate	Standard error	t statistic	P value	95% CI of the estimate	Odds ratio (OR)	95% confidence interval of the OR
(a)							
Intercept	2.58	1.22	2.11	0.03	0.027 to 5.09		
Birth weight	-1.27	0.35	-3.65	<0.001	-2.00 to -0.63	0.28	0.13 to 0.53
(b)							
Intercept	3.17	1.74	1.83	0.07			
Birth weight	-1.33	0.37	-3.59	<0.001	-2.10 to -0.64	0.27	0.12 to 0.53
Multiple birth	-0.42	0.86	-0.49	0.6	-1.97 to 1.56	0.66	0.14 to 4.75

but below 5 kg there is an increasing probability of dying as birth weight decreases.

The logit function also has the very great advantage that the parameter estimates can be easily converted to **odds ratios**, making interpretation of the results quite straightforward. If β_k denotes the logistic regression parameter for the explanatory variable x_k , then the corresponding odds ratio (OR), is:

$$\text{OR}_k = e^{\beta_k}.$$

The associated **95% confidence interval** of the odds ratio can therefore be calculated as:

$$95\% \text{ confidence interval } (\text{OR}_p) = e^{\beta_k \pm 1.96 \times se_k},$$

where se_k is the standard error of the parameter estimate, β_k .

Therefore, for the example in Table 22.8a:

$$\text{OR}_{\text{birth weight}} = e^{-1.27} = 0.28,$$

$$\begin{aligned} 95\% \text{ confidence interval } (\text{OR}_{\text{birth weight}}) \\ = e^{-1.27 \pm 1.96 \times 0.35} = 0.13, 0.53. \end{aligned}$$

This states that, according to the fitted model, the odds of a lamb dying before 12 weeks of age changes, by a factor estimated to be 0.28 for each kilogram increase in birth weight. As the 95% confidence interval of this estimate (0.13 to 0.53) does not include one, it can also be concluded that the effect of birth weight on the risk of dying is statistically significant (as also indicated by the P value for the regression parameter) and that increased birth weight reduces the risk of dying.

Categorical variables can be included as input variables in logistic regression models by using dummy variables, in the same way as for general linear models (see earlier). Hence, in the example, if the aim was to investigate the effect of birth weight on the risk of dying before 12 weeks of age, after allowing for the effect of multiple births, a dummy variable to identify twin and triplet lambs could be included in the logistic regression model. The results (Table 22.8b) suggest that, after allowing for birth weight, the best estimate of the ratio of the odds of dying for multiple-birth lambs compared with singleton lambs is 0.66. However the 95% confidence interval (0.14, 4.75) includes one; hence these data do not provide good evidence against the null hypothesis (H_0 : OR = 1) of no effect of multiple births.

Linear regression is also a special case of a GLM, which in this case uses the **identity** link, $g(\mu) = \mu$, where μ denotes the mean of Y . GLMs can be extended to other types of data. For example, analysis of count data (such as the number of farms diagnosed with bovine tuberculosis in a region each month) often uses a logarithmic link, i.e., $g(\mu) = \log(\mu)$, in conjunction

with a Poisson assumption for the distribution of the outcome variable.

One important area of application for the logistic regression model is in the analysis of data from unmatched case-control study designs. As noted in Chapters 15 and 16, analysis of matched case-control studies should account for the matching that has occurred and this requires a modified form of the logistic model, called **conditional logistic regression**. Conditional logistic regression analyses cannot estimate the effect of the matching variables (or any other variables that do not vary within the matched sets). Not all statistical software programs perform conditional logistic regression. Both *STATA* (using the clogit command) and *R* (using the clogit function, available in the *survival* package) can perform conditional logistic regression on $n:m$ matched data. *SAS* can undertake conditional logistic regression on 1:1 matched data using the *LOGISTIC* procedure or on $n:m$ matched data using the *PHREG* procedure.

Model selection for logistic regression models

As noted earlier, selection of variables for inclusion in a logistic regression model proceeds in a similar manner to that described above for general linear models, with assessment of whether or not a term significantly improves the explanatory power of the model being assessed using either of two tests associated with the likelihood function, namely the Wald test or the likelihood ratio test (LRT). The **Wald test** statistic for inclusion of an input variable x_k is:

$$W_k = \frac{\beta_k}{se(\beta_k)},$$

where β_k is the regression parameter estimate and $se(\beta_k)$ its associate standard error. An absolute value of W_k bigger than 1.96 is equivalent to $p < 0.05$. The Wald test provides rapid assessment of the likely significance of single terms in a model, and most packages routinely include it in their standard output. However, the likelihood ratio test is preferred as it has slightly better theoretical properties in finite samples; in large samples, the two tests usually give very similar results (Hauck and Donner, 1977; Jennings, 1986).

The **likelihood ratio test statistic** (G) is the change in the deviance between the two models being compared. The deviance of a GLM is analogous to the residual sum of squares in linear regression and is calculated as:

$$D = -2 \times \log \left[\frac{\text{likelihood of the current model}}{\text{likelihood of the saturated model}} \right],$$

where the saturated model is one that contains as many explanatory variables as there are observations.

The likelihood ratio statistic to compare two models is:

$$G = D_1 - D_0,$$

or equivalently:

$$G = 2 \times \ln \left[\frac{\text{likelihood of model 1}}{\text{likelihood of model 0}} \right],$$

where model 0 is a special case of model 1, that is, model 1 with one or more terms removed. The likelihood ratio statistic follows a χ^2 distribution with degrees of freedom equal to the difference in the number of parameters in the two models.

For example, the deviance for the model in Table 22.8a is 117.10 and for the model with a single additional term (*multiple birth*; Table 22.8b) is 116.87. Therefore, $G = 117.10 - 116.87 = 0.23$. From Appendix IX, $P > 0.05$, and from a statistical software program, $P = 0.6$. Hence, there is strong evidence to suggest that inclusion of the variable *multiple birth* does not improve the model.

Alternative approaches to comparing models include the AIC (Akaike, 1974) and Schwarz's BIC (Schwarz, 1978). Whereas the likelihood ratio test only can be used to compare nested models (that is, where the terms included in one model are a sub-set of those in the other model), AIC and BIC can be used to compare both nested and non-nested models. However, test-based methods (F -test, likelihood ratio test) are the preferred methods for comparing nested models. In addition, only models based on exactly the same data sets can be compared using AIC or BIC. There are several (slightly different) methods of calculating AIC and BIC; including:

$$\text{AIC} = -2 \times \ln(\text{likelihood}) + 2(k + 1),$$

$$\text{BIC} = -2 \times \ln(\text{likelihood}) + (k + 1)(\ln(n)),$$

where k is the number of parameters in the model and n is the number of observations (Burnham and Anderson, 2004). Conventionally, models are compared by direct comparison of these values for each model, and models with lower AIC or BIC are preferred. As a general rule, a change in AIC or BIC between 2 and 6 provides moderate evidence, a change of 6–10 provides strong evidence and a change greater than 10 provides very strong evidence in favour of the preferred model. Inspection of the definitions of G , AIC and BIC makes it clear that all three use a similar principle, namely that the correct way to compare two models is by the difference between their respective log likelihoods. They differ only in the threshold at

which they favour the more complicated model. If we use G , the threshold is determined by the critical value for statistical significance as judged by a χ^2 distribution, which in turn requires a choice of *which* critical value to use – convention would suggest the value corresponding to $P = 0.05$. If we use AIC, the threshold is just the difference between the numbers of parameters in the two models. If we use BIC, the threshold increases with the number of observations, n . The inescapable conclusion is that model building is something of a 'black art'; our own preference is to use likelihood ratio tests tempered by contextual information.

Diagnostic checking of logistic regression models

As with generalized linear regression models, diagnostic checking is an important part in the development of a logistic regression model, or of any other GLM. A number of approaches are available (Dohoo *et al.*, 2009), but important considerations include:

- checking that the data are consistent with the assumptions of model;
- identifying influential observations.

The assumption of linearity can be assessed using similar approaches to those described for continuous outcome variables, but taking account of the assumed link function (e.g., exploration using models that include: transformation of input variables; piece-wise terms; categorized input variables; or, alternatively, generalized additive models (see later)). In particular, a plot of standardized residuals, against fitted values is still one of the most useful kinds of diagnostic plot, but with an important qualification, as follows. Note that for a binary outcome $Y = 0$ or 1 , the fitted value is p , the probability that $Y = 1$. Then, the fitted variance of Y is $V = p(1 - p)$, and the standardized residual is therefore:

$$r = \frac{Y - p}{\sqrt{V}}.$$

Because Y can take only two values, the same applies to each residual and even in a well-fitting model, the plot looks far from the random scatter described earlier for well-fitting linear models. If values of x are replicated, a more useful plot is obtained by summing both the numerator and denominator in the above definition of r , hence if m values of Y have identical fitted values p , we write Y^+ for the sum of these m values and define a single residual:

$$r^+ = \frac{Y^+ - mp}{\sqrt{mV}}.$$

If the values of x are not replicated, we can achieve a similar result by ordering the Y values from smallest to largest and dividing into groups of, say, 10 consecutive values, and within each group summing Y , p and V , hence:

$$r^+ = \frac{Y^+ - p^+}{\sqrt{V^+}}.$$

To be useful as a graphical method, this requires a reasonably large data set, say $n \geq 100$ to give at least 10 plotted points. The method is also the basis of the Hosmer–Lemeshow goodness-of-fit test (Hosmer and Lemeshow, 1980; Lemeshow and Hosmer, 1982; Hosmer *et al.*, 1988). Table 22.9 provides the observed and expected number of deaths in each of four groups. Four groups are used here, rather than 10, because of the relatively low number of events (deaths) in the data, which also rules out an effective graphical presentation. The data presented in Table 22.9 are calculated as follows, using the information on the number of dead lambs in the lowest risk quartile, group 1. The observed number of dead lambs is calculated as the sum of the observed outcome values (0 or 1) for the individuals included in this group, in this case one out of the 53 lambs in this group (with 52 remaining alive). The expected number of dead lambs is calculated as the sum of the predicted probabilities of dying for each individual in this group, in this case 0.9. Hence, for this group, and for the other groups, the observed and expected frequencies of events are similar, suggesting the data are a reasonable fit to the model. The Hosmer–Lemeshow χ^2 statistic for these data is 0.97 with two degrees of freedom. Therefore, from Appendix IX, $P > 0.05$, or from a statistical software package, $P = 0.6$. Hence, there is insufficient evidence to reject the null hypothesis that there is no difference between the observed and predicted values, implying that the data are a reasonable fit to the model.

Table 22.9 Observed and expected number of events (deaths) within each quartile for each outcome (alive, dead) based on logistic regression model from Table 22.8a.

Group	Alive		Dead	
	Observed	Expected	Observed	Expected
1	52	52.10	1	0.90
2	50	50.93	4	3.07
3	45	43.25	4	5.75
4	34	34.72	12	11.28

Influential observations may be identified using delta-beta ($\Delta\beta$) values, which provide an estimate of the effect of each observation on the estimated coefficients (hence the name, delta = change, beta = coefficient). Plots of delta-beta values for each observation can be used to identify influential points. Observations with large delta-beta values may, for exploratory purposes, be deleted from the data and the model re-run to ascertain the extent to which these observations influence the parameter estimates (Hosmer *et al.*, 2013). Delta-beta values are sometimes considered moderately large if greater than 0.5 in absolute value, and very large if greater than 1 in absolute value. Figure 22.7d illustrates the delta-beta values for the fitted logistic regression model (Table 22.8a). All are small, suggesting that no observations have excessive influence on the parameter estimate.

Generalized additive models

The **generalized additive model** (GAM) extends the general linear model in exactly the same way as the additive model extends the linear model. In a GLM for binary data with link function $g(p)$, the fundamental assumption is that:

$$g(p) = \alpha + \beta_1 x_{1i} + \beta_2 x_{2i} \dots + \beta_p x_{pi} : i = 1, \dots, n.$$

For the logistic model,

$$g(p) = \log \left[\frac{p}{1-p} \right].$$

The corresponding GAM allows any or all of the linear terms, $\beta_k x_{ki}$ to be replaced by arbitrary smooth functions, to give:

$$g(p) = \alpha + s_1(x_{1i}) + s_2(x_{2i}) + \dots + s_p(x_{pi}) : i = 1, \dots, n.$$

One of a number of uses of a GAM is to provide an alternative graphical check on the goodness of fit of an assumed GLM. Most software implementations of GAMs allow the user easily to plot each fitted smooth function $s_k(x)$ together with approximate 95% confidence limits. If the fitted model for the term involving x , that is, βx for a linear fit, $\beta_1 x + \beta_2 x^2$ for a quadratic fit, and so on, is added to this plot, it should lie mostly within the envelope formed by the confidence limits. What is meant by ‘mostly’ has to be somewhat vague, not least because of the approximate nature of the confidence limits associated with the GAM fit.

Modelling clustered data

The methods described above assume that the residuals, Z_{ip} , are independent. However, this is not the

case when the data are clustered. Clustering is common in epidemiological data sets and may arise under a wide range of circumstances. Clustering occurs when observations within a cluster, or group, are more similar to each other in terms of the outcome of interest than would be expected by chance. Hence, risk factors can be considered to be clustering variables. For example, if *sex* was a risk factor for the average rate of occurrence of a particular disease, the probability of being diseased would be more similar for two males (or two females) than would be expected by chance. Where the effect of such variables can be identified and measured, these should be included in the model as explanatory variables, as described in the preceding sections. Such variables are called **fixed effects**. When a variable of this kind has not been measured it can still be included in a model by treating it as an unobserved random variable, called a **random effect**. For example, suppose that the probability that the weight, Y , of a lamb at a specified age depends on both its birth weight and its gestation time. A suitable linear regression model would specify that:

$$Y = \alpha g + \beta x + Z,$$

where g denotes gestation time, x birth weight and Z the residual, assumed to be Normally distributed with a mean of zero and variance of σ^2 . Note that g takes the same value for all lambs born to the same ewe. Now suppose that the gestation time for each lamb is not available, only its birth weight. We can no longer fit the model above to the data, but could instead specify an alternative model:

$$Y = A + \beta x + Z,$$

where now A is an unobserved random variable. The usual assumption about A would be that it varies amongst lambs according to a Normal distribution with mean α and variance v^2 . This alternative model is called a **linear mixed model**: mixed, because it treats birth weight as a fixed effect and unobserved gestation time as a random effect. Now suppose that the survival probability depends on a third variable, also unavailable in the data. It might be tempting to extend the model to:

$$Y = A_1 + A_2 + \beta x + Z,$$

where now both A_1 and A_2 are unobserved random variables. But this would be futile, because if both A_1 and A_2 are unobserved we have no way of knowing how much variation to ascribe to A_1 and how much to A_2 . So we revert to the previous model with a single random effect A representing the sum of A_1 and A_2 . This argument can be repeated with any number of unobserved variables. It follows that a random effect

can be thought as a proxy for the combined effect of all relevant, but unobserved, explanatory variables.

Why is it that we cannot untangle the contributions of A_1 and A_2 , but we can untangle the contributions of A and Z ? This is possible only if the data include multiple births. Then, although the values of A for each lamb are unknown, we do know that lambs of the same ewe share the same value of A . This implies that the correlation between values of Y for two lambs of the same ewe is:

$$r = \frac{v^2}{v^2 + \sigma^2}.$$

Because Y and x are observed for every lamb, r can be estimated, and hence so can the relative contributions of A and Z to the total variation of Y about the fitted regression line, $\alpha + \beta x$.

In addition to its occurrence due to individual characteristics, clustering may arise because of the way animals live and the way data are collected. For example, populations of interest to veterinary epidemiologists are often kept in managed groups (clusters): farms, herds, sheds, kennels, households and so on. Hence, these animals share a common environment and other exposures that may cause animals within a cluster to be more similar to one another, with respect to the outcome, than to animals in other clusters. Alternatively, repeated measurements of, say, heart rate, made on animals are clustered with each animal. These scenarios lead to models with multiple random effects.

The *structure* of clustered data can vary from quite simple (such as *dogs* within *households*) to more complex. Complexity may arise because there may be many more levels of clustering in the data and/or because the relationship between the levels may be more complex.

Nested data structures occur when the levels of one factor vary only with the levels of one cluster of each higher level. The example used in much of this chapter, of lambs within ewes, is such a case, because each ewe had between one and three lambs. More complex examples may exist. For example, *Figure 22.8a* illustrates the clustering in a hypothetical study of the presence of antibodies to *Salmonella* in pigs:

Level	Name of level	Description of level
6	Farms (F)	Farms in a region are randomly selected for inclusion in the study
5	Houses (H)	Houses are randomly selected from all houses on the selected farm
4	Pens (P)	Pens are randomly selected from each selected house

3	Animal (A)	Animals are randomly selected from each selected pen
2	Sample time (T)	Each pig has blood drawn for testing each week for four weeks
1	Lab measurement (M)	Each sample is tested for antibodies three times

Crossed data structures occur when each lower level may be related to more than one higher-level unit (i.e., whenever the data are not fully nested). *Figure 22.8b* illustrates clustering in a hypothetical study investigating the risk factors for the detection of bovine tuberculosis in cattle at post-mortem; individual cows (C) may not cluster within single farms (F) if they move during their lives. Similarly, individual farms may not cluster within particular abattoirs if their stock are slaughtered at more than one facility. In this example, cows 1 to n all spent time on farm 1, but cow 3 also moved to farms 4 and 5 during her life. Also, farm 1 sent animals to abattoirs (A) 1 and 2. Hence, the levels are not arranged hierarchically, but rather are *crossed*.

Where the number of clustering groupings is reasonably small compared with the number of observations, it is possible to account for clustering by these variables by including them as fixed effects in a model.

For example, if data were collected from 100 animals on each of four farms, a categorical variable identifying the farm for each observation could be included as a fixed effect.

Alternatively, cluster groupings can be thought of as arising from a larger, population-wide, set of clusters. The difference between the clusters is attributed to random variation, and the clustering variables are called random effects. Hence, the variation between two observations may be due to random variation between individuals, random variation between the clusters they belong to, or to variation in fixed effects – that is, the explanatory variables of interest. As noted above, models that include both fixed and random effects are often called **mixed-effects models**. Other names include **random-effects models**, **multilevel models** and **hierarchical models**, although the latter only really applies to nested mixed-effects models. Generalized linear models that include both fixed and random effects are often called **generalized linear mixed models** or GLMMs. Each fixed effect has associated with it a regression parameter, and the model is used to estimate the regression parameters from the data. In contrast, the random effect is a random variable – in a strict technical sense, it does not make sense to estimate a random variable. Instead, parameters, typically variances, are estimates that describe the *distribution* of the random effects. Having estimated the model parameters, we can *predict* the realized values of the random effects

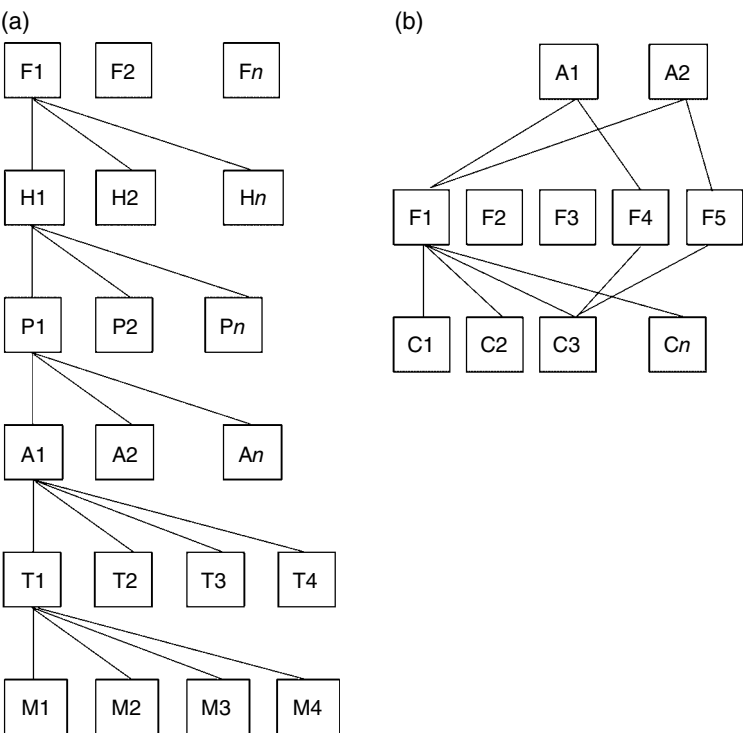


Fig. 22.8 Schematic representations of: (a) a nested data structure, and (b) a crossed data structure.

if these are of interest; specifically, the **minimum mean square error predictor** of the realized value of a random effect is the expected value of each random effect conditional on the data. Typically, these predicted values are much less precise than are parameter estimates because each prediction draws largely on the information provided by the data from a single cluster, whereas parameter estimates pool information across all clusters.

Mixed models account for the influence of the random effects by partitioning the error in the model into the different levels of the random effects. For example, a mixed-effects model of the data arising from the observation of lambs would partition the error (i.e., the residuals) into two components, the first due to differences between ewes and the second due to differences between lambs of the same ewe.

A two-level linear mixed model (LMM) with a single random effect takes the form:

$$Y_{ij} = \alpha + \beta_1 x_{1ij} + \beta_2 x_{2ij} + \dots + \beta_p x_{pij} + W_i + Z_{ij}.$$

In this model, W_j is the residual error due to the random effect (e.g., due to ewes being different from one another), and Z_{ij} is the residual error due to the individual (e.g., due to lambs differing from one another). Notice that each parameter is now identified by the subscripts i and j , with i identifying each random-effect group and j identifying each observation. Hence, x_{1ij} is the measurement of variable x_1 for lamb j from ewe i .

The simplest LMM, incorporating only the intercept (i.e., the **random intercept model**), gives as its result the overall mean of the outcome variable. If a random effect is included, this simple model is written as:

$$Y_i = \alpha + W_j + Z_{ij},$$

which can be rearranged as:

$$Y_i - \alpha = W_j + Z_{ij}.$$

In this equation, the amount that each observation differs from the mean value is now partitioned into the two random effects, as depicted in Figure 22.9a for the three lambs of ewe 6 in the example data.

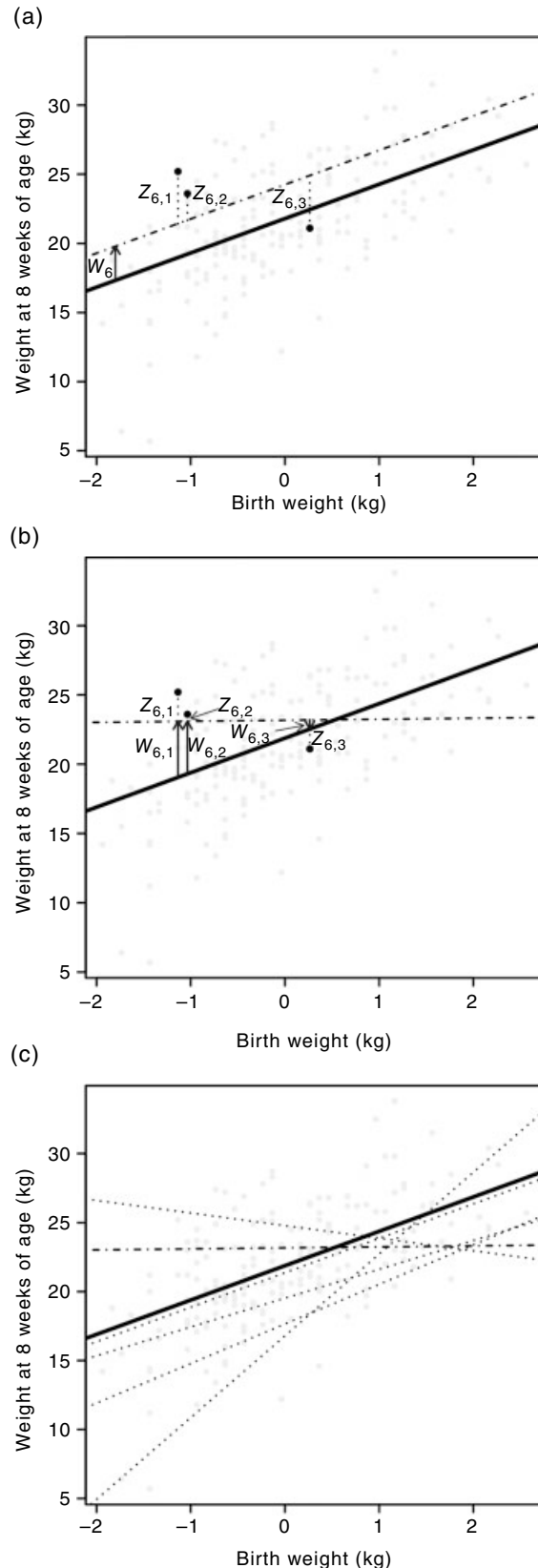


Fig. 22.9 Plot of results of a mixed-effects regression analysis of the relationship between birth weight and weight at eight weeks of age, in which observations (on lambs) are clustered within ewes. (a) Random intercept model: comparison of results for ewe 6 (---) with the average ewe (thick solid line). W_6 is the residual error due to the effect of ewe 6, $Z_{6,1}$, $Z_{6,2}$, $Z_{6,3}$ are the residual error associated with each of ewe 6's three lambs. (b) Random slope and intercept model: comparison of results for ewe 6 (---) with the average ewe (thick solid line). W_6 is the residual error due to the effect of ewe 6, $Z_{6,1}$, $Z_{6,2}$, $Z_{6,3}$ are the residual error associated with each of ewe 6's three lambs. (c) Random slope and intercept model: comparison of results for ewe 6 (---), the average ewe (thick solid line) and the ewes with the 1st, 25th, 50th, 75th and 100th percentile values for the slope (dotted lines).

The above LMM allows the intercept of the regression model to vary between clusters, but assumes that the slope of the regression line is constant across the groups. In the example, this leads to the assumption that the impact on weight at eight weeks of, say, a 1 kg difference in birth weight is the same for all ewes. However, it is not unreasonable to imagine that the effect of having low compared with high birth weight may be greater for twin lambs of some ewes compared with others, perhaps due to mothering ability, milk production and so on. That is, it may be that the effect of birth weight differs between ewes or, in other words, the slope of the regression line might vary between ewes; so-called **random slopes**. Random slopes are similar to interaction terms between two fixed effects. *Figure 22.9b* illustrates the regression line for ewe 6 in a model in which the effect of birth weight is allowed to vary between ewes; for lambs from this ewe the association between birth weight and weight at eight weeks of age is approximately horizontal. *Figure 22.9c* illustrates, in addition to the average ewe and ewe 6, the regression lines for the ewes with the 1st, 25th, 50th, 75th and 100th percentile values for the slope. This suggests considerable variation between ewes in terms of the effect of their lambs' birth weights on weight at eight weeks of age; comparison of these models using the likelihood ratio test ($g_2 = 10.3$) indicates that inclusion of the random slope improves the model (from Appendix IX, $0.01 < P < 0.001$, or from statistical software, $P = 0.006$).

Partitioning of the residual variation into that due to different random effects enables estimation of the proportion of the total variation that is due to each random effect. This proportion is the correlation between two responses within the same cluster, also called the **intra-class correlation coefficient (ICC)**. For a two-level model this can be expressed as:

$$ICC = \frac{\sigma_w}{\sigma_w + \sigma_z},$$

where σ_z is the variance of Z_{ij} , and σ_w is the variance of W_j .

When the $ICC = 0$ all the variation between observations is due solely to variation between observations (as $W_j = 0$), in which case there is no need to account for clustering. In contrast, if the $ICC = 1$, all the variation between observation is due to variation between clusters, and there is no random variation between observations within each cluster.

The value of the ICC can reveal useful information. For example, consider the data for the weight of lambs at eight weeks of age. In the intercept-only model, the variance attributable to the random effect was 13.96, and the residual variance was 5.92. Therefore, the

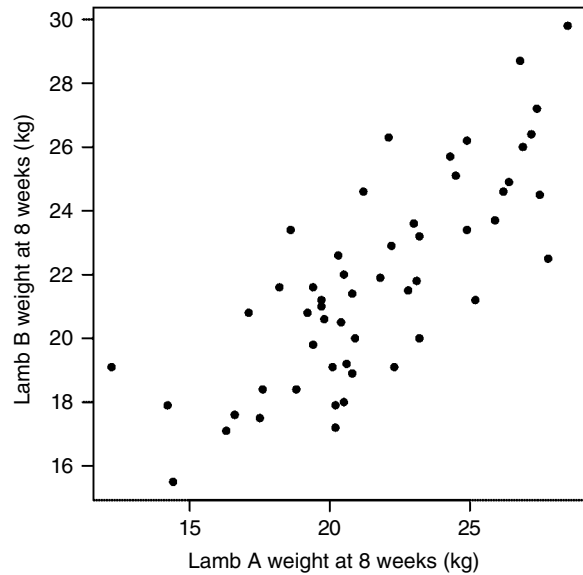


Fig. 22.10 The relationship between the weights at eight weeks of age of twin lambs. Within each pair of twins, one lamb was randomly selected to be lamb A, and the other lamb B.

proportion of the variance due to the random effect is $13.96/(13.96 + 5.93) = 0.7$. Hence, considerable variation is attributable to variation between ewes, and the weight of sibling lambs at eight weeks of age is highly correlated. This is evident in *Figure 22.10*, which shows the relationship between the weights at eight weeks of age of twin lambs.

There are many reasons why the lambs of some ewes may be smaller at eight weeks of age compared with those of other ewes. For example, litter size is a ewe-level variable (i.e., all of the lambs from a single ewe have the same response for the variable, *litter size*) and it is reasonable to suspect that twin (and triplet) lambs will, on average, be smaller than singletons. In other words, variation attributed to the random effects is, at least in part, due to fixed effects *not* included in the model. Therefore, it is not surprising that inclusion of the fixed effect *litter* in the model results in reduction of the variance attributed to the ewe level (*Table 22.10*). Note in *Table 22.10* that although the estimates of the coefficients are not greatly different to those calculated without inclusion of the random effect (*Table 22.5b*), the standard errors of the estimates are somewhat larger, and hence the t statistics are smaller. An important effect of failure to account for clustering within data is to change the standard errors of the estimated fixed-effects regression parameters. As a consequence, an analysis that ignores the clustering structure is incorrect, and typically underestimates the standard errors, potentially leading to spurious claims of statistical significance.

Table 22.10 Output of a linear mixed-effects regression model of the effect of litter size (singleton, twin or triplet) on weight of lambs at eight weeks of age. *Ewe* is included as a random effect (W_{ewe}).

	<i>Estimate</i>	<i>Standard error</i>	<i>t statistic</i>	<i>P value</i>	<i>95% confidence interval</i>
Intercept	24.94	0.64	39.10	<0.001	23.69 to 26.2
Twin	-3.39	0.79	-4.30	<0.001	-4.93 to -1.85
Triplet	-6.32	1.13	-5.62	<0.001	-8.53 to -4.12
$W_{ewe} = 9.99$					
$Z_i = 5.47$					

Other types of outcome variable can be modelled by inclusion of random effects in generalized linear models, for example, a **mixed-effects logistic regression model** can be used to model binary outcomes whilst accounting for clustering due to random effects. Let p_{ij} denote the probability of a positive response from the j th member of the i th cluster. A mixed logistic regression model with a single explanatory variable x_{ij} assumes that:

$$\log\left(\frac{p_{ij}}{1-p_{ij}}\right) = \alpha + W_i + \beta x_{ij}.$$

There is no term Z_{ij} in the model, because the variance of a binary response is necessarily equal to $p_{ij}(1-p_{ij})$. Nor is there a simple expression for the correlation between two binary responses in the same cluster. However, as in the analogous linear mixed model, this correlation is positive, and its value is directly related to the variance of the random effect W . Analogues of ICC are described, for example, in Goldstein and others (2002), including the binary linear approach and the latent variable approach.

The **binary linear approach** to the calculation of ICCs assumes that the outcome is, in fact, linear

and therefore models the untransformed outcome. The **latent variable approach** assumes that the variation at the level of the outcome equals $\pi^2/3$. The latent variable approach is most appropriate when the binary response (0,1) is derived from an underlying continuum. For example, categorizing an ELISA optical density result as positive or negative on the basis of a cut-off value, or of pass/fail on the basis of the results of an examination, represents binary categorization of an underlying continuous variable. This approach is less appropriate for truly discrete variables, such as *dead* or *alive*.

Model checking for mixed-effects models should be conducted to test the model assumptions. For linear models it is useful to examine the residuals, recalling that the total residual error is partitioned. Methods for model checking for mixed-effects logistic regression models are not well developed.

Further discussion of the theory and practicalities of modelling clustered data are beyond the scope of this book. Twisk (2006), Crawley (2007), Dohoo and others (2009) and Snijders and Bosker (2012) provide more detailed information.

Further reading

- Diggle, P.J. and Chetwynd, A.G. (2011) *Statistics and Scientific Method*. Oxford University Press, Oxford
- Dohoo, I., Martin, W. and Stryhn, H. (2009) *Veterinary Epidemiologic Research*, 2nd edn. VER, Charlottetown
- Hosmer, D.W., Lemeshow, S. and Sturdivant, R.X. (2013) *Applied Logistic Regression*. 3rd edn. John Wiley and Sons, Hoboken
- Kleinbaum, D.G., Kupper, L.L., Nizam, A. and Rosenberg, E. (2013) *Applied Regression Analysis and Other Multivariable Methods*, 5th edn. Cengage Learning, Boston
- Snijders, T.A.B. and Bosker, R.J. (2012) *Multilevel Analysis: An Introduction to Basic and Advanced Multilevel Modeling*, 2nd edn. Sage Publishers, London
- Twisk, J.W.R. (2006) *Applied Multilevel Analysis*. Cambridge University Press, Cambridge

23

Mathematical modelling

Modelling is the representation of physical processes, designed to increase appreciation and understanding of them. Thus, two causal models were described in Chapter 3, and two models of interaction in Chapter 5. Similarly, the Normal distribution (see Chapter 12) sometimes is termed the Normal probability model. A more specific meaning of modelling is the representation of events in quantitative **mathematical** terms, which may allow predictions to be made about the events¹. Modelling, in this sense, is applied to many disciplines, including economics, engineering, agriculture, finance, meteorology and medicine. In epidemiology, it provides a useful means of investigating diseases where experiments and field observations are impracticable. Models are constructed to attempt to explain and predict patterns of disease occurrence and what is likely to happen if various alternative control strategies are adopted. An introduction to this approach has been given already in Chapter 8 in which the basic reproductive number (R_0) was introduced as a measure of the tendency of an infection to spread in a population, and in which the Reed–Frost model of a propagating epidemic was described. Accurate models can be useful guides to choosing the most efficient disease-control techniques as well as increasing understanding of the life-cycles of infectious agents. This chapter describes the main types of model, giving examples relating to infectious diseases.

Modelling dates back to the 18th century, when Daniel Bernoulli applied a simple life table to French smallpox data, which indicated that variolation² was efficacious and conferred lifelong immunity (Bernoulli, 1766; Dietz and Heesterbeek, 2002). Records of human deaths were maintained in parish registers in the UK, a notable example being those

of the 1665–1666 epidemic of plague in the village of Eyam in Derbyshire (Creighton, 1965). John Graunt's compilation of 17th-century English mortality data, and the French Royal Society of Medicine's recording of 18th-century statistical data on animal and human epidemics, have already been mentioned in Chapter 1. Friendly Societies, founded in the UK early in the 19th century, recorded information on disease and deaths among their members (Ratcliffe, 1850). Thus, morbidity and mortality data on diseases such as smallpox and plague were readily available by the middle of the 19th century, and mathematical models were proposed by medical statisticians to explain the observed mortality rates (Greenwood, 1943). William Farr fitted a lognormal curve (see Chapter 12) – initially enunciated by De Moivre in 1733 – to smallpox data in England and Wales (Farr, 1840), and predicted the course of the 1866 British rinderpest epidemic using a similar approach (Brownlee, 1915; Spinage, 2003)³.

The early models, which founded general theory, described natural epidemics of human infectious diseases. Only within the last 60 years has significant attention been paid to the modelling of animal diseases. Since the early work, there has been a division into the theoretical approach, which is concerned with epidemic theory, and the practical approach, which attempts to be of direct value to disease-control campaigns. Successive models have attempted to become

¹ For the distinction between broad mathematical models and statistical models (described in Chapter 22), see Chapter 2 and Appendix I.

² Variolation is the obsolete process of inoculating a susceptible person with material from the vesicle of a patient with smallpox.

³ Farr also studied cholera, and his quantitative investigations were in contrast to the qualitative observations of John Snow (see Chapter 3), the latter of which, however, provided a clearer explanation of the disease's cause than the former (Eyler, 2001). Nonetheless, Farr exerted a major influence on 19th-century medicine in the UK (Susser and Adelstein, 1975), and the fundamental principle that epidemics follow a natural sequence in which they spread and subside in a pattern that approximates a symmetrical curve (sometimes termed 'Farr's Law') has been applied subsequently to a wide variety of conditions, ranging from AIDS (Bregman and Langmuir, 1990) to drug overdose (Darakjy *et al.*, 2014).

more realistic, incorporating the effects of control techniques, such as vaccination, quarantine and treatment, in order to evaluate alternative strategies for disease control (e.g., Arino *et al.*, 2007; Brauer, 2008). The effects of economic constraints and implications have, of necessity, been incorporated in the more recent formulations. Most techniques use computers to simulate situations, although they are not always necessary.

Types of model

Density and prevalence models

Veterinary modelling has been directed towards infectious diseases, although non-infectious ones also can be modelled. Infectious agents can be classified into two groups according to their generation dynamics: **microparasites** (e.g., viruses and bacteria) and **macroparasites** (e.g., helminths and arthropods) (see Chapter 7), and the two different dynamic patterns lend themselves to two different types of modelling.

Density models consider the absolute number of infectious agents in each host, and are commonly used in macroparasitic infections, where numbers of infectious agents can be estimated either in the host or in the environment. Microparasitic infections also can be modelled using density models when microparasite numbers can be enumerated, but are frequently studied using **prevalence models**, which consider the presence or absence of infection in various host cohorts; for example, young and mature, immune and susceptible⁴. The density model is potentially the more refined of the two techniques because it attempts to enumerate the number of infectious agents with which a host is challenged.

Deterministic and stochastic models

In many models, the values of input parameters can be fixed, and the results obtained do not take account of random variation (i.e., variability). Such mathematical descriptions are examples of **deterministic** models. In contrast, some models describe processes or events subject to random variation. Additionally, there may be uncertainty stemming from the modeller's lack of knowledge of the parameters (see also Chapter 24).

⁴ A specific class of models, termed **SIR models** (Allen, 1994), computes the theoretical number of individuals infected with a transmissible infectious disease in a closed population over time. The name of this class of models derives from the fact that they involve coupled equations relating the number of susceptible individuals, *S*, the number of infected individuals, *I*, and number of individuals who have recovered, *R*. One of the simplest **SIR** models is the **Kermack–McKendrick model** (see also Chapter 8: 'Kendall's Threshold Theorem'), which was proposed to explain the pattern of epidemics of plague and cholera (Kermack and McKendrick, 1927).

Variability and uncertainty lead to outcomes with a probability. These models are **stochastic**, the word being derived from the Greek *stochastikos*, meaning 'skilful in aiming at, able to guess'. Stochastic models often enable probability distributions and intervals (see Appendix I) to be associated with the outputs.

It may not always be clear whether a model is deterministic or stochastic. For example, the basic Reed–Frost model (see Table 8.1 and Figures 8.7 and 8.8) includes a probability of an animal infecting another, yet the predicted outcome has no measure of variation attached to it. This model is therefore deterministic.

Density and prevalence models often can be formulated deterministically or stochastically, using a variety of approaches.

Modelling approaches

Deterministic differential calculus modelling

Differential calculus is a mathematical technique for finding small (theoretically infinitesimal) rates of change. Models based on this procedure generally establish equations in terms of the rate of change of either the number of parasites or the number of hosts, or subsets of these populations, with respect to time.

Deterministic exponential decay paradigm

One of the earliest and simplest examples of differential calculus models is that of **exponential decay**. The instantaneous rate at which susceptibles in a population become infectious individuals, denoted by dx/dt (where dx is the change in the population in the small time interval dt), is assumed to be directly proportional to x (the number of susceptibles in the population):

$$\frac{dx}{dt} = -\alpha x.$$

In the model, α is a positive number that remains constant and is therefore a parameter of the model. It is preceded by a minus sign because the number of susceptibles decreases as they become infectives. If an input condition is known, such as the number of susceptibles in the population at time zero, then solving the equation leads to:

$$x_t = Ne^{-\alpha t},$$

where x_t and N are the numbers of susceptibles in the population at times t and 0 respectively and e is the universal exponential constant, 2.718. If x_t is plotted for different values of t , a curve is obtained showing x_t decreasing rapidly for small values of t and thereafter more slowly.

Sheep vaccination paradigm

The following example demonstrates how immunity could be included in the simple exponential decay model. Suppose a flock of sheep is constantly under challenge from an infectious agent, infection with which usually causes death. The rate at which animals die is once again assumed to be in direct proportion to the number of animals that are under challenge and that are susceptible to infection. If a certain proportion of the animals show signs, and can be treated with a vaccine that provides temporary immunity, then these animals will not die. Once the protection of the vaccine ceases, these immune animals become susceptible again and risk mortality. The system is illustrated by the two-compartmental representation shown in Figure 23.1.

The top left-hand compartment represents the susceptibles under challenge. Animals in this compartment may either die at rate γ or be identified for vaccine treatment and so become transferred at rate α to the right-hand compartment, which represents the immune animals. When immunity ceases, these animals return to the susceptible group at rate β . The model consists of two equations, one for the rate of change of susceptible animals, dx/dt , and one for the rate of change of immune animals, dy/dt :

$$\frac{dx}{dt} = -\alpha x - \gamma x + \beta y,$$

and:

$$\frac{dy}{dt} = \alpha x - \beta y.$$

Losses from the susceptible group due to mortality and vaccination are in proportion to the number in the group and are the terms $-\alpha x$ and $-\gamma x$ on the right-hand side of the equation for dx/dt . Animals returning to the susceptible group, however, lose immunity in proportion to the number in the immune

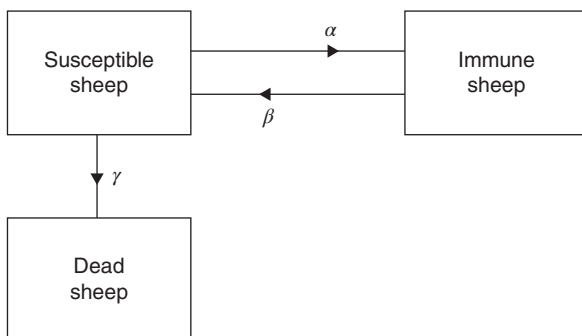


Fig. 23.1 Compartmental representation of a sheep vaccination model.

group. This movement into the susceptible group is denoted by $+\beta y$. Similarly, the rate of change of animals in the immune group is the net result of the gain αx and loss $-\beta y$. Standard methods of solution enable expressions for x and y to be obtained in terms of t :

$$x = \frac{N}{(a-b)} \{ (\beta-b)e^{-bt} - (\beta-a)e^{-at} \},$$

and:

$$y = \frac{N\alpha}{(a-b)} (e^{-bt} - e^{-at}),$$

where:

$$a = \frac{1}{2} \left[(\alpha + \beta + \gamma) + \{ (\alpha + \beta + \gamma)^2 - 4\gamma\beta \}^{1/2} \right],$$

$$b = \frac{1}{2} \left[(\alpha + \beta + \gamma) - \{ (\alpha + \beta + \gamma)^2 - 4\gamma\beta \}^{1/2} \right],$$

and N is the size of the flock at time zero. Figure 23.2 shows the trends in the numbers of susceptible and immune animals. These are only *general* trends; the *exact* trend will depend on the values assigned to the parameters, α , β and γ . It can be seen that the numbers in the susceptible group decrease rapidly whereas the numbers in the immune group rise to a peak before falling. Eventually the numbers in both groups will be zero because all animals will die unless some other control action is undertaken.

The early models of simple epidemics were based on the differential calculus approach, and usually contained simplistic assumptions; for example, that no infectious individuals were removed from the population during the course of the epidemic. This assumption was incorporated into the Reed–Frost model, which formed the basis of many early models. Although this assumption sometimes may be valid in human epidemics, it is frequently untrue of animal

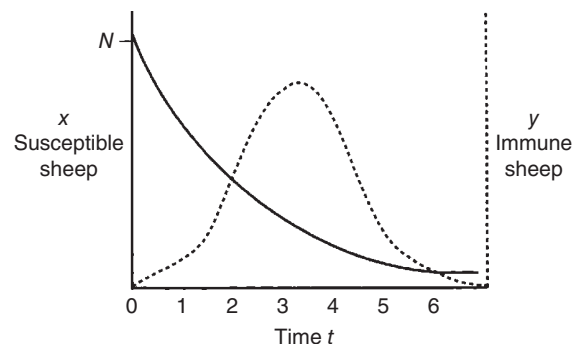


Fig. 23.2 Trends showing the changing number of sheep in the susceptible and immune groups in the sheep vaccination model paradigm.

epidemics, where infectious animals may be removed by culling (Mollison, 1984)⁵.

The early models assumed that a group was of a given size with homogeneous mixing and that a susceptible individual was infectious as soon as it was infected (i.e., the infection had no incubation or latent period). Most infectious diseases have latent periods and, in many situations, mixing is rarely homogeneous and individuals may become immune. Models have been produced that take account of these factors (e.g., Brauer, 2008, 2012).

Additionally, only a few diseases are characterized by inter-epidemic periods when no cases of disease occur (e.g., foot-and-mouth disease in the UK). Most diseases are endemic to a varying degree, and it is possible to have recurrent epidemics, requiring different models (Earn, 2008).

The earlier models considered epidemics as processes that occur in continuous time; these were **continuous time** models. **Discrete time** models have been designed, which portray disease patterns in fixed intervals of time. For example, if an epidemic starts with a single individual or several simultaneously-infected individuals, then new cases will occur in a series of stages separated by time intervals equal to the disease's incubation period. A further discrete time development has been of models that consider individuals as occurring in a variety of states (**multistate** models), for instance susceptible, immune, infected and dead. Models that consider how individuals can move from one state to another are called **state-transition** models⁶. These are frequently prevalence models.

Tuberculosis in badgers

Bovine tuberculosis continues to be a substantial problem in the south and west of England, at least in part

because infected badgers are considered to be a source of infection to cattle. A possible method of disease control is to reduce the badger population density to a level below the threshold level (see Chapter 8) for maintenance of the infection.

An early simple model of the dynamics of bovine tuberculosis in badgers used a deterministic, state-transition, differential calculus, prevalence model (Trehwella and Anderson, 1983) which includes three states: susceptible animals, animals incubating the disease, and infected animals (*Figure 23.3*). There is no need to include an immune state because badgers show no immunity to bovine tuberculosis. Parameters include density-dependent regulation of the badger population, natural and disease-induced death rates, the incubation period of the infection, and the rate of transmission.

The model predicts that prevalence increases with badger density – the predicted values being supported by field observations – and that prevalence oscillations occur with a periodicity of 18 years. The model suggests that, in areas of high badger density, considerable effort would be required to control the infection. Since then, many other proposed models have considered how best to control bovine tuberculosis (Brooks-Pollock *et al.*, 2014). However, the mode of transmission is still unclear, and so such models cannot be fully exploited.

Rabies in foxes

Foxes are hosts of rabies in North America and Europe, and constitute a serious obstacle to control of the disease. The infection became established in foxes in Poland towards the end of the Second World War. The epidemic spread slowly westwards at a rate

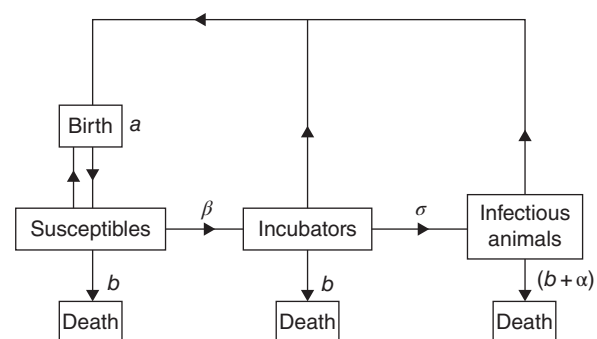


Fig. 23.3 Flow chart of the deterministic model of bovine tuberculosis in badgers. The flow of individual hosts between states is controlled by the rate parameters: a , the per capita birth rate; b , the natural death rate; β , the disease-transmission coefficient; σ , the rates at which incubating individuals become infected; α , the disease-induced death rate. (Adapted from Trehwella and Anderson, 1983.)

⁵ Arthropod vectors also may be culled to control some human diseases (Gourley *et al.*, 2007).

⁶ State-transition models exemplify a class of models termed **population-level models**, in which a population is split into logical, non-spatial compartments (this can include more than one population and species; for example, when considering the spread and control of zoonotic influenza: Dorjee *et al.*, 2012, 2016). The basic Reed–Frost model is another example. Such models generally assume homogeneity of a population and therefore do not account for the variability and interactions that can exist between individual members of a population (D'Souza *et al.*, 2009). These characteristics can be explored by simulating the behaviour of individuals in **individual-level models** (Deardon *et al.*, 2010), which, for example, can be integrated with geographical information systems for broad spatial coverage (Kennedy *et al.*, 2009). Bradhurst and others (2013) contend that the complexities of the spread of livestock diseases nationally justify a 'hybrid approach' utilizing both classes of model.

of about 30 km/year. The standard method of control was slaughter of foxes, but results were disappointing. A mathematical model (Macdonald and Bacon, 1980) suggested that control, other than by slaughter of foxes, would be more successful. The model has two components:

1. prediction of the course of the disease in fox populations;
2. evaluation of different control policies.

The model of the disease in fox populations makes plausible assumptions about the host and parasite. Foxes breed once a year in the autumn, and fox mortality is highest in the winter, resulting in an annual fluctuation in fox numbers. The virus has a long incubation period and can therefore survive in hosts of high, changing and low densities (see Figure 6.4); in the last circumstance it can exist for a long time in individuals.

If rabies enters a fox population, the future of the host and parasite will be affected by the number of healthy foxes that are infected by rabid foxes; expressed as a ratio, this is the **contact rate**. If the disease is modelled for various contact rates, there are different predicted outcomes; these are shown in Figure 23.4a. The upper lines of the graphs represent the total fox population, the lower lines the healthy foxes, and the shaded areas the number of rabid foxes. The horizontal lines represent the number of foxes that, theoretically, can be carried by the habitat. A contact rate of 0.5 (one rabid fox infecting half a healthy fox) will, according to Kendall's Threshold Theorem (see Chapter 8), be insufficient to allow the infection to become established; the model supports this. Higher contact rates result in fluctuation in the fox population and in the number of rabid foxes. A contact rate of 1.4 allows the disease to persist, oscillating annually. A contact rate of 1.9 produces epidemics every four years that are severe enough to reduce the population to a level that will not support infection. The infection again becomes epidemic when the fox population recovers. Field surveys have shown that this periodicity is demonstrated in European foxes. Higher contact rates would lead to extinction of the fox population.

The second component of the model considers three control techniques (Figure 19.4b):

1. slaughter;
2. temporary sterilization;
3. bait vaccination of foxes.

In case A, a single cull is instituted when rabies is at its earliest detectable level. Although slaughter initially decreases the prevalence of the disease, the latter soon increases again and then follows a pattern similar to

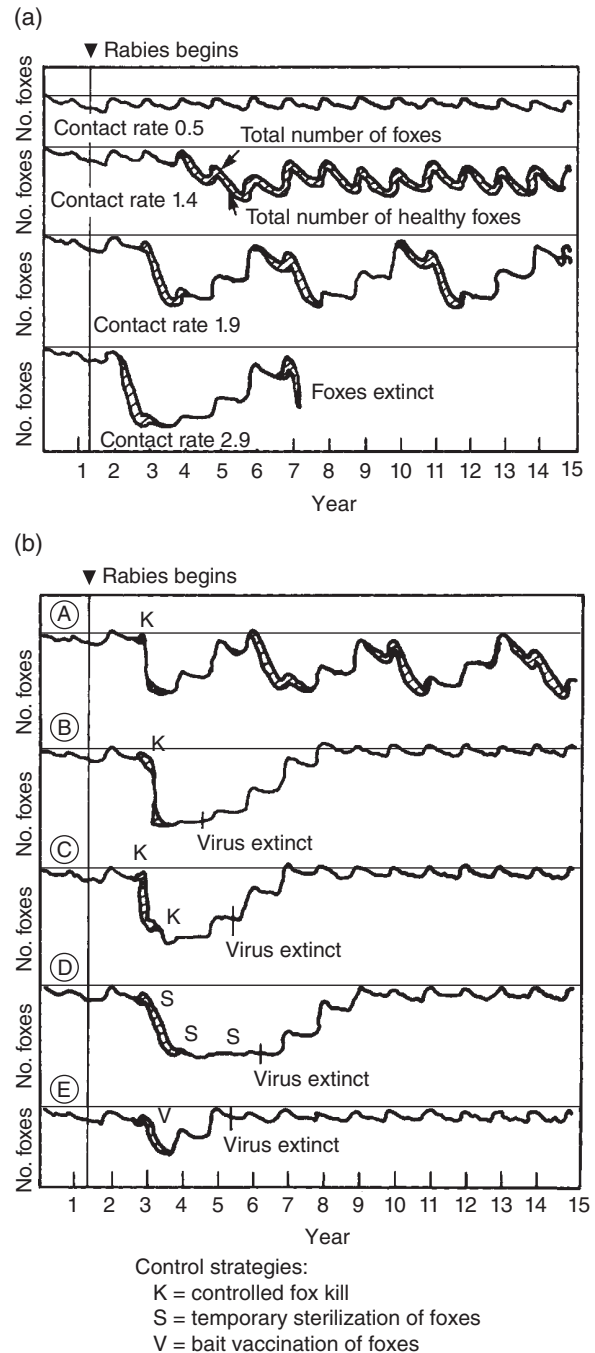


Fig. 23.4 (a) Merlewood model of rabies in foxes. (b) Merlewood model of alternative control strategies for controlling rabies in foxes. In each graph, the initial level of the fox population is the same. (Source: Macdonald and Bacon, 1980.)

that in the graph in Figure 19.4a depicting a contact rate of 1.9.

In case B, killing takes place later, probably when rabies is more likely to have been detected. Paradoxically, although initially there are more cases, the

disease and the kill work together because more of the foxes that are not killed are incubating rabies and therefore will die. The virus thus becomes extinct as the fox population is dramatically reduced.

Case C represents two killings, separated by six months. Again, the virus becomes extinct, but the fox population is reduced below the levels in A and B.

Three applications of temporary sterilizing agent are illustrated in case D. The virus again is extinguished, but the fox population is reduced further.

Vaccination of foxes using vaccine-laden bait, shown in case E, offers the best results: removing the virus from the population and maintaining the number of healthy foxes (with 60% of foxes assumed to be immunized).

This model therefore suggests a more efficient and ecologically acceptable way of controlling fox rabies than slaughter. This type of oral vaccination is being used successfully in Europe in foxes and raccoons (Müller, 1991; Pastoret and Brochier, 1998; Mähl *et al.*, 2014), where it can be complemented by the control of fox populations (Aubert, 1994). Oral vaccination also is being applied to other wild-animal hosts in North America (Sterner *et al.*, 2009).

A criticism of simple models based on differential calculus is the common assumption that parameters remain constant throughout the period of operation; for example, that the survival rate of infective organisms does not change during a season, whereas, in reality, climatic variation may alter survival rates from day to day. Some models do have time-dependent parameters, but this may lead to a model for which a solution is unobtainable or make the operation of the model clumsy. A major feature of such models is that they enable the long-term behaviour of the parasite population to be studied. The population may either become extinct, or increase indefinitely, or reach a *steady state*. It is often whether or not a steady state exists and the nature of the steady state that is of interest, although for many diseases of production animals the initial progression may be of paramount importance if economic losses are to be minimized.

Stochastic differential calculus modelling

The first epidemic models considered that *the course of an epidemic must depend on the number of susceptibles and the contact rate between susceptible and infectious individuals*; this is the basic assumption underlying deterministic models. In a deterministic model, the future state of an epidemic process can be predicted precisely if the initial number of susceptible and infectious individuals is known (e.g., Figures 8.7 and 8.8).

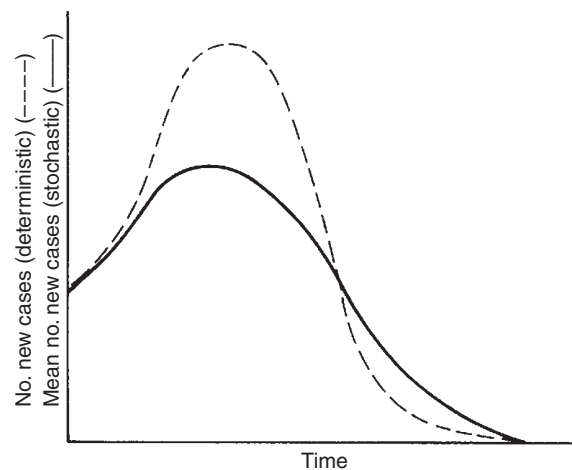


Fig. 23.5 Deterministic and stochastic model curves for a simple infectious epidemic. (Adapted from Bailey, 1975.)

Later, it was realized that the deterministic approach was not always applicable: variation and choice (of contact between susceptible and infected individuals) should be considered as part of the epidemic process. **Stochastic** modelling, which includes the **probability of infection**, therefore evolved. This leads to results that have a probability distribution from which means, variances and probability intervals can be derived. The deterministic and stochastic approaches may produce a different result when modelling a simple epidemic (Figure 23.5); the deterministic curve represents the absolute point estimates. The stochastic curve represents the mean of all the values generated by the various probabilities.

Stochastic exponential decay paradigm

A stochastic analogue of the deterministic model for the changing number of susceptibles in a population, described above, can be formulated. As before, x_t denotes the number of susceptibles in the population at time t , and N the number at time zero. It is now assumed that x_t is a random variable and the probability of r susceptibles at time t is denoted by $p_r(t)$. A differential calculus approach, similar to that used in the deterministic model, can be applied to obtain an expression for the rate of change of $p_r(t)$. This rate of change, $dp_r(t)/dt$, will be influenced by flows in from the state $r+1$ and flows out from the state r . For state $r+1$ to have a flow in, there must be two events: first, there must be $r+1$ susceptibles at time t ; secondly, one of these susceptibles must leave. The probability of these two events is $\alpha(r+1)p_{r+1}(t)$. For state r to have a flow out, there must be r susceptibles at time t and one susceptible must leave. The probability of these

two events is $\alpha p_r(t)$. The instantaneous rate of change of $p_r(t)$ is therefore:

$$\frac{dp_r(t)}{dt} = \alpha(r+1)p_{r+1}(t) - \alpha p_r(t),$$

where α is a parameter.

The solution to such a differential equation may not be easily obtained, but standard methods for solving it are available. These methods lead to an expression for $p_r(t)$ in terms of t :

$$p_r(t) = {}^N C_r (1 - e^{-\alpha t})^{N-r} e^{-\alpha t r},$$

where ${}^N C_r$ is a mathematical shorthand notation for $\frac{N!}{r!(N-r)!}$.

The expression for $p_r(t)$ is known as the time-dependent binomial probability distribution, from which it can be deduced that x_t has mean value $Ne^{-\alpha t}$ and variance $Ne^{-\alpha t}(1 - e^{-\alpha t})$. Comparing these results with those of the deterministic model, it can be seen that the mean value of x_t is identical to the solution obtained before. This is often, but not always (Figure 23.5), true of deterministic and stochastic models. However, an important distinction between deterministic and stochastic models is that the latter provides a variance; therefore, the extent to which population susceptible numbers fluctuate at each point in time can be deduced. Figure 23.6 illustrates this point, where the mean of the number of susceptibles at each point is shown along with the 2.5th and 97.5th percentiles (see Chapter 12) of the distribution of the number of susceptibles. This provides a 95% probability interval for the

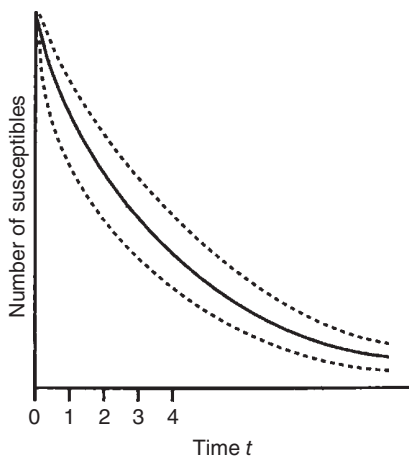


Fig. 23.6 Mean (—) and 95% probability interval (---) for the number of susceptibles in the stochastic exponential decay paradigm.

number of susceptibles. The wider the probability interval, the greater is the range of observed deviations from the mean number of susceptibles.

Empirical simulation modelling

The goal of this technique is **simulation** of the performance of parasites or diseases in relation to conditions that change either deterministically or stochastically. Although simulation models do not always require a computer for implementation, their power and success have been closely linked to advances in computer technology. Many simulations undertaken today would have been impossible 50 years ago.

Successful simulation models have the potential to accurately *forecast* disease incidence. These forecasts, like those possible using time series analysis (see Chapter 8), are of value in selecting suitable prophylactic procedures.

Empirical models utilize *indicators that are obtained by analysing the relationship between morbidity and any associated variables*. Frequently-used variables are those relating to climate. These models are not strictly mathematical models because they do not attempt to analyse the dynamics of agents' life-cycles, but simply quantify associated phenomena. They sometimes are referred to as 'black-box' models because the relationship between data that are fed into the model and the results that are generated cannot be satisfactorily explained.

Fascioliasis

Fascioliasis has been modelled empirically in Britain (Ollerenshaw and Rowlands, 1959; Ollerenshaw, 1966; Gibson, 1978). The life-cycle of *Fasciola hepatica* is complex, involving stages inside a final and intermediate host, and on herbage. Two important meteorological factors in the development of the parasite are temperatures above 10 °C and the presence of free water. In the late 1950s, Ollerenshaw suggested that development is therefore usually impossible during the winter (too cold) and that there may be insufficient water during some of the summer months (too dry). This is the basis of the 'Mt' forecasting system for fascioliasis. Mt is a monthly index of wetness given by:

$$(R - p + 5)n,$$

where, on a monthly basis:

R = rainfall in inches;

p = potential transpiration;

n = number of rain days.

Table 23.1 Associations between ΣMt values* and losses owing to fascioliasis in England and Wales. (Data from Ollerenshaw, 1966.)

ΣMt		Losses
North-west England, south-east England and north Wales	Other parts of England and Wales	
<300	<400	No losses
300–450	400–450	Some losses
>450	>450	Heavy losses

* See text for explanation.

Observations suggested that, because parasite development is also temperature-dependent, the rate of development is similar in June, July, August and September, but is halved in May and October, when the Mt index should therefore be halved.

A seasonal summation of Mt indices (ΣMt) can be calculated by adding the Mt values for the six-month period May to October. This sum simulates the progression of the disease in relation to changing meteorological conditions and so can be used to predict losses owing to fascioliasis, so that suitable prophylactic measures can be undertaken (Table 23.1).

This prediction model is deterministic because no element of randomness is included in the formulation. Its simple approach enables its execution without a computer. The model was adapted for use in France (Leimbacher, 1978) and Northern Ireland (Ross, 1978), and is considered to have been very successful (Smith, 2011). Similar indices have been developed for human fascioliasis (Fuentes *et al.*, 1999).

Process simulation modelling

Many mathematical models that describe the dynamics (i.e., biological processes) of parasite and host populations have been formulated. These more refined techniques allow the course of a disease to be simulated. They include models for forecasting fluke morbidity (Hope-Cawdery *et al.*, 1978; Williamson and Wilson, 1978; Wang, 2012), the airborne spread of foot-and-mouth disease (Gloster *et al.*, 1981, 2010; Casal *et al.*, 1997; Sørensen *et al.*, 2000) and the occurrence of clinical ostertagiasis.

Bovine ostertagiasis

The level of pasture contamination by infective *Ostertagia ostertagi* larvae can be predicted by simulating the course of events experienced by cohorts of parasite eggs deposited on pasture (Gettinby *et al.*, 1979). This

involves estimating the proportion of eggs that proceed to the first, second and third larval stages using **development fractions**, which quantify the rate of development of the parasite from one stage to the next according to the temperatures that it experiences. In addition, parameters associated with infectivity, fecundity and migratory behaviour of the larvae must be included.

Thus, suppose a calf commences grazing on contaminated grass on 1 April. The number of infective larvae, L , ingested on 1 April can be estimated from known pasture contamination levels and the daily herbage intake of the calf. Not all larvae become established. The number of adult worms, A , to be expected in the abomasum of the calf 21 days later on 22 April is modelled using:

$$A = (K - A_0)(1 - e^{-\alpha L}) + A_0,$$

where A_0 is the number of adults already in the abomasum. The curve of A for different values of L is sigmoidal, reflecting the assumed density-dependent relationship between larval challenge and establishment of adult worms (see also Figure 7.4). The parameters K and α control the rate of establishment so that the proportion established is high for low levels of challenge and low for high levels of challenge. The adult worms will produce eggs on 1 April and thereafter. The number of eggs, E , produced on 22 April is estimated from empirical data relating egg output to adult worm burden. These eggs undergo development. The time to the appearance of infective larvae is estimated by calculating from daily temperatures the fraction of development to take place each day and summing these fractions until all development has occurred n days later:

$$\frac{1}{D_1} + \frac{1}{D_2} + \dots + \frac{1}{D_n} = 1,$$

where the D s are the number of days that would be required to complete development under conditions of constant temperature. Adding n to 22 April gives the earliest day on which the infective larvae can appear. Not all developing eggs and larvae survive and so the number of eggs that avoid mortality is the proportion, p'' , of the egg output on 22 April. The parameter p is an estimate of the daily survival rate. If the values of n and p'' are determined for each day during which the calf grazes, then it is possible to estimate the expected totals of infective larvae on pasture and the number of adult worms infecting the calf. This type of simulation requires iterative calculations, which can only be performed in a reasonable time by using a computer.

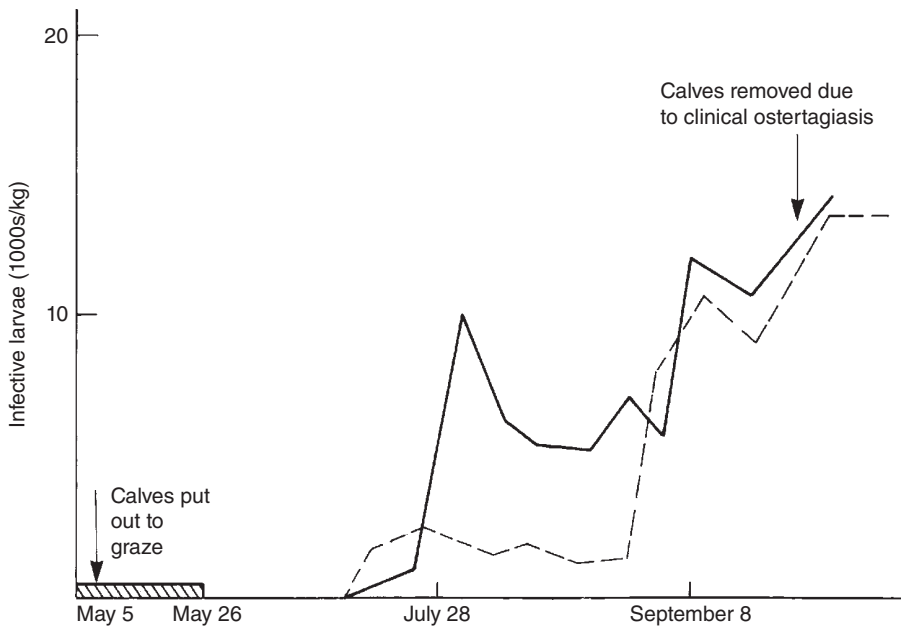


Fig. 23.7 Observed and predicted counts of infective *Ostertagia ostertagi* larvae on pasture in 1975. — Observed pasture count; - - - predicted pasture count; [shaded area] overwintered infective larvae. (Source: Gettinby *et al.*, 1979. Reproduced with permission of BMJ Publishing Group Ltd.)

Figure 23.7 shows the results of such a simulation for calves that grazed on an experimental pasture from May to September 1975. Comparison of predicted and observed larval counts shows a high degree of similarity.

A prediction of herbage infective larval burdens using this type of simulation model can facilitate optimum use of anthelmintics, and movement of animals to safe pasture before challenge by large numbers of infective larvae, thereby preventing clinical ostertagiasis. A similar approach has been successfully applied to ovine ostertagiasis (Paton *et al.*, 1984) and tick infestations of sheep (Gardiner and Gettinby, 1983).

Monte Carlo simulation modelling

In many cases deterministic and stochastic models can be formulated for which no analytical solution is known. Alternatively, finding the solution may be extremely difficult or tedious. In such circumstances, simulation methods are increasingly being undertaken. Since simulation studies attempt to mimic the physical process being modelled, they can be very informative and therefore are often preferred. In these methods, random processes are simulated using random numbers in order to decide whether or not an event takes place. This is somewhat akin to gambling; hence the term **Monte Carlo** simulation.

Sheep tick paradigm

Suppose a model is to be formulated for the outcome of adult ticks of the species *Ixodes ricinus* mating on the sheep host. In particular, a measure of the total number

of female ticks is required so that future population numbers can be anticipated. Suppose a field study indicates that the total number of engorged ticks found on individual sheep is 0, 1, 2 or 3, and suppose that the respective probabilities are 0.4, 0.3, 0.2 and 0.1. The field study also suggests that male and female ticks are present in equal proportions. Using a fair 10-sided die with faces labelled 1 to 10, and a fair two-sided coin with faces labelled M for male and F for female, it is possible to simulate and obtain the number of mated female ticks on each sheep. The die is thrown and, depending on which of the sets {1,2,3,4}, {5,6,7}, {8,9} and {10} the outcome belongs to, the simulated number of adult engorged ticks on the sheep is taken to be 0, 1, 2 or 3, respectively. The above sets are chosen because the outcomes 0, 1, 2 and 3 will occur with probabilities 0.4, 0.3, 0.2 and 0.1, respectively, which is consistent with the results of the field study. Suppose the outcome of the throw of the die is a 10, the simulated number of adult engorged ticks on the sheep is then three. The coin is now tossed three times and the outcomes used to simulate the sex of the three adult engorged ticks. In the event of all three ticks having the same sex (i.e., outcomes MMM or FFF), there will be no mated females. All other outcomes will lead to at least one male and one female on a sheep and, assuming that one male mates only with one female, there will be only one mated female. Table 23.2 shows the possible combinations of the outcomes of the throw of the die and the coin tossing.

The procedure can be repeated for each sheep to obtain a series of zeros and ones reflecting the outcomes of the attachment and sex distribution of the

Table 23.2 Possible number of mated female *Ixodes ricinus* on one sheep, resulting from simulated attachment and sex distribution of the tick population.

Outcome from one throw of a 10-sided die	Simulated number of engorged ticks on a sheep	Simulated sex distribution of engorged ticks from coin tosses	Number of mated females
{1,2,3,4}	0	–	0
{5,6,7}	1	M	0
		F	0
{8,9}	2	MM	0
		MF	1
		FF	0
		FM	1
{10}	3	MMM	0
		MMF	1
		MFM	1
		FMM	1
		FFF	0
		FMF	1
		MFF	1
		FFM	1

tick population on the sheep flock. To summarize the results of the simulation: the proportion of the flock with one or zero mated female ticks could be reported. When the simulation is carried out, it is found that, on average, only 18 out of every 100 sheep are hosts to one mated female tick. A sensitivity analysis⁷ could also be undertaken to test the effects of different assumptions or parameter estimates on the outcomes. For instance, if the ratio of male to female ticks was no longer 1:1 but biased towards females, in the ratio 1:2, then the study could be repeated using a biased coin that would give an M outcome with probability 0.33 (1/3) and an F outcome with probability 0.67 (2/3) when tossed. The simulation then leads to an average of 15 out of every 100 sheep hosting one mated female tick. Comparison of the results suggests that the analysis is not very sensitive to changes in the sex distribution; consequently further field studies to accurately determine the sex ratio are not warranted. Similarly, if it was

thought that male ticks could mate with several females, then the simulation could be repeated. If, for example, attachment led to the combination of one male and two female ticks on a sheep, then this would produce two, rather than one, mated females.

In modern simulation studies the computer takes over the role of the die and the coin. Thus, the results of these 'lotteries' are produced by replacing throws of a 10-sided die with an instruction to the computer to produce integer numbers from one to 10 in random order such that a large series of generated numbers would produce each of these numbers in equal proportions.

Sheep tick control

Monte Carlo simulation can be used to investigate the behaviour of a stochastic model for the incidence of tick mating which could be of value to control strategies (Plowright and Paloheimo, 1977). Field investigations (Milne, 1950) revealed that tick occurrence was patchy: a pasture may have a heavy burden whereas an adjacent field separated from the first only by a fence may have no ticks. This suggested that ticks have problems with effective dispersal. One reason may be that at low densities tick population growth is inhibited by a low rate of mating. The model proposed makes several assumptions: that each adult only mates if it encounters an individual of the opposite sex on the sheep to which it is attached; that each adult only mates once; that each tick has an equal probability of attaching to a sheep; and that all ticks have an equal probability of encountering one another. The last two assumptions are dubious but do not affect the model appreciably.

The total number of matings at various sheep densities, and with varying numbers of ticks on each sheep, can be modelled by applying a Poisson distribution (see Chapter 12). Table 23.3 lists the results and demonstrates the difference between the stochastic and deterministic output. By including a rate of survival in the model, it is possible to predict the growth rate of the tick population for different levels of tick population size and sheep density. When the sheep population is low, the rate of tick population increase is insensitive to changes in the size of the tick population, but highly sensitive to changes in the size of the sheep population. Conversely, when the tick population is low, the rate of tick population increase is relatively insensitive to changes in sheep numbers, but sensitive to changes in tick numbers. This supports the hypothesis that it is difficult for ticks to establish themselves in new pastures. It also suggests that a reduction in host density may not be an effective means of controlling tick infestation because the rate

⁷ The extent to which changes in values of an input parameter affect output parameters is assessed by **sensitivity analysis** (Saltelli *et al.*, 2008). If minor changes in values of an input parameter induce major changes in output parameters, then the model is highly sensitive to that input parameter. Conversely, if major changes in an input parameter induce only minor changes in output parameters, then the model is relatively insensitive to the input parameter.

Table 23.3 Comparison of the results of deterministic and stochastic models of incidence of mating in *Ixodes ricinus* for various parameter values. (From Plowright and Paloheimo, 1977.)

<i>p</i>	Number of ticks	Number of sheep	Total number of matings	
			Deterministic model	Stochastic model (mean of 600 replications)
0.05	40	10	3.3357	3.4400
0.05	40	20	4.7532	4.7833
0.05	40	40	5.1271	5.1533
0.05	40	80	3.9683	3.8395
0.05	40	160	2.2143	2.2117
0.05	80	10	8.9590	9.0883
0.05	80	20	13.2866	13.4567
0.05	80	40	15.3686	15.5733
0.05	80	80	12.6488	12.8450
0.05	80	160	7.9323	8.0050
0.01	40	10	0.3021	0.2933
0.01	40	20	0.5528	0.5567
0.01	40	40	0.9282	0.9017
0.01	40	80	1.3279	1.2733
0.01	40	160	1.4345	1.4633
0.01	80	10	1.0342	1.0017
0.01	80	20	1.8993	1.9483
0.01	80	40	3.2270	3.0550
0.01	80	80	4.7108	4.5867
0.01	80	160	5.2683	5.3367

p, probability of a tick attaching to a sheep.

of tick population increase does not always depend on sheep density. The model also predicts that extinction of the tick population takes place over a narrow range of tick population sizes, corroborating field observations of patchy tick distribution.

Models also can be constructed to explore the effects of tick-control strategies. For example, treatment of deer with acaricides is predicted to increase red grouse density by controlling ticks and louping ill virus (a pathogen of grouse for which ticks are vectors), thereby swelling grouse numbers for hunting purposes (Porter *et al.*, 2013).

Matrix population modelling

The use of **matrices** to describe population changes became firmly established when Leslie (1945) first published, and subsequently refined (1948), his Leslie

matrix. Matrices often take the form of a rectangular array containing numbers of hosts or parasites in a defined state or stage of development, known as the **state vector**, or containing reproduction and survival rates of hosts or parasites in different states or stages known as the **transition matrix**. In this way, it is possible to obtain the state of the system from one point in time to another.

Fascioliasis

The life-cycle of *Fasciola hepatica* can be used to illustrate the formulation of a simple matrix model. The parameters have been estimated from field studies. It is assumed that eggs from the adult fluke develop to miracidia after four weeks, that miracidia that penetrate the molluscan host, *Limnea truncatula*, develop and emerge as metacercariae eight weeks later, and that the metacercariae can survive up to three weeks before desiccation. The weekly survival rates of the adult flukes, the developing eggs, the stages in the snail, and the metacercariae are 0.95, 0.3, 0.5 and 0.8, respectively. Each adult fluke is assumed to produce 2500 eggs weekly, and each miracidium to penetrate a snail with probability 0.005. The phases of development in the snail are simplified by labelling all of the asexual stages *s*. It also is assumed that reproduction occurs in the last intra-molluscan stage, and the fecundity is 4.3. A further simplification is that each metacercaria in each week of development has probability 0.02 of becoming an adult worm.

Let *a* be the number of adult flukes and *e_i*, *c_i* and *m_i* be the number of eggs, cercariae and metacercariae, respectively, in the *i*th week of development. The number of adult flukes from week *t* to week *t* + 1 will be those adults in week *t* that survive plus those metacercariae that are ingested and become established:

$$a(t+1) = 0.95a(t) + 0.02m_1(t) + 0.02m_2(t) + 0.02m_3(t).$$

The number of eggs at time (*t* + 1) in the first week of development will be those produced by adults in the previous week:

$$e_1(t+1) = 2500a_1(t).$$

The number of eggs at time (*t* + 1) in the second week of development will be those surviving from the previous week:

$$e_2(t+1) = 0.3e_1(t).$$

The change from week to week of all the stages can conveniently be summarized in matrix form (Table 23.4). In shorthand notation, this is written:

$$\underline{x}_{t+1} = \underline{P}\underline{x}_t,$$

Table 23.4 Matrix formulation of the life-cycle of *Fasciola hepatica*.

a		0.95	0	0	0	0	0	0	0	0	0	0	0	0.02	0.02	0.02	a
e_1		2500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	e_1
e_2		0	0.3	0	.	.								.	0	e_2	
e_3		.		0.3												e_3	
e_4		.			0.3											e_4	
s_1						0.002										s_1	
s_2							0.5									s_2	
s_3								0.5								s_3	
s_4									0.5							s_4	
s_5										0.5						s_5	
s_6											0.5					s_6	
s_7												0.5				s_7	
s_8													0.5			s_8	
m_1		.												4.3		m_1	
m_2		.													0.8	m_2	
m_3	$t + 1$	0	.	.											0.8	0	m_3

where $\underline{x}_t$ and $\underline{x}_{t+1}$ are the state vectors and correspond to times t and $t + 1$, and $\underline{P}$ is the transition matrix. Characters are underlined to denote a matrix. Note that to retrieve the first equation, the first element in the state vector (a_{t+1}) is equated with the sum, after the first row of the transition matrix has been turned on its side and each element of this row multiplied by the corresponding elements of the column state vector $\underline{x}_t$ at time $t + 1$; that is:

$$\begin{aligned}
 a = & + 0.95 \times a \\
 & + 0 \times e_1 \\
 & + 0 \\
 & 0 \\
 & 0 \\
 & . \\
 & . \\
 & . \\
 & + 0.02 \times m_3.
 \end{aligned}$$

Other relationships are similarly retrieved. For example, if the number of metacercariae in the first week of development at time $(t + 1)$ is required, then the row transition matrix corresponding to this element is turned on its side and corresponding elements in $\underline{x}_t$ multiplied to give:

$$m_1(t + 1) = 4.3s_8(t).$$

The matrix model proposed for the life-cycle of *F. hepatica* is similar to that proposed by Leslie (1945) for populations in general. Leslie suggested that members of a population can be divided into exclusive age classes of fixed duration, so that every member of a class faces the probability of surviving and the same probability of reproducing. If the vector containing the number in each class during a certain time interval is multiplied by the Leslie matrix describing the population dynamics, then the vector containing the number in each class during the following time interval is obtained. For example, if there are k age classes:

$$\begin{bmatrix} n_1 \\ n_2 \\ n_3 \\ \vdots \\ n_{k-1} \\ n_k \end{bmatrix}_{t+1} = \begin{bmatrix} f_1 & f_2 & \cdots & f_{k-1} & f_k \\ p_1 & 0 & \cdots & 0 & 0 \\ 0 & p_2 & \cdots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \cdots & p_{k-1} & 0 \end{bmatrix} \begin{bmatrix} n_1 \\ n_2 \\ n_3 \\ \vdots \\ n_{k-1} \\ n_k \end{bmatrix}_t$$

where $n_i(t+1)$ is the number in the i th class at time $t+1$, and f_i and p_i are respectively the fecundity and survival rates for age class i .

The advantage of the matrix approach is that, once $\underline{P}$ and the numbers for the state vector at time zero are known, then the population sizes at any future time can be predicted. For example, the population numbers after four units of time could be obtained from the successive calculations:

$$\begin{aligned} \underline{x}_1 &= \underline{P}\underline{x}_0, \\ \underline{x}_2 &= \underline{P}\underline{x}_1, \\ \underline{x}_3 &= \underline{P}\underline{x}_2, \\ \underline{x}_4 &= \underline{P}\underline{x}_3. \end{aligned}$$

The matrix equations described above have many interesting properties from which the salient features of the population can be investigated. Details of these are given by Leslie (1945).

A more realistic matrix representation of the life-cycle of *F. hepatica* was discussed by Gettinby and McClean (1979). This is a state-transition model with five states: mature flukes (in sheep); eggs (on grass); rediae (in snails); metacercariae (on grass); and immature flukes (in sheep). A mortality rate is attached to all stages, and fecundity to the adult fluke and redia, which reproduce sexually and asexually, respectively. The matrix includes probabilities of transition from one stage to the next, and fecundity, based upon available field data. The first part of the model describes the natural infection in sheep in Britain and Ireland. The second part investigates and compares various control strategies: the use of flukicides, molluscicides and land drainage. There are four conclusions. Molluscicides are most effective when applied in early spring. Flukicides eradicate the infection when given monthly and control it when given at two-monthly intervals. If dosing is only annual, then it is best given in August. Good drainage is an effective means of control. Again, the model only indicates possible outcomes, in the absence of accurate field data to support the values of the input parameters in the model.

Network population modelling

The inability of many other models to cope with changing inputs during the period of operation of the model can be circumvented using a **network** representation of a parasite's life-cycle. Network models, although extensively exploited by control engineers, have been largely overlooked in the life sciences, with some exceptions (Pearl, 2000). The same problem often can be formulated using a network and a matrix approach. The network formulation is particularly attractive when time delays are a feature of the life-cycle being modelled, and when the output response of a biological system is to be measured for a given input. On the other hand, matrix formulations are attractive when the behaviour of several states of a population is of interest at successive points in time. Some elementary examples will demonstrate the symbolism used in the network approach.

Protozoal paradigm

Consider a population of identical protozoa that reproduce by binary fission. A constant T units of time occurs between successive fissions. The probability that any one protozoon survives this time is P . To construct the network shown in Figure 23.8, which represents the life-cycle, consider the flow of newly-formed protozoa in the population. After a time delay, T , only a proportion, P , of these survives. This results in the flow after the time delay being scaled by a factor P . This is immediately succeeded by fission, which results in the flow doubling and so a further scaling by a factor 2 is required. The network convention is to denote time delays by squares and scaling parameters by circles. Since the products of fission are newly-formed cells, this flow is connected back into the time delay. This is a very simple network and consists of one loop and no alternative paths.

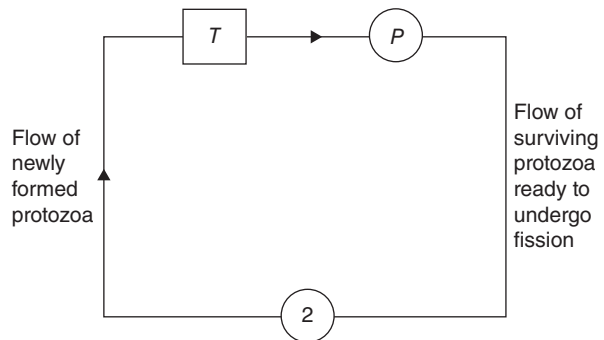
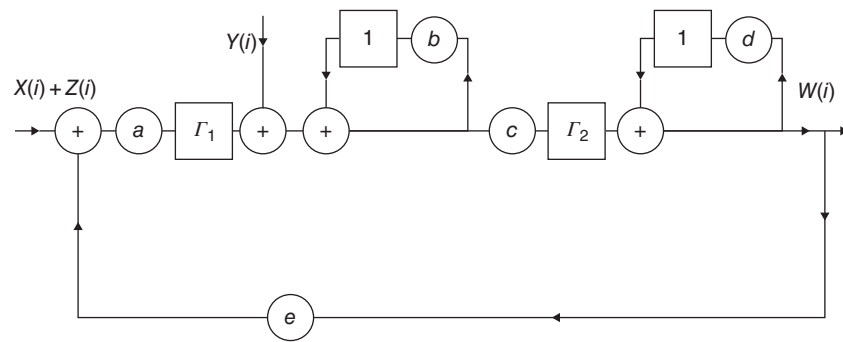


Fig. 23.8 Network representation in the protozoal paradigm.

Fig. 23.9 Network representation of the life-cycle of *Ostertagia circumcincta* showing adult parasite output, W , derived from ewe egg input, X , lamb egg input, Z , and overwintered infective larval input, Y . The constants a , b , c , d and e are parameters of survival, infectivity and fecundity; Γ_1 and Γ_2 are time delays representing parasitic development times on the pasture and in the lamb, respectively. (Adapted from Paton and Gettinby, 1985.)



Ostertagiasis

A network can be constructed for the life-cycle of *Ostertagia circumcincta*, an important parasite responsible for outbreaks of parasitic gastroenteritis in sheep (Paton and Gettinby, 1983). The network is shown in Figure 23.9. The unit of time is one week. Ewe and lamb egg inputs, $X(i)$ and $Z(i)$, voided out to the pasture in week i , undergo a time delay of Γ_1 (gamma) weeks before infective L_3 larvae appear. The proportion that survive is a . During the following week, these L_3 larvae, plus available overwintered infective larvae $Y(i)$, either become ingested by the sheep to become adults at rate c , or accumulate on the pasture at rate $1 - c$. Those that accumulate are delayed for one week, during which their survival rate is b , before joining the flow of new infective larvae. The L_3 larvae that were ingested are delayed for Γ_2 weeks before reaching the adult egg-laying stage. Half of these adults will be females, which reproduce at a rate of e eggs per week. From successive egg cohorts, female adults will accumulate from week to week. To facilitate the accumulation, the existing females must enter a feedback loop in which the weekly survival rate is d , and which connects them with the flow of new female adult worms in the sheep. The network therefore consists of a forward loop and two feedback loops.

When the model is operated, the effects of various anthelmintic strategies can be investigated by altering components of the network that will be affected by the particular strategy. For example, dosing of lambs will reduce the lamb egg input, and dosing of ewes will decrease the ewe egg input. The simulation suggests that regular dosing of lambs at four-weekly intervals for the first six months of life is very effective. Similarly, dosing lambs three times in July and August is effective. The single administration of an anthelmintic to ewes at lambing time is the least effective of the anthelmintic strategies.

Contact-network modelling

The network concept also may be applied to models of the interactions between individual animals within a

population (e.g., Keeling and Eames, 2005), which represent potential transmission pathways for infectious diseases; for example, by direct contact between animals (Turner *et al.*, 2008) or between groups of animals when, for instance, they move between farms (Kiss *et al.*, 2006). Moreover, investigation of livestock trade networks can be an aid to animal health surveillance (Hardstaff *et al.*, 2015).

Networks may be analysed in order to identify the characteristics of contact at the level of individuals, sub-groups and the networks themselves (Wasserman and Faust, 1994; Newman, 2003; Boccaletti *et al.*, 2006; Dubé *et al.*, 2009; Martínez-López *et al.*, 2009; Danon *et al.*, 2011).

The potential for spread of disease can be explored either by assessing 'connectedness' of a farm network or by quantifying potential for transmission (e.g., by estimating R_0 ; Kao *et al.*, 2006).

The application of contact-network modelling has been facilitated by the large-scale storage of animal movement data, such as those in the British Cattle Tracing System (see Chapter 11).

Contact networks may be illustrated as graphs, but numerical representations are used for analysis. It is common to represent simple contact networks as $n \times n$ matrices⁸, in which the rows and columns represent the n individuals in the population and the cell values provide a measure of contact. These may be binary (indicating the presence or absence of contact), nominal (specifying the type of contact), weights (designating the strength of contact), or they may denote the probability of contact. The contact networks may be static; alternatively, the contact-network patterns may change as a simulation progresses in order to capture changes (e.g., seasonal variation in contact).

⁸ However, particularly where the average number of contacts, k , is relatively few, it is more efficient to use a *contact list* of length $n \times k$, in which each row identifies the individuals involved in the contact; hence, only contacts that occur are recorded in the contact list (in contrast to the inclusion of zeros in an $n \times n$ matrix). If required, a weight or other value can be assigned to each row of data.

Recent examples of network-based models of live-stock disease transmission (notably of foot-and-mouth disease and scrapie) are those of Kiss and others (2006), Kao and others (2006, 2007) and Volkova and her colleagues (2010). Danon and co-workers (2011) review the role and application of network science to modelling infectious diseases.

Systems modelling

'Modelling' now has a broad remit, including the conceptual representation of any real event in mathematical terms. Models that assess the cost of disease and its control have also been designed; some of these have been reviewed by Beal and McCallon (1983) and Dykhuizen (1993). Models of livestock nutrition have been formulated (Theodorou and France, 2000; France and Kebreab, 2008), and Sørensen and Enevoldsen (1992) review models of herd health and production. Increasingly, models are being linked together to produce large-scale **systems models**. Examples are *EpiMAN* (see Chapter 11) and, more recently, the *Australian Animal DiSease* model (AADIS: Bradhurst *et al.*, 2013).

A bibliography of veterinary models, including a discussion of model classification, is given by Hurd and Kaneene (1993).

The rational basis of modelling for active disease control

Many veterinary models have been used to explore the dynamics of disease in order to produce options that might be selected to control disease (e.g., Medley, 2003). However, they may not have faced the critical test of being applied when disease actually occurs. In some cases, they *have* served as policy guides during actual outbreaks of disease. For example, the 2001 epidemic of foot-and-mouth disease in the UK was characterized by a policy to pre-emptively cull cattle on premises contiguous to infected premises, on the basis of model formulations that predicted that this would be an efficacious tactic for control (Ferguson *et al.*, 2001; MAFF, 2001)⁹. A consideration of the conditions under which the validity and appropriateness of a model may be judged is therefore a necessary complement to knowledge of the types of models that have been described.

⁹ Haydon and others. (2002), Kao (2002), Keeling (2005) and Mansley and his colleagues (2011) review, compare and criticize the models used in that epidemic.

Available knowledge, and the functions of models

The validity of a model is determined by the level of **epidemiological knowledge** about a disease's natural history, and the **quality and quantity of data** relating to the disease (Graat and Frankena, 2001; Taylor, 2003). For example, models of airborne transmission of infection (see Chapter 6) require information on microbial output from hosts and host susceptibility (epidemiological knowledge), and details of stock distribution (data quality and quantity) in order to predict transmission accurately. However, methods to assess the quality of data and knowledge are highly variable, often subjective and open to interpretation (Christley *et al.*, 2013).

Levels of knowledge can be conveniently classified as 'poor' and 'good', so that a general framework for the application of models can be identified (*Table 23.5*). This defines four main applications of models:

1. development of hypotheses;
2. hypothesis testing;
3. elementary explanation of past events;
4. detailed representation of past events, and prediction.

The applications span not only the model formulations outlined in this chapter but also other numerical analytical procedures such as observational studies (Chapter 15). In reality, levels of knowledge are continuous, and so a considerable degree of judgement may be required in assessing the particular role of a model.

Development of hypotheses

Hypotheses can be developed when epidemiological knowledge is poor and good data are not available. For example, case-control studies using retrospective veterinary clinic data that cannot be validated might

Table 23.5 Uses of models in the context of epidemiological knowledge and data quality and quantity. (Adapted from Taylor, 2003; based on Holling, 1978.)

<i>Epidemiological knowledge</i>	<i>Data quality and quantity</i>	
	<i>Poor</i>	<i>Good</i>
Poor	Exploration of hypotheses	Hypothesis testing
Good	Simplified representation of past events, and <i>guarded</i> use for prediction of future events	Detailed representation of past events, and prediction of future events

be used to explore possible intrinsic determinants of disease, such as breed, age and sex; these studies may then be succeeded by prospective studies with careful control over the quality of the data that are collected. However, this is an uncommon modelling strategy because most modellers usually begin at least with formulations that depend on an underlying hypothesis. For example, the sheep vaccination paradigm is based on the hypothesis that the number of susceptible sheep decays exponentially (see Figure 23.2).

Hypothesis testing

Availability of good data allows hypotheses to be tested by fitting observations to associations or relationships that are hypothesized to exist. Prospective observational studies frequently follow this approach, carefully validating data as they accrue.

Elementary explanation of past events

A sound understanding of the natural history of disease may be attenuated by lack of high-quality data. For example, the vulpine rabies model described earlier incorporates the major characteristics of the transmission of the disease. However, accurate data on contact rates usually are not available. Thus, the model may be used to explain previous patterns of disease with assumptions about putative contact rates. However, its use to predict future disease patterns would need to be *very guarded* because of the lack of information on current parameter levels in the field.

Detailed representation of past events, and prediction

The availability of high-quality data, linked to a detailed understanding of the functioning of a system, enables both the accurate representation of past events quantitatively, and sound predictions of future events to be made. A good non-veterinary example is the use of aircraft flight simulators to train pilots. However, this is an example from the physical sciences, which are subject to fewer laws and are usually less complex than biological systems, which display considerable variability. Notwithstanding the weaknesses of inductive reasoning, on which much of science is based (see Chapter 3), in practice the accurate prediction of future complex biological events (including disease occurrence) is difficult, although the past may be modelled accurately. The difference between theory, hypothesis and fact – with which models might be labelled – and the strength of evidence and criteria required to shift from theory to fact, should therefore be appreciated.

From theory to fact¹⁰

Scientific theories

A theory is a supposition that explains something. It cannot be proved in the sense that propositions in logic and mathematics can. However, *certain* scientific theories are held with much more confidence than others. The confidence one has in any particular theory, such as the Second Law of Thermodynamics, the atomic theory of matter, or the microbial theory of the cause of infectious disease (see Chapter 1), depends on several factors, including how **well-tested** the theory is, and how **often** and how seriously this testing has refuted the theory. For example, the theories behind weather forecasting – particularly long-range forecasting – are quite often refuted, thereby reducing the confidence held in future forecasts.

The '**testedness**' of a theory is difficult to define closely: the concept implies more than just a large number of repetitive tests. For example, one might well make 10 000 observations of the Sun, and never observe an eclipse. Yet one would not hold with any confidence the theory that 'the path of the moon never lies between the Earth and the Sun'¹¹. To be well-tested, a theory should have given predictions of what should happen in a variety of different circumstances. If these predictions were extensively tested over a wide range of conditions (either in the field or experimentally), then the theory can be called well-tested.

Besides 'testedness', a theory can be characterized by other attributes, although there is no general agreement on what these are. In addition to 'testedness', four have been listed (Davies, 1973):

1. **generality**: that is, the unification of existing concepts that is achieved; the greater the unification, the more 'fundamental' the theory is said to be;
2. **simplicity**: that is, the ability of a theory to be easily tested;
3. **precision**: that is, its ability to generate precise predictions;
4. '**refutedness**': that is, the extent to which it is inconsistent with previous tests, or its inconsistency with established data¹².

¹⁰ This section may be considered as a logical continuation of Chapter 3. Moreover, it is applicable to all areas of epidemiological investigation (e.g., observational studies and clinical trials). It is presented here because of its particular relevance to models that may claim to predict future events, and, in so doing, might be interpreted as factual descriptions of the future.

¹¹ This is reminiscent of Russell's 'inductivist turkey' (see Chapter 3).

¹² There are other aspects of a theory, which tend to be somewhat abstract and more suited to pure science. Thus, Albert Einstein was concerned with the 'inner perfection' of a scientific theory: an

Bertrand Russell viewed 'refutedness' in terms of 'external confirmation': the theory must not contradict empirical facts. However evident this demand may appear in the first place, its application turns out to be quite delicate. For it is often – perhaps even always – possible to adhere to a general theoretical foundation by securing the adaptation of the theory to the facts by means of artificial additional assumptions. This point of view is concerned with the confirmation of the theory by the available empirical facts.

The confidence that one has in a theory clearly depends on the relative importance attached to these characteristics¹³. Karl Popper (see Chapter 3), for example, focussed on 'testability' in situations of potential refutation. In epidemiology generally, emphasis has been placed on precision and 'refutedness', whereas some mathematical models might also suggest generality (e.g., R_0 ; see Chapter 8).

The relative importance of these characteristics also may be determined by the two broad functions of models: **strategic** or **tactical** (Holling, 1966). Strategic models explore general issues (e.g., the principles underlying spread of disease within populations), whereas tactical models address specific problems (e.g., the practical control of a specific epidemic). Strategic models sacrifice detail for generality (Levins, 1966), whereas tactical models may be loaded with so much detail that they become difficult to use and interpret (Thulke *et al.*, 1999). Moreover, the structure of strategic models may not assist the tactical modeller.

Thus, the value of a mathematical model, as a theory, should be judged in the context of these five attributes ('testedness', generality, simplicity, precision, 'refutedness') and its function. It is only after such judgement that a model may be deemed to be more than mere hypothesis.

intellectual elegance, which is aesthetic rather than utilitarian. Similarly, theories underpinned by Euclidian geometry, Newtonian dynamics and the mathematical form of Maxwell's electromagnetism are deemed to be 'beautiful' (Penrose, 2004). 'Testedness' and 'refutedness' are not involved in this idea of 'inner perfection'; however, generality and simplicity are. This is relevant to assessing if one theory is 'better' than another. The better one requires a high degree of confidence, and the 'inner perfection' should be as high as possible (i.e., the generality, simplicity and 'predictiveness' should be as high as possible). Thus, progress in pure science aims at an improvement of 'inner perfection', while also maintaining a reasonably high confidence in the theory. The goal of modern theoretical physics, for example, is to identify a 'Theory of Everything', which will rationalize the various laws operating in the Universe in a simple, elegant unity (Laughlin and Pines, 2000).

¹³ Formulations have been developed to 'measure' these attributes, and therefore the impact of their various combinations on a theory's merit (Davies, 1973).

Hypotheses and laws

Hypotheses and scientific laws are special cases of theories. A **hypothesis** is a theory of low generality and low 'testedness'. Many initial formulations of models of specific diseases therefore involve hypotheses.

A scientific **law** is a theory that is reasonably general, simple, and well tested (and its 'inner perfection' therefore is consequently never low). Although some mathematical models may appear to fulfil the first two criteria (e.g., the exponential decay paradigm), they often fail the last criterion.

Facts

A **fact** is held with very high confidence. Theories that are neither very general nor very precise can be held with great confidence if they are well tested but never refuted; they therefore are facts (e.g., the fact that the Earth is round). Theories therefore may become facts if, after much further testing, they were repeatedly confirmed but never refuted. Using these criteria, currently no mathematical models constitute facts.

Model building

Model building requires several steps (Figure 23.10), detailed by Dent and Blackie (1979), Martin and others (1987), Taylor (2003) and Woolhouse and others (2011).

First, the **objectives** of the model must be clearly specified. Thus, a model of the wind-borne transmission of Aujeszky's disease may have as its goal prediction of the farms likely to be exposed to infective virus plumes.

Secondly, details of the **data** and **knowledge** to be included as input parameters to the model are needed. Meteorological variables determining conditions favourable to long-distance transmission of virus, levels of virus excretion from infected pigs, and location of susceptible pigs, for example, would be required in the Aujeszky's disease-transmission model. This will then enable the main framework of the model, including the relationships between all of the parameters, to be determined. It is important at this preliminary stage for dialogue to occur between the modellers and those with expert biological knowledge of the candidate disease (e.g., microbiologists, field veterinarians, and epidemiologists)¹⁴.

¹⁴ A meeting of various experts, designed to produce a consensus on all available relevant knowledge, is sometimes termed a 'Delphi conference' (named after the Classical Greek oracle at Delphi). Modern Delphi conferences usually have well-defined rules of engagement for the purpose of knowledge elucidation.

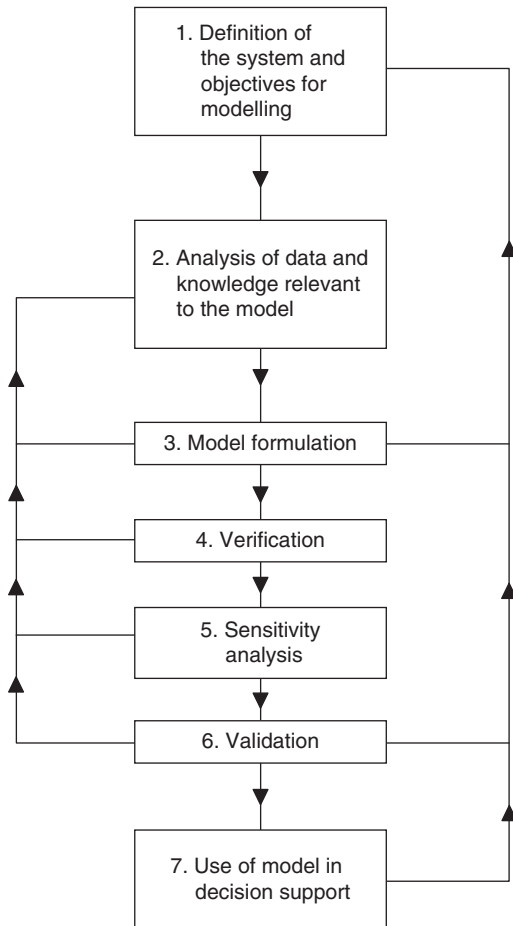


Fig. 23.10 Stages in model building. (Adapted from Taylor, 2003.)

Thirdly, the model is **formulated**; for example, using some of the modelling approaches described earlier in this chapter.

Fourthly, the model is **verified** by undertaking checks to confirm that the type of output anticipated by its design is generated.

A **sensitivity analysis** is then undertaken (see earlier in this chapter). This is particularly important if the model is sensitive to input variables based on data of doubtful quality, because they could lead to erroneous predictions. Additionally, sensitivity analysis is of benefit in determining the stability of the model in relation to the known variability of input parameters (e.g., the ratio of male to female ticks in the sheep tick paradigm, above).

The model then needs to be **validated**¹⁵. Initially, affirmative answers to four questions will support a model's validity:

1. have all the known determinants that influence occurrence of the disease been included?
2. can the value of these determinants be estimated with accuracy?
3. does the model make biological 'common sense'?
4. does the model behave in a mathematically reasonable way (i.e., is it sensitive to biologically relevant variables)?

Validation therefore involves establishing if the model behaves like the actual biological system that it is designed to mirror; for this, two conditions must be met (Spedding, 1988):

1. the model is assessed against data not used in its construction;
2. the precision of the model is specified in advance, noting that there is likely to be variability in the behaviour of the biological system.

The first condition may be difficult to fulfil; notably, when there is a paucity of data (e.g., if rare epidemics are being modelled). This also will limit its 'testedness'. Alternative approaches often are used, including recreation by the model of key features of the input data or by comparing results with those of other models (Woolhouse *et al.*, 2011). However, neither of these approaches provides true validation (Woolhouse *et al.*, 2011, Christley *et al.*, 2013).

The second condition is eased by stochastic formulations, which provide probability intervals for outputs as measures of error bounds.

The validity of a model ultimately may be assessed in terms of its usefulness (Green and Medley, 2002; Hugh-Jones, quoted by Salman, 2004), the key feature being whether decisions made with the model are more correct than those made without it (Dent and Blackie, 1979). However, such assessments can involve subjectivity and selectivity, and therefore may be contested. For example, the models used to inform control of the 2001 foot-and-mouth disease epidemic in the UK considered a narrow range of policy options, making it difficult '*to make the argument that mathematical models showed that implementation of widespread and intensive culling was the only tenable option*' (Haydon *et al.*, 2004), with subsequent analysis of field data refuting this argument (Mansley *et al.*, 2011).

Some authors have contended that the usefulness of a model sometimes may be primarily based on its function within the social and political environment in which it is set, rather than being based on, or reflecting,

¹⁵ The importance of validation cannot be emphasized enough, particularly when models' consequences have a profound impact on

society, as was the case with those relating to the 2001 foot-and-mouth disease epidemic in the UK (Kitching, 2004; Pfeiffer, 2004; Mansley *et al.*, 2011).

the quality of the model and its conclusions (Christley *et al.*, 2013) although this may not address relevant ethical issues (Boden and McKendrick, 2017).

If the model is deemed to be adequate, it can be used to **support decisions**, or become part of a larger decision support system (e.g., the *EpiMAN*: see Chapter 11), noting its suitability to particular roles (Table 23.5).

The model-building strategy addresses the main issues in assessment of a theory: generality, simplicity, precision, 'refutedness' and 'testedness'. The extent to which models perform against these criteria determines the degree to which they may be legitimately and widely applied.

There also is the risk of ignoring the wider potential fallacy of *petitio principii* ('begging the question') of assuming that a model can be considered to be 'mathematically successful', yet fails to explain the biological

truth. The extent to which a mathematically successful theory can fully explain reality (the belief that it can, being termed the 'Sin of Galileo': Mumford, 1970) has been tendentiously redacted elsewhere in the wider scientific community (Smolin, 2006).

Finally, models cannot stand alone in determining efficient control strategies, but should be used in conjunction with accurate field data and experimentally-derived data relating to diseases' natural history. The dangers of applying modelling in isolation from traditional field observation have been noted, both in human epidemiology – in the context of a reappraisal of Snow's classical investigation of cholera (Cameron and Jones, 1983) – and in veterinary epidemiology (Hugh-Jones, 1983). Indeed, models should incorporate veterinary knowledge and experience, and, as such, should be a 'collective veterinary brain'. Otherwise, they may become exercises in mathematical sophistry.

Further reading

- Ackerman, E., Elveback, L.R. and Fox, J.P. (1984) *Simulation of Infectious Disease Epidemics*. Charles C. Thomas, Springfield
- Anderson, R.M. and May, R.M. (1991) *Infectious Diseases of Humans: Dynamics and Control*. Oxford University Press, Oxford. (Includes a comprehensive description of the basic models)
- Anderson, R.M. and Nokes, D.J. (1991) Mathematical models of transmission and control. In: *Oxford Textbook of Public Health*, Vol. 2, 2nd edn. Eds Holland, W.W., Detels, R. and Knox, G., pp. 225–252. Oxford University Press, Oxford. (An introduction to mathematical modelling of infectious diseases and their control, with an emphasis on human infections)
- Bailey, N.T.J. (1975) *The Mathematical Theory of Infectious Diseases and its Applications*, 2nd edn. Charles Griffin, London. (A seminal text)
- Barnes, B. and Fulford, G.N. (2002) *Mathematical Modelling with Case Studies*. Taylor and Francis, London and New York. (An introduction to modelling using differential equations, including some medical and veterinary examples)
- Becker, N.G. (1989) *Analysis of Infectious Disease Data*. Chapman and Hall, London. (An introduction to models of infectious diseases)
- Black, F.L. and Singer, B. (1987) Elaboration versus simplification in refining mathematical models of infectious disease. *Annual Review of Microbiology*, **41**, 677–701
- Boden, L.A. and McKendrick, I.J. (2017) Model-based policymaking: a framework to promote ethical "good practice" in mathematical modelling for public health policymaking. *Frontiers in Public Health*, **5**, 68; doi: 10.3389/fpubh.2017.00068
- Brauer, F. and Castillo-Chavez, C. (2008) *Mathematical Models in Population Biology and Epidemiology*, 2nd edn. Springer, New York
- Brauer, F., van den Driessche, P. and Jianhong, W. (Eds) (2008) *Mathematical Epidemiology*. Springer-Verlag, Berlin and Heidelberg. (An introduction to types of mathematical models, with examples)
- Caswell, H. (2000) *Matrix Population Models: Construction, Analysis, and Interpretation*, 2nd edn. Sinauer Associates, Sunderland
- Christley, R.M., Mort, M., Wynne, B., Wastling, J.M., Heathwaite, A.L. *et al.* (2013) "Wrong, but useful": negotiating uncertainty in infectious disease modelling. *PloS ONE*, **8**, e76277; doi 10.1371/journal.pone.0076277. Correction: Christley, R.M., Mort, M., Wynne, B., Wastling, J.M., Heathwaite, A.L. *et al.* (2014) Correction: "Wrong, but Useful": negotiating uncertainty in infectious disease modelling. *PLoS ONE*, **9**, doi 10.1371/annotation/240b4b75-2b98-4c22-bf3b-f700369f594b
- Daley, D.J. and Gani, J. (1999) *Epidemic Modelling: An Introduction*. Cambridge University Press, Cambridge. (A general introduction, mostly in the context of bacterial and virus diseases of human populations)
- De Jong, M.C.M. (1995) Mathematical modelling in veterinary epidemiology: why model building is important. *Preventive Veterinary Medicine*, **25**, 183–193
- Diekmann, O. and Heesterbeek, J.A.P. (2000) *Mathematical Epidemiology of Infectious Diseases*.

- Model Building, Analysis and Interpretation*. John Wiley, Chichester
- Fournié, G., Walker, P., Porphyre, T., Métras, R. and Pfeiffer, D. (2012) Mathematical models of infectious diseases in livestock: concepts and application to the spread of highly pathogenic avian influenza virus strain type H5N1. In: *Health and Animal Agriculture in Developing Countries*. Eds Zilberman, D., Otte J., Roland-Holst, D. and Pfeiffer, D., pp. 183–205. Springer, New York
- Giordano, F.R., Weir, M.D. and Fox, W.P. (2009) *A First Course in Mathematical Modeling*, 4th edn. Brookes/Cole, Belmont. (A general introduction to mathematical modelling)
- Halloran, M.E. (1998) Concepts of infectious disease epidemiology. In: *Modern Epidemiology*, 2nd edn. Rothman, K.J. and Greenland, S., pp. 529–554. Lippincott-Raven, Philadelphia. (A quantitative introduction to the transmission of infectious disease)
- Hethcote, H.W. (2000) The mathematics of infectious diseases. *SIAM Review*, **42**, 599–653
- Hurd, H.S. and Kaneene, J.B. (1993) The application of simulation models and systems analysis in epidemiology: a review. *Preventive Veterinary Medicine*, **15**, 81–99
- Isham, V. (2005) Stochastic models for epidemics. In: *Celebrating Statistics. Papers in Honour of Sir David Cox on the Occasion of his 80th Birthday*. Eds Davison, A.C., Dodge, Y. and Wermuth, N., pp. 27–54. Oxford University Press, Oxford
- Keeling, M.J. and Rohani, P. (2008) *Modeling Infectious Diseases in Humans and Animals*. Princeton University Press, Princeton and Oxford
- Kitching, R.P., Thrusfield, M.V. and Taylor, N.M. (2006) Use and abuse of mathematical models: an illustration from the 2001 foot and mouth disease epidemic in the United Kingdom. *Revue Scientifique et Technique, Office International des Epizooties*, **25**, 293–311
- Lanzas, C., Lu, Z. and Gröhn, Y.T. (2011) Mathematical modelling of the transmission and control of foodborne pathogens and antimicrobial resistance preharvest. *Foodborne Pathogens and Disease*, **8**, 1–10
- Mansley, L.M., Donaldson, A.I., Thrusfield, M.V. and Honhold, H. (2011) Destructive tension: mathematics versus experience – the progress and control of the 2001 foot and mouth disease epidemic in Great Britain. *Revue Scientifique et Technique, Office International des Epizooties*, **30**, 483–498 (A cautionary discussion of the application of mathematical models to determine disease-control policy)
- Manton, K.G. and Stallard, E. (1988) *Chronic Disease Modelling: Measurement and Evaluation of the Risks of Chronic Disease Processes*. Charles Griffin, London/Oxford University Press, New York. (A comprehensive description of models of human chronic diseases and ageing)
- Messud-Petit, F. and Dubois, M.A. (2010) Modelling in epidemiology: an introduction. In: *Infectious and Parasitic Diseases of Livestock*, Vol. 1. Eds Lefèvre, P.-C., Blancou, J., Chermette, R. and Uilenberg, G., pp. 81–92. Lavoisier, Paris
- Meyer, W.J. (1984) *Concepts of Mathematical Modeling*. McGraw-Hill, New York. (An old, but broad and readable, introduction to mathematical modelling)
- Nunn, M., Garner, G. and White, D. (Eds) (1993) Animal health. *Agricultural Systems and Information Technology*, **5** (1), 4–43. (An overview of epidemiological modelling and examples of systems models)
- Proceedings of the Mathematical and Information Models for Veterinary Science Workshop, Wednesday 22 December 1993, Moredun Research Institute, Edinburgh, UK*. Moredun Research Institute/University of Strathclyde/Scottish Agricultural Colleges Veterinary Services, Edinburgh
- Scott, M.E. and Smith, G. (Eds) (1994) *Parasitic and Infectious Diseases: Epidemiology and Ecology*. Academic Press, San Diego. (An introduction to modelling, with case studies of infections in humans, livestock and wild animals)
- Squire, G.R. and Hamer, P.J.C. (Eds) (1990) *United Kingdom Register of Agricultural Models 1990*. AFRC Institute of Engineering Research, Wrest Park, Silsoe. (A list and summary of agricultural models, including some veterinary ones)
- Thornley, J. and France, J. (2006) *Mathematical Models in Agriculture: Quantitative Methods for the Plant, Animal and Ecological Sciences*, 2nd edn. CABI Publishing, Wallingford. (Includes discussions of models of animal diseases, animal husbandry and animal products)
- Vynnycky, E. and White, R.G. (2010) *An Introduction to Infectious Disease Modelling*. Oxford University Press, Oxford
- Willeberg, P. (Ed.) (2011) Models in the management of animal diseases. *Revue Scientifique et Technique, Office International des Epizooties*, **30**, 381–643

24

Risk analysis

Risk analysis is a formal procedure for estimating the likelihood and consequences of adverse effects occurring in a specific population, taking into consideration exposure to potential hazards and the nature of their effects. This includes the management (usually reduction) of the likelihood of exposure. It is used widely in finance (e.g., to control either an individual's or a company's chances of losing on an investment), environmental science (e.g., to estimate hazards associated with contaminants or other environmental conditions, as they affect exposed humans, animals, or selected elements of an ecosystem) and engineering (e.g., to study the safety of nuclear reactors).

The impetus for risk analysis was global industrialization, which accelerated after the Second World War, presenting new hazards (e.g., novel synthetic isotopes) with which society was unfamiliar (Rowe, 1977). New anxieties associated with man-made risks were rapidly disseminated via the media, and created perceptions that some risks were unacceptably high, resulting in confrontations in the courts, referenda and regulation. There was a need to address these problems (including the more subjective issue of how risks are deemed to be acceptable or unacceptable) in a regular and consistent manner. Formal risk analysis consequently emerged in the nuclear and chemical industries in the 1960s and 1970s, respectively¹. The need to understand and communicate what risks signify was realized in the 1980s, but remains the least appreciated component of risk analysis.

¹ Awareness of risk, however, is much older. The Biblical dietary laws of the Pentateuch are considered by some (Townley, 1827), but not all (Milgrom, 1992), scholars to be among the earliest documented example of a risk analysis, designed to improve food hygiene.

Definition of risk

There is no single definition of risk (*Table 24.1*). Risk has two components: the **likelihood** of an event occurring, and the **severity** (i.e., impact, magnitude or seriousness) of the consequences of the event. The latter may range from relatively mild (e.g., most cases of staphylococcal food poisoning) to catastrophic (e.g., a nuclear accident or a tsunami).

Qualitative descriptions commonly rank risk as 'very low', 'low', 'medium' or 'high'². In addition, risk may be characterized as 'negligible' (generally assumed to be 'below very low') or 'non-negligible'. Descriptions with elements combined from each of these levels also may be used.

A quantitative description often focusses on likelihood expressed as the **probability** (see Chapter 12) of an event (exposure to a hazard) occurring over a specified period of time (hence the use of 'risk' as a synonym for cumulative incidence: see Chapter 4). This probabilistic notion of risk (also sometimes described as 'objective' or 'real' risk) is found in risk analysis in engineering, toxicology and actuarial studies (Renn, 1992), and constitutes a 'physical, technical' conception of risk, in contrast to social outlooks (Bradbury, 1989a)³.

² This implicitly accepts that in most contexts 'zero risk' is extremely difficult, if not impossible, to achieve (Nakayachi, 1998), although the notion is very attractive to the general public (Tversky and Kahneman, 1981), as material comfort and individualism in Western societies appear to have increased sensitivity to risk.

³ It follows that, in pursuing the probabilistic notion, decisions on how to respond to risk are determined by 'technical' (i.e., scientific) evidence, which may become central to regulatory mechanisms (the 'technocratic model': Weingart, 1999), rather than being subordinate to political considerations (the 'Weberian decisionist mode': Weber, 1958). In practice, interpretation of risk, and therefore responses, may be based on a mix of scientific and non-scientific factors, including values, interests and politics (Wintle and Cleland, 2012).

Table 24.1 Some terms used in risk analysis.

<i>Term</i>	<i>Definition/description</i>	<i>Source</i>
Acceptable risk	Risk level judged by each <i>OIE</i> Member to be compatible with the protection of animal and public health within its country	Murray <i>et al.</i> (2010b)
	A level of risk that is so small in terms of likelihood of occurrence or consequences that, in comparison with the expected benefits, stakeholders are willing to accept it	Jakob-Hoff <i>et al.</i> (2014)
Average (<i>of 'likelihood'</i>)	The usual amount, extent, rate	<i>Oxford English Dictionary</i> (1971)
Consequence assessment	Description of the relationship between specified exposures to a biological agent and the consequences of those exposures	OIE (2013)
Critical control point	A location, practice, procedure or process at which control can be exercised over one or more factors, which, if controlled, could minimize or prevent a hazard	Gracey <i>et al.</i> (1999)
Entry assessment	Description of the biological pathway(s) necessary for an importation activity to introduce pathogenic agents into a particular environment, and estimating the probability of that complete process occurring	OIE (2013)
Exposure assessment	The qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant	Codex Alimentarius Commission (1999)
	Description of the biological pathway(s) necessary for exposure of animals and humans in importing countries to the hazards (in this case pathogenic agents) from a given risk source, and estimating the probability of the exposure(s) occurring	OIE (2013)
Extremely (<i>of 'likelihood'</i>)	Outermost; furthest from the centre; situated at either end; the highest or most extreme degree of anything	<i>Oxford English Dictionary</i> (1971)
Hazard	A situation that could occur during the lifetime of a product, system or plant that has the potential for human [<i>or animal</i>] injury, damage to property, damage to the environment, or economic loss	The Royal Society (1992)
Hazard characterization	The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with the hazard	Codex Alimentarius Commission (1999)
High (<i>of 'likelihood'</i>)	Extending above the normal or average level	<i>Oxford English Dictionary</i> (1971)
	Event is likely to occur	Moutou <i>et al.</i> (2001)
Insignificant (<i>of 'likelihood'</i>)	Unimportant, trifling (<i>cf.</i> ' <i>significant</i> ')	<i>Oxford English Dictionary</i> (1971)
Likelihood	Probability; the state or fact of being likely [†]	<i>Oxford English Dictionary</i> (1971)
Likely	Probable; such as might well happen or be true; to be reasonably expected	OIE (2013)
Low (<i>of 'likelihood'</i>)	Less than average, coming below the normal level	<i>Oxford English Dictionary</i> (1971)
	Event may occur in some cases	Moutou <i>et al.</i> (2001)
Negligible (<i>of 'likelihood'</i>)	That need not be considered	<i>Oxford English Dictionary</i> (1971)
	Probability so low as to be effectively discounted or event likely only under rare circumstances	Moutou <i>et al.</i> (2001)
	Insignificant (<i>cf.</i> ' <i>significant</i> ')	<i>Oxford English Dictionary</i> (1971)
Remote	Slight, faint	<i>Oxford English Dictionary</i> (1971)
Risk	A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard	The Royal Society (1992)
	The amalgam of the probability of an event occurring and the seriousness of the event's consequences	Cameron and Wade-Gery (1995)
	The probability that an event will occur and the seriousness of that event for the environment and human [<i>and animal</i>] health	CEPS (2002)
	The potential for realization of unwanted, negative consequences of an event	Rowe (1977)

(Continued)

Table 24.1 (Continued)

Term	Definition/description	Source
Risk analysis	A process consisting of three components: risk assessment, risk management and risk communication	Codex Alimentarius Commission (1999)
Risk assessment	A process consisting of four components: hazard identification, risk assessment, risk communication, risk management	OIE (2013)
	A scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment and (iv) risk characterization	Codex Alimentarius Commission (1999)
	A scientifically based process consisting of the following steps: entry assessment, exposure assessment, consequence assessment and risk estimation	OIE (2013)
	A tool for extrapolating from statistical and scientific data to arrive at a value which people will accept as an estimate of the risk attached to a particular activity or event	UKILGRA (1996)
Qualitative	A risk assessment based on data which, while forming an inadequate basis for numerical risk estimations, nonetheless, when conditioned by prior expert knowledge and identification of attendant uncertainties permits risk ranking or separation into descriptive categories of risk	Codex Alimentarius Commission (1999)
	An assessment where the outputs on the likelihood of the outcome or the magnitude of the consequences are expressed in qualitative terms such as high, medium, low or negligible	Murray <i>et al.</i> (2010b)
Quantitative	A risk assessment that provides numerical expressions of risk and indication of the attendant uncertainties	Codex Alimentarius Commission (1999)
Risk characterization	The process of determining the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment	Codex Alimentarius Commission (1999)
Risk communication	The interactive exchange of information and opinions concerning risk and risk management among risk assessors, risk managers, consumers and other interested parties	Codex Alimentarius Commission (1999)
Risk estimate	Output of risk characterization	Codex Alimentarius Commission (1999)
Risk estimation	Integration of the results from entry assessment, exposure assessment, and consequence assessment to produce overall measures of risks associated with the hazards identified at the outset	OIE (2013)
Risk management	The process of weighing policy alternatives in the light of the results of risk assessment and, if required, selecting and implementing appropriate control options, including regulatory measures	Murray (2002)
Safety	... a judgment of the acceptability of risk; a thing is safe if its risks are judged to be acceptable (<i>see 'acceptable risk'</i>) (<i>contrast with the usual dictionary definition of 'safe' as 'not exposed to danger'</i>)	Lowrance (1976)
Significant (<i>of 'likelihood'</i>)	Noteworthy, important, consequential (<i>cf. 'insignificant'</i>)	<i>Oxford English Dictionary</i> (1971)
Uncertainty	The lack of precise knowledge of the input values which is due to measurement error or to lack of knowledge of the steps required, and the pathways from hazard to risk, when building the scenario being assessed	Murray <i>et al.</i> (2010b)
	An assessor's lack of knowledge (level of ignorance) about parameters that characterize the physical system that is being modelled (<i>cf. 'variability'</i>)	Vose (2008)
Variability	The fact or quality of being variable in some respect; tendency towards, capacity for, variation or change	<i>Oxford English Dictionary</i> (1971)
	A real-world complexity in which the value of an input is not the same for each case due to natural diversity in a given population	Murray <i>et al.</i> (2010b)
	A phenomenon in the physical world to be measured, analysed and where appropriate explained (<i>cf. 'uncertainty'</i>)	Cox, quoted by Vose (2008)

[†]This broad definition is relevant to the way in which the term is generally used in risk analysis. In formal statistics, 'likelihood' has a specific meaning (see Appendix I and Everitt and Skrondal, 2010).

Risk defines neither a current problem nor a future certainty. It is the potential for future harm. Thus, it can only be estimated, not measured (Gould *et al.*, 1988).

Risk analysis and the 'precautionary principle'

The '**precautionary principle**' argues that if there is scientific uncertainty regarding a risk and its consequences (i.e., cause and effect relationships are not fully established) then preventive measures can be justified in the absence of proof of such a risk. The term emerged in Germany in the 1970s, and attained international credibility in the 1980s (Cameron and Wade-Gery, 1995). It has been cited mainly in the context of emerging perceived hazards, such as environmental pollution, genetically modified foods and other food-safety hazards, where uncertainty remains (CEPS, 2002).

A precautionary approach has a useful protective role as an initial response to new potential threats⁴. It also may be the only reaction to complex serious problems such as global warming (Iverson and Perry, 2011) and loss of biodiversity (Cooney and Dickson, 2005), where traditional, formal risk analysis is not (and, indeed, may never be) adequate⁵. A precautionary approach also may comfort politicians and the public, who are frequently slow to be reassured in the face of uncertainty. However, it tends towards inertia and tardiness in responding to new policy options, and can indefinitely mask political decisions (Starr, 2003)⁶. In contrast, risk

analysis provides an operational framework for determining policy, using accruing knowledge⁷.

Risk analysis in veterinary medicine

Risk analysis is now applied to a wide range of veterinary issues (Table 24.2). A major area is **import risk analysis**. Although diseases may occur at low levels and be adequately controlled (or eradicated), there may be a risk of importing them from other countries. Such a risk can only be eliminated completely if importation is totally prohibited. However, current political pressures in the world favour free trade⁸. Thus, the 'unquantified' risk of introduction of a disease cannot now be presented as a trade barrier, exemplified by concerns that the liberalization of trade under the *General Agreement on Tariff and Trade* (and the resultant *World Trade Organization* (WTO)) may be associated with countries applying domestic standards that are less stringent than those applied to imported goods, thereby restricting trade (Scudamore, 1995).

Additionally, it may be untenable to adopt a 'zero-risk' approach. For example, it is unrealistic to aim for absence of pathogens in livestock, and so effective control of food-borne pathogens must be tackled by identification of those animals that pose a risk, management of the risk at critical stages in the production process, and hygiene (Kühne and Lhafi, 2005). This introduces the second major area of veterinary-related risk analysis: **food-safety risk analysis**. In contrast to import risk analysis, most food-safety risk analyses relate to domestic issues focussing on the food-production processes and risk-reduction or mitigation strategies.

Import risk analyses frequently report risk in terms of likelihood (for example of introduction into an importing country), but exclude severity from the assessment. This often is because severity may be very difficult to assess (see later: 'What level of risk is acceptable?').

Veterinary risk analysis therefore has evolved to assess, as objectively as possible, the risks associated with particular diseases, rather than only relying on the somewhat subjective judgements of individual scientists or parties (Morley, 1993a; Murray *et al.*,

⁴ Precautionary regulation can be considered as a continuum, being more precautionary the earlier it takes effect and the more stringently it restricts suspected sources of risk (Wiener and Rogers, 2002). Thus, it may range from regulation in the absence of complete evidence about a particular risk to forbidding risky activities until they are shown to pose no (or an acceptable) risk.

⁵ However, a precautionary approach and a 'scientific' risk-based approach should not be viewed as being mutually exclusive: key elements of the former are consistent with responses to intractable issues in risk analysis, such as uncertainty (Stirling, 2001). Moreover, each approach should take its appropriate place in determining policies to deal with particular issues (Stirling, 2010).

⁶ Nevertheless, it has been applied as a very effective temporary trade barrier (e.g., the ban on the importation of British beef into France between 1996 and 2002, in relation to bovine spongiform encephalopathy), and its acceptance by the European Union (CEC, 2000) has led to disagreement between Europe and North America over trade in genetically modified commodities, although it arguably has no basis in international law (Trouwborst, 2002). It has also been formulated in international treaties and agreements, including the *Montreal Protocol on Substances that Deplete the Ozone Layer* (1987), the *Third North Sea Conference* (1990), the *Rio Declaration on Environment and Development* (1992), the *Framework Convention on Climate Change* (1992), the *Treaty on European Union (Maastricht Treaty)* (1992) and the *Cartagena Protocol on Biosafety* (2000) (Harremöes *et al.*, 2001).

⁷ Thus, precautional quarantine became the standard method of preventing the entry of rabies into the UK early in the 20th century, when little was known of the disease's cause and vaccines were not available. However, increased knowledge of the natural history of rabies, and the development of vaccines, facilitated risk analyses (MAFF, 1998; Dye, 1999) leading to the 'PETS' travel scheme (AHVLA, 2012), which allows vaccinated dogs and cats to enter the UK without the lengthy restriction of quarantine.

⁸ See Irwin (1996) for a discussion of the case for free trade. The issue dates back to the 18th century, with the publication of Adam Smith's '*Wealth of Nations*' (Smith, 1776), but is not without its problems (Stiglitz, 2006).

Table 24.2 Some veterinary and associated risk analyses.

Topic	Source
Animal health risks associated with wildlife products	Bengis (1997)
Antimicrobial resistance originating in food animals	Kelly <i>et al.</i> (2004); Snary <i>et al.</i> (2004); Bartholomew <i>et al.</i> (2005)
Biosecurity risks associated with imported livestock testing serologically-positive in quarantine	Pharo (1999)
<i>Campylobacter</i> spp. infection of humans from consumption of poultry	Rosenquist <i>et al.</i> (2003); Nørrung (2006)
Crimean Congo haemorrhagic fever virus introduction into Europe through migratory birds	Gale <i>et al.</i> (2012)
Disease transmission by artificial insemination and embryo transfer	MacDiarmid (1993); Suttmoller and Wrathall (1995, 1997); Eaglesome and Garcia (1997); Wrathall (1997)
Emerging zoonotic potential of animal diseases	Palmer <i>et al.</i> (2005)
<i>Escherichia coli</i> O157 in pasteurized milk	Clough <i>et al.</i> (2006)
Exposure of the public to bovine spongiform encephalopathy from beef-on-the-bone	Comer and Huntley (2004)
Exposure of the public to bovine spongiform encephalopathy from burial and burning of cattle infected with foot-and-mouth disease	Comer and Huntley (2003)
Exposure of the public to bovine spongiform encephalopathy from drinking water potentially contaminated by rendering-plant effluent	Gale <i>et al.</i> (1998)
Importation of animal diseases via live animals and animal products	Morley (1993b)
Importation of brucellosis into GB via breeding cattle	Jones <i>et al.</i> (2004a)
Importation of exotic infectious diseases in smuggled meat	Wooldridge <i>et al.</i> (2006)
Importation of foot-and-mouth disease in South American beef	Astudillo <i>et al.</i> (1997)
Importation of foot-and-mouth disease into Australia in animal feed	Doyle (1995)
Importation of foot-and-mouth disease into Australia in milk products	Heng and Wilson (1993)
Importation of foot-and-mouth disease into New Zealand in passenger luggage	Pharo (2002b)
Importation of foot-and-mouth disease into Spain by live animals	Martínez-López <i>et al.</i> (2008)
Importation of foot-and-mouth disease into the UK from South Africa	DEFRA (2003)
Importation of rabies into GB	MAFF (1998); Dye (1999); Jones <i>et al.</i> (2002, 2005); Laurenson <i>et al.</i> (2002); Kosmider <i>et al.</i> (2006); Ramnial <i>et al.</i> (2010); Goddard <i>et al.</i> (2012)
Importation of rabies into New Zealand	MacDiarmid and Corrin (1998)
Introduction of African swine fever into Europe	De Vos <i>et al.</i> (2011); Costard <i>et al.</i> (2013a)
Introduction of <i>Echinococcus multilocularis</i> into the UK via imported beavers	DEFRA (2012d)
Introduction of equine infectious anaemia into GB via movement of horses	DEFRA (2011b)
Introduction of exotic animal diseases into the US via cruise-ship waste	McElvaine <i>et al.</i> (1993)
Introduction of exotic diseases of fish into New Zealand via imported salmon	MacDiarmid (1994)
Introduction of exotic diseases of pigs via uncooked swill	Corso (1997); Horst <i>et al.</i> (1997)
Introduction of foot-and-mouth disease into the US via imported beef	USDA (2013)
Introduction of henipavirus into the UK	Snary <i>et al.</i> (2012)
Introduction of infectious bursal disease virus and Newcastle disease virus in chicken meat	MAF (2000)
Introduction of Newcastle disease virus in hatching eggs	Pharo (2001)
Introduction of porcine reproductive and respiratory disease virus into New Zealand	Cobb <i>et al.</i> (2015)
Introduction of West Nile virus into GB by migrating birds	Bessell <i>et al.</i> (2014)
Introduction of wild boar into Denmark	Alban <i>et al.</i> (2005)

(Continued)

Table 24.2 (Continued)

Topic	Source
Lyme disease in relation to vector habitat	Guerra <i>et al.</i> (2002)
Methicillin-resistant <i>Staphylococcus aureus</i> acquisition in dogs	Heller <i>et al.</i> (2010, 2011)
Microbial contamination in poultry production and processing	Kelly <i>et al.</i> (2003); Cox <i>et al.</i> (2005); Kelly (2005)
Microbial contamination of beef carcasses	Jordan <i>et al.</i> (1999); Mellor <i>et al.</i> (2004)
New outbreaks of foot-and-mouth disease associated with burning pyres	Jones <i>et al.</i> (2004b)
New outbreaks of foot-and-mouth disease associated with public access to footpaths during a foot-and-mouth disease epidemic	Taylor (2002)
Potential animal health risks from imported sheep and goat meat	MacDiarmid and Thompson (1997); Rapoport and Shimshony (1997)
Potential for bovine spongiform encephalopathy in the US	Cohen <i>et al.</i> (2001)
Public health and animal health risks associated with highly pathogenic avian influenza viruses in Europe	DEFRA (2005); EFSA (2005); Mettenleiter (2005); ECDC (2006)
Prion risk from restocking goat herds	Ortiz-Pelaez <i>et al.</i> (2012)
Residues in poultry and eggs	Donoghue (2005)
Risk of foot-and-mouth disease associated with faulty vaccine production	Cané <i>et al.</i> (1995)
Risks and hazards associated with the handling and consumption of game birds	Coburn <i>et al.</i> (2005)
Risks associated with reduction in routine swabbing techniques for the detection of <i>Taylorella equigenitalis</i> (the cause of contagious equine metritis)	Wood <i>et al.</i> (2005)
Risks associated with release of abalone from hatcheries into the open sea	Jones and Fletcher (2012)
<i>Salmonella</i> spp. exposure in Ireland from consumption of eggs	Murchie <i>et al.</i> (2008); Kelly <i>et al.</i> (2009)
<i>Salmonella</i> spp. infection of humans from consumption of eggs and broiler chickens	FAO/WHO (2002)
Tick-borne encephalitis in Sweden in relation to landscape ecology	Zeimes <i>et al.</i> (2014)
Transmission of bovine spongiform encephalopathy to humans and cattle through the application of sewage sludge to agricultural land	Gale and Stanfield (2001)
Transmission of bovine tuberculosis from badgers to cattle	Gallagher <i>et al.</i> (2003)
Transmission of bovine tuberculosis from wild animals (relative to badgers) to cattle	Delahay <i>et al.</i> (2007)
Transmission of foot-and-mouth disease in milk and dairy products	Donaldson (1997)
Transmission of shrimp viruses via live and frozen shrimp	Lightner <i>et al.</i> (1997)
Transmission of zoonoses during taxidermy	Thrusfield (2006)
Vector-borne disease introduction and the effects of climate change	Gale <i>et al.</i> (2012)
West Nile virus emergence in the UK	Brown <i>et al.</i> (2012a)

2004c, 2010b). (This squares well with the probabilistic, 'technical' notion of risk.) All aspects of international animal disease-control policy are now based on risk analysis, which therefore has become a routine veterinary procedure.

Components of risk analysis

Guidelines have been published to assist in undertaking import risk analysis and food-safety risk analysis. For import risk analysis, the *World Organisation for Animal Health* (OIE) has produced a general

handbook on risk analysis (Murray *et al.*, 2010b) and a handbook specifically on quantitative risk assessment (Murray *et al.*, 2004c) as well as the Terrestrial Animal Health Code, in which a chapter is dedicated to risk analysis (OIE, 2013). For food-safety risk analysis, the recognized guidelines were published by the *Codex Alimentarius Commission* (CAC) (1999).

Following the OIE (2013) guidelines, there are four main components of import risk analysis: **hazard identification**, **risk assessment**, **risk management** and **risk communication** (Figure 24.1). These are preceded by definition of the **risk question** (i.e., the issue to be addressed).

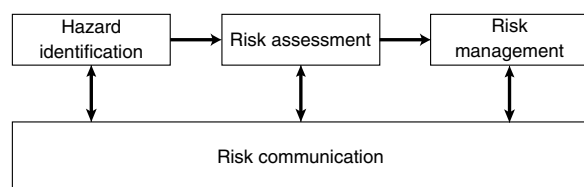


Fig. 24.1 The components of risk analysis. (Source: Terrestrial Animal Health Code 2016. Reproduced with permission of World Organisation for Animal Health (OIE).)

Hazard identification

Hazard identification is simply the definition of hazards (e.g., a pathogen). This occasionally may be eased by published information (e.g., the list of diseases to be notified to *OIE*), but frequently requires scrutiny of literature, and discussion with experts and those potentially affected by the hazards (e.g., the hazards associated with handling and consumption of wild game, which are discussed later in this chapter). In food-safety risk analysis, hazard identification is part of risk assessment, and includes a review of all information relating to the food product, the pathogen and the consumer, as well as details of outbreaks.

Risk assessment

The *CAC* (1999) and *OIE* (2013) frameworks describe risk assessment as comprising a number of different steps. In the *CAC* framework, the steps are:

- hazard identification;
- hazard characterization;
- exposure assessment;
- risk characterization.

The steps in the *OIE* framework are:

- entry assessment;
- exposure assessment;
- consequence assessment;
- risk estimation.

The main difference between these frameworks is that hazard identification is considered part of risk assessment in the *CAC*'s structure. In both cases, the process should involve comprehensive scrutiny of the relevant literature and may include formal meta-analysis (see Chapters 17 and 19), as well as eliciting expert opinion.

Hazard characterization This is the qualitative or quantitative evaluation of the nature of the adverse health effects associated with hazards. For chemical agents, a dose–response assessment should be

performed. For biological or physical agents, a dose–response assessment should be undertaken if the data are obtainable.

Entry assessment Entry assessment (previously known as release assessment) is the determination of the likelihood of a commodity (e.g., an imported animal) being contaminated or infected with hazards, and a description of the pathways by which the hazards can be introduced into the environment. The survival of organisms also is an important component of microbial entry assessment. A **scenario tree**, graphically portraying the release pathways (e.g., *Figure 24.2*), often is constructed.

Exposure assessment This is the estimation (qualitatively or quantitatively) of the magnitude, frequency, duration and route of exposure of humans or animals to hazards. The assessment also should describe the size and nature of the exposed population. An assessment of the risk associated with the unrestricted importation of animals or animal products, for example, would consider the prevalence of pathogens in the source population, the probability of the pathogens surviving during importation, and the probability of the pathogens coming into contact with local livestock after importation. Examples include the risk of introducing maedi-visna and scrapie into New Zealand (MacDiarmid, 1993). In a food-safety risk analysis, all stages in the food-production process would be considered, and the effects of environmental factors such as temperature on the prevalence and number of organisms would be taken into account. The route of exposure can be presented graphically in an **exposure pathway**.

Consequence assessment Consequence assessment is similar to hazard characterization. However, it also can include indirect effects; for example, in import risk analysis, it may include surveillance and control costs, compensation costs and trade losses.

Risk characterization and risk estimation These are descriptions of the nature and magnitude of risk, either to health or to the environment. Each description combines results of the previous steps. In a quantitative analysis, it includes the probability and severity of the predicted risk.

Comparing the two frameworks, it can be seen that they are, essentially, very similar. Their terms and definitions relate to the different types of problem that they have been designed to address.

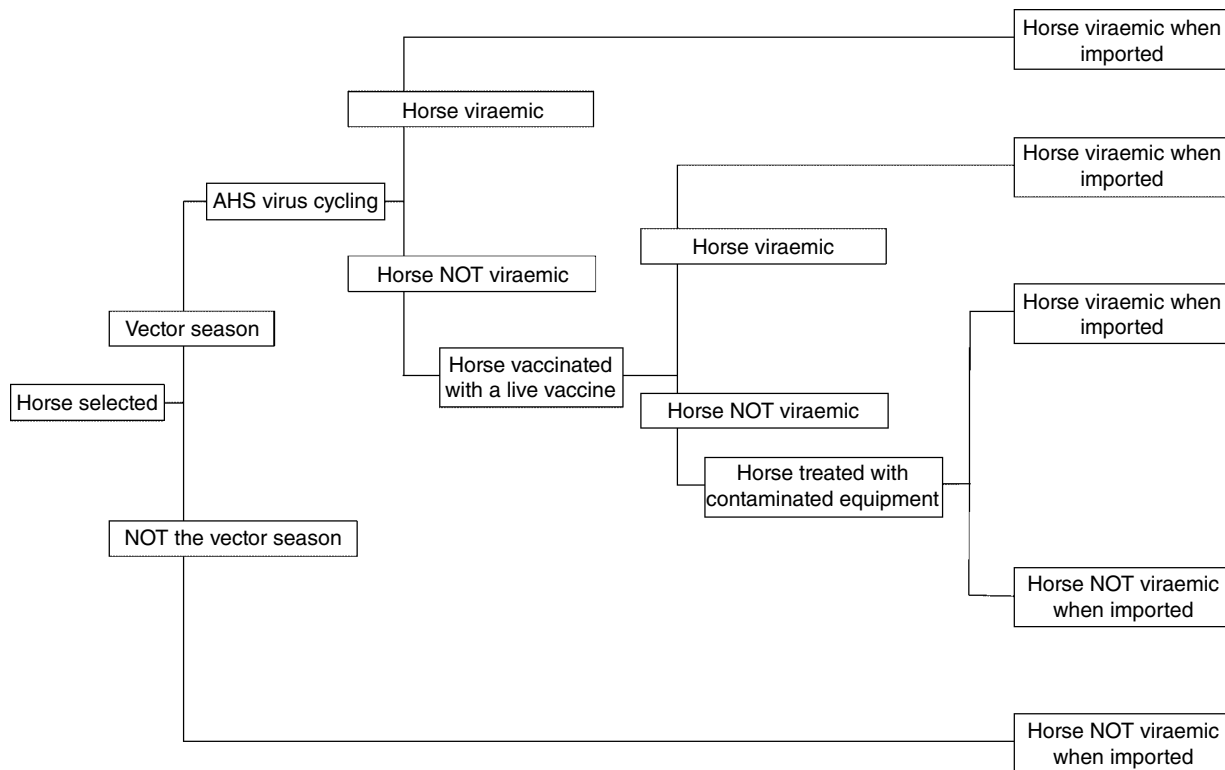


Fig. 24.2 Example of a scenario tree for an entry assessment: the biological pathways necessary for a horse to become infected with, and harbour, African horse sickness (AHS) virus when imported. (Source: Murray, 2002.)

Variability and uncertainty

The risk assessment procedure is accompanied by two characteristics: **variability** and **uncertainty** (Table 24.1). The former is the inherent variation in biological systems, which can be managed by standard statistical procedures such as the use of distributions (see Chapter 12). The latter, in contrast, indicates ignorance (e.g., lack of knowledge of the disease status of a country). Variability will exist, even if there is complete knowledge (e.g., it may be known that disease is present in a country, but the prevalence may be based on a sample survey, giving rise to a point estimate and a confidence interval for the prevalence). Thus, uncertainty may be reduced with time as more data are collected and more research is completed. In contrast, variability cannot be reduced by further study because it expresses the inherent variability of a system.

Variability sometimes is termed 'stochastic uncertainty' or 'aleatory uncertainty'⁹, whereas uncertainty, as defined above, is termed 'epistemic uncertainty'. The combination of variability and uncertainty is termed 'total uncertainty' or 'indeterminability' (Vose, 2008).

⁹ Aleatory = 'depending on the throw of a dice' (Latin: *aleator* = 'dice player').

Risk characterization and estimation must include an indication of variability and uncertainty.

Subjectivity

A degree of **subjectivity** also is attached to both qualitative and quantitative assessments (Redmill, 2002). The questions to be asked in risk analysis involve judgement, which is subjective (Kasper, 1980). If data are sparse, expert opinion may be sought (either individually, or by a formal Delphi conference; see Chapter 23 and Van Der Fels-Klerx *et al.*, 2002). In some cases, the opinion may be little more than a guess (e.g., the cattle-to-human species barrier of bovine spongiform encephalopathy: Comer and Huntley, 2004)¹⁰. Consequently, the results obtained by one risk analyst are unlikely to be obtained by others.

¹⁰ The consequences of selecting the wrong opinion can be disastrous. For example, following the tragedy of the space shuttle, *Challenger*, in 1986, Feynman (1988) noted that organizational decision making was conducted as though the *National Aeronautics and Space Administration's* management's estimate of a shuttle failure (one in 100 000 – equivalent to a daily launch with an average of 300 years between accidents) was accurate, whereas an engineer's estimate was, more realistically, as low as one in 100. A subsequent quantitative risk analysis identified the weaknesses in the booster-rocket seal performance, which was responsible for the accident (Dalal *et al.*, 1989).

Likelihood	High	Non-pathogenic <i>Escherichia coli</i> Some endoparasites	Endoparasites	Ectoparasites of farmed fish (e.g., sea lice)	Bluetongue
	Medium			Introduction of anthelmintic-resistant strains of helminths	
	Low	Exotic strains of non-pathogenic organisms		Introduction of equine viral arteritis in horses due to movement of horses from the continent	Widespread dissemination of foot-and-mouth disease with new measures (e.g., 'standstill' procedures*) in place
	Very low				Introduction of contagious bovine pleuropneumonia
		Very low	Low	Medium	High
		Severity			

Fig. 24.3 Example of a risk matrix: exposure to some animal diseases in the UK. Darker shading indicates higher levels of risk. * 'Standstill' requirements direct that, when livestock are moved onto a farm, a minimum period of time must lapse before any livestock can leave the farm.

Risk analyses, therefore, should always be fully **documented**, including details of analytical methods, the data that are used, assumptions and references¹¹. Finally, they should be subjected to independent **peer review**¹².

Risk management

Risk management is the selection of the options for controlling risks, taking into account social values, legal requirements and costs of control. It is not about future decisions, but about the future of decisions

¹¹ Comprehensive documentation enables all aspects of the analysis to be scrutinized. This is termed **transparency** (from 'transparent' = 'easily seen through'; Latin: *transparens* = 'shine through').

¹² Peer review of a quantitative analysis should include not only examination of input parameters but also deconstruction of the model so that its structure is revealed.

that must be taken now; that is, about speculative or uncertain aspects of the outcomes of decisions (Charette, 1989).

Management can be prioritized by construction of a **risk matrix (probability–impact matrix)**, which ranks risks according to their two components: likelihood and severity of consequences (Donoghue, 2001; Peeler *et al.*, 2015). *Figure 24.3* illustrates the technique for exposure to some animal diseases in the UK. Note that risks associated with some hazards (e.g., foot-and-mouth disease and contagious bovine pleuropneumonia) may be ranked relatively high, although likelihood is low (or, in the case of contagious bovine pleuropneumonia, very low); this is because of the severity of the consequences of exposure to these hazards. The construction of risk matrices is assisted by appropriate software (*Risk Matrix*: see Appendix III and Lansdowne, 1999). Cox (2008) criticizes some properties of risk matrices.

Hazard Analysis Critical Control Point

Hazard Analysis Critical Control Point (HACCP) is a procedure for identifying, evaluating and controlling hazards associated with particular stages in a process. Control occurs by action at key stages in a process – the **critical control points (CCPs)**. HACCP is therefore an important risk-management tool. It was initially developed in the 1960s for the US's *National Aeronautics and Space Administration* to ensure safe food for the first manned space missions¹³. It is now an internationally recognized technique for ensuring food safety (Brown, 2000); impetus for its adoption in the UK, for example, followed an outbreak of food poisoning due to *Escherichia coli* O157 in the 1990s (Stationery Office, 1997).

The stages of HACCP are:

- identify the **hazards** that must be prevented, eliminated or controlled;
- identify the **CCPs**;
- establish the **critical limits** (measurable target levels (e.g., temperature, pH) that indicate that a product is at risk from an identified hazard) at the CCPs; each CCP must have at least one critical limit;
- **monitor** procedures to ensure that each CCP is under control;
- establish any **corrective actions** that are required;
- confirm that the HACCP plan is effective (**validation**);
- confirm that the HACCP plan is being followed (**verification**);
- **document** the procedure with an effective record-keeping system.

The CCPs can be subdivided (Collins and Huey, 2015) thus:

- CCP1: where hazards can be prevented or eliminated; that is, control is effective;
- CCP2: where hazards can be minimized, reduced or delayed; that is, control is not absolute;

alternatively:

- CCPe: where hazards are eliminated and no further problem exists;
- CCPp: where hazards are prevented but not necessarily eliminated;
- CCPr: where hazards are significantly reduced, minimized or delayed.

¹³ Its antecedent was the 'Prescott–Meyer–Wilson strategy', which was developed in the UK and US in the 1930s, and was aimed at taking preventive and remedial measures, rather than merely examining samples of the final product (Mossel, 1989).

If HACCP is applied before and during slaughter of red-meat animals, for instance, hazards would include bacterial contamination of raw meat (biological hazards), contamination by disinfectants and cleansing agents (chemical hazards), and contamination with metal, glass, plastic, bone splinters and string (physical hazards). Critical control points would include dirty animals, hide removal, evisceration, chill and storage, despatch and transport, and scalding (Figure 24.4). Thus, at the stage of evisceration, contamination of carcasses by food-poisoning bacteria (e.g., *Salmonella* spp.) can occur due to rupturing of the stomach and intestines, and control involves sealing of the oesophagus and rectum to minimize spillage. Likewise, during the receipt, storage, cutting and transport of poultry meat, growth of *Salmonella* spp. may occur due to inadequate temperature regulation; the control measure therefore involves keeping poultry meat at, or below, 4°C.

Figure 24.4 also indicates that contamination of animals from feeds, the environment and other animals can occur during production. Thus, emphasis is now being placed on food-safety assurance during this so-called 'pre-harvest' stage (Smulders and Collins, 2002). The HACCP approach can be applied on the farm to improve hygiene in an attempt to reduce contamination by chemicals, microorganisms and parasites (Edwards, 2002), and is therefore relevant to the 'paddock to plate' approach to food quality (see Chapter 1). Microbial risk analysis, which estimates the magnitude of microbial exposure at various stages of the production chain, has already been outlined briefly (see Chapter 2). Additionally, the international use of HACCP by food manufacturers has a role in standardizing international food-safety regulations and in reducing trade barriers arising from food safety (Caswell and Hooker, 1996).

Hazard Analysis Critical Control Point procedures also can be applied to veterinary preventive medicine more generally. Table 24.3 presents a basic HACCP programme for the control of mastitis in dairy herds. Several microorganisms constitute the hazards, and risk is managed at three CCPs (in the calving pen, at drying off and in the milking parlour), reflecting the multifactorial nature of the disease. A more detailed programme is presented by Beekhuis-Gibbon and others (2011a,b). Noordhuizen and Frankena (1999), Johnston (2000), Bonde and Sørensen (2004), Boersema and others (2008) and Noordhuizen and others (2008) discuss the use of HACCP on the farm.

The elegance of HACCP lies in the known reduction of risk at the CCPs. Risk can only rise subsequently through external contamination of the process.

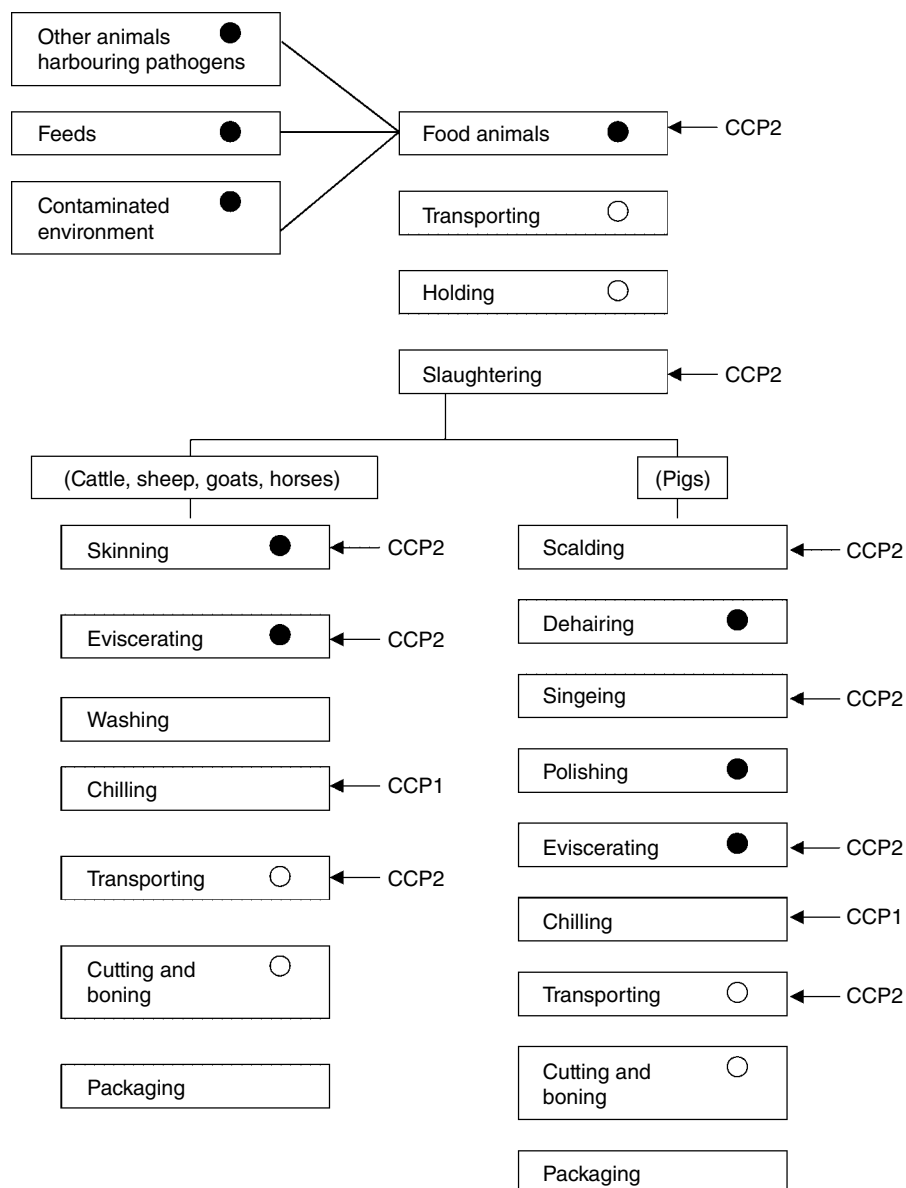


Fig. 24.4 Sources of contamination and critical control points before and during slaughter and processing of red-meat animals. ○: site of minor contamination; ●: site of major contamination; CCP1: effective; CCP2: effect not absolute (see text). (Expanded and reprinted from Collins and Huey (2015). Reproduced with permission from John Wiley and Sons, Inc.)

Table 24.3 Application of HACCP to the control of bovine mastitis. (Based on, and expanded from, Cullor, 1995.)

Hazards	Critical control points	Action	Critical limits
Mastitis-inducing organisms on or in the udder	Calving pen	Changing of bedding, and disinfection of pens at appropriate periods	
	Drying off	Apply five-point plan (see Chapter 26)	Liner-use limits: 2500 milkings/six months
	Milking parlour	Apply five-point plan (see Chapter 26), plus pre-milking dipping/spraying	Milking-machine standards: 14–16" mercury (high milk lines); 12.5–13.5" mercury (low milk lines) Milk:rest ratio between 40:60 and 60:40

Risk communication

Risk communication is concerned with disseminating information about the hazards and risks to all interested parties (e.g., livestock owners, the general public and politicians) and encouraging dialogue between them. Risk communication is particularly important because the perception of risk by risk analysts often is different from that of the general public. The former may argue that risk should be determined objectively by the 'data alone', whereas the latter may 'irrationally' colour their perception of risk by subjective factors (Schrader-Frechette, 1991)¹⁴. Collectively termed '**outrage factors**' (Sandman, 1987), these include '**dread**' (e.g., AIDS and cancer may be perceived as more serious risks than emphysema, although all may be lethal), the '**involuntary**' nature of the risk (involuntary exposure to a risk is viewed more seriously than if an individual has control over exposure) and '**familiarity**': 'exotic' risks tend to provoke outrage more than familiar ones (e.g., ingestion of meat potentially infected with the bovine spongiform encephalopathy prion, compared with electrocution by faulty electrical appliances in the home: Setbon *et al.*, 2005; Hueston, 2013). Thus, the public's concern over the risk of importation of rabies into the UK may be excessive in terms of the actual risk (MAFF, 1998); and reaction to the risk of Ebola virus infection – with consequent excessive use of quarantine (see Chapter 27) – appears to be unjustified (PHE, 2015).

'Outrage factors' are not distortions of the public's perception of risk, they are intrinsic parts of what risk means. They may be intensified by extensive media coverage, strong public concerns and institutional attention, resulting in '**socially amplified**' risks (Kasperson *et al.*, 2001) – bovine spongiform encephalopathy is a good example. Since society generally reacts more to outrage than 'mere hazard', an important part of risk communication is to make serious hazards 'more outrageous', and modest hazards less so. Gruesome graphic government campaigns highlighting the dangers associated with driving under the influence of alcohol or drugs are examples of increasing outrage. The extent to which the public accepts risks is clearly related to the degree of outrage¹⁵.

¹⁴ For a discussion of the psychology of risk evaluation by the layman, and the set of judgemental rules (heuristics) that are employed, see Kahneman and others (1982) and Gilovich and others (2002). Heuristics make assessments or judgements of probability simpler, but can lead to serious biases.

¹⁵ See Bedford and Cooke (2001) for the criteria by which the public judges the acceptability of various risks.

Risk communication should not be an afterthought (Covello *et al.*, 2001). Consideration of communication of the results of a risk assessment is essential in both defining the hazard and the risk question, as well as formulating the approach to the whole risk analysis. Otherwise, the exercise may be nugatory.

Qualitative or quantitative assessment?

There may be a temptation in contemporary society – where a degree of intellectual superiority is often attached to numerical outputs – to assume that a quantitative assessment is 'better' than a qualitative one¹⁶. However, qualitative assessment is appropriate for the majority of risk assessments, and is the commonest type of assessment to support routine decisions. As such, it is '*... a reasoned and logical discussion of ... a hazard where the likelihood of its release and exposure, and magnitude of its consequences are expressed using non-numerical terms such as high, medium, low or negligible*' (Murray, 2002). Quantification may be required to explore a problem more fully and to compare control measures (e.g., removal strategies relating to test-positive imported animals: see Table 20.25). This involves the construction of a mathematical model, with the attendant difficulties of the quality and availability of data, choice of appropriate statistical distributions, assumptions and validation (see Chapter 23). Such models may not yield to easy interpretation, noting that '*... mathematicized theory in science is rarely so pellucid or so rigorous that its significance and bearing can be grasped immediately by distant readers*' (Porter, 1995). Consequently, quantitative assessments may not stand up well in an adversarial climate.

Semi-quantitative risk assessment

Semi-quantitative risk assessment usually involves assigning numerical (or non-numerical) scores to qualitative estimates, thereby generating ordinal data (see Chapter 10). For example, an on-farm risk assessment evaluating the risk of Johne's disease might include scores for particular management practices, thus (VTCHIP, 2003):

multiple animal use in the calving areas: 0 = never; 3 = rarely; 5 = occasionally; 7 = frequently; 10 = always; manure build up in the calving areas: 0 = none; 3 = minimal; 5 = moderate; 7 = considerable; 10 = extensive.

This avenue often is followed because of concerns over the subjectivity of a purely qualitative approach

¹⁶ Recall that '*... all numbers pose as true*' (see Chapter 1).

and the aforementioned perception that numbers are 'better'. However, the weightings attached to scores are usually arbitrary and therefore subjective. Semi-quantitative assessments therefore provide an illusion of objectivity and precision that is unwarranted. This may be acceptable if the aim is to prioritize risks in a non-contentious context (e.g., the on-farm risk of Johne's disease, outlined earlier), but offers no advantage over qualitative assessments in determining realistic estimates of risk, especially in controversial matters. Thus, semi-quantitative methods, by augmenting qualitative assessments with a quantitative dimension, add superfluous complexity. Murray (2002) concludes that the best 'antidote' for 'too much subjectivity' is thorough documentation and peer review of qualitative assessments.

The term 'semi-quantitative' also applies to quantitative assessment of parts of a process; notably, when the detail (of both data and mechanisms) is known for only small parts of the exposure pathway (e.g., time/temperature inactivation curves for microbes), which therefore may be modelled quantitatively. This is of value in identifying and confirming critical control points.

Qualitative risk analysis

Framework for qualitative risk assessment

Example of a qualitative risk assessment: hazards and risks posed by wild game

The framework for a qualitative risk assessment is best demonstrated by an example: hazards and risks posed by wild game, which is detailed by Coburn and others (2003) and summarized by Coburn and others (2005).

Risk question The risk question, to be answered qualitatively, was: 'Under current UK law, what is the risk to human health (particularly of human infection with a food-borne pathogen) from handling/consumption of

Table 24.4 Hazards considered for wild-game species in a qualitative risk assessment. (From Coburn *et al.*, 2005.)

Hazard	Wild-game species
<i>Escherichia coli</i> O157	All
<i>Salmonella</i> spp.	All
<i>Campylobacter jejuni</i>	All
<i>Mycobacterium avium</i>	Game birds, wild ducks, wild deer
<i>Chlamydophila psittaci</i>	Game birds
<i>Clostridium botulinum</i>	Wild ducks
<i>Mycobacterium bovis</i>	Wild deer
<i>Yersinia pseudotuberculosis</i>	Wild lagomorphs
Lead shot	Game birds, wild ducks, wild lagomorphs

wild-game meat and how would the currently proposed European Union hygiene proposals affect the risk?

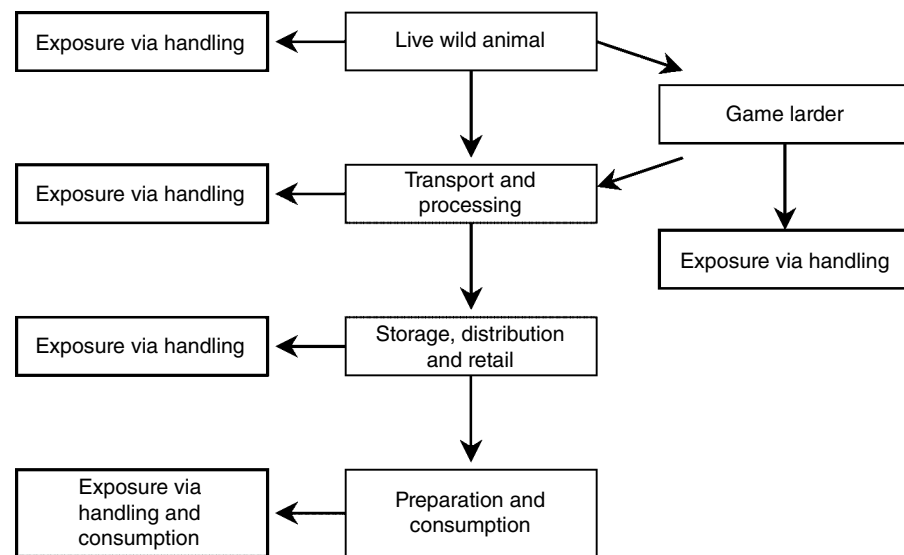
Hazard identification A search of the literature and expert opinion indicated that the relevant hazards were *Escherichia coli* O157, *Salmonella* spp., *Campylobacter jejuni*, *Chlamydophila psittaci*, *Mycobacterium avium*, *Clostridium botulinum*, lead shot, *Mycobacterium bovis* and *Yersinia pseudotuberculosis*. The relevant wild game comprised game birds, wild ducks, wild deer and wild lagomorphs, the particular species to be included being based on the number shot (e.g., pheasant were included, but capercaillie and ptarmigan were not). Table 24.4 lists these in relation to the hazards.

Hazard characterization Hazard characterization included investigation of the infectivity of organisms. For example, only a few viable cells are required to initiate human salmonellosis (D'Aoust, 2001). Dose-response relationships were available for some microorganisms (e.g., Table 24.5). Lead poisoning

Table 24.5 Results of a dose-response experiment with *Campylobacter jejuni*, strain A3249, in healthy volunteers. (Simplified from Black *et al.*, 1988.)

Dose (number of ingested bacteria)	Number dosed	Number infected	Percentage infected	Number with gastrointestinal symptoms	Percentage of infected with gastrointestinal symptoms
800	10	5	50	1	20
8 000	10	6	60	1	17
90 000	13	11	85	6	55
800 000	11	8	73	1	13
1 000 000	19	15	79	1	7
10 000 000	9	9	100	2	22

Fig. 24.5 Human exposure pathway for hazards from wild game. (Source: Coburn *et al.*, 2005. Reproduced with permission of BMJ Publishing Group Ltd.)



occurs with the consumption of high-dose or low-dose sources; retention of metallic lead (e.g., lead shot) in the stomach is a high-dose source (Beers and Berkow, 1999).

Entry assessment Entry assessment focussed on the qualitative likelihood of a carcass releasing the hazard into the environment. This depends on the prevalence of infection in the live animals and the survival of microorganisms in the carcass. In many cases, there was meagre evidence on prevalence (e.g., only one publication citing the prevalence of *E. coli* O157 in wild birds as 0.34%: Rice *et al.*, 2003); consequently, there was a high level of uncertainty. However, there was more published information on microorganisms' survival (e.g., *C. jejuni* survives best at refrigeration temperature), supplemented by expert opinion (e.g., chlamydiae survive in a killed pigeon, as long as the carcass is fresh).

Exposure assessment The exposure pathways are presented in Figure 24.5. Exposure can occur when handling live animals and their carcasses at all stages in the distribution and marketing chain, and when they are consumed. However, the degree of exposure in the marketing chain is highly variable. For example, the conditions under which deer and birds are transported (outlined by expert opinion) vary, some being suspended, and others being piled on vehicle floors. Similarly, the mode of storage in game larders varies, with temperature fluctuations occurring (Barnes *et al.*, 1973). Since these factors affect the likelihood of cross-contamination, there is the capacity for

substantial variability in the degree of cross-contamination.

Risk characterization and estimation The risk estimation defined risk qualitatively as 'negligible', 'very low', 'low' and 'non-negligible'. The criteria for characterization included the infectivity and survival of the microorganisms; the likelihood of cross-contamination during storage, distribution and retail; and the prevalence of infection in the various species. Thus, the risk of *Salmonella* spp. infection through handling and consumption of wild deer was considered to be very low, based on the very low prevalence of infection in deer. In contrast, the risk of *C. jejuni* in wild ducks was considered to be non-negligible (the highest ranking) based on a high prevalence in wild waterfowl. The results of the overall risk assessment are presented in Figure 24.6.

Risk management The second part of the risk question refers to management of the risks – namely, to what extent would new European Union proposals on hygiene affect the risk? These proposals are threefold:

1. training of hunters;
2. control of hazards on game-meat plants using HACCP principles;
3. veterinary post-mortem inspection.

The risk analysis was concerned mainly with the second and third proposals, and drew mixed conclusions. Thus, using HACCP, adequate trimming and rejection of deer carcasses contaminated with rumen contents would reduce the amount of cross-contamination

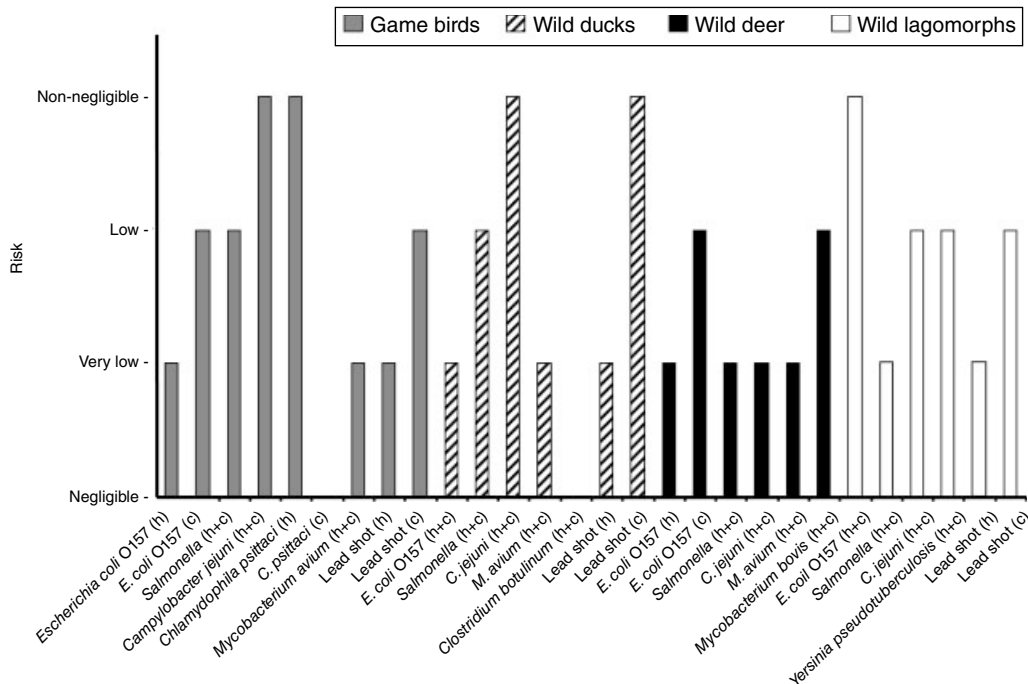


Fig. 24.6 Assessed risk to human health from identified hazards in wild game. (c): consumption of game; (h): handling game. (Source: Coburn *et al.*, 2005. Reproduced with permission of BMJ Publishing Group Ltd.)

during processing, thereby reducing the risk of *Salmonella* spp. exposure, either by handling or consumption. Consequently, the adverse risk to human health would be reduced. Likewise, veterinary post-mortem examination would additionally ensure the rejection of septicaemic or toxaemic carcasses, thereby further reducing risk. In contrast, although application of HACCP to *C. jejuni* infection of deer would reduce the risk of cross-contamination, veterinary post-mortem examination would provide no additional benefit because the infection is carried without visible lesions.

Small wild game (rabbit and hare) infected with *E. coli* O157, *Salmonella* spp., *C. jejuni*, *Y. pseudotuberculosis*, and contaminated with lead shot, would benefit from reduced risk associated with HACCP (including the removal of obviously unfit carcasses and residue testing), but veterinary post-mortem inspection would not result in added benefit.

Although there was clear uncertainty attached to several of the hazards, this analysis provided a profile of risks associated with wild game, and gave a useful indication of where veterinary post-mortem examination (an additional cost to marketing) would be of benefit, and where it would not.

Qualitative risk assessment during epidemics

An important component of the control of major epidemic diseases, such as classical swine fever and

foot-and-mouth disease, is the identification of all susceptible livestock that may have been exposed to infection. These animals may be incubating disease, which, at some future date, could result in the appearance of clinical signs accompanied by virus excretion and subsequent spread. Such animals are termed 'dangerous contacts' (see also Chapter 27) and their removal during the incubation period of infection, before the release of virus associated with the appearance of clinical disease, may be necessary to manage (i.e., reduce) the risk of transmission of infection. This involves undertaking a qualitative veterinary risk assessment of all susceptible livestock on premises where the risk of exposure to infection may be high. This first requires a thorough knowledge of the characteristics of the relevant microbe – notably, its survival and modes of transmission. Foot-and-mouth disease virus, for example, is commonly transmitted by direct contact between infected and susceptible animals, and by short-range aerosol spread. However, the PanAsia strain is not associated with significant aerosol spread (Sakamoto and Yoshida, 2002), which is estimated to be less than 100 m under farming conditions in the UK (Donaldson *et al.*, 2001). Moreover, the ability of the virus to survive in the environment (see Table 6.8) facilitates spread on footwear, clothing, vehicles and other fomites, and the virus also can be transmitted atmospherically (see Chapter 6), noting that this can be modified by the characteristics of

the virus strain (see *Table 6.6*). Thus, potential high-risk premises include those contiguous (adjacent) to premises with confirmed infection in animals (termed infected premises (IPs)), those potentially exposed to infection via the movement of livestock (both infected and uninfected), vehicles and personnel from IPs onto other farms, and those under a virus plume, if one forms.

Example of a contiguous premises assessment

The assessment of animals on contiguous premises is a major component of dangerous-contact assessment. It should be considered on a case-by-case basis, according to their species and locations relative to animals on the IP to which they are adjacent. Consider, for instance, the premises and animals in *Figure 24.7*. The IP comprises 200 cattle at pasture, many of which are clinically affected by the PanAsia strain of foot-and-mouth disease virus.

Farm A The sheep on Farm A are separated from the IP by a wood, and so are unlikely either to have direct contact with the infected cattle or to be exposed to short-distance aerosols. The cattle on Farm A are not at pasture, but are housed some distance from the IP and so, similarly, are unlikely to be exposed to infection. Thus, animals on this farm would not be declared to be dangerous contacts. However, to

manage any potential risk, the animals should be isolated, the farm placed under movement restrictions, and regular veterinary surveillance inspections mounted (traditional epidemic risk-management techniques: HMSO, 1969).

Farm B The sheep on Farm B may have had direct contact with the infected cattle across a common boundary, and so are deemed to be dangerous contacts, and are subjected to precautionary slaughter.

Farm C The sheep on Farm C, in common with those on Farm A, are separated from the IP by a wood. Therefore, they should not be classed as dangerous contacts, but should be isolated, the farm placed under movement restrictions, and regular veterinary surveillance inspections undertaken.

Farm D The sheep on Farm D are at grass some distance from the IP, and the cattle are housed. Therefore, again, animals on this farm are not considered to be dangerous contacts.

Farm E The pigs on Farm E are at pasture some distance from the farm's boundary with the IP. Although there may have been some vehicular movement close to the boundary, the cattle on the IP are pastured some distance from the boundary. Moreover, pigs are hard

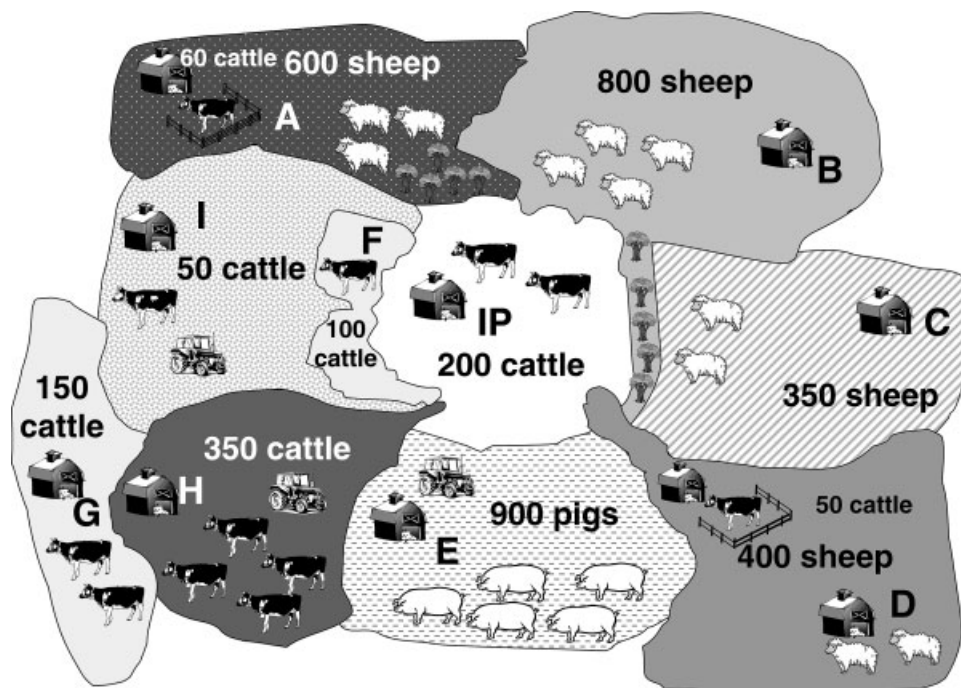


Fig. 24.7 Location of animals around a premises infected with foot-and-mouth disease (IP). (Similar shading indicates premises under common ownership.)

to infect by aerosol, infection by ingestion being the commonest route (Donaldson and Alexandersen, 2002). Thus, the pigs on this farm are not classified as dangerous contacts. However, because of the large amounts of virus that can be produced by pigs, if infected (Donaldson, 1987), regular veterinary surveillance inspections and the other traditional epidemic risk-management strategies (movement restrictions and isolation of animals) should be undertaken. Alternatively the pigs may be assessed as dangerous contacts and slaughtered as a precaution, to minimize the potential risk posed by the (uncontrollable) spread of airborne virus if the pigs succumbed to infection and produced a virus plume.

Farm F The cattle on Farm F are in close proximity to the infected animals, and thus may have had contact with them across the fence. They should therefore be classified as dangerous contacts, and slaughtered as a precaution (like Farm B).

Farm G Farm G and Farm F have the same owner. However, the livestock on G have had no contact with the stock on F; therefore they are not classified as dangerous contacts, but are subjected to regular surveillance.

Farm H Cattle on Farm H are not grazing close to the infected cattle, and there is very little common boundary with the IP, justifying their not being classified as dangerous contacts, although they should be subjected to surveillance.

Farm I Farm I also has very little common boundary with the IP, and its cattle are also grazing some distance away from cattle on the IP, and on Farms G and H. This suggests that these are at low risk and therefore should not be classified as dangerous contacts but, again, should be subjected to surveillance.

Note that case-by-case veterinary risk assessment – which has been shown to be effective in identifying premises at high risk (Honhold *et al.*, 2004b) – is a more selective approach than the blanket culling of susceptible animals on all premises that are cartographically contiguous to an IP, irrespective of stock location and details of movements of vehicles and personnel (see Chapter 27). In this example, the selective approach results in 1100 animals (including those on the IP) being culled (2000, if the pigs on Farm E are slaughtered), whereas blanket culling results in 3860 being slaughtered.

Quantitative risk analysis

Framework for quantitative risk assessment

Quantitative risk assessment follows the same general framework as qualitative assessment (indeed, qualitative risk assessment is the foundation on which quantitative assessment is built, and in many cases a quantitative assessment will follow a qualitative assessment), but numerical values are attached to the various stages on the entry and exposure pathways; a numerical estimate of risk is thereby generated. This frequently involves the use of **simulation models**, and methods may be either **deterministic**, providing single point estimates of risk as outputs, or **stochastic**, whereby probability distributions are obtained as outputs. (See Chapter 23 for a fuller discussion of models and modelling terms.)

A deterministic assessment of the risk associated with the unrestricted importation of animals or animal products, for example, would consider, as input parameters, the prevalence of pathogens in the source population and the probability of the pathogens surviving during importation as part of the entry assessment, and the probability of the pathogens coming into contact with local livestock after importation as part of the exposure assessment. The output would be the single point estimate of the annual probability of an infected animal or animal product introducing the pathogen. In contrast, a stochastic assessment would be based on the probability distributions of the pathogens surviving during importation and coming into contact with local livestock after importation, thereby presenting a range of values for the annual probability of introduction of the pathogen.

Deterministic prediction of the risks of importing infected animals under various general importation rules has already been discussed in detail in Chapter 20 ('Diagnostic tests in import risk assessment').

Example of a deterministic risk assessment: introduction of anthrax into New Zealand in imported unprocessed Australian hides

New Zealand imports from Australia unprocessed ('green') hides, which may be contaminated with anthrax. A deterministic risk assessment was therefore undertaken to answer the risk question: '*What is the probability of the introduction of anthrax into New Zealand in unprocessed Australian hides?*' (Cox and Ryan, 1998).

The following entry and exposure pathways can be identified:

- unprocessed hides and skins, which may be contaminated with anthrax spores, are imported into New Zealand for processing in tanneries;
- in some tanneries, wastewater is discharged without treatment; thus, waterways downstream from the tanneries could be contaminated with anthrax spores;

- during floods, spores that escape could be carried onto pasture and infect livestock.

Table 24.6 details the data and calculations used in the assessment. Note that some input parameters are based on expert opinion (e.g., the survival of spores, p_s), whereas others are little more than guesses (e.g., the number of officially approved tanneries with a risk of contaminating pasture during flood periods, t). Other assumptions include:

- a contaminated hide will not cross-contaminate other hides during handling, transportation and storage, except (possibly) other hides processed on the same day at the same tannery;
- the probability that New Zealand livestock will be infected with anthrax if spores escape from a 'risk tannery' on a flood day is 1;
- infected hides are randomly distributed among all hides imported from Australia;
- spores on a hide can only escape into rivers on the day it is processed in a 'risk tannery';
- all 23 tanneries in New Zealand process the same number of hides in each year;
- each tannery processes a constant number of Australian hides each working day.

The assessment concludes that the annual probability of anthrax introduction in Australian hides is 1.2×10^{-2} ; that is, approximately one in 82. Thus, one (or more) outbreaks of anthrax can be expected to occur once every 82 years¹⁷.

A more realistic analysis of the risk would require improved knowledge of the pattern of the disease in Australia, handling of the hides during shipment, processing of hides, and management of wastewater. The assumption that escape of spores from a 'risk tannery' during a flood always leads to infection should also be examined further.

More detailed information on the variability and uncertainty associated with the input parameters would give rise to a stochastic approach. Thus, the assessment could be refined by attaching a range of values to the input parameters. The probability that anthrax spores survive processing, for example, could be represented using a point estimate and upper and lower limits, generating three final estimates of risk. However, there are two disadvantages to this approach. First, no weighting is attached to the three values of probability, yet the point estimate may be more likely than the limits. Secondly, analyses become

cumbersome as the number of parameters increases. If, for example, six input parameters were being considered, each with a point estimate and upper and lower limits, there would be 729 (3^6) output scenarios. These problems can be overcome by **Monte Carlo simulation modelling** (see Chapter 23). This is commonly undertaken using the dedicated risk-analysis software, *@RISK* (see Appendix III).

Probability distributions

To develop a risk analysis using Monte Carlo simulation modelling, probability distributions of input parameters are first required. If a substantial amount of empirical data is available, standard statistical methods, such as the construction of histograms and box plots (see Chapter 12) may be used to explore the distribution. There also are statistics that explore the 'goodness-of-fit' of data to defined distributions; these are given in standard statistical texts. If data are sparse (but representative), bootstrapping (see Chapter 12) can be used to derive a distribution. If data are rare or unrepresentative, then expert opinion may need to be sought (with its attendant uncertainty). Expert opinion also can be combined with empirical data (Murray, 2002).

Some distributions that are applied in risk analysis are listed in Table 24.7. Key ones are the binomial, Poisson and hypergeometric¹⁸. The **triangular distribution** (Gupta and Nadaraja, 2004) (Figure 24.8) is popular in risk analysis as a proxy for the beta distribution, which – although a suitable model in risk analysis because it provides a wide variety of distributional shapes over a finite interval – is not readily understood and its parameters are not easily estimated (Johnson, 1997). The triangular distribution requires only the mode (most common value or values) and end-points of a distribution (i.e., does not require description of its shape). It can be constructed easily using the point estimate of a parameter as the most likely value, and the lower and upper limits to define the range of the distribution. It is therefore attractive because credible input data may not be available, but experts may be relied upon to provide figures for the mode and two extreme values¹⁹. Probabilities associated with any

¹⁸ This is found when random sampling is undertaken 'without replacement' (see Chapter 13 and Appendix X).

¹⁹ Note, however, that experts tend to narrow the range of a distribution; that is, subjective estimates – especially extreme estimates – provided by experts are liable to potential bias (Hudak, 1994). One reason for this is 'anchoring' (Morgan and Henrion, 1990): experts usually select the most likely value first (the 'anchor') and then adjust the minimum and maximum estimates from this value. However, the adjustment rarely includes the extremes that could occur; that is, the estimates of the extremes are biased towards the most likely value.

¹⁷ The expression of frequency in this form (rather than the probability, 1.2×10^{-2}) is easy to understand by the layman, and so is often an appropriate figure for communicating risk.

Table 24.6 Deterministic quantitative risk assessment of the introduction into New Zealand of anthrax in unprocessed Australian hides and skins. (Based on Cox and Ryan, 1998, with parameters extracted from MacDiarmid, 1993.)

p_i	= probability that a hide contains anthrax spores = 9.94×10^{-7} (maximum annual incidence of anthrax in Australia, estimated at 40 cases/year; 40.23 million cattle and sheep slaughtered annually; therefore $p_i = 40/4.023 \times 10^7$);
p_s	= probability that anthrax spores survive to processing in New Zealand = 0.9 (estimate based on the known resistance of anthrax spores);
n	= number of Australian hides processed annually in New Zealand = 0.92×10^6 (extracted from official records);
g	= number of officially approved tanneries = 23 (data from Ministry of Agriculture and Fisheries records);
t	= number of officially approved tanneries with a risk of contaminating pasture during flood periods = 5 (approximately 20% of g – a guessed estimate, in the absence of data);
d	= annual number of processing days at each tannery = 235 (365 days minus weekends and holidays);
v	= average number of days per year on which flooding occurs downstream of tanneries = 25 (estimated from a range of 20–30 days);
p_f	= probability that there is a flood on any day at any of the ‘risk tanneries’ = $v/365$ = 0.0685;
p_a	= probability that an Australian hide in New Zealand is contaminated = $p_i \times p_s$ = $9.94 \times 10^{-7} \times 0.9$ = 8.946×10^{-7} ;
h	= number of Australian hides processed each day by each tannery = $(n/g)/d$ = $(0.92 \times 10^6/23)/235$ = 170;
p_x	= probability, on any given day, at any given tannery, that there is both a flood and at least one infected hide processed = $p_f \times p_1$
where:	$p_1 = [1 - (1 - p_a)^h]$ = $(1 - 0.999848)$ = 0.000152;
thus:	$p_x = 0.0685 \times 0.000152$ = 1.0412×10^{-5} ;
P_E	= annual probability of anthrax introduction in Australian hides = $[1 - \exp(-p_x \times t \times d)]^*$ = $[1 - \exp(-1.0412 \times 10^{-5} \times 5 \times 235)]$ = $[1 - \exp(-0.0122341)]$ = $[1 - 1/(\exp 0.0122341)]$ = $1 - 0.9878$ = 0.012 = 1.2×10^{-2}

* This assumes that that annual number of floods (and hence number of flood days when at least one infected hide is processed) follows a Poisson distribution (see Chapter 12), where the annual mean number of anthrax introductions, λ , = $p_x \times t \times d$. Thus, using the formula and notation in Chapter 12, the probability that there are no anthrax introductions in a year = $(e^{-\lambda} \lambda^0)/0! = e^{-\lambda}$. Therefore, the probability of at least one anthrax introduction in a year, P_E , is one minus the probability that there are no introductions = $1 - e^{-\lambda} = [1 - \exp(-p_x \times t \times d)]$.

range between the lower and upper limits then can be derived using linear interpolation. The triangular distribution is considered to be a good approximation to distributions such as the Normal distribution (see Chapter 12). However, it should not be applied when useful information, suggesting a distribution other than the triangular, is available.

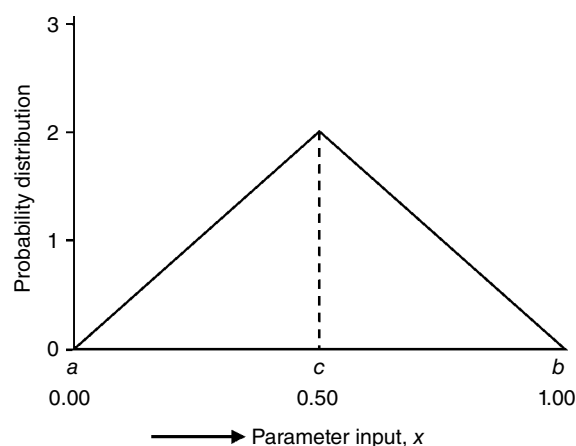
Variability and uncertainty

Variability and uncertainty both need to be addressed in stochastic simulations. Fortunately, both of these can be explored by Monte Carlo simulation, and it is suggested that they should both be identified individually so that the contribution of each to the total uncertainty can be noted (Vose, 2008). Variability

Table 24.7 Some distributions applied in risk analysis*. (Adapted from Murray *et al.*, 2004c.)

Discrete distributions	Continuous distributions
Binomial [†]	Beta [§]
Discrete	Cumulative
Discrete uniform	Exponential
Geometric	Gamma
Hypergeometric	Histogram
Negative binomial	Lognormal [†]
Poisson [†]	Normal [†]
	Pert [§]
	Triangular
	Uniform (rectangular)

* See Evans and others (2000) for full details.

[†] Described in Chapter 12.[§] See also Chapter 20.**Fig. 24.8** The triangular distribution. *a*: minimum; *b*: maximum; *c*: mode. (The distribution can be skewed.)

can be addressed by the adoption of distributions, such as some of those listed in *Table 24.7*, for undefined parameters. Uncertainty associated with parameter inputs is often more difficult to address, but may be tackled by either assuming a distribution, or using a range of parameter values to investigate the sensitivity of outputs to the parameter input (i.e., a 'sensitivity analysis' is undertaken, see Chapter 23).

Example of a stochastic risk assessment: introduction of rabies into Great Britain in dogs and cats

Rabies prevention in Great Britain was traditionally based on quarantine of susceptible species, notably dogs and cats. However, in the 1990s, it became clear that the policy should be reviewed because of the reduced incidence of rabies in the European Union and the availability of vaccines. Accordingly, a stochastic risk assessment was undertaken to answer the risk question: 'What is the risk of an imported dog or cat developing rabies if various policies to prevent the entry of rabies into Great Britain are implemented?' (MAFF, 1998).

The various policy options are listed in *Table 24.8*. Risk of release in relation to all options, the possible routes of entry of imported dogs and cats, and points of interception, are given in *Figure 24.9*. This gives rise to eight possible pathways (the numbered columns in the figure). The most important outcomes under the quarantine system in force in the 1990s are represented by the black squares in columns 1 and 5: an infected animal develops rabies in quarantine, or an illegal infected animal develops rabies outside quarantine, respectively.

The number of infected or uninfected animals that are expected to enter Great Britain by each pathway can be estimated. This requires several parameters

Table 24.8 Policies to prevent the entry of rabies into Great Britain. (Adapted from MAFF, 1998.)

Policy current in the 1990s (option (a))	Quarantine 6 months
Option (b)	Reduced quarantine period (e.g., 1 month)
Option (c)	Vaccination of animals >3 months old at a fixed time (e.g., 6 months), or during a fixed interval (e.g., 6–12 months), before entry to GB; followed by serological testing; permanent marking for identification
Option (d)	As (c), but checking (of certificates and blood test results) to be done away from port of entry
Option (e)	As for (c) or (d), but without quarantine; animals not satisfying requirements to be destroyed or refused entry
Option (f)	As for (e), but with all cats and dogs in GB vaccinated

		Pathways							
		1	2	3	4	5	6	7	8
Outside GB	Infected at entry	■	■	■	■	■	□	□	□
	Checked at entry	□	□	■	■	□	□	■	□
Inside GB	Quarantined (voluntary)	■	■	□	□	□	■	□	□
Outcomes	Rabid in quarantine	□	■	□	■	□	□	□	□
	Legal immigrant, rabies +	■	□	■	□	□	□	□	□
	Legal immigrant, rabies –	□	□	□	□	□	■	■	□
	Illegal immigrant, rabies +	□	□	□	□	■	□	□	□
	Illegal immigrant, rabies –	□	□	□	□	□	□	□	■

Fig. 24.9 Pathways by which imported dogs and cats can enter Great Britain, accounting for the different control measures and points of interception. □: smuggled animals not discovered; ■: smuggled animals checked and placed in quarantine; ■: pathways by which the majority of rabid animals enter Great Britain. Each numbered column represents a different sequence of events and outcomes, which, in the upper part of the diagram, are chronological from top to bottom. There are eight possible pathways, hence eight columns. When considering the risk of a cat or dog developing rabies in Great Britain, these pathways lead to five relevant outcomes (the bottom part of the diagram). (Adapted from MAFF, 1998.)

(Table 24.9). For example, the number of infected animals entering in pathway 1 is:

$$N_1 = Iq(1 - r_q),$$

using the notation in Table 24.9.

The data that were required to estimate the variables and parameters were obtained from published and unpublished sources, and ranged from verifiable objective data to guesses. Thus, the number of animals imported under the quarantine system was obtained from official government records, whereas the number of animals expected to be imported if control was changed to one based on vaccination without quarantine was a guess.

Taking account of the variability and the uncertainty in the risk assessment, modelled using the various triangular distributions, the results concluded that there would be very little increase in the risk of rabies being imported into Great Britain if quarantining of animals from the European Union was abandoned and was replaced by a control strategy based on animal identification, vaccination and serological testing. This risk assessment therefore provided evidence in favour of a policy change to the latter strategy.

Following the development of the rabies risk assessment and the subsequent change in policy, the PETS travel scheme was introduced in Great Britain. Subsequent risk assessments were undertaken after

the establishment of the PETS scheme, to include other countries (e.g., North America) and ferrets (AHVLA, 2012). In 2012, there was a further major change in policy whereby the European Union Pet Movement Policy (Europa, 2003) was adopted. Again, the change in policy was based on the results of a quantitative risk assessment (Goddard *et al.*, 2012).

The rabies story is a good example of the progressive use of risk assessments to assist governments in their decisions on policy changes.

What level of risk is acceptable?

The results of risk assessments need to be meaningfully interpreted so that appropriate risk-management strategies can be adopted. The latter implicitly depend on the level of risk that is deemed to be acceptable (more strictly, on the options that entail a specific level of risk). This is a major concern in the context of the international trade in animals and animal products, where epidemic diseases and zoonoses may be imported. The *Sanitary and Phytosanitary (SPS) Agreement* of the WTO (OIE, 1997) requires that each participating country specifies its appropriate level of protection or acceptable level of risk.

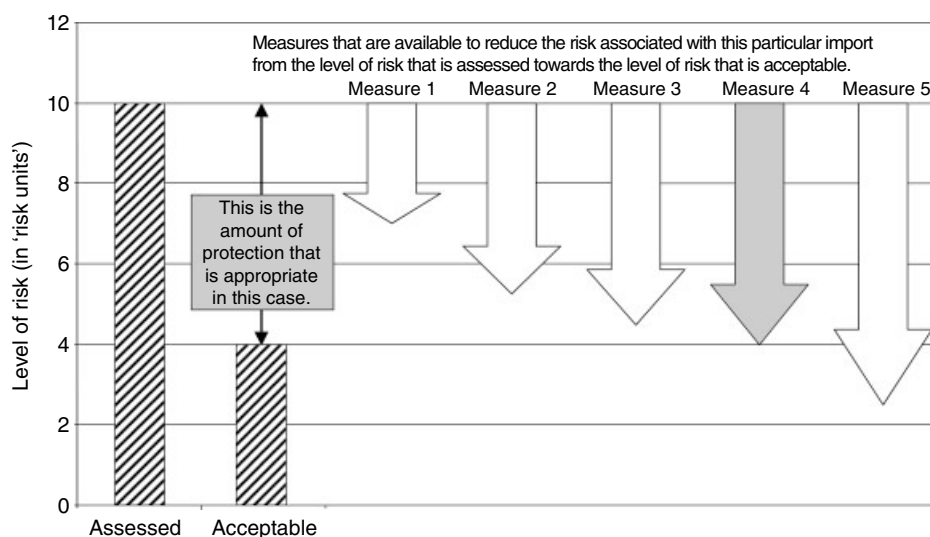
Figure 24.10 depicts a conceptual framework for the acceptable level of risk in relation to SPS control measures (e.g., quarantine) for importation of animals or

Table 24.9 Parameters and variables required for a stochastic quantitative risk assessment of the importation of rabies into Great Britain. (From MAFF, 1998.)

Symbol	Definition	Value*
N_x	Number of animals (dogs + cats) entering GB annually via pathway x	Variable; 8914 (7267, 12 901) for present policy
I	Number of rabies-infected, prospective imported animals (dogs + cats) into GB/year	Varies with country of origin
b	Rabies incubation period (years); used to calculate prevalence of infection among imported animals	0.115 (0.055, 0.164)
c	Fraction of animals checked at port of entry; animals that do not meet requirements of the policy in question are apprehended, and then quarantined (options (c),(d)), destroyed (options (e),(f)) or do not enter GB (withdrawn)	Varies with policy
q	Fraction of animals entering quarantine, voluntarily or through enforcement after non-compliance	Varies with policy and compliance
r_q	Fraction of rabies-infected animals that develop rabies in quarantine; determined by incubation period; varies according to quarantine period	0.9 (0.86, 0.95) for six months quarantine; 0.5 (0.45, 0.53) for one month
r_p	Fraction of rabies-infected animals that develop rabies during a given, statutory pre-entry period	0.9 (0.86, 0.95) six month pre-entry period; 0.95 (0.91, 1.0) for 12 months
v	Fraction of animals vaccinated before entering GB	Varies with compliance
s	Fraction of vaccinated animals that is blood tested	0.98 (0.8, 0.998)
t_+	Fraction of rabies-infected animals that test positive following vaccination	0.95 (0.9, 0.99)
t_-	Fraction of uninfected animals that test positive following vaccination	0.95 (0.9, 0.99)
p_+	Fraction of vaccinated, uninfected, blood test positive that is protected	0.99 (0.98, 0.999)
p_-	Fraction vaccinated, uninfected, blood test negative that is protected	0.5 (0.2, 0.8)
w	Fraction blood test negative that is withdrawn	0.15 (0.05, 0.25)
d_q	Duration of quarantine (years)	0.083 (option (b)) or 0.5 (other options)
d_p	Time between vaccination and entry (years); options (c)–(f)	0.5 or 1.0
e	Vaccine efficacy; proportion of vaccinated animals that is protected against infection = $t_+p_+ + (1 - t_+)p_-$	0.9666 (0.902, 0.997)

*The three values are best (low, high) defining the points of triangular distributions used in variability and uncertainty analysis.

Fig. 24.10 The relationship between assessed risk, acceptable level of risk, the appropriate level of protection, and sanitary and phytosanitary measures, for import of a specific commodity. (Source: Pharo, 2004.)



their products. Noting that risk has two components – the probability of an event's occurrence and the severity (impact) of its consequences – the risk units are best considered in economic terms, because probability is dimensionless, but consequences of introduction of disease have an economic impact (e.g., in disease losses and control, and loss of trade). This framework assumes that risk can be estimated objectively and accurately (ideally quantitatively), and that each country can set its level of acceptable risk. Thus, if the current level of disease risk is 10, and the acceptable level of disease risk is 4, Measure 4 comprises the optimum combination of control measures. Measures 1–3 are inadequate, whereas Measure 5 is excessive²⁰. Note that the acceptable level of risk and the appropriate level of protection are subtly different. The former is about acceptable economic losses to the national economy, whereas the latter is the level of economic losses avoided by the application of safeguards.

However, noting the steps in risk assessment (see 'Components of risk analysis' earlier in this chapter), Pharo (2004) concludes that neither the scientific assessment of risk nor the risk-reduction effect of safeguards can be estimated as objectively as the SPS framework anticipates. Entry assessment in import risk assessment, for example, usually computes the probability of an imported animal being affected. However, this is based on the prevalence of infection in the exporting countries, many of which may have poor surveillance systems and consequently poor prevalence data. Additionally, predicting the volume of trade (and therefore the number of imported animals) may be difficult. If the imported commodity is an animal product rather than a live animal (e.g., hatching eggs), the exposure pathway and infectious dose may be complex and difficult to ascertain. If bulk commodities such as animal feeds are being imported, the choice of trade unit size is somewhat arbitrary and microbial inactivation curves for various stages of the production process are usually unknown. Diagnostic tests may be used to estimate the risk of importing affected test-negative animals, but there is often a lack of precision attached to the estimate because of the attendant lack of precision associated with the relevant parameter (sensitivity), reflected in the width of its confidence interval (see Chapter 20).

The consequences of exposure also may be refractory to objective assessment, with the opinions of experts being no more than personal belief. Infections

pose an additional problem when their zoonotic impact is uncertain (e.g., highly pathogenic avian influenza virus). Additionally, unknown factors may also confound prediction. For example, the magnitude of the foot-and-mouth disease epidemic in the UK in 2001 (see *Figure 4.1b*) was determined by widespread dissemination of the virus by subclinically infected sheep (Mansley *et al.*, 2003) – a feature that was impossible to predict before the epidemic. Moreover, economic impact is partly related to control policy, which may be modified unpredictably in the future. Thus, the traditional foot-and-mouth control procedures were modified in the UK in 2001 by a more aggressive culling policy only after the epidemic had begun, thereby incurring higher control costs than could be predicted (Kitching *et al.*, 2006)²¹.

Market reaction also can be unpredictably extreme. A single case of bovine spongiform encephalopathy in Canada in 2003, for instance, resulted in cessation of exports, and the country's \$7 billion per year cattle and beef industry suffered a 34% drop in value (Carter and Hule, 2004). Since Canada is a major exporter of beef to the US, the export ban also resulted in a substantial increase in the price of US beef (Hanrahan and Becker, 2004).

In summary, there usually is a lack of quantitative data with which to assess precisely the probability and magnitude (severity) of the consequences of importation. Moreover, if acceptable risk is viewed monetarily, then it may be framed in terms of the level of economic losses that can be tolerated to optimize the benefits of international trade – but the perception and reaction of the public and politicians cannot be discounted. The determination of acceptable levels of risk in import risk assessment therefore is complex, in common with acceptable risk in the wider context, which involves economic (Starr, 1969) and psychological (Fischhoff *et al.*, 1978) factors. This indicates that the final decision on the acceptable level of risk usually involves social, economic and political, rather than scientific, considerations (Slovic, 2000; Sjöberg, 2001; see also Chapter 25).

Veterinarians need to be aware of the limitations of risk analysis, which may pose more questions than it answers. (Indeed, an important function of risk analysis is to identify what is not known.) The results of risk analyses may be founded on invalid assumptions, and frequently there may be uncertainty attached to the

²⁰ If the risk units are expressed in monetary terms, there is thus a clear similarity between risk analysis and cost-benefit analysis (see Chapter 25 and Lave, 1996).

²¹ The full impact involved more than the cost of control. It also included losses to the leisure and tourist industry (Fawcett and Head, 2001a,b; Thompson *et al.*, 2002; Blake *et al.*, 2003), as well as psychological damage to individuals (Mort *et al.*, 2005, 2008).

hazards and processes for which risk is being assessed (Ballard, 1992). Moreover, it is often possible only to produce a relative ranking of the likelihood of events, rather than accurate assessments (Ansell, 1992), noting that numerical outputs are likely to be crude (Schneiderman, 1980). Nevertheless, risk analysis has

a valuable role to play in identifying and managing risk in many areas of veterinary medicine, rather than attempting to prosecute a 'zero-risk' strategy, which is not amenable to changes in the light of new knowledge, economic circumstances and political requirements.

Further reading

- Ahl, A.S., Acree, J.A., Gibson, P.S., McDowell, R.M., Miller, L. *et al.* (1993) Standardization of nomenclature for animal health risk analysis. *Revue Scientifique et Technique, Office International des Epizooties*, **12**, 1045–1053
- Amass, S.F. (2005) Biosecurity: stopping the bugs from getting in. *The Pig Journal*, **55**, 104–114. (*Guidelines for qualitative risk assessment of farm biosecurity to prevent entry of pathogens*)
- Amass, S.F. (2005) Biosecurity: reducing the spread. *The Pig Journal*, **56**, 78–87. (*Guidelines for qualitative risk assessment of farm biosecurity to prevent within-farm spread of infections*)
- Beier, R.C., Pillaie, S.D. and Phillips, T.D. (2004) *Preharvest and Postharvest Food Safety: Contemporary Issues and Future Directions*. Blackwell Publishing, Oxford. (*Includes discussion of food-safety risk analysis, including the HACCP system*)
- Codex Alimentarius Commission (1999) *Principles and Guidelines for the Conduct of Microbial Risk Assessment*. CAC/GL-30. Food and Agriculture Organization of the United Nations, Rome
- Covello, V.T. and Merkhofer, M.W. (1993) *Risk Assessment Methods: Approaches for Assessing Health and Environmental Risks*. Plenum Press, New York and London
- Covello, V.T., McCallum, D.B. and Pavlova, M.T. (Eds) (1989) *Effective Risk Communication: The Role and Responsibility of Government and Nongovernment Organizations*. Plenum Press, New York
- Garforth, C.J., Bailey, A.P. and Tranter, R.B. (2013) Farmers' attitudes to disease risk management in England: a comparative analysis of sheep and pig farmers. *Preventive Veterinary Medicine*, **110**, 456–466
- ICMSF (1988) *HACCP in Microbiological Safety and Quality. Micro-organisms in Food, 4, Application of the Hazard Analysis Critical Control Point (HACCP) System to Ensure Microbiological Safety and Quality*. The International Commission on Microbiological Specifications for Foods (ICMSF) of the International Union of Microbiological Societies. Blackwell Scientific Publications, Oxford
- ICMSF (1998) Potential application of risk assessment techniques to microbiological issues related to international trade in food and food products. International Commission on Microbiological Specifications for Foods (ICMSF) Working Group on Microbial Risk Assessment. *Journal of Food Protection*, **61**, 1075–1086
- Jacob-Hoff, R.M., MacDiarmid, S.C., Lees, C., Miller, P.S., Travis, D. *et al.* (2014) *Manual of Procedures for Wildlife Disease Risk Analysis*. World Organisation for Animal Health (OIE [Office International des Epizooties]), Paris/International Union for Conservation of Nature, Gland. (*Also includes a comprehensive discussion of general principles*)
- Kindred, T.P. (1996) Risk assessment and its role in the safety of foods of animal origin. *Journal of the American Veterinary Medical Association*, **209**, 2055–2056
- MacDiarmid, S.C. and Pharo, H.J. (2003) Risk analysis: assessment, management and communication. *Revue Scientifique et Technique, Office International des Epizooties*, **22**, 397–408
- Moos, M. (1995) Models of risk assessments for biologicals or related products in the European Union. *Revue Scientifique et Technique, Office International des Epizooties*, **14**, 1009–1020
- Morley, R.S. (Coordinator) (1993) Risk analysis, animal health and trade. *Revue Scientifique et Technique, Office International des Epizooties*, **12**, 1001–1362
- Murray, N. (2002) *Import Risk Analysis: Animals and Animal Products*. Ministry of Agriculture and Forestry, Wellington
- Murray, N., MacDiarmid, S.C., Wooldridge, M., Gummow, B., Morley, R.S. *et al.* (2004) *Handbook on Import Risk Analysis for Animals and Animal Products, Volume II. Quantitative Risk Analysis*. Office International des Epizooties, Paris
- Murray, N., MacDiarmid, S.C., Wooldridge, M., Gummow, B., Morley, R.S. *et al.* (2010) *Handbook on Import Risk Analysis for Animals and Animal Products, Volume I. Introduction and Qualitative Risk Analysis*, 2nd edn. Office International des Epizooties, Paris
- North, D.W. (1995) Limitations, definitions, principles and methods of risk analysis. *Revue Scientifique et Technique, Office International des Epizooties*, **14**, 913–923

- OIE (2013) Risk analysis. In: *Terrestrial Animal Health Code*, Section 2, 22nd edn. World Organisation for Animal Health, Paris
- OIE (2015) *OIE Standards, Guidelines and Resolution on Antimicrobial Resistance and the Use of Antimicrobial Agents*. World Organisation for Animal Health, Paris. (Includes recommendations for analysing the risks to animal and human health from antimicrobial-resistant microorganisms of animal origin)
- O'Riordan, T., Cameron, J. and Jordan, A. (Eds) (2001) *Reinterpreting the Precautionary Principle*. Cameron May, London. (A discussion of the current evolution of the precautionary principle in science, public affairs, the public understanding of science, and international law and trade)
- Peeler, E.J., Murray, A.G., Thebault, A., Brun, E., Giovaninni, A. *et al.* (2007) The application of risk analysis in aquatic animal health management. *Preventive Veterinary Medicine*, **81**, 3–20
- Peeler, E.J., Reese, R.A. and Thrush, M.A. (2015) Animal disease import risk analysis – a review of current methods and practice. *Transboundary and Emerging Diseases*, **62**, 480–490
- Pfeiffer, D.U. (2006) Communicating risk and uncertainty in relation to development and implementation of disease control policies. *Veterinary Microbiology*, **112**, 259–264
- Roberts, T., Ahl, A. and McDowell, R. (1995) Risk assessment for foodborne microbial hazards. In: *Tracking Foodborne Pathogens from Farm to Table: Data Needs to Evaluate Control Options*. Conference Proceedings, 9–10 January 1995, pp. 95–115. United States Department of Agriculture Miscellaneous Publication No. 1532
- Rogers, C.J. (Ed.) (2001) *Risk Analysis in Aquatic Animal Health*. Office International des Epizooties, Paris
- Stärk, K.D.C. and Salman, M.D. (2001) Relationships between animal health monitoring and the risk assessment process. *Acta Veterinaria Scandinavica*, **94**, Suppl., 71–77
- United States Environmental Protection Agency (2012) *Microbial Risk Assessment Guideline: Pathogenic Microorganisms with Focus on Food and Water*. EPA/100/J-12/001. United States Environmental Protection Agency, Washington DC
- Verdonck, F., Chauzat, M.-P., Mosbach-Schulz, O., Ritter, W., Rortais, A. *et al.* (2014) An improved method for qualitative risk assessment – validation and application to a bee pest. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Dublin, 26–28 March 2014. Eds Mintiens, K. and Parkin, T.D.H., pp. 260–270
- Vose, D.J. (1997) Risk analysis in relation to the importation and exportation of animal products. *Revue Scientifique et Technique, Office International des Epizooties*, **16**, 17–29
- Vose, D. (2008) *Risk Analysis: A Quantitative Guide*, 3rd edn. John Wiley, Chichester
- Webber, A.D. and Hill, C.M. (2014) Using participatory risk mapping (PRM) to identify and understand people's perceptions of crop loss to animals in Uganda. *PLoS One*, **9** (7), e102912; doi 10.1371/journal.pone.0102912. (A participatory approach to risk assessment)
- Wooldridge, M., Clifton-Hadley, R. and Richards, M. (1996) 'I don't want to be told what to do by a mathematical formula' – overcoming adverse perceptions of risk analysis. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Glasgow, 27–29 March 1996. Eds Thrusfield, M.V. and Goodall, E.A., pp. 36–46
- Zander, J. (2010) *The Application of the Precautionary Principle in Practice*. Cambridge University Press, Cambridge

25

Economics and veterinary epidemiology

The popular notion of economics is that it is concerned exclusively with financial values. In the context of veterinary epidemiology, usually these are what purport to be the costs of disease and its control in animal populations. However, economics is far deeper than this common misconception suggests.

Economics is a novel way of looking at the world, founded on a rigorous, logical theoretical framework that addresses a particular class of decision problems. The discipline exists because people must make choices about the allocation of scarce resources; without scarcity, there would be no need for economics. Moreover, economics' focus is on the well-being of society as a whole, and criteria for the allocation of society's scarce resources to extract maximum **value**. As discussed below, value is *intangible*, and so is not susceptible to direct apprehension by the senses – an unusual perspective for natural scientists such as veterinarians, whose focus is the observable world. A unique contribution of economics is to enable intangible value to be translated into units that can be quantified.

Mathematics and statistics are important tools for modelling and quantifying economic relationships, which is what is meant by 'econometrics'. However, if economic models and analytical results are to make sense, the essential logic of economic principles (theory) must always come first; 'no measurement without theory' should be the inviolable rule. Indeed, many decision problems in economics preclude all but the most simple quantification, whether on grounds of time or data constraints. Consequently, the following discussion concentrates on general economic concepts and principles, which are not just relevant to veterinary medicine.

General economic concepts

Economists investigate how people's well-being in society depends on the choices they make collectively

about use of **scarce resources** to **supply** things they want to **consume**. Thus, economists also study **demand**: the nature and consequences of people's behaviour as consumers. In the context of epidemiology, the supply side is the more important. It is the first stage of an **economic process** of transforming **resources** into **products** for people's **consumption** (Figure 25.1). Expressed alternatively, it concerns study of input–output relationships as a basis for taking decisions about how best to supply products for people's benefit.

From the perspective of economics, animals are resources used in production¹. Animals benefit people by providing them with **goods** in the form of food products and raw materials, and **services** such as transport, traction, sport, companionship, assistance, and even the satisfaction derived from simply knowing that particular animals exist. In general, quantities of goods and services that are produced increase as a result of using more of the resources necessary to produce them.

Production functions

The generic term for any technical relationship between resources that are inputs to production and the goods and services comprising output is a **production function** (Figure 25.2). Livestock farming is an example, where resources are both natural (e.g., land) and man-made in that raw-material resources are first transformed into other forms (e.g., buildings, machinery, farm livestock, compound feeds) necessary to produce the desired output. Therefore, resources undergo physical transformation (e.g., animal feed into body protein) during a production process. Labour and

¹ Note that it makes no difference to the nature of the relationship that, in a technical sense, resources and products are essentially identical when animals are used to breed replacements or add to numbers of stock. It simply means that existing resources are used to produce outputs destined to be new resources.

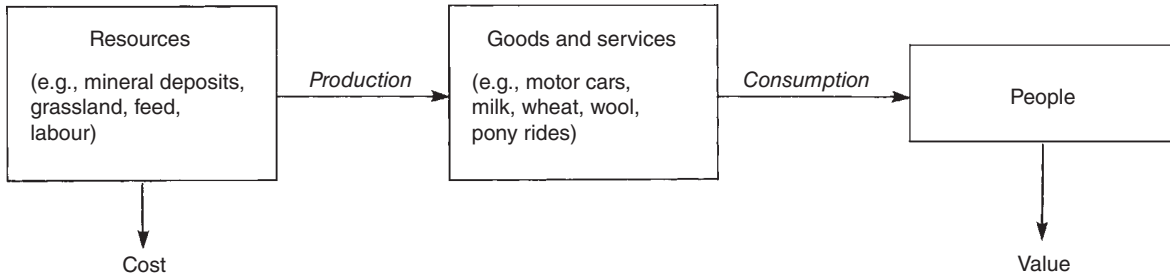


Fig. 25.1 The basic economic model.

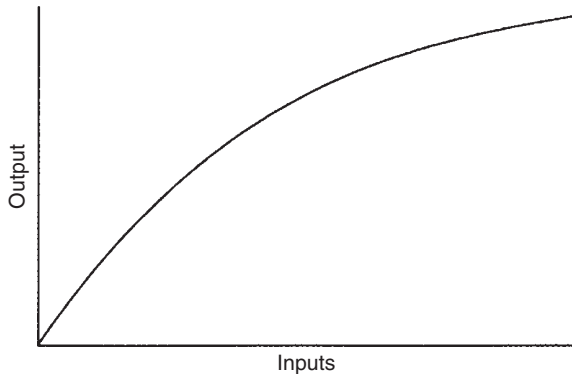


Fig. 25.2 The shape of a general production function, plotting output against variable inputs, others fixed.

management are separately categorized resources, each in its own way facilitating a **physical transformation process**. Labour is any kind of physical effort associated with production, whereas management involves all decisions about what to produce, how much to produce, and how to produce it².

Importantly, empirical evidence shows that because certain inputs are fixed – in the short term, at least (e.g., land, managerial expertise of the farmer) – the relationship is non-linear for increases in variable inputs³. Typically, beyond a certain point, successive increases in variable inputs (e.g., numbers of animals that are kept) are associated with less than proportionate increases in output. This is encapsulated in the **law of diminishing returns**, which is so-called because it is a widely observed phenomenon in nature (Heady and Dillon, 1961; Dillon and Anderson, 1990).

² No distinction is made here between entrepreneurship, which is about responsibility for making major production decisions and accepting the associated risks, and management, which is about implementing production decisions effectively. In farming, the two aspects typically overlap.

³ A variable input is defined as an input whose use varies with the planned level of production.

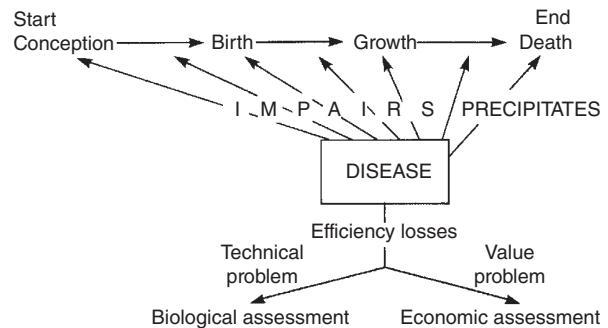


Fig. 25.3 The physical transformation process.

Disease and animal production functions

Livestock production is a specific example of a physical transformation process (Figure 25.3). Disease impairs this process (i.e., reduces output) in a variety of ways (McInerney, 1996):

- destruction of basic resources (death of breeding and productive animals);
- reduction in the physical output of a production process or its unit value (e.g., lowered milk yield or quality);
- lowering of the efficiency of the production process and the productivity of resources used (e.g., reduced rates of growth or feed conversion).

It has wider effects, too, including:

- lowering the suitability of livestock products for processing, or generating additional costs in the distribution chain (e.g., warble-fly larval damage to hides; drug residues);
- affecting human well-being directly (e.g., zoonotic infections such as salmonellosis and brucellosis);
- causing more diffuse economic effects that reduce the value of livestock to society (e.g., constraints on trade and tourism; concerns for poor food quality and animal welfare).

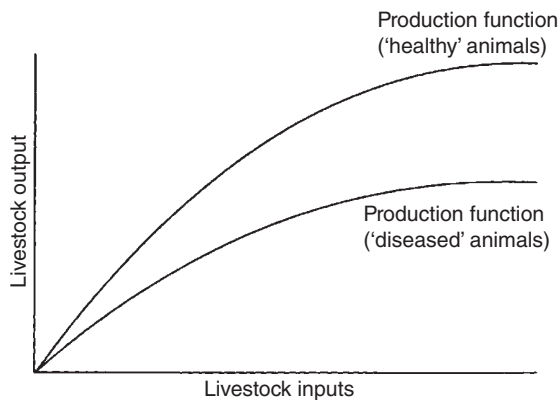


Fig. 25.4 Production functions for 'healthy' and 'diseased' animals.

All of the above effects represent efficiency losses, because scarce resources available to society – note, not exclusively for animal production – produce less output than is feasible in the absence of disease. *Figure 25.4* shows **lost technical efficiency** as the vertical difference between the stylized production functions of 'healthy' and 'diseased' animals. Disease acts as a **negative input**, so that the relationship between inputs and output is shifted downwards: lower output is obtained for given inputs in diseased animals compared with disease-free animals. The concept of efficiency loss is therefore a relationship, not a number, and is smaller under low-input, low-output production systems than under more intensive systems. It follows that the potential economic importance of disease varies between farms, regions and countries, and therefore that control measures justified in one situation may not be justified in another.

Value and money

Economics is concerned with **value**, and the gains (benefits) and losses (costs) that result from the choices people make. Importantly, value is intangible, a meta-physical (i.e., abstract) concept. What people value is based on their physical and emotional feelings of needs and wants. This varies with each individual person, his or her personal tastes and preferences, ethics, culture and religious beliefs. Fortunately, people reveal what, and how much, they value by their observable behaviour, including decisions about how to allocate money they have available to spend. Because benefit means anything that promotes well-being, anything that detracts from it must be a 'dis-benefit'; that is, a cost. Thus, output losses are a cost of animal disease.

In an everyday sense, people tend to equate value with monetary worth, which depends on prices; for example, of a litre of milk, a kilogram of meat, or an

hour's horse-riding lesson. But not everything of value has a monetary price; for example, people's pleasure that animals are kept healthy and well-cared-for. Note especially that, so far, there has been no mention of monetary prices for output and inputs. Until now, this has been unnecessary, and is a reminder that people do not live by money, but by the real resources and real products that money enables them to acquire. For instance, food, housing, health and education are considered basic needs. That is why a distinction is made between the **real economy** and the **money** or **financial economy**.

Money is a device that evolved to facilitate the exchange of real resources and products by people and institutions without the inconvenience of their different wants having to coincide. Thus, without money, a veterinarian wanting food for her family would need to find someone who was able to supply her with the food products that she requires and who also wanted her services (e.g., to treat a sick pet). Without that **coincidence of wants**, there is no basis for exchange. But with money, the food supplier receiving the money knows that, in turn, someone else similarly will take it for providing real goods or services that she wants. Thus, money is a **medium of exchange**, a device which greatly facilitates transactions in any economy. Crucially, money works entirely on trust. If, for any reason, people lose confidence that others will accept a money payment for something real, the system collapses. Fortunately, this rarely happens.

Money and prices

Given a certain sum of money, people reveal how much they want something by how much they choose to spend on it; that is, their **willingness-to-pay**. In other words, money expended represents a kind of vote or signal to guide resource allocation in the economy according to the weights of preferences expressed. This is so, whether for people as individuals or in organizations such as private-sector companies and government departments. In general, the more a person or organization wants (values) something, the more they are prepared to pay for it. But when money is limited to a fixed amount, as is typically the case, the more that is spent on one thing inevitably means that less is available to spend on anything else. Thus, money helps one to quantify and compare **relative values** attributed to technically dissimilar resources and products; for example, animal disease control with school education.

For sources of value without an observable money price (e.g., animal welfare), people show their

preferences by other means, such as by how they cast their political votes, or by demonstrating their support (e.g., by donating money or volunteering with animal charities). By donating money, a person explicitly expresses his or her willingness to pay for (i.e., how much he or she values) the output of animal well-being. In the absence of actual prices or money spent, economists resort to **contingent valuation** or **choice experiment** techniques designed to elicit people's willingness-to-pay (Bennett and Larson, 1996; van Kooten and Bulte, 2000; Bennett and Blaney, 2003; Bennett and Willis, 2008; Kumar *et al.*, 2011; Bennett *et al.*, 2014). Although sometimes there is no alternative, results from such techniques always should be treated with caution. People are inconsistent. In principle, they may be willing to pay higher prices for food produced under high-welfare conditions; in practice, they may nevertheless continue to buy cheaper products obtained under low-welfare conditions. The relationship between prices and consumer perceptions of animal welfare is complex (e.g., Harper and Henson, 1998; IGD, 2007; Clark *et al.*, 2016).

Opportunity cost

Economics exists only because resources are scarce. If resources were unlimited, anyone could have whatever they wanted, in as much quantity as they wanted, whenever they wanted it. But real life is not like that. In practice, if we expend an amount of our personal resources (typically money) on one thing, we forego the opportunity to spend it on something else. For example, given a budget constraint, spending money on acquiring and keeping a family pet dog could mean that something else that is wanted must be sacrificed – perhaps a holiday. Similarly, if a farmer contemplates having more beef cattle on his holding, he may have to reduce the size of his sheep flock to release sufficient land to keep them; or if a young person decides to train as a veterinary epidemiologist, she cannot simultaneously become a medical general practitioner (GP). Making a choice precludes other possibilities.

Loosely interpreted, value lost as a result of deciding to use resources for one thing and not an alternative is called the **opportunity cost**; but strictly interpreted, it is the **loss from the best paid alternative**. Thus, if a would-be veterinary epidemiologist has the qualifications to train as a GP instead, and no other occupation would yield as much money income as a GP over her lifetime, the opportunity cost of becoming an epidemiologist is her potential life-time earnings as a GP. The same principle applies when decisions are made by governments on behalf of society as a whole. Given scarce resources, always and inevitably there are **trade-offs** between alternatives: comparing the benefits against costs in making

any decision, and choosing the option that maximizes positive net benefits. Such considerations are central to identifying the criteria for economic efficiency.

Technical and economic efficiency

Importantly, **technical efficiency** and **economic efficiency** are different. The explanation is this. The lesson of Figure 25.4 is that animal disease makes people worse off, because without disease they obtain more of something they value from any given quantity of inputs. In Figure 25.5, curve 1 corresponds to the 'healthy' animals' production function, and curve 2 to the 'diseased' animals' production function, of Figure 25.4. Only one output – liveweight gain per day – is considered in relation to all non-veterinary inputs, variable and fixed.

For an arbitrary quantity of non-veterinary variable inputs, say x_1 , used with a fixed number of animals, point G_1 indicates the liveweight gain per day when they are 'healthy' animals (curve 1); G_2 identifies the liveweight gain if they are 'diseased' animals (curve 2). Thus, $G_1 - G_2$ is the loss in technical efficiency. One option is to control the disease exclusively by veterinary intervention, thus restoring G_2 to G_1 . However, reduction of disease commonly does not depend *exclusively* on veterinary services and medicines. An economist regards these as just particular types of inputs that may have substitutes in the form of greater managerial input, use of more land to reduce stocking rate, and so on, all of which may reduce disease. Using $x_2 - x_1$ additional non-veterinary inputs also restores G_2 to G_1 . In practice, the option most commonly adopted corresponds to a movement along some intermediate curve, say curve 3. Production is then lifted from G_2 to G_1 in two parts. The proportion of $G_1 - G_2$ given by RS is achieved by veterinary expenditures, while the

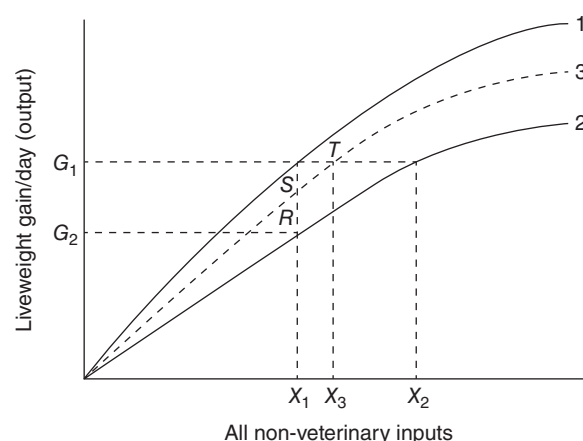


Fig. 25.5 The recovery of planned output utilizing veterinary and non-veterinary inputs. (Adapted from Howe, 1985.)

remainder (climbing from S to T) is achieved by increasing non-veterinary inputs from x_1 to x_3 . The main goal of economic evaluation is to identify the path corresponding to RST , which enables G_1 , rather than G_2 , to be obtained most cheaply; that is, identifying the *combination* of veterinary and other inputs that will minimize the costs of recovery – a key criterion of economic efficiency. Thus intermediate curve 3 is technically inferior to curve 1, but identifies the economic optimum (Howe, 1985).

Positive and normative economics

Economists must be divorced from personal values when analysing a problem. Given information about the technical characteristics of disease, and prices of resources and products, their professional task is to identify the best solution according to agreed economic criteria. **Positive economics** describes the way things are, whereas **normative economics** elucidates judgements about the way things ought to be. The latter has two elements: first, when, according to accepted economic criteria, analysis shows that a current situation could be improved (i.e., by increasing economic efficiency); and secondly, when an existing situation is deemed unacceptable in terms of fairness.

Thus, there are both technical and ethical aspects to economic decisions, and they must not be confused. For example, on conventional benefit–cost criteria (see ‘Social cost–benefit analysis (CBA)’ later in this chapter), control of bovine tuberculosis in the cattle population of England and Wales by culling badgers was shown to be highly inefficient (ISG, 2007), but nevertheless became part of UK government policy. Political factors doubtless played a part. However, an element of those factors is likely to have been recognition that the immense stress experienced by farm families affected by repeated herd inspections and losing cattle to tuberculosis was thought to be unacceptable. Non-economic factors overrode results from economic analysis. Economic analysis only provides information for decision makers. It cannot determine what a decision should be, except in terms of its own specific rigorous criteria. But that is economics’ unique and essential contribution to policy making at any level. It enables the consequences of decisions to be quantified in value terms and, as discussed here, to reveal the implicit weightings given to different group interests in society.

Levels of aggregation

The overriding concern of economics is the well-being of society as a whole. Society is typically defined as the population in a given country, but equally might be a

larger geographical region such as the European Union (EU), or even the world. The well-being of sub-groups, such as livestock farmers, companion-animal owners, or the horse-racing industry, similarly is of interest. This is also true of individual persons, mainly because their behaviour in sum determines aggregate economic effects.

The relationship of epidemiology to economics is that economics also is concerned with the effects of disease in animal populations, but specifically on the well-being of people. Moreover, economics has close affinities with epidemiology in respect of levels of aggregation (Howe, 1989). Both are concerned with different levels of aggregated individual units. For instance, epidemiology often focusses on disease in national (country) and international (regional or global) animal populations, including entire sectors of specific animal species (e.g., a national dairy herd or poultry flock, domesticated cats, or wild animals such as badgers). Lower levels of aggregation include production animals in herds and flocks, and sub-regional or sub-country localities affected by particular disease problems (e.g., bovine tuberculosis in south-west England). Importantly, like economics, epidemiology is not fundamentally concerned with individuals. That is the province of clinical veterinary science, except insofar as understanding disease aetiology in individual animals contributes to understanding implications for some aggregate level of population.

At all levels, epidemiology is about the technical aspects of disease, and economics is concerned with these aspects’ implications for people’s well-being. Epidemiologists investigate the nature of disease as a technical phenomenon, embracing its biological and environmental origins, and the scientific and institutional aspects of its control. Economists’ role is to ask if measures that are proposed are worth it, taking into account the costs and benefits (i.e., value losses and gains). The level of aggregation of an animal population subject to economic analysis must be explicitly taken into account, because it affects the nature and scope of economic effects. The economic consequences of disease in a national farm livestock population, for example, is not the sum of individual farm, herd or flock effects (a nationwide epidemic of foot-and-mouth disease, for instance, may affect tourism: Blake *et al.*, 2003).

Disease contained at farm level

Animal disease contained at farm level affects only **private costs and benefits**; that is, those impacts experienced only by the farmer as decision maker and recipient of the profits that reward his skill as entrepreneur and manager. By virtue of their close association,

the farm family and perhaps hired labour also may be affected. Depending on the specific animal population and disease, physical output commonly will fall while expenditures are incurred on veterinary services and medicines to restore animals to health. Thus, revenue falls as financial costs rise, reducing profit margins and therefore the availability of financial and real resources for consumption and investment to benefit the farm family and other dependents. In this example, other farms and farmers are not affected; that is, there are no *third-party (externality)* effects.

Disease not contained at farm level

An **externality** is a cost or benefit incurred by a third party that is not taken into account by a decision maker. From an economics perspective, externalities are the focus of many of the most important problems in epidemiology. Classic examples are outbreaks of foot-and-mouth disease, Newcastle disease, avian influenza, classical swine fever, and bovine tuberculosis. For example, a farmer's failure to report signs of foot-and-mouth disease in a north of England pig herd in 2001 led to a national epidemic (Mansley *et al.*, 2003). What began as an individual farm problem was replicated many times, and so created **negative externalities**. Moreover, costs extended far beyond the farm sector because disruption caused to society at large resulted in attendant social costs. The National Audit Office (2002) estimated that compensation and other payments to farmers were expected to total nearly £1.4 billion; direct costs of measures to deal with the epidemic, including the purchase of goods and services to eradicate the disease, were nearly £1.3 billion; other public sector costs were £0.3 billion.

In the private sector, the areas most affected by the epidemic were agriculture, the food chain and supporting services, incurring net costs of £0.6 billion; tourism and supporting industries lost revenues of between £4.5 billion and £5.4 billion. However, the UK Treasury estimated that the net economic effect of the epidemic amounted to less than £2 billion, or just 0.2% of Gross Domestic Product (GDP), because many of the losses suffered by individuals and firms in agriculture and tourism led to equivalent amounts of money being spent in other sectors of the economy. Importantly, the nature of economic activity is such that costs incurred by one sector may be offset to a greater or lesser extent by benefits to another. In that specific sense, veterinarians are a group who benefit financially from animal disease, although sometimes suffering dis-benefits, particularly as a result of trauma from confronting extreme disease events (Makita *et al.*, 2015).

Another example of negative externalities is that of undesirable side-effects on unknowing consumers of antibiotic residues in milk. If they were aware of the risks, they would be prepared to pay less for the milk (if, indeed, they would buy it at all). To protect consumers, and to 'internalize' the external effects, legislation limiting the use of antibiotics and affected milk may be necessary, instead of reliance on a deficient price mechanism. By contrast, farmers who invest in biosecurity measures to protect their own animals from infection create **positive externalities** by simultaneously, and unintentionally, protecting other farms from infection; their actions in their own private interest create an obstacle to their own herds or flocks becoming reservoirs of infection and a potential threat to other farms.

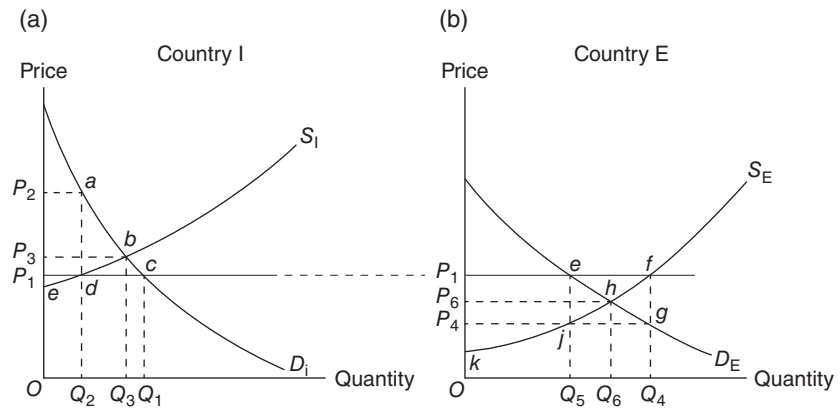
In summary, a *social cost or benefit* is a *private cost or benefit plus a negative or positive externality*.

Zoonotic disease

Zoonotic diseases add another dimension to externality effects. In part, zoonotic epidemics have similar economic effects to those confined to animal populations as outlined earlier in this chapter, but there are significant contrasts. Thus, the World Bank Group (2015) report on the Ebola virus outbreak in Liberia, Sierra Leone and Guinea emphasizes its macroeconomic effects⁴. The lower revenues and increased expenditure because of the disease were expected to result in a fiscal deficit of US\$262 million, an excess of government expenditure over tax receipts of about 12.8% of GDP compared with 1.2% in the year before the crisis. For less-economically-developed countries, such deficits amount to constraints on the ability of governments to provide health, education and national infrastructure from domestic resources, all essential foundations for achieving economic growth and so improved human well-being in the longer term. The emerging One Health perspective linking animal and human disease issues should stimulate greater attention than hitherto to analysis of the economic implications of zoonoses, taking a broader, more comprehensive perspective.

⁴ Macroeconomics is about the economy as a whole. Its main components are aggregate supply and demand, money and banking, monetary and fiscal (i.e., tax) policy, inflation, employment, exchange rates, international trade, and economic growth. Note that sometimes in the economics of animal health literature reference is made to 'macroeconomic' in a different, and strictly erroneous, sense. There, it really means 'aggregate' or 'sector level' in a microeconomic context. Microeconomics concerns decisions made by individuals or groups of consumers, and firms.

Fig. 25.6 The economic implications of an import ban for: (a) Country I, importer, and (b) Country E, exporter. (See text for explanation.)



Disease at international level

Diseases of international concern are exemplified by transboundary airborne pathogens such as vesicular stomatitis and Aujeszky's disease (see Chapter 6), those occurring as a result of infective materials recycled in the food chain through national and international trade (e.g., bovine spongiform encephalopathy), or spread by people migrating: pathogens respect no political frontiers. At one level, diseases of international concern are the equivalent of single-country effects experienced on a larger geographical scale. At another level, policy responses to such diseases add new dimensions; **import bans** by trading countries aiming to avoid introducing infection from exporting countries are a case in point. There are consequences for both importers and exporters, illustrated in Figure 25.6, which also takes explanation of economics' unique contribution a step further. It enables *financial effects* to be distinguished from *economic welfare effects*; the former are what people normally think of as economic effects, whereas the latter are what really matter in terms of value and people's well-being.

Financial effects of international trade bans

Assume only one country, Country E, which exports potentially infected animals or potentially contaminated animal products to only one importer, Country I. For simplicity, regard these exports as a single product⁵. Figure 25.6a summarizes the supply and demand situation in Country I as yet unaffected by disease, and therefore before it bans imports. A **supply**

curve, S_I , shows the relationship between quantity of domestic product supplied and its price; it has a positive slope because the higher the domestic price, the greater the incentive for domestic producers to increase output. Domestic supply also depends on the quantities of resources available, such as land, labour, buildings, machinery, and the quality of technology embodied in them, which determines their productivity (output per unit of resource)⁶. In contrast, people normally consume (demand) more in response to falls in price. Thus a **demand curve**, D_I , which shows the relationship between the quantity of a product consumed and its price, has a negative slope. Domestic demand also depends on the size and age structure of the population, people's incomes, tastes and preferences. When the focus is on supply and demand curves, for analytical purposes these other variables are regarded as constant.

Under free trade, prices will equalize between countries at P_1 , because as long as prices differ there is an incentive to move products from a lower price to a higher price location. At price P_1 , Country I's consumers demand OQ_1 quantity of the product. Of the total, domestic production is OQ_2 , and imports ($Q_1 - Q_2$), equal to $(Q_4 - Q_5)$ exports from Country E

⁵ Economic analysis has been described as about applying 'powerful simplicity', building models on a foundation of simple assumptions up to a level of complexity judged the minimum necessary to illuminate a real-world context. To begin from trying to model the real world in all its complexity is a recipe for confusion and analytical disaster.

⁶ Note that a production function is parent to a supply curve, as follows. Along a production function, the gradient at each point shows the marginal amount of output produced, dQ , from the last unit increment of a variable input, say dX . The law of diminishing returns means that dQ/dX diminishes as input X is increased. Thus, its inverse, $1/(dQ/dX) = dX/dQ$, gets larger. But we are not interested in this inverse physical relationship for its own sake. What matters is how it helps us find the *variable cost of producing each unit addition to output*. So, multiply the unit increment of input, dX , by its per unit price, P_x , giving $P_x \times dX/dQ$. This is the expression for the **marginal cost curve** (i.e., supply curve) for the product. As long as marginal cost is less than the price per unit of output, P_Q , it pays to increase output; total gross margin, from which fixed costs must be deducted to get profit, is for that level of total product where $P_Q = P_x \times dX/dQ$.

in Figure 25.6b. Free trade enables countries to obtain the **gains from trade**. Here, Country E is the more efficient producer, because its equilibrium market price P_6 is lower than equilibrium market price P_3 in Country I before trade takes place. The **market equilibrium** is so called because only at this combination of price and quantity does supply equal demand. At any price higher than the equilibrium level, producers supply more than consumers are prepared to consume at that price; at lower than equilibrium price, consumers demand more than producers are prepared to supply. The consequence of achieving more efficient international resource use by allowing free trade between the two countries is to increase aggregate production available for consumption. Country E's more efficient producers are able to provide both for Country E's own consumers, OQ_5 , and to export a surplus of $(Q_4 - Q_5)$ to Country I as well.

By imposing an import ban as protection against a threat of importing disease from Country E, Country I restricts its consumers to supplies exclusively from domestic production, OQ_2 . The immediate impact of induced scarcity is to inflate domestic market price to P_2 , shown from the demand curve, D_1 . Financial expenditure by Country I's consumers changes from P_1Q_1 to P_2Q_2 . If the ban is retained for a long period, at price P_2 Country I's producers have an incentive to increase output. This requires use of more resources, and adjustment takes time. But as producers increase output, equivalent to moving up the supply curve, S_1 , its greater abundance needs price to fall for consumption to increase. By how much is determined from the demand curve until, in the long term, price and quantity stabilize for Country I at P_3Q_3 .

Figure 25.6b shows the corresponding effects for Country E. With Country I's import ban, products formerly exported from Country E are retained on its home market. The increase in supply benefits Country E's consumers because it induces a fall in market price to P_4 ; now consumers pay P_4 per unit for OQ_4 quantity purchased. Instead of $P_1 \times Q_4$, producers in aggregate now receive only $P_4 \times Q_4$, or $(P_1 - P_4)$ less per unit sold. In summary, if the ban is short-lived, price effects therefore are to the advantage of consumers in Country E and to the disadvantage of consumers in Country I, and the contrary for their respective producers.

If Country I's import ban is retained for a long time, Country E's producers will also adapt by reallocating resources to other uses. As domestic production falls in response to price reductions, simultaneously there are demand-side effects. Specifically, increasing product scarcity causes prices paid by consumers to rise, cushioning the extent of supply-side adjustments.

Eventually a new domestic market equilibrium for price and quantity in Country E is established at P_6Q_6 .

The above price and quantity effects are all financial in the 'everyday sense' – values of transactions expressed in actual money resulting from market activity. Also financial, the foreign exchange cost of imports to Country I before the ban is $P_1 \times (Q_1 - Q_2)$, with equivalent receipts $P_1 \times (Q_4 - Q_5)$ to Country E. With no international trade, obviously there are no foreign exchange implications.

Morgan (2001) for bovine spongiform encephalopathy, and, for foot-and-mouth disease, Blayney and others (2006) and Junker and others (2009), explore the wider implications of trade bans for international trade in animals and livestock products, including when many countries are affected.

Economic welfare effects of international trade bans

The important distinction between financial and economic effects should always be made clear in economic policy analysis. Economic, or, more precisely, economic welfare effects, have components explained here using the short-term and long-term effects of an import ban as an example. The principles expounded underpin the econometric studies by Jin and others (2004), Panagiotou (2008), Junker and others (2009), Mutondo and others (2009), Dabalen and Nguyen (undated) and Kutlina-Dimitrova (2015).

Short-term production effects From Figure 25.6a, at price P_1 under free trade, Country I's supply curve shows that its own producers of animals and related products choose to supply OQ_2 quantity. As a result of imposing an import ban, Country I's demand curve, D_1 , shows that, in the short term, price increases to P_2 for OQ_2 , a bonus of $(P_2 - P_1)$ per unit for Country I producers.

Producer surplus is a key concept in **welfare economics**. It is defined as the excess of producer well-being represented by the difference between the same higher price actually received by all producers from sale of a total quantity of product and the different lower prices at which individual producers would have been prepared to sell their share of the total quantity. Each point on the supply curve for domestic producers tells us the additional or **marginal cost** of producing the Q th unit of product (see footnote 6). In Country I, for example, price P_1 is just sufficient to cover the cost of producing precisely the Q_2 th unit; thus, at price P_1 , all units produced up to the Q_2 th earn more than their respective marginal costs of production. Adding up the marginal costs of each unit produced up to that unit where the price received for it

just covers its cost of production gives the total (variable) costs for the total quantity produced⁷.

In effect, producer surplus is a component of profits: here it is a sector-level gross margin (total gross output minus total variable costs), from which fixed costs also must be deducted to obtain sector-level profits. Under free trade, producer surplus in Country I was area P_1de ; thus rectangle P_2adP_1 is additional value, or **gain in producer surplus**, on quantity OQ_2 , which Country I's producers now receive as a result of an import ban, but which they were prepared to supply anyway at the lower price, P_1 .

By contrast, from *Figure 25.6b*, when Country E exported under conditions of free trade, all OQ_4 production was sold at price P_1 per unit. With imports suddenly excluded by Country I, the price in Country E must fall to P_4 per unit if its own consumers are to clear the market of all OQ_4 produced. Given the definition of producer surplus, free-trade producer surplus in Country E of P_1fk falls after Country I's import ban to P_4jk , a net **loss in producer surplus** of P_1jfP_4 . But that is not all: in the very short run – and so without scope for Country E's producers to adjust their supplies – fgj is additional loss. The reason is as follows: relative to the new situation when product price is P_4 per unit, at the former higher price of P_1 per unit producers had the incentive to increase output by another $(OQ_4 - OQ_5)$ units. This required resource expenditures amounting to Q_5jfQ_4 additional costs of production. But of that total, only area Q_5jgQ_4 of the additional costs incurred equates to revenue earned from sale of $(OQ_4 - OQ_5)$ output at price P_4 per unit. The costs not recouped are therefore given by area fgj . Unless the reduced producer surplus (i.e. sector gross margin) after the price fall, area P_4jk , exceeds the residual variable costs, area fgj , sufficient to leave at least some positive margin, sector losses will be such that not only is producers' economic survival jeopardised in the very short term but there is also no prospect of them being able to make a contribution to payment of fixed costs – an unsustainable situation. Importantly, this example demonstrates how economic principles point analysts in the direction of knowing which relationships must be quantified in any given real-world problem context.

Short-term consumption effects Just as each point along a domestic supply curve tells us about the cost of its corresponding unit of product, so each point along a demand curve tells us the value a consumer

attributes to the corresponding unit of product. From *Figure 25.6a*, before Country I imposed an import ban, its consumers purchased OQ_1 of product *in total* at unit price P_1 . As shown by the demand curve, this included quantity OQ_2 from Country I producers, for which its consumers were prepared to pay at least P_2 . Thus, before the import ban, under free-trade price P_1 for all units of product, those consumers who value units of OQ_2 enough to pay at least P_2 per unit, received a kind of 'value bonus'. To be precise, the 'value bonus' specifically on the Q_2 th unit of product is given by $(P_2 - P_1)$, higher than for all subsequent intermediate units, which also contain a 'value bonus' up to the Q_1 th. This relationship is consistent with everyday behaviour: whenever someone considers a purchase to be 'good value' or 'a bargain', they mean implicitly that their subjective valuation of their purchase is greater than the price they actually pay for it.

Consumer surplus is another key concept in welfare economics. It is defined as the excess of consumer well-being represented by the difference between the same lower market price actually paid by all consumers to obtain a total quantity of product and the different higher prices individual consumers would have been prepared to pay for obtaining individual units of that total product. In estimating the effect of Country I abandoning free trade by imposing an import ban, the following can be noted. Again from *Figure 25.6a*, for each i th unit of product, Q_i , between the Q_2 th and Q_1 th, the demand curve shows a 'value bonus' $(P_i - P_1)$ less than $(P_2 - P_1)$. Summing $(P_i - P_1)$ for each individual unit of product between the Q_2 th and the Q_1 th we obtain area acd below the demand curve. Adding this to the above-mentioned area relating to the $(P_2 - P_1)$ price increase when Country I consumers are constrained to buy OQ_2 from Country I producers, gives area P_2acP_1 . This is the total loss of 'value bonus' by Country I consumers as an immediate result of the import ban, or **loss in consumer surplus**.

Given knowledge of the demand curve, the consumer surplus loss can be interpreted as follows. First, all consumers who value the product at prices between P_1 and P_2 per unit (namely units from OQ_2 to OQ_1), buy nothing at P_2 ; at the relatively high price, P_2 , they value the product, but not that much. Consequently, they benefit nothing from now zero consumption. Secondly, consumers who do value the products sufficiently to buy OQ_2 at price P_2 also lose benefit from the import ban, because when imports supplemented domestic production they paid only the lower price P_1 per unit for it.

By contrast, Country E's consumers benefit from Country I's import ban. In the short term, they gain

⁷ Students of calculus will recognize this procedure as equivalent to integrating a differential equation describing the supply curve from zero production up to the Q_2 th unit.

consumer surplus of P_1egP_4 (Figure 25.6b). This similarly comprises two parts. First, OQ_5 formerly purchased at price P_1 is now obtained at lower price P_4 per unit; secondly, retained exports ($Q_4 - Q_5$) are additionally consumed at home because, as the demand curve shows, at price P_4 every additional unit above the Q_5 th up to the Q_4 th is valued by consumers at least that much.

Long-term production and consumption effects If Country I's import ban is retained for a sufficiently long period, producers and consumers in both countries will make adjustments to resource use with implications for prices, and quantities produced and consumed.

In Figure 25.6a, for sustained price P_2 , Country I producers have an incentive to use more resources, increasing production above OQ_2 ; in other words, producers move up the supply curve. But as supply increases, price must fall with respect to the demand curve if consumers are to buy the extra production. Simultaneously, supply and demand adjust until a new domestic market equilibrium is reached at point b , with coordinates P_3 and Q_3 . Relative to free trade, the long-term producer surplus gain is area P_3bdP_1 , and consumer surplus loss area P_3bcP_1 .

In Figure 25.6b, at sustained price P_4 Country E's producers will contract output; that is, move down the supply curve from point f . As a result of increasing scarcity of products, the demand curve shows that price recovers until, at point h , a new domestic market equilibrium is established with coordinates P_6 and Q_6 . Relative to before Country I's import ban, Country E's producer surplus loss is area P_1fhP_6 and consumer surplus gain is area P_1ehP_6 .

Whole-society effects and policy implications For economic policy analysis, people are categorized separately as entities of consumers, producers and taxpayers (see following section) to investigate the overall consequences for economic welfare of changing from an existing to a preferred situation. This is an analytical device, because it is understood that people actually belong to one or more of the groups at any given time. Whole-society effects are obtained as the sum of all three.

In the short run, for reasons explained above, an import ban initially causes a shock price effect. This is temporary and soon dissipates if the ban is removed. However, even short-term effects can be problematical: consumers in Country I who spend a large proportion of their income on animal products, especially food items, tend to be the poorest people in society and so suffer disproportionately from price increases

(Dabalén and Nguyen, undated). In Country E, a sudden reduction in product price may cause cash flow problems for farmers. Note that, as drawn in Figure 25.6b, the initial price reduction to P_4 per unit represents such a severe drop in short-term gross revenue from sales that a proportion of resource costs, given by area fgj under the supply curve are not being covered.

If a ban is maintained, the long-term economic welfare effects endure. From inspection of Figure 25.6a, in Country I, the producer surplus gain from an import ban, P_3bdP_1 , is less than the consumer surplus loss, P_3bcP_1 , a net economic welfare loss to society equal to the area bcd . From Figure 25.6b, after full adjustment to loss of its market in Country I, the Country E producer surplus loss, P_1fhP_6 , exceeds the consumer surplus gain, P_1ehP_6 , a net economic welfare loss to society equal to the area of triangle efh . Therefore, a sustained import ban causes net economic welfare losses in both countries. Society as a whole, defined as people in both Country I and Country E, is made worse off.

The outcome described explains why international trade restrictions ostensibly implemented for the protection of domestic producer or consumer health, or both, may be undesirable as a policy instrument in the long term. In principle, a comprehensive economic analysis would also assess the implications for economic welfare of allocating resources to disease control in both countries, and identify the better option. In practice, time and research capacity limits analysis to what is feasible.

Also, it explains the political sensitivity to trade barriers when it is thought that justification for their implementation is made on dubious grounds. For example, in 2000 Egypt contentiously introduced an import ban on all EU beef and live cattle in response to bovine spongiform encephalopathy⁸. The *World Trade Organization* (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (WTO, 2011)⁹ seeks to strike a balance between the right of WTO members to protect health and the need to allow the smooth flow of goods across international borders. The Agreement recognizes the right of WTO members to adopt legitimate measures to protect food safety and animal and plant health, while ensuring that these measures are not applied in an unnecessary manner for protectionist purposes.

⁸ See http://eeas.europa.eu/delegations/egypt/eu_egypt/trade_relation/import_into_egypt/sps/index_en.htm (accessed 5 August 2017).

⁹ Also see https://www.wto.org/english/docs_e/legal_e/15sps_02_e.htm (accessed 5 August 2017)

Finally, note that Country I's import ban causes resources allocated to animal and related production to be increased in the less efficient producer (Country I) and reduced in the more efficient producer (Country E) – resources that have opportunity costs in terms of their potential returns from use in other sectors of the respective economies. The magnitudes of resource adjustments are given by the areas below the supply curves. For Country I, the money value of additional resources after long-run adjustment to its own import ban is area Q_2dbQ_3 ; for Country E, the area of resource reduction is Q_6hfQ_4 . Recall that a supply curve is a marginal cost curve (see footnote 6), and the sum of marginal costs over a range of increments in output equals total variable costs over that range.

Evaluating disease-control policies

A similar economic welfare approach applies to the economic assessment of sector-level disease-control policies in farm livestock production (Ebel *et al.*, 1992; Howe, 1992; Kristjanson *et al.*, 1999). From the previous discussion of animal disease and production functions, disease acts as a negative input: it shifts animal production functions downwards, indicating reduced productivity of conventional resources. The technical efficiency of transforming resources into products is diminished. But, if resources are devoted to reducing the negative effects of disease, the production function shifts upwards. Given that a supply curve is derived mathematically from a production function, reducing disease has the effect of shifting the supply curve to the right.

Figure 25.7 illustrates the general relationships, including the corresponding demand curve for

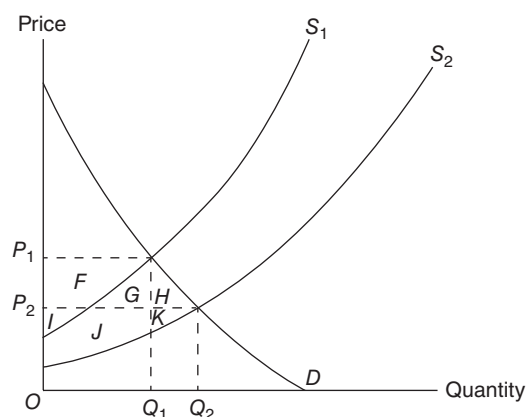


Fig. 25.7 Consumer and producer surpluses after implementing a disease-control programme. (See text for explanation.)

products. Drawing a single demand curve implies that the existence of disease does not affect people's attitudes negatively towards the products, which is not always the case (e.g., bovine spongiform encephalopathy, *Salmonella enteritidis*). For example, food-safety scares associated with food poisoning will shift the relevant product demand curve to the left. P_1Q_1 is market equilibrium price and quantity 'with disease'; P_2Q_2 is equilibrium 'with disease control'. The capital letters label areas on the figure to aid explanation of the economic consequences of disease control.

After disease control, the combination of more output available for consumption at a lower price gives a consumer surplus gain of area $(F + G + H)$. The interpretation of supply effects for producers is less straightforward. With disease, producer surplus is area $(F + I)$; with disease control, $(F + I)$ is lost and replaced by area $(I + J + K)$. The 'with disease' supply curve, S_1 , is no longer relevant, having been replaced by the 'with disease control' supply curve S_2 . But we need to refer to S_1 to compare the 'before' and 'after' situations. Table 25.1 summarizes the distribution of economic welfare and the outcome for society as a whole from Figure 25.7.

Three caveats apply. First, the model shown takes no account of the value of resources devoted to the disease-control programme, whether for implementation in terms of personnel, equipment, veterinary services and medicines used, or research and development necessary to make control possible. The aggregate money value of such resources should be set against net benefit $(J + K + G + H)$ to assess whether investment in disease control still leaves society better off overall. Where a control programme is state-funded, it represents a **taxpayer cost**. The resources have an opportunity cost elsewhere in the economy, as also they do if funded by the private sector.

Secondly, unambiguously, consumers gain as a result of disease control. For farm producers, the outcome is not clearcut. It depends on the monetary value of area $(J + K)$ gain relative to area F loss. While economic principles point the analyst towards the key relationships to be investigated, they also indicate which must be quantified most carefully before

Table 25.1 Summary of the consequences for economic welfare of disease control illustrated in Figure 25.7.

	Gain	Loss	Net
Producers	$I + J + K$	$F + I$	$J + K - F$
Consumers	$F + G + H$	(zero)	$F + G + H$
Society	$F + G + H + I + J + K$	$F + I$	$J + K + G + H$

substantive conclusions can be drawn. Critical variables here are the **price elasticity of demand** and respective **elasticities of supply** (Begg *et al.*, 2014) for the products, parameters, which measure the sensitivity of consumption and production to price changes, as well as estimates of the magnitude of the **supply curve shift** (and therefore production function shift) with disease control.

Thirdly, welfare economics analysis must be strictly neutral in the sense that one unit of economic welfare to a consumer equals one unit of economic welfare to a producer equals one unit of economic welfare to a taxpayer. Importantly, the above discussion illustrates the power of economic analysis to go far beyond people's usual focus – and misunderstanding – that economics is only about money values in an everyday sense. But here the limits of economics are reached, too.

For illustration, suppose that the sum of consumer and producer gains estimated to arise from implementing a disease-control policy is insufficient to offset taxpayer losses in the ratio 1:3. In simple terms, the policy looks too expensive. Does this mean that the policy should not be implemented? On the bare figures, the answer is 'yes'. But if political judgement is that consumer and producer well-being is considered three times, or more, as important as that of taxpayers, then tax revenue should be spent to fund the intervention. The key words are 'political judgement' – the outcome of a decision process that is qualitative in nature, and not obviously open to quantification. Economics only provides a particular kind of information as an input to a decision process; there is no 'economic formula' which says 'this is what must be done'. At the same time, economics can reveal the implicit weights accorded to the interests of different segments of society when a decision is taken. That is economics' unique contribution.

Components of disease costs

Fundamental questions to be answered for disease-control policy are how much does disease cost, and to what extent is it worth trying to do something about it? A welfare economics analysis is one approach, but can be demanding in terms of data requirements, research time constraints, and expertise needed for model estimation. Other methods often may be considered more practical, and based on conventional financial costs and benefits. Whichever method is applied, it must be remembered that there is no single cost of disease, only different kinds of costs relevant to decision making in different problem contexts (Pritchett *et al.*, 2005).

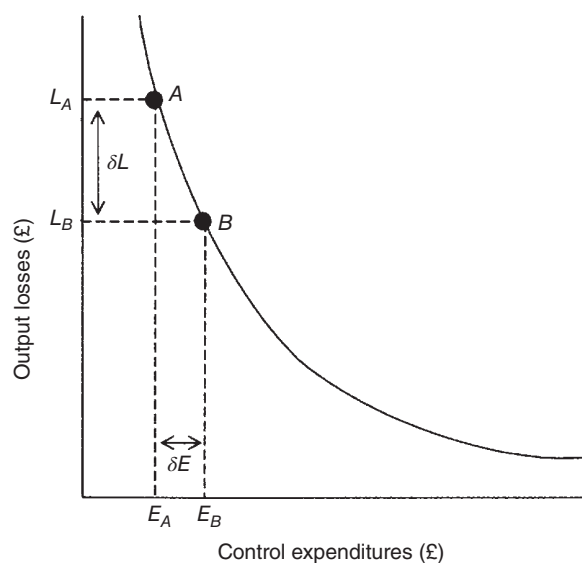


Fig. 25.8 The general relationship between output losses, L , and control expenditures, E . (Source: Schepers, 1990. Reproduced with permission.)

The total economic cost of disease can be estimated as the sum of **output losses** and **control expenditures**. A reduction in output is a loss because it is a benefit that is either taken away (e.g., when milk containing antibiotic residues is compulsorily discarded) or unrealized (e.g., decreased milk yield). Expenditures, in contrast, are increases in resource inputs, and are usually those associated explicitly with disease control. Examples of control expenditures are veterinary intervention and increased use of agricultural labour, both of which may be used either therapeutically or prophylactically.

Figures 25.4 and 25.5 use the basic model of a production function to illustrate the implications of disease and its control for technical and economic efficiency. A closely-related approach is to explore the general relationship between control expenditures and output losses as defined by a curve, which again reflects the law of diminishing returns (Figure 25.8). The curve is also an 'efficiency frontier' if it defines the lowest disease losses that can be attained for any level of control expenditure, or the lowest possible expenditures for restricting losses to a specified level¹⁰. Combinations of output losses (L) and control expenditures (E) to the 'south west' of the curve are unattainable, whereas combinations observed to the 'north east' result from technical deficiencies in livestock management. Two control programmes, A and B,

¹⁰ This curve relates to persistent or recurrent conditions, such as bovine mastitis. If the disease of interest could be eradicated (e.g., swine fever), the frontier would intersect the horizontal (E) axis.

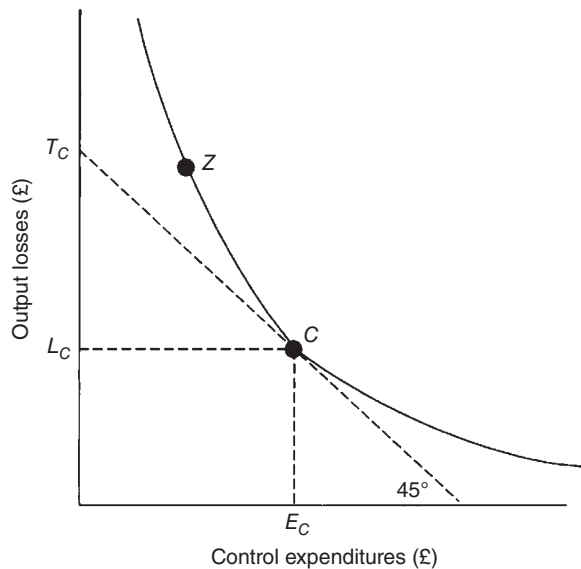


Fig. 25.9 Defining the economically optimum control programme. (Source: Schepers, 1990. Reproduced with permission.)

are identified in the figure. A change from programme A to programme B involves an increase in control expenditures of $\delta_E = E_B - E_A$, and a decrease in output losses of $\delta_L = L_A - L_B$. It is worth increasing the level of control expenditures by δ_E because δ_L is greater than δ_E . However, it becomes increasingly expensive to achieve incremental reductions in output losses.

Optimum control strategies

The **optimum** control strategy is defined by point C in Figure 25.9. At this point, the total costs, $T_C = (L_C + E_C)$ are the lowest that can be attained (i.e., the avoidable costs = £0). To the left of C, £1 of control expenditures reduces output losses by more than £1; to the right of this point, £1 of control expenditures reduces output losses by less than £1. This is another illustration of the importance of **marginal analysis** in economics and, specifically, the principle that resources should be used up to the point where the expenditure on the last unit of resource is just recouped by the additional returns.

An example of identifying an optimum control strategy: bovine subclinical mastitis in the UK (McInerney *et al.*, 1992)

Bovine mastitis is considered to be the most important disease affecting dairy cattle in many more-economically-developed countries. In the UK, a national mastitis survey conducted in 1977 provided detailed information on the prevalence of subclinical infection

and control procedures practised in over 500 herds (Wilson and Richards, 1980; Wilson *et al.*, 1983). The latter include:

- teat dipping and spraying;
- dry-cow therapy;
- annual testing of milking machines.

The losses due to subclinical mastitis include:

- decreased milk yield;
- changed milk composition;
- decreased milk quality (e.g., antibiotic residues);
- accelerated replacement of dairy cows.

Sometimes there are offsetting savings in some input expenditures such as decreased feed intake because of loss of appetite in affected cows.

Control expenditures relate to the three control techniques, and sufficient published information was available to attach financial values to these expenditures and the losses. The prevalence was expressed as the percentage of quarters that were subclinically infected per day, and annual incidence was estimated assuming that subclinical infection lasts an average of 0.6 years (Dodd and Neave, 1970).

Table 25.2 lists the predicted disease incidence and the economic costs associated with the various combinations of control strategy. The lowest costs that can be attained are associated with control strategy 1 (teat dipping throughout the year, administering dry-cow therapy to all eligible animals, and annual testing of milking machines), where the total costs = £3006 per 100 cows per year. The specific relationship between control expenditures and output losses is displayed in Figure 25.10, and is consistent with the general case (Figure 25.9). The curve identifies the 'best practical' options from a technical point of view, and so the corresponding economic optimum must be somewhere along its length.

If these results are extrapolated to the national herd, they suggest that if strategy 1 were implemented in all dairy herds, the overall cost of mastitis to the nation could be reduced from £172.7 million to £159.6 million, at which level the avoidable costs = £0. It is these costs that are relevant to decisions about resource allocation and disease control – not the costs measured from a base of zero, which it may be impossible to reach with current control techniques. The costs cannot be reduced further unless investment is made into research to improve methods of mastitis control.

Bennett (2003) and Bennett and Ijpelaar (2005) have applied and extended this model to over 30 endemic diseases in Great Britain.

Table 25.2 Predicted mastitis incidence and economic costs associated with 18 different control strategies employed by dairy herds in the National Mastitis Survey, UK, 1977. (Adapted from McNerney *et al.*, 1992.)

Control method			Group no.	Effect on incidence		Loss/expenditure coordinates		
Teat dip/spray (period)	Dry-period therapy (cows)	Testing milk machine		Predicted incidence rate (/100 cows/year)	Decrease in incidence (relative to no control)	Control expenditures (£/100 cows/year) (E)	Output losses (£/100 cows/year) (L)	Total economic cost (C)
All year	All	Yes	1	18.5	24.8	710	2296	3006
		No	2	22.3	21.0	670	2770	3440
	Some	Yes	3	23.4	19.9	490	2899	3389
		No	4	27.2	16.1	450	3373	3823
	None	Yes	5	27.8	15.5	270	3446	3716
		No	6	31.6	11.7	230	3923	4153
Part of year	All	Yes	7	33.7	9.6	615	4148	4799
		No	8	37.6	5.7	575	4657	5232
	Some	Yes	9	38.6	4.7	395	4786	5181
		No	10	42.4	0.9	355	5260	5615
	None	Yes	11	43.0	0.3	175	5333	5508
		No	12	46.8	-3.5	135	5807	5942
Not used	All	Yes	13	30.3	13.0	480	3751	4231
		No	14	34.1	9.2	440	4225	4665
	Some	Yes	15	35.1	8.2	260	4354	4614
		No	16	38.9	4.4	220	4827	5047
	None	Yes	17	39.5	3.8	40	4900	4940
		No	18	43.3	0	0	5374	5374

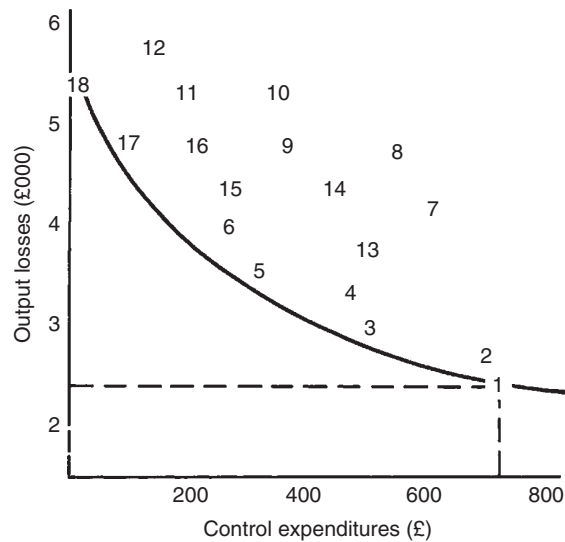


Fig. 25.10 Output losses and control expenditures of farms in the National Mastitis Survey. (Numbers refer to the control strategies listed in Table 25.2.) (Source: McInerney *et al.*, 1992. Reproduced with permission of Elsevier.)

Partial budgets

Partial farm budgets have been used to assess the suitability of control strategies, notably against endemic diseases such as mastitis (Swinkels *et al.*, 2005) and internal parasitism on individual farms. A partial budget is an accounting approach to describe the financial consequences of particular changes in farm-management procedures, of which disease-control programmes are a part. 'Partial' indicates that assessment is restricted to the factors that are likely to change as a result of the changes; it has been described as '*a rough form of marginal analysis*' (Barnard and Nix, 1973).

There are four main components:

1. additional revenue realized from the change, r_1 ;
2. reduced costs stemming from the change, c_1 ;
3. increased costs as a result of the change, r_2 ;
4. cost of implementing the change, c_2 .

If $(r_1 + c_1) > (r_2 + c_2)$, then the proposed change is justified. The calculations may also be based on gross margin analysis (Rushton, 2009).

For example (Erb, 1984), if a new programme to control subclinical mastitis comprised maintenance of milking machines, routine intramammary dry-cow therapy and teat dipping, then:

- r_1 = sales due to increased milk production;
- c_1 = savings stemming from fewer cases of clinical mastitis to treat;

- r_2 = increased feed costs due to increased milk production;
- c_2 = costs of implementation (disinfectants, dry-cow intramammary preparations).

A one-year partial farm budget for this programme produced values of $r_1 = £397$, $c_1 = £18$, $r_2 = £77$, $c_2 = £246$. Thus:

$$r_1 + c_1 = £415,$$

$$r_2 + c_2 = £323,$$

and so there is a net benefit of £92, indicating that the farm should increase its annual profits by that amount if it invests in the control campaign.

Social cost-benefit analysis (CBA)

Social cost-benefit analysis (CBA) developed as a means of assessing large-scale investment projects. It evolved in the public sector, as an aid to resource allocation in areas where markets do not exist, where there are no clear market signals to guide the size and direction of investments, and where governments are responsible for determining the shape of services (Burchell, 1983; H.M. Treasury, 2003). It has been used widely in veterinary medicine to assess national animal disease-control campaigns against infectious diseases; for example, swine fever (Ellis *et al.*, 1977), rinderpest (Felton and Ellis, 1978; Tambi *et al.*, 1999), rabies (Aubert, 1999), bovine virus diarrhoea (Valle *et al.*, 2000) foot-and-mouth disease (Berentsen *et al.*, 1992a,b.) and bovine trypanosomiasis (Shaw *et al.*, 2015) (see also Table 25.4).

Principles of CBA

Social cost-benefit analysis appeals to non-economists because of its superficial simplicity. It is based on two core ideas: (1) resources expended (costs) and what they produce (benefits) are commonly spread over time; and (2) money in hand today is worth more than an identical amount of money at some future time. Because interest is a payment in return for foregoing the opportunity to spend money now, costs and benefits accruing over time must be discounted before they can be compared on the basis of **net present value**. Social cost-benefit analysis attempts to quantify the social advantages and disadvantages of a policy in monetary terms, and incorporates economic concepts discussed above (Mishan and Quah, 2007; Boardman *et al.*, 2011).

For example, the building of a road will incur costs to society arising from the resources expended on construction and maintenance, the undesirable side-effects of pollution, increased noise levels and spoiling of the

landscape. The benefits include savings in travelling time, reduced congestion and decreased noise levels in a town if the road is a bypass. Some of the costs or benefits (e.g., construction outlays – costs) are expressed easily in pecuniary values. Other costs or benefits (e.g., decreased noise level – a positive externality), however, are much more difficult to translate into monetary terms. Only by using the common denominator of money is it possible to aggregate the gains and losses that ultimately interest society as the benefits and costs perceived in *real* terms; that is, as adding to or reducing people's sense of well-being. Consequently, it is important to quantify, in monetary units, all important factors as comprehensively as possible. Any problem areas should be made explicit, especially with valuation of externalities, positive and negative.

If a disease-control programme were initiated, costs would include those of manpower, drugs, vaccines, quarantine buildings, compensation for slaughter, transportation and training programmes. The benefits would include increased productivity, decreased animal and (in the case of zoonotic diseases) human suffering, increased trade and the psychological well-being accompanying the decreased disease incidence. The prefix 'social' to CBA is often dropped from the name but is important. It emphasizes that CBA is used by an organization to maximize the *net benefits to society*, rather than maximizing its own purely private benefits (Pearce and Sturmeay, 1966).

Discounting

Control campaigns may operate over several years. If the interest rate is 5% per annum, then £100 now is worth £105 compound in one year's time, £110.25 in two years, and so on. If costs and benefits, spread over several years, are to be compared, then they must be adjusted back to their value now. The calculation uses a rate of discount that is usually defined by governments. Cost-benefit analysis is performed in real terms, which means that the rate of interest used in the calculations is adjusted to exclude the effects of price inflation.

Shadow prices

The social value of a benefit may not always be the market price. For example, a litre of milk is valued by the farmer at its market price. However, when governments use trade barriers to increase product prices for domestic farmers as, for example, happened in the EU before production quotas were introduced, a milk surplus may develop. Then the real economic value to society of the excess supply must be less than its (supported) market price. A national disease-control campaign that resulted in increased milk surpluses under

price support therefore would use the value of the excess milk, termed a **shadow price**, as a better estimate of its true economic value to society. This will be the international market price if supplies are disposed of on the world market.

Uncertainty

Any project is accompanied by uncertainty. The results of a control campaign cannot be known with certainty, but it is necessary to have an idea what the outcome might be. There are two approaches to dealing with uncertainty. First, if a model is constructed, the 'most probable outcome' can be defined; a sensitivity analysis (see Chapter 23) can then be conducted to determine whether changes in the model's parameters can produce major changes in the outcome. Alternatively, the likelihood of the various outcomes can be judged using probability theory (Reutlinger, 1970).

Summary criteria for CBA selection of a control campaign

Three important measures of economic efficiency used as criteria for selecting a control campaign are:

1. net present value (*NPV*);
2. benefit:cost ratio (*B/C*);
3. internal rate of return (*IRR*).

The **net present value** (*NPV*) is the value of the stream of discounted benefits less costs over *n* time periods. It is given by:

$$NPV = \frac{B_0 - C_0}{(1+r)^0} + \frac{B_1 - C_1}{(1+r)^1} + \dots + \frac{B_n - C_n}{(1+r)^n}$$

$$= \sum_{t=0}^n \frac{B_t - C_t}{(1+r)^t},$$

where:

C_t = value of costs incurred in time *t*,
 B_t = value of benefits gained in time *t*,
 r = discount rate,
 n = life of project.

A project is considered to be viable if the *NPV* is positive.

The **benefit:cost ratio** (*B/C*) is the ratio of the present value of benefits to that of costs. It is given by:

$$B/C = \frac{\sum_{t=0}^n \{B_t / (1+r)^t\}}{\sum_{t=0}^n \{C_t (1+r)^t\}}$$

$$= \sum_{t=0}^n \frac{B_t}{C_t}.$$

A project is viable if the ratio is greater than or equal to one.

The **internal rate of return (IRR)** is the rate of discount that equates the present value of the costs with the present value of the benefits. It is given by solving for r such that:

$$NPV = \sum_{t=0}^n \frac{B_t - C_t}{(1+r)^t} = 0.$$

If the internal rate of return of an investment project is greater than the actual interest rate, the project is economically worthwhile.

An example of CBA: alternative policies for the prevention, control and eradication of infestation with *Chrysomya bezziana* in Australia (Cason and Geering, 1980)

Chrysomya bezziana, the Old World screw-worm fly (SWF), causes serious economic losses in livestock in Africa, Asia and Papua New Guinea. Damage caused by burrowing larvae can result in a case fatality rate of up to 50% in young animals, and can cause loss of condition, occasional deaths and sterility (if genitalia are struck) in adults. The disease is not present in Australia, but if the fly entered Australia, potential losses have been estimated at around \$100 million per annum. The most likely method of entry into Australia would be by movement of infested livestock across the Torres Strait islands from Papua New Guinea, although entry could also occur either by the direct flight of the flies or by transmission of the flies by migratory birds and aircraft.

Strategies to prevent the introduction and establishment of SWF in Australia include:

- improved quarantine surveillance, including training of local inhabitants and education programmes;
- better control of livestock in danger areas, possibly including total destocking of the Torres Strait islands – although this would probably be socially unacceptable to the islanders;
- the development of a SWF monitoring system, facilitated by a specific chemical bait ('Swormlure');
- the construction of a clinic in the Torres Strait islands to neuter dogs and cats (potential hosts of SWF);
- eradication of SWF from Papua New Guinea.

The major strategy to control and eradicate an outbreak of infestation with SWF in Australia is ground control of the fly, consisting of restriction of movement of livestock, dipping and spraying with insecticides, the use of a sterile insect release method, by which sterile male flies compete with fertile male flies

for each mating female, and the use of a screw-worm adult suppression system (SWASS), in which poisoned baits are released by aeroplane over infested areas. The quickest response to an outbreak of SWF infestation would be obtained if a sterile SWF facility were built in advance, but not opened until an outbreak occurred.

Table 25.3 shows the benefit:cost ratios for the various strategies over a 20-year period, assuming that infestation occurred after one, 10 or 20 years. In all cases the benefit:cost ratios are greater than one, indicating that all techniques are economically justifiable.

In the example, the benefits do not derive from increased production, but from decreasing the risk of infestation. Although the benefit:cost ratios are high, they have to be weighed against the probability of infestation occurring when a particular policy either is or is not adopted. These and other considerations are discussed by the authors of the study.

Some problems associated with CBA

There are several general problems related to CBA. The technique assumes that the preferences and priorities of society are known; this may not always be true. The technique inevitably also applies current social preferences. Additionally, as indicated earlier, the costs and benefits of externalities and intangibles may be difficult to assess.

There are specific problems relating to disease-control policy formulation (Schepers, 1990). A CBA refers to only one combination of control expenditures and output losses. Thus, point Z in Figure 25.9 represents a benefit:cost ratio greater than one; that is, the reduction in output losses exceeds the control expenditures. Indeed, the combinations of expenditures and losses furthest to the left of the curve have the greatest benefit:cost ratios, and there may be the temptation fallaciously to conclude that the larger the benefit:cost ratio, the more economically efficient the control technique. However, the economically optimum control programme is indicated by point C; this will be identified by a CBA only if the CBA happens, by chance, to be concerned with evaluating a control policy corresponding to point C.

Moreover, CBA is a relatively sophisticated technique, but may be applied in situations – especially in the less-economically-developed countries – where accurate basic data are lacking (Grindle, 1980, 1986). For example, there may be inadequate information on livestock numbers, disease morbidity and the actual economic impact of disease. It may also be impossible to predict future market prices (e.g., of beef and milk), which need to be included in analyses. The sophistication of the economic techniques therefore needs to be balanced with the quality of the data that are available.

Table 25.3 Cost-benefit analysis of strategies for the prevention, control and eradication of infestation with *Chrysomya bezziana* in Australia. (The analysis is in present values over a 20-year period, and assumes that the methods prevent Australia-wide losses commencing in year 1, year 10 and year 20; discounted at 9.5%). (Adapted from Cason and Geering, 1980.)

Prevention method	Year infestation prevented					
	1		10		20	
	Benefit: \$389m		Benefit: \$164m		Benefit: \$69m	
	Cost (\$000)	Benefit: cost ratio	Cost (\$000)	Benefit: cost ratio	Cost (\$000)	Benefit: cost ratio
Strategic eradication from Papua New Guinea	2 962	131	11 734	14	16 123	4
Mothballed factory and maintenance colony	1 394	279	1 670	98	1 809	38
Ground control*	2 523	154	1 065	154	449	155
Destocking of Torres Strait islands	657	592	776	212	836	83
Improving quarantine	124	3 137	417	394	563	123
'Swormlure' trapping and myiasis monitoring	30	12 966	171	961	241	288
Training	26	14 961	69	2 383	91	763
Extension	13	29 923	83	1 980	119	583
Torres Strait clinic	7	55 571	40	4 110	56	1 239

* Includes the SWASS technique (see text).

However, although CBA and other techniques of project evaluation are, at best, approximate (Gittinger, 1972), they play a useful role in economic evaluation. Despite its limitations, CBA is a rigorous approach to project evaluation, and its application can help towards better informed decisions regarding the efficient use of scarce resources in disease-control programmes.

Summary of methods

Partial budgets, CBA and other economic methods used to support decisions on animal disease control, are summarized in *Table 25.4*.

Note that the methods set out in *Table 25.4* illustrate the range of quantitative techniques found useful for the economic evaluation of disease control, ranging from budgets to complex mathematical programming. But to reassert an essential message from the beginning of this chapter, quantification always must be underpinned by a broad understanding of economic theory, and which principles apply in any given context. It is only by approaching a problem first by specifying conceptually the relationships to be estimated (a theoretical model) that empirical analysis or mathematical modelling should take place. In other words, economic principles are the essential foundation for

quantitative analysis; without recourse to those well-established general understandings about how variables relate to one other, there can be no assurance that the numbers emanating from quantitative analysis make sense.

Further study

Veterinary epidemiology with economics makes a formidable combination as the foundation for policy analysis and implementation for animal disease control and the promotion of animal welfare. Worldwide, currently a minority of people share this particular expertise. At the time of writing, a new International Society for Economics and Social Sciences of Animal Health is being inaugurated. It aims to improve animal health and welfare policies, programmes and projects through more nuanced use of concepts and tools available in economics and social science disciplines. Until specialist programmes in the economics of animal health are devised, graduate courses in agricultural or health economics are recommended for in-depth training, as well as general economics courses with a significant microeconomics content. Such programmes extend principles to account for the risk and uncertainty that pervade real-world decision

Table 25.4 Economic methods commonly used to support decisions on animal disease control. (Adapted and updated from Otte and Chilonda, 2011.)

<i>Method</i>	<i>Level of analysis</i>	<i>Basic concept</i>	<i>Choice indicators</i>	<i>Examples</i>
Partial budgets	Farm/herd	Partial budget set up over defined period (often one year). For longer periods, cost–benefit analysis may be appropriate	Marginal benefit/gross margin	Mukhebi <i>et al.</i> (1989); Rougoor <i>et al.</i> (1994); Lane <i>et al.</i> (1997); Munoz del <i>et al.</i> (2007)
Enterprise budgets	Farm/herd	Gross margin analysis to compare profitability of different enterprises	Gross margin	Okello-Onen <i>et al.</i> (1998); Sebei <i>et al.</i> (2004)
Decision tree analysis	Farm/higher level of aggregation	Computation of the value of different courses of action by incorporating risk (probabilities), and attitudes towards risk, in the analysis	Optimal choice based on criteria (e.g., expected monetary value)	Fetrow <i>et al.</i> (1985); Ngategize <i>et al.</i> (1986); Parsons <i>et al.</i> (1986); Carpenter <i>et al.</i> (1987); Rodrigues <i>et al.</i> (1990); Bennett <i>et al.</i> (2010)
Linear programming and variants	Farm	Obtaining the best solution for competing activities when constraints exist	‘Optimal’ solution	Christiansen and Carpenter (1983); Habtemariam <i>et al.</i> (1984); Jalvingh (1993); Stott <i>et al.</i> (2003)
Dynamic programming	Farm	Obtaining optimal solution	‘Optimal’ solution	Van Arendonk (1985); Cha <i>et al.</i> (2010)
Simulation	Farm/higher level of aggregation	Simulation of the risk and dynamic aspects of livestock disease in production systems	Variable	James (1977); Dijkhuizen <i>et al.</i> (1987); Van der Kamp <i>et al.</i> (1990); Houben <i>et al.</i> (1994)
Cost–benefit analysis	Industry/national	Comparison of benefits and cost covering more than a year	Net present value (<i>NPV</i>), internal rate of return (<i>IRR</i>), benefit:cost ratio (<i>B/C</i>)	Power and Harris (1973); Cason and Geering (1980); Berentsen <i>et al.</i> (1992b); Karki <i>et al.</i> (2015)
Cost-effectiveness analysis	All	Production of a desired technical output at least cost	Cost-effectiveness (e.g., number of animals vaccinated per £ spent)	Tambi <i>et al.</i> (1999); Detilleux and Serteyn (2005); Benedictus <i>et al.</i> (2009)

making. Econometrics and mathematical programming for empirical analysis and quantitative modelling are also core elements of such programmes. However, initial simplicity is always a virtue, especially as the foundation on which to add such building blocks of

greater complexity as prove necessary to understand real-world problems. Under all circumstances, acquiring firm grounding in economic principles is essential. This chapter has aimed to give a flavour of what is entailed, in the context of veterinary medicine.

Further reading

- Babo Martins, S. and Rushton, J. (2014) Cost-effectiveness analysis: adding value to assessment of animal health, welfare and production. *Revue Scientifique et Technique, Office International des Epizooties*, **33**, 681–689
- Dijkhuizen, A.A. (1993) Modelling animal health economics. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Exeter, 31 March–2 April 1993. Ed. Thrusfield, M.V., pp. ix–xx
- Ellis, P.R. and James, A.D. (1979) The economics of animal health – (1) Major disease control programmes. *Veterinary Record*, **105**, 504–506
- Ellis, P.R. and James, A.D. (1979) The economics of animal health – (2) Economics in farm practice. *Veterinary Record*, **105**, 523–526
- Erb, H. (1984) Economics for veterinary farm practice. *In Practice*, **6**, 33–37
- Galligan, D. (2006) Economic assessment of animal health performance. *Veterinary Clinics of North America: Food Animal Practice*, **22**, 207–227
- Hill, B. (1990) *Introduction to Economics for Students of Agriculture*, 2nd edn. Pergamon Press, Oxford. (A general introduction to agricultural economics)
- Howe, K.S. (1985) An economist's view of animal disease. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Reading 27–29 March 1985. Ed. Thrusfield, M.V., pp. 122–129
- Howe, K.S. (1992) Epidemiologists' views of economics – an economist's reply. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Edinburgh, 1–3 April 1992. Ed. Thrusfield, M.V., pp. 157–167
- Howe, K.S. and Christiansen, K. (2004) The state of animal health economics: a review. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Martigny, 24–26 March 2004. Eds Reid, S.W.J., Menzies F.D. and Russell, A.M., pp. 153–165
- Howe, K.S. and McInerney (Eds) (1987) *Agriculture. Disease in Farm Livestock: Economics and Policy*. Proceedings of a symposium in the Community programme for coordination of agricultural research, 1–3 July 1987, Exeter. Report EUR 11285 EN.
- Commission of the European Communities, Luxembourg. (A study of economic losses due to animal disease in the European Union, and methods for the economic assessment of disease control)
- Howe, K.S., Häslér, B. and Stärk, K.D.C. (2013) Economic principles for resource allocation decisions at national level to mitigate the effects of disease in farm animal populations. *Epidemiology and Infection*, **141**, 91–101. (Develops the principles explained in this chapter, with suggestions for research)
- Jackson, E.L., Waret-Szkuta, A., Raboisson, D., Niemi, J., Aragrande, M. *et al.* (2016) Europe needs consistent teaching of the economics of animal health. *EuroChoices*, **15** (2), 42–49
- Jin, H.J., Skripnitchenko, A. and Koo, W.W. (2004) *The Effects of the BSE Outbreak in the United States on the Beef and Cattle Industry*. Center for Agricultural Policy and Trade Studies, Department of Agribusiness and Applied Economics, North Dakota State University, Fargo
- Marsh, W. (1999) The economics of animal health in farmed livestock at herd level, *Revue Scientifique et Technique, Office International des Epizooties*, **18**, 357–366
- McInerney, J.P. (1996) Old economics for new problems – livestock disease: Presidential Address. *Journal of Agricultural Economics*, **47**, 295–314
- McInerney, J.P., Howe, K.S. and Schepers, J.A. (1992) A framework for the economic analysis of disease in farm livestock. *Preventive Veterinary Medicine*, **13**, 137–154
- Mishan, E.J. (1982) *Cost-Benefit Analysis: An Informal Introduction*, 3rd edn. Allen and Unwin, London
- Morris, R.S. and Dijkhuizen, A.A. (Eds) (1997) *Animal Health Economics: Principles and Applications*. Postgraduate Foundation in Veterinary Science, University of Sydney, Sydney
- Ngategize, P.K. and Kaneene, J.B. (1985) Evaluation of the economic impact of animal diseases on production: a review. *Veterinary Bulletin*, **55**, 153–162. (A review of some analytical techniques)
- OIE (1999) The economics of animal disease control. *Revue Scientifique et Technique, Office International des Epizooties*, **18**, 295–561
- Putt, S.N.H., Shaw, A.P.M., Woods, A.J., Tyler, L. and James, A.D. (1987) *Veterinary Epidemiology and*

- Economics in Africa*. ILCA Manual No. 3, International Livestock Centre for Africa, Addis Ababa
- Rich, K.M. and Perry, B.D. (2011) The economic and poverty impacts of animal diseases in developing countries: new roles, new demands for economics and epidemiology. *Preventive Veterinary Medicine*, **101**, 133–147
- Rushton, J. (2009) *The Economics of Animal Health and Production*. CABI, Wallingford
- Rushton, J. (Ed.) (2017) The economics of animal health. *Revue Scientifique et Technique, Office International des Epizooties*, **36**, 1–384
- Rushton, J. and Redmond, E.F. (2015) Application of economics to equine health and welfare. *Equine Veterinary Journal*, **47**, 633–634
- Rushton, J., Viscarra, R., Otte, J., McLeod, A. and Taylor, N. (2007) Animal health economics – where have we come from and where do we go next? *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, **1**, 1–10
- The World Bank (2012) *People, Pathogens and Our Planet, Vol. 2: The Economics of One Health*. Report No. 69145-GLB. The World Bank, Washington DC

26

Health schemes

Private health and productivity schemes

The traditional role of the veterinarian has been to attend individual sick animals when requested to do so by the owner: such attention has been called 'fire brigade' treatment. This approach was useful when most diseases, such as the classical epidemic infectious diseases, had a predominantly single cause and responded to a simple course of treatment. However, an appreciation during the 1960s of the multifactorial nature of many diseases, which coincided with intensification of animal industries in the more-economically-developed countries, with a relative decrease in the value of individual animals, resulted in a change in attitude towards the management of diseases in live-stock units (see Chapter 1). First, it became clear that diseases needed to be controlled by simultaneously manipulating all determinants: those associated with agent, host and environment. The veterinarian's objective should be to prevent, rather than just to treat, disease. Secondly, it became necessary to consider disease in terms of its contribution to reduced performance (and therefore profitability) of a herd¹.

The first change stimulated the development of **preventive medicine programmes** in the early 1960s (Woods, 2013a). The second change resulted in the evolution of comprehensive **herd health and productivity schemes**, encompassing preventive medicine and the assessment of productivity (Blood *et al.*, 1978; Cannon *et al.*, 1978; Ekesbo *et al.*, 1994). These programmes and schemes may be run by one or more

general practitioners, with data stored by the practitioners (the 'bureaux' approach); alternatively, the data may be managed entirely 'on farm' (Etherington *et al.*, 1995). All are concerned with problems on individual farms; they are therefore **private** schemes.

Structure of private health and productivity schemes

Objectives

The goals of a health and productivity scheme were initially summarized by Blood (1976). They should:

- identify disease and productivity constraints and problems on a farm;
- rate the problems in order of importance, with reference to technical and economic criteria²;
- initiate suitable control techniques and measure their success, not only technically but also with regard to the economic efficiency of the utilization of resources at the national and individual farm level, thereby indicating which technique should be increased and which reduced.

The scope of service offered by the veterinarian in a comprehensive health and productivity scheme (Grunsell *et al.*, 1969; Ribble, 1989) now includes:

- the diagnosis and prevention of the major epidemic diseases;
- the diagnosis and control of endemic diseases;
- an emergency service for individual animals;
- the prescribing and supply of drugs;
- advice on environmental determinants (nutrition, housing and management);
- advice on production techniques and general policies of livestock farming.

¹ *Performance-related diagnosis* was introduced in Chapter 1; and an example of multifactorial 'disease', defined in terms of a production shortfall in a population, was given in Figure 5.2. The concept of a 'sick' population is not confined to veterinary medicine, though. Medical epidemiologists also search for characteristics of 'sick' populations; for example, to answer the questions 'Why is hypertension common in London but absent from Kenyan nomads?' and 'Why is coronary heart disease common in Finland but rare in Japan?' (Rose, 1985).

² The financial impact of production diseases can be measured in terms of relative health indices such as *Healex* (Esslemont and Kossaibati, 2002), which compares direct losses due to disease with those in 'top' herds.

This scope is broad and indicates that the veterinarian requires more than just a knowledge of the diagnosis and treatment of clinical disease. In many cases, the veterinarian may need to enlist further expert help from nutritionists, building advisors and management specialists who have some knowledge of farm economics.

Components

There are differences between the schemes applied to different species, but the principles are the same. The main components of a scheme are:

- the recording of a **farm profile** comprising details of animal numbers, buildings and feeding systems, stocking density, nutrition, usual management practices, disease status and current levels of production;
- identification of production shortfalls;
- monitoring of all aspects of production;
- identification of the major disease problems;
- routine prophylaxis against the major disease risks;
- definition of **production targets** that are suitable for the system of management operating on the particular livestock units and for the aims of the farmer³;
- advice on management and husbandry, to achieve the predetermined targets;
- detection of unacceptable shortfalls in production (and therefore in profitability);
- correction of the shortfalls by eliminating the defects associated with agent, host and environment, or revising the production targets in the light of experience;
- identification of farmers' perception of strengths and weaknesses in health, fertility and nutrition.

Regular visits to farms are an important part of health schemes. A **health plan** is drawn up containing details of procedures (e.g., routine treatments and vaccination) to be followed during the year.

Health and productivity schemes require accurate records to be kept. Early schemes used longhand records, but contemporary systems store data on microcomputers where the data can be analysed rapidly. The first computerized systems were run on mainframe computers⁴ and were organized by central advisory bureaux. There is now a trend towards complete decision support systems (see Chapter 11), complemented by mathematical models (see Chapter 23),

which support farmers in herd management (Pià, 2007). For example, *CHESS* (Computerized Herd Evaluation System for Sows) comprises a decision support system that assesses performance in pig breeding herds, and three expert systems that attempt to identify strengths and weaknesses in the enterprises in an economic context (Huirne and Dijkhuizen, 1994). All of these systems are essentially microscale (see Table 11.4).

Targets

The variables that are used to determine production targets are described below in relation to the different species. Targets usually have been defined as measures of position (e.g., mean age of dry sows) or as upper or lower limits (e.g., maximum calving interval or minimum first service pregnancy rate in dairy cattle). Measures of location do not indicate the dispersion of values in a herd and can be misleading; measures of dispersion, such as the standard deviation (when a variable is Normally distributed) or the semi-interquartile range are more informative (see Chapter 12). Thus, if a farmer bred some cows very soon (less than 35 days) after calving, then the measure of position would be reduced, but the measure of dispersion would be increased. This can eliminate the economic benefit that owners think they are achieving, because the economic benefit results from most cows calving with intervals close to 365 days (Morris, 1982); the benefit will not exist with a large dispersion.

Some variables that are used as production indices have frequency distributions that are skewed, in which case the mean may be far from the peak of the distribution. For example, the frequency distribution of calving to conception intervals is positively skewed; typically the median may be 10 days less than the mean. More appropriate measures of position and dispersion therefore would be the median and semi-interquartile range, respectively. Logarithmic transformation of values (see Chapter 12) may be undertaken because this may convert skewed distributions to Normal ones. Morant (1984) discusses the appropriateness of measures in relation to dairy fertility, giving examples. In practice, mean values usually are used because they are understood by farmers. This approach works, provided they also appreciate the notion of dispersion of values.

No single set of standards can be set as production targets because satisfactory performance varies with type of farm and management practices (Olson, 2004). **Internal standards** can be set, using a farm's historical data (say, over the previous three years). Additionally, **external standards**, such as the mean or median performance (with the associated standard

³ The veterinarian's awareness of the aims of a farmer is important in both communication between the two of them, and compliance of the latter (Derks *et al.*, 2013b).

⁴ Mainframe computers are networked configurations of workstations, but the early ones were large devices, housed in metal frames.

deviation or quartiles, respectively) of similar herds, may be specified. In The Netherlands, for example, such standards are published for pig breeding herds (Baltussen *et al.*, 1988) and in the UK for dairy herds (Whitaker *et al.*, 2000, 2004).

Measuring shortfalls in production

Shortfalls in production are defined by an **action level** (also termed an **interference level**). This is the level at which the recorded production variable ceases to be acceptable in relation to its target level. Action levels are often identified by experience, based on financial criteria; and defined as levels beyond which there is unacceptable financial loss. When the measurement of location of a variable (the mean commonly has been used), recorded over a period of time, is beyond the action level, corrective measures are undertaken. This method does not consider the effects of random variation. These can be accommodated by defining the action levels as statistical parameters of the target level; the standard error is a suitable parameter for Normally-distributed data. Suitable techniques for continuous data are the construction of cusums and Shewhart charts (see Chapter 12).

Justification

A herd health and productivity scheme must be justifiable. Participation in schemes is associated with improved farm performance, relative to that on non-participating farms (Derks *et al.*, 2014; McDougall *et al.*, 2014). The economic justification of these schemes also is well documented (e.g., Williamson, 1980, 1987, 1993; Ifende *et al.*, 2014). Pharo and others (1984) derived a benefit:cost ratio of approximately 3:1 for a computerized dairy herd health and productivity scheme in Britain over a five-year period, and a similar value has been estimated for a scheme in the US (Williamson, 1987)⁵. Similarly, Brand and others (1996) demonstrated that herd health schemes improved farms' economic results in The Netherlands⁶. Additionally, schemes may assist in reducing use of antibiotics (Trevisi *et al.*, 2014).

⁵ Demonstration of benefits requires analysis of appropriate data from herds, some of which may be large. Chamberlain and Wassell (1995) suggest that, for most variables, adequate precision (see Chapters 10 and 13) to assess poor herd performance is achieved from a sample of animals in a herd (approximately 40 cows from a 200-cow herd, and 20 cows from a 100-cow herd), but that it is not achieved for some variables (e.g., annual culling rate), in which circumstance data from the whole herd should be monitored.

⁶ Despite the financial advantages and the benefits perceived by veterinarians, uptake by farmers has been slow in some sectors. In the early 1990s, for example, about one-third of dairy practices in the UK had health schemes, but only a small proportion of dairy clients were using them – possibly because of doubts about 'new

Details of schemes for the various species given here are presented only as introductory examples and therefore are not comprehensive descriptions of the systems. Eddy (1992) gives a concise introduction to these schemes, and Rueg (2001) describes them in detail.

Dairy health and productivity schemes

Dairy health and productivity schemes were developed in the 1950s and since then have received considerable attention. The main object of a dairy scheme is to improve welfare and productivity by maximizing health, milk yield and milk quality under the particular system of management on the farm. Optimum milk yield and quality are achieved by:

- efficient reproduction;
- decreasing important diseases – especially mastitis and lameness;
- optimum feeding – both nutritionally and economically.

Targets

Some suggested targets for efficient reproduction for a dairy enterprise in the UK are listed in *Table 26.1*. North American targets are given by Fetrow and others (1997). A target mean calving to conception interval of 85 days often is recommended. This facilitates an annual reproductive cycle because the cow's gestation period is approximately 280 days, with a calving interval (the interval between calvings for an individual cow) of 365 days, and a calving index (the mean calving interval for all cows⁷) with a similar value.

The index variables used in measuring reproductive performance are complex, and need to be interpreted with care (Eddy, 1992). The calving to conception

technology' (Wassell and Esslemont, 1992) – and demonstration of schemes' cost-effectiveness continues to be a main concern for both veterinarians and farmers (Mee, 2007; Leach *et al.*, 2010a; Hall and Wapenaar, 2012). Additionally, uptake by farmers may be influenced by the skill of the veterinarians in explaining the benefits of schemes (Derks *et al.*, 2013a) as well as other factors that motivate farmers individually (Espetvedt *et al.*, 2013; Paton *et al.*, 2015). Kleen and others (2011) highlight the need for appropriate communication between veterinarian and farmer.

⁷ Although the mean calving interval is a useful *goal* of a dairy herd health programme, it is not an adequate means of *monitoring* performance because it requires two consecutive calving dates, and these could be as much as two years apart; additionally, it introduces bias by excluding culled cows, first-lactation animals and animals that are pregnant but have not yet calved (Fetrow *et al.*, 2007)

Table 26.1 Suggested reproductive performance targets for a dairy herd in the UK. (Adapted from Eddy, 1992.)

Index variable	Target	Interference level
Mean calving to conception interval	85 days	95 days
Mean calving to first service interval	65 days	70 days
Mean first service to conception interval	20 days	25 days
Pregnancy rate to first service (%)	60	50
Pregnancy rate to all services (%)	60	50
Overall culling rate (%)	<18	>23
Per cent served of cows calved	95	<90
Per cent conceived of cows calved	85	<80
Per cent conceived of cows served	95	<90
Per cent of interservice intervals 18–24 days	60	45
Per cent of interservice intervals <18 days	8	12
Submission rate* (%)	90	<75

*The number of cows or heifers served within a 21-day period, expressed as a percentage of the number of cows or heifers at or beyond their earliest service date at the start of the 21-day period.

interval, for example, is related to the calving to first service interval and to the first service to conception interval. The calving to first service interval, in turn, depends on the restoration of ovarian function, uterine involution, oestrus detection and farm policy on mating; whereas the first service to conception interval is affected by oestrus detection efficiency and the pregnancy rate⁸. Moreover, no single index should be used as an indicator of reproductive efficiency. Thus, the calving to conception interval and culling rate need to be considered jointly because they are inversely related; a reduced culling rate on fertility grounds will result in an increased calving to conception interval, and vice versa (Eddy, 1992). Some authorities recommend assessing fertility performance in a single financial index (*Fertex*), which combines the calving interval, culling rate for failure to conceive, and

Table 26.2 Some suggested disease and production targets for a dairy herd in the UK. (Calculated from the 'top' 10 herds out of 42 herds.) (Adapted from Esslemont and Kossaibati, 2002.)

Index variable	Target
Twinning (cases/100 cows)	2.5
Calf mortality (cases/100 calves born)	4.4
Aid at calving (cases/100 cows)	3.9
Retained fetal membranes (cases/100 cows)	2.2
Milk fever (cases/100 cows)	2.2
Vulval discharge including endometritis	
(a) % of cows affected (initial cases/100 cows)	5.7
(b) average number of cases per affected cow	1.3
(c) total cases per 100 cows ($a \times b$)	7.4
(d) extra cases per 100 cows ($c - a$)	1.7
Oestrus not observed	
(a) % of cows affected (initial cases/100 cows)	15.6
(b) average number of cases per affected cow	1.3
(c) total cases per 100 cows ($a \times b$)	20.3
(d) extra cases per 100 cows ($c - a$)	4.7
Mastitis	
(a) % of cows affected (initial cases/100 cows)	14.1
(b) average number of cow-cases per affected cow	1.2
(c) total cow-cases per 100 cows ($a \times b$)	17.3
(d) extra cow-cases per 100 cows ($c - a$)	3.2
Lameness	
(a) % of cows affected (initial cases/100 cows)	6.0
(b) cases per affected cow	1.4
(c) total cases per 100 cows ($a \times b$)	8.4
(d) extra cases per 100 cows ($c - a$)	2.4

pregnancy rate (Esslemont, 1993a). This may be difficult to translate into appropriate action because it is first necessary to identify which of its component parameters is deficient.

Targets for a wider range of problems and production parameters can be defined according to the performance of the 'top' herds for which data are available (*Tables 26.2 and 26.3*).

Routine visits

Regular farm visits are required to achieve reproductive targets (Morrow, 1980). De Kruif (1980) recommended visits to cows:

- that have calved during the previous three to six weeks and have abnormal histories of parturition and of the early post-partum period;
- not seen in oestrus by 50–70 days post-partum;
- with abnormal discharges or irregular oestrous cycles;

⁸ **Pregnancy rate (pregnancy risk)** is the percentage of cows eligible to become pregnant, in a given time frame, that actually do become pregnant (Niles *et al.*, 2001; Cook, 2010). A logical time frame is 21 days – the typical length of an oestrus cycle – and some authors recommend the 21-day pregnancy rate as an appropriate single parameter (Cabrera, 2014).

Table 26.3 Some targets and performance indicators derived from analysis of 500 randomly-selected National Milk Records dairy herds in the UK. (Adapted from Hanks and Kossaiati, 2013.)

<i>Index variable</i>	<i>Median</i>	<i>First-third quartile</i>	<i>Interquartile range</i>	<i>Target*</i>
Culling rate	25%	20%–30%	10%	20%
Culling/death rate in first 100 days of lactation	5%	3%–7%	4%	3%
Age at exit (years)	6.5	5.8–7.2	1.4	7.2
Age at exit by lactations	3.8	3.3–4.4	1.1	4.4
Percentage served by day 80	53%	41%–63%	22%	63%
Percentage conceived 100 days after calving	29%	18%–38%	20%	38%
Calving to first service interval (days)	87	77–108	31	77
Calving interval (days)	414	402–433	31	402
Age at first calving (years)	2.4	2.2–2.6	0.4	2.2
Conception rate	32%	26%–39%	13%	39%
Percentage service intervals at 18–24 days	32%	25%–38%	13%	39%
Percentage service intervals >50 days	26%	18%–36%	18%	18%
Percentage eligible for service that served	32%	23%–45%	22%	45%
Percentage eligible for service that conceived	11%	6%–15%	9%	15%
Lifetime milk/cow/day (kg)	11	9–13	4	13
Milk/cow/year (kg)	7769	6825–8752	1927	8752
Average milk protein percentage	3.24%	3.17%–3.31%	0.14%	3.31%
Average milk fat percentage	4.00%	3.87%–4.16%	0.29%	4.16%
305-day milk yield (kg)	7577	6727–8257	1530	8257

* The target is set at the level achieved by the best 25% of herds. Thus, depending on the index variable, it is either the first quartile or the third quartile.

- inseminated three or more times without becoming pregnant;
- served 35–60 days earlier (for pregnancy diagnosis).

Modern practice in the UK would anticipate visits every one or two weeks (depending on breeding season), and the post-partum period over which cows not seen in oestrus necessitating visits reduced to 42 days. Moreover, an additional item would be included; namely, visits to cows:

- not having received a first service by 63 days post-partum;

with pregnancy diagnosis undertaken from 30 days after being served.

Some veterinarians only conduct pregnancy diagnosis and give advice on management and nutrition, spurning the use of all treatments to influence the timing of oestrus and conception rate.

The introduction, by milk purchasers, of Quality Assurance schemes (Red Tractor Farm Assurance Dairy Scheme, 2013), which require a Herd Health Plan, recording of disease rates and trends, written Action Plans, and annual review of herd health and

performance by a veterinarian, has provided additional opportunities for improvements in health, welfare and productivity.

Decreasing important diseases

Mastitis is a major cause of loss of milk yield (Kossaiati and Esslemont, 1997; Kossaiati *et al.*, 1998). A recommended five-point control programme, recognizing the disease's various component causes, was developed by Kingwill and colleagues (1970). It includes:

- 1 the dipping of teats in a suitable disinfectant immediately after milking;
- 2 antibiotic therapy of clinical cases;
- 3 antibiotic therapy of all cows at 'drying off';
- 4 culling of recurrent cases;
- 5 improved general husbandry, including milking-machine maintenance.

This remains a useful and valid template, which has developed in its detail (Brand *et al.*, 1996; Erskine, 2001; Blowey and Edmondson, 2010; Green and Bradley, 2013).

Pedal lameness also is an important cause of reduced production, especially when chronic (Kossaibati and Esslemont, 1997; Barker *et al.*, 2010)⁹. 'Lameness' is a disease definition based on presenting signs (see Chapter 10), which result from a range of lesions including heel horn erosions, white line disease, sole bruising, sole ulceration, axial-wall fissure, digital dermatitis, interdigital necrobacillosis and interdigital hyperplasia (Atkinson, 2013). Component causes relate to host, agent and environment. Digital dermatitis, for example, depends on the presence of an infection; whereas pododermatitis has a genetic component associated with sires (Peterse and Antonisse, 1981) and therefore might be controlled partially by sire selection. Laminitis and the related conditions of white line disease and sole ulcer are caused primarily by lack of comfortable lying time (Weaver *et al.*, 2005). Control of lameness therefore depends on identification of the major lesions and manipulation of their component causes (Mahendran and Bell, 2015). The survey illustrated in Chapter 11 (*Figure 11.2*) was undertaken to identify the lesions associated with pedal lameness and their importance. Weaver and others (2005) describe hoof lameness and its control. Refaai and colleagues (2013) review infectious causes of lameness, focussing on digital dermatitis.

Optimum feeding

The correct nutritional balance is important in maintaining optimum milk yield, milk quality and fertility. The possibility of deficiencies or imbalances arising can be estimated in theory by feed analysis, but unrecognized variations in individual food intake within groups of cows can limit the value of conclusions. Blood analysis can be of value in diagnosing some mineral deficiencies (e.g., magnesium, selenium and copper), but failure to appreciate the relationships between dietary content, intake and blood levels can lead to the wrong conclusions.

A popular means of assessing the nutritional balance in dairy herds is the **metabolic profile**, initiated in the 1970s (Payne *et al.*, 1970). This involves estimating the levels of blood constituents that are important components of metabolism related to milk yield, health and fertility. Such constituents include some minerals, protein metabolites and measures of energy balance.

Much has been learned since the initial use of metabolic profiles in dairy cattle. It has become apparent

that planning of blood sampling according to feed changes and calving patterns is necessary, and that care must be taken when selecting cows 'typical' of the herd. Useful and sensible interpretation of biochemical results can only be carried out with access to background information on the cows' level of performance, stage of lactation and diet. With these caveats, some authors conclude that practical information can be obtained on the important aspects of the nutritional status of animals (Whitaker, 2000; Kelly and Whitaker, 2001; Kida, 2003).

Assessment of 'rumen health' also is required to determine how cows are utilizing their diet, as milk yields increase and animals therefore need to be fed energy-dense feedstuffs (Atkinson, 2009).

Pig health and productivity schemes

Intensively-reared pigs are housed in large units, sometimes with over 100 sows in a herd (see *Table 1.11*).

There are four areas of concern:

- 1 reproduction in the dry sow;
- 2 production in the suckling sow;
- 3 performance of the growing pig;
- 4 performance of the boar.

Different constraints operate in each area. The dry sow's reproductive potential is governed by the service programme (the rate of reproduction), by the fertility of the sow (the sow's efficiency) and by disease. Productivity of the suckling sow is a function of the farrowing rate, the number of piglets born alive, piglet mortality, diseases of the sow, and the sucking piglets' growth rate. Performance of the growing pig is affected by the type of feed, feed costs, food conversion efficiency, disease and mortality. Boar performance, which affects the reproductive capacity of the sow, is affected by the age of the boar, the boar:sow ratio, the frequency of mating, and the litter size and litter scatter¹⁰ attributable to the boar.

The basic variables that should be recorded to produce and regularly update the profile of a pig farm are listed in *Table 26.4*.

Targets

Table 26.5 lists target and interference levels (the latter based on experience) for dry sows, suckling sows, growing pigs and boars in an intensively-reared pig

⁹ The true extent of lameness is difficult to measure. In the UK, a recent estimate puts its incidence at an average of 50 'limb-cases' per 100 cow-years at risk (Archer *et al.*, 2010).

¹⁰ Litter scatter is the percentage of litters of eight or less.

Table 26.4 Recommended variables to be recorded in a pig herd. (From Deen *et al.*, 2001.)

Data recorded in the breeding herd
Sow identity
Birth date or date of entry
Service date and boar used
Abortion date
Farrowing date
Number born alive
Number born dead
Number mummified
Total litter weight at birth
Piglet deaths (recorded as they occur)
Weaning date and number weaned
Weight at weaning (total litter or individual pigs)
Weaner deaths (recorded as they occur)
Sow culling date
Feed consumed by breeding females and by piglets in nursery
Data recorded in the feeder-finishing unit
Identity of group
Date of entering nursery-feeder-finishing unit or date at which 30 kg liveweight reached
Group weight at entry
Date sold off farm
Weight at date of sale
Total amount of feed consumed to determine feed conversion ratio
Death losses (recorded as they occur)

herd. Targets again will vary according to management practices on a particular farm (Muirhead *et al.*, 2013). Alternatively, targets may be set with reference to the performance profile of a number of herds (Table 26.6), which provides a standard against which specific herds can be compared (a 'benchmark'). Deen and others (2001) discuss performance monitoring and target levels in detail.

Routine visits

A schedule for regular visits to a pig herd is recommended by Muirhead (1980). Table 26.7 lists components of monthly visits to a herd of 150–300 sows. Visits comprise a standard procedure and discussion of special topics. The standard procedure involves:

- pre-visit preparation, when previous reports, current investigations and clinical problems are studied;
- a clinical examination of the reproductive and service areas, dry sows, the farrowing and weaning areas, and fattening pigs;
- a discussion with the farmer.

Comprehensive examination of a pig herd is described by Muirhead and others (2013). General measures to prevent disease on a farm are described

by Douglas (1984); and Pritchard and others (2005) outline methods of impeding entry of disease onto farms. Prevention of specific diseases is detailed by Straw and others (2006).

Sheep health and productivity schemes

The major areas of concern in sheep health schemes, like schemes in other species, are reproductive efficiency, losses due to disease, and suboptimal production (which may be caused by subclinical disease).

Sheep schemes are designed primarily for lowland flocks. Examples include those developed in England (Hindson, 1982; Holland, 1984; Morgan and Tuppen, 1988; Scott, 2001; Lovatt, 2004; Spedding *et al.*, 2007; Winter and Clarkson, 2012), Australia (Bell, 1980; Morley *et al.*, 1983), New Zealand (McNeil *et al.*, 1984), The Netherlands (Konig, 1985) and Germany (Ganter, 2008). The potential for health plans to improve health, welfare and productivity is reviewed by Scott and others (2007). Kaler and Green (2013) review farmers' perceptions of flock health schemes, including barriers to their implementation; while Bellet and others (2015) report on veterinarians' perceptions of their ability to engage in preventive medicine on sheep farms.

An initial visit should be undertaken to generate a general farm profile (West *et al.*, 2009), including:

- location of property;
- elevation (flat, hill-country);
- soil type;
- a farm plan;
- livestock profile (Table 26.8).

A detailed history of management practices and levels of disease morbidity should also be documented.

Targets

Records of past performance of a flock enable the setting of suitable production targets. Examples of targets are given in Table 26.9. In common with other health schemes, accurate records must be kept so that productivity can be assessed and corrective action taken if shortfalls are detected.

Routine visits

Advisory visits are made at important stages of sheep management. In Britain, three to six visits per year are advised (Table 26.10).

Condition scoring, examination of udders, mouths, feet and legs, and assessment of fertility are important components of the health scheme. Rams should be assessed, to ensure that they are sound for breeding (Sargison, 2008). Nutrition at all stages, and for all

Table 26.5 Suggested targets and interference levels for an intensive pig unit in north-east England. (From Muirhead, 1978.)

<i>Index variable</i>	<i>Target</i>	<i>Interference level</i>	<i>Index variable</i>	<i>Target</i>	<i>Interference level</i>
(a) The dry sow			Piglet mortality (%):		
Average number of sows in herd	As determined	Fluctuation of 30% of target	Laid on	5	7
Average age	24 months	30 months	Congenital defects	0.5	1.5
Ratio of average number of maiden gilts:sows	1:15		Low viability	1.5	3
Service programme	As determined	>10% variation	Starvation	1	3
Weaning to service interval average	7 days	9 days	Scour	0.5	2
			Miscellaneous	3	5
			Pigs reared/sow/year	21	19
			Sow feed (tonnes/year)	1.1	1.2
			(c) The growing pig		
Normal repeat service (%)	5	8	Mortality (%)	2.5	3.5
Abnormal repeat service (%)	3	4	Feed conversion from weaning:		
Abortions (%)	1	2.5	Pork (60 kg)	2.7	2.9
Sows infertile not in pig (%)	<2	5	Cutter (80 kg)	2.9	3.2
Sow deaths (%)	2	3	Bacon (90 kg)	2.9	3.2
Sows culled due to disease (%)	<2	4	Heavy (115 kg)	3.6	3.8
Farrowing rate (%)	85–89	80	Feed cost/kg liveweight gain	Variable with feed costs	Comparative figures
Litters/sow/year*	2.25	2.0			
			(d) The boar		
(b) The farrowing/suckling sow			Average age	20 months	30 months
Number of piglets born alive/litter	10.9	10	Boar:sow ratio	1:20	1:30
Number of piglets born dead (%)	5	7	Matings/week	4	5
Number of mummified piglets (%)	0.5	1	Matings/sow	2	3
Number of piglets weaned/litter	9.6	9	Mating interval	12 hours (variable)	–
Deaths until weaning (%)	8–12	13	Boar litter scatter (%)	15	25
Litter scatter (%)†	10	18	Litter size	9.8–10.8	Obtained by boar comparison
			Conception to first service (%)	>90	85

* Assuming five-week weaning

† Litter of eight or less (see text for definition)

types, of production (maintenance, conception and pregnancy, lactation, wool growth and meat production) is important (Freer and Dove, 2002), and metabolic profiles, based on butyrate examination of several sheep (Russel, 1985), are valuable.

Specific disease problems should be identified during visits (Winter and Clarkson, 2012). In Britain,

problem diseases can include infection by orf, infectious abortion, mastitis, swayback, pregnancy toxæmia, mineral and trace element deficiencies, ecto-parasitism and endo-parasitism (Aitken, 2007) and lameness (Green, 2015). Infection by *Clostridium* spp., however, now is controlled effectively by routine vaccination.

Table 26.6 PigCHAMP breeding herd summary data, based on 355 farms in the US, 2012. (From PigCHAMP, 2013.)

<i>Index variable</i>	<i>Mean</i>	<i>Median</i>	<i>Upper 10%</i>	<i>Lower 10%</i>
Breeding performance				
Repeat services	310.74	219.00	709.00	65.00
Repeat services (%)	8.47	7.64	13.91	3.96
Total services	4 256.37	2 711.00	8 676.00	1 101.00
Farrowing performance				
Farrowings	3 638.17	2 226.00	7 397.00	862.00
Farrowing rate (%)	83.04	84.91	90.18	75.90
Total born	4 9031.47	28 708.00	108 742.00	11 230.00
Total born/litter	13.22	13.17	14.64	11.92
Total liveborn	4 3241.69	26 040.00	94 007.00	10 159.00
Liveborn/litter	11.78	11.79	12.76	10.91
Liveborn/female/year	26.11	26.60	29.29	22.39
Total stillborn	3 126.75	2 014.00	7 357.00	518.00
Stillborn/litter	0.86	0.85	1.27	0.50
Total mummified	1 331.52	556.00	3 014.00	43.00
Mummified/litter	0.29	0.24	0.56	0.04
Weaning performance				
Sows weaned	3 713.07	2 231.00	7 966.00	863.00
Piglets weaned	37 381.30	23 120.00	78 483.00	8 660.00
Piglets weaned/litter	10.52	10.56	11.21	9.76
Total losses of liveborn (%)	12.80	12.65	17.00	8.73
Average litter weaned weight (lb)	126.52	136.15	161.20	37.90
Piglet age at weaning (days)	20.51	20.55	22.09	18.74
Piglets weaned/sow/year	23.83	24.18	26.78	20.47
Pigs weaned/female/year	22.70	23.16	25.83	19.57
Population				
Total boars	8.74	3.00	12.00	0.00
Sows culled or sold	760.65	420.00	1 744.00	175
Cull/year (%)	45.43	44.49	68.38	31.40
Sows died	134.42	74.00	312.00	26.00
Sow deaths/year (%)	8.12	7.83	11.90	4.67
Total sows	1 534.59	954.00	3 130.00	392.00

Beef health and productivity schemes

The beef cattle industry comprises a range of production systems, including breeding (cow-calf, suckler) and fattening (feedlot) enterprises, which are managed under differing conditions. In the US, for example, breeding enterprises tend to utilize rangeland and pasture, whose only economic alternative use is recreation and wildlife. This contrasts with the UK, where beef cattle may be reared as a matter of choice¹¹.

¹¹ Mixed dairy and beef enterprises were common, but there is now a trend towards specialized enterprises.

In the US, beef health and productivity schemes are not common, but, when they do exist, focus on reproductive efficiency (Engelken *et al.*, 2007). In Europe, the economics of beef production have become so poor that beef health and productivity schemes hardly exist. Where routine veterinary attention is sought, it is usually confined to investigating possible fertility constraints when failure to conceive has resulted in an unacceptable adult-cow disposal rate. Nutritional causes of this are common, and monitoring through metabolic profile testing in late pregnancy and soon after calving, early enough in the following season, can allow preventive measures to be taken (Whitaker, 2000). Thus, although financial margins are small,

Table 26.7 A 12-monthly preventive medicine programme for a herd of 150–300 sows on monthly visits. (From Muirhead, 1980.)

Visit no.	Special topics
(1) Standard procedure*	Herd security
(2) Standard procedure	Stock introduction; methods of gene movement; herd-replacement policies; gilt selection requirements
(3) Standard procedure	Economic losses in the herd; feed costs and utilization
(4) Standard procedure	External/internal parasites; vermin control; vaccination programme
(5) Standard procedure	Fertility; boar management
(6) Standard procedure	Herd security check; farrowing-sow diseases; parturition, mastitis, etc.
(7) Standard procedure	Piglet problems
(8) Standard procedure	Diseases, mortality and management of the weaned and fattening pigs
(9) Standard procedure	Housing utilization; alterations; associated diseases
(10) Standard procedure	Man management, disease and productivity
(11) Standard procedure	Slaughterhouse monitoring; pathological tests
(12) Standard procedure	12-monthly appraisal and analysis

* See text for details.

programmes to maintain and enhance efficiency can be useful.

Targets

Beef health and productivity schemes are designed to attain optimum reproductive efficiency and to reduce morbidity and mortality in the stock that are being fattened. Traditionally, the number of calves reared is the sole measure of production. The production target of beef rearing is to produce one calf per cow per year. This objective is somewhat optimistic, requiring a breeding season restricted to six to nine weeks, a 95% pregnancy rate, and a 100% reared-calf crop during each year, none of which usually occurs. In practice, in the UK, a breeding season of 9–12 weeks is considered to be a reasonable target.

Targets depend on the type of production system. *Table 26.11* lists some variables that are suggested as

Table 26.8 An information sheet recording a farm profile, and illustrating some recorded variables in a lowland sheep flock health programme. (From Holland, 1984.)

Information sheet
(1) Rams – Nos. and breed(s)
– rams
ram lambs
(2) Ewes – Nos. and breed(s)
– ewes
– ewe lambs
(3) Ewes to tup
nos. and date
(4) Ewes lambed
(5) Lambs born
– alive
– dead
(6) Lamb deaths between birth and weaning
(7) Lambing commenced – Date
(8) Lambing completed – Date
(9) Ewes housed/housed at lambing/not housed
(10) Ration
(a) ewes
(b) lamb creep
(c) lamb fattener
(11) Lamb sales
(a) commenced
(c) completed
(12) Comments on:
(a) results
(b) problem areas
(13) Comments on mastitis in ewes
(a) known cases this year
(b) previous culling percentage
(14) Condition scoring fully practised and understood?

indices of reproduction and production, with target levels, for a North American cow-calf enterprise; whereas *Table 26.12* lists variables under two types of production system in the UK.

Table 26.9 Some suggested production targets for sheep flocks. (Reduced and modified from Scott, 2001. Reproduced with permission of Elsevier.)

<i>Index variable</i>	<i>Target</i>
Lambing rate	
Number of lambs born per ewe exposed to the ram per breeding period	1.5–2.00 (varies with breed and the purpose of the flock)
Oestrous cycling index	
Percentage of females bred in the first 17 days of breeding period	100%
Breeding index	
Percentage of females bred (marked by ram) per breeding period	100% (for a two-cycle breeding season)
Length of breeding period	35 days
Ram: ewe ratio	
Depends on the age of the ram, breeding environment (pen or field), ram behaviour, and whether synchronized oestrus is used	
• ram lamb	1:20
• yearling ram	1:25–30
• mature ram	1:40
Pregnancy rate	
Percentage of females pregnant per female exposed to the ram per breeding period	98%
Abortion rate	
Percentage of females that abort	<2%
Weaning rate	
Number of lambs weaned per female exposed to the ram per breeding period	1.5–1.7
Ewes weaning lambs	
Percentage of ewes that wean at least one lamb	95%
Average daily gain	
Measured from birth to weaning and adjusted for sex and multiple birth	0.3–0.5 kg/day (dependent on breed and management of the flock)
Age at first breeding/lambing	
Intensive systems	7–12 months
Extensive conditions	18 months
Culling rate	20%
Reasons for culling should be age, not chronic disease such as mastitis and footrot	
Ewe mortality rate	
Percentage of ewes that die annually	<2%
Perinatal lamb mortality rate	
Percentage of lambs born alive that die from birth to weaning	<5%
Stillbirth rate	
Percentage of lambs born dead	<2%

Routine visits

Routine visits should coincide with major events. Chenoweth and Sanderson (2001) suggest that, in breeding herds in North America, the reproductive year should be divided into four periods for spring-calving herds:

- 1 late winter–early spring;
- 2 late spring–early summer;

- 3 late summer–early autumn;
- 4 late autumn–early winter.

Relevant management procedures and prophylaxis are carried out, and advice is given, during each period. For example, in late winter to early spring, heifers are dewormed and vaccinated, and bulls checked for vibriosis; whereas in late spring and early summer,

Table 26.10 Plan of visits to monitor a sheep health programme. (Adapted from Clarkson and Winter, 1997.)

Visit	Timing of visit	Jobs
Visit 1*	Two months before tupping	1 Rams (all): Condition score, check fertility and feet. Ensure that the farmer/shepherd is able to condition score rams and ewes 2 Ewes (100 selected): Condition score, cull (teeth, age, udders) 3 Store lambs (a selection): Condition score 4 Take samples of blood and faeces for copper, B12, selenium, fluke and worms and abortion profile (<i>Toxoplasma</i> and enzootic) 5 Check hay or silage and take samples for fibre, protein and energy estimates 6 Inspect pasture, examine for snail habitats 7 Check and discuss Farm Questionnaire (sent and hopefully returned in advance) 8 Discuss 'clean grazing' 9 Discuss farmer's objectives and possible targets for production
Prepare draft programme following this visit and receipt of laboratory results		
Visit 2	Approximately one month later	Discuss draft programme with farmer and finalize programme Condition score 100 ewes Advise about feeding (including trace elements) Treat clinical cases
Visit 3*	Six weeks before lambing	Condition score 100 ewes Advise about housing facilities, colostrum, lambing and recording Treat clinical cases
Visit 4	Two weeks before lambing	Clinical events Recording of lambs Advise about hypothermia and starvation, <i>Escherichia coli</i> , coccidia and worming
Visit 5*	At peak lambing time	
Visit 6†	12 weeks after lambing	Check growth rates

*Visits in subsequent years.

†When more than one flock is involved with widely different lambing dates, more than six visits will be necessary, although it is probable that some can be combined. In addition to the six planned visits, advice will be available by telephone.

external parasites are controlled in cows, heifers and bulls, and calves are castrated, dehorned and vaccinated.

Beef health schemes are reviewed by Caldwell (1984) and discussed in detail by Chenoweth and Sanderson (2001) and Smith and others (2001).

National schemes

Some schemes operate at the national (and sometimes regional) level and may be subject to legislation (Rees and Davies, 1992). They are frequently part of national control and eradication campaigns (see Chapter 27) directed at diseases that the farmer cannot control alone (e.g., tuberculosis and brucellosis) or at zoonotic infections that are of greater significance to human, than to animal, health (e.g., avian salmonellosis). In Australia, for example, national schemes are available

for sheep to improve the control of the notifiable endemic diseases, paratuberculosis (Johne's disease) and pulmonary adenocarcinoma (jaagsiekte), and, in New South Wales, virulent footrot is notifiable and subject to an eradication scheme (OECD, 2012). Similarly, in The Netherlands, there is a national scheme to control bovine mastitis (Lam *et al.*, 2013). National schemes therefore may include both notifiable diseases and non-notifiable diseases that are of such significance that structured voluntary schemes to improve their control are desirable. Thus, they contrast with the private schemes, which are involved with problems that can be controlled by the stockowner on individual farms (e.g., bovine mastitis).

Accredited/attested herds

Herds or flocks can be voluntarily certified free from *specified* diseases; they are then described as

Table 26.11 Suggested reproductive performance and production variables for a beef enterprise in the US. (From Spire, 1990.)

<i>Index variable</i>	<i>Target</i>	<i>Interference level</i>
Reproduction		
Calf crop (%)	>90	<85
60-day pregnancy rate (%)	>95	<90
First 20-day pregnancy rate (%)	>65	<50
Herd		
Average cow age (years)	5–6	<4, >7
Body condition score		
Mid-gestation	4.5–6.0	<4
Calving	5–6	<4.5
Adult dystokia (%)	<5	>8
Heifer dystokia (%)	<15	>25
Losses during pregnancy (%)	<2	>3
Perinatal losses (%)	<5	>10
Annual cow loss (%)	<2	>5
Culling rate (%)	15–20	<10, >25

Table 26.12 Targets for beef suckler herds in the UK (continental sire).

<i>Index variable</i>	<i>Target: upland/ low-ground herds</i>		<i>Target: hill herds</i>	
	<i>Autumn calving</i>	<i>Spring calving</i>	<i>Autumn calving</i>	<i>Spring calving</i>
Calving period (max. days)	90	85	100	90
Calves weaned/ 100 cows bulled	92	92	90	90
Calf mortality	<5%	<5%	<5%	<5%
Barren cows	<6%	<6%	<8%	<8%
Weaning weight of calf (kg)	330	250	270	225

accredited or **attested** (Latin: *testis* = 'witness'). Such schemes include rules for testing animals and maintaining disease-free status. For example, in the UK, the Deer Health Scheme provides a pool of deer herds of tuberculosis-attested health status (ADAS, 1989). Herds must pass three successive tuberculin tests before they are accredited (see Table 20.22: 'Negative-herd retesting'), after which deer entering the herd must be isolated and tested. Disease status is monitored by regular periodic testing and compulsory post-mortem examination of

deer that die. Members of the scheme must identify all animals, maintain an approved herd record, and report all movements into the herd. Adequate security (e.g., boundary fences and walls) must also be maintained.

Accredited/attested herd schemes are frequently the initial component of eradication campaigns; for example, accreditation of herds was the first step in eradication of bovine virus diarrhoea in Scandinavia (Lindberg, 2003). Several European countries have voluntary bovine virus diarrhoea eradication schemes, with or without vaccination (depending on each country's regulations), although success depends on compliance of participating farmers (Lindberg *et al.*, 2006).

Voluntary schemes often are followed by compulsory test-and-slaughter programmes (usually when the disease has been voluntarily removed from 80–90% of herds). For example, the first attested herd scheme in the UK was introduced in 1935 as the initial stage of a national tuberculosis eradication campaign. Subsequently, a similar approach was adopted to eradicate brucellosis in the UK (MAFF, 1983) and US (Ragan, 2002). Incentives frequently are offered to increase participation (e.g., milk bonuses for participating dairy herds).

Health schemes

National health schemes establish a pool of herds or flocks of recognized health status. They may be directed at one or more *specific* diseases that may be of economic importance to the livestock industry, but may not currently be suitable candidates for eradication. Alternatively, they may have the *general* aim of improving health and productivity. A consequence is enhancement of the market potential of animals for national and international trade. National health schemes often are run by government veterinary services in collaboration with authorized laboratories and private practitioners.

Cattle Health Certification Standards (CHeCS) is a self-regulatory body for cattle health schemes in the UK, which applies a set of standards to which all licensed cattle schemes must adhere (Duncan, 2000). These standards ensure that herd health status in one scheme is equivalent to that in other schemes, and that schemes in the UK are compatible with those in other countries. Registered CHeCS schemes provide accreditation programmes for the monitoring, control and eradication of specific diseases (Table 26.13) and certification when a herd meets agreed national health standards. Various accredited government and private laboratories establish criteria, and

Table 26.13 Cattle Health Certification Standards accreditation in the UK. (From Statham, 2011a.)

Disease	How to achieve accreditation
Bovine virus diarrhoea	A check test is carried out on five calves from each management group (ages nine to 18 months). A second check test is carried out 12 months later. Accredited status is obtained if all results are negative
Johne's disease	Subject to herd history, two or three herd tests are carried out on all animals aged two years and older at intervals of 12 months. Accredited status is obtained if all results are negative
Infectious bovine rhinotracheitis	Two herd tests are carried out at an interval of between four weeks and 12 months. All animals aged 12 months and older, plus non-homebred younger animals, must be sampled. Accredited status is obtained if all results are negative
Leptospirosis	Two herd tests are carried out at an interval of between five and 12 months. All animals aged over 24 months, plus any stock aged 12–24 months that are intended for breeding, must be tested. Accredited status is obtained if all results are negative

provide facilities, for testing and accrediting herds (Statham, 2011a).

Milk-recording and commercial organizations also have developed schemes for managing multifactorial diseases such as mastitis, lameness, calf pneumonia and calf diarrhoea (Statham, 2011b).

Also in the UK, the Premium Sheep and Goat Health Schemes offer maedi-visna/caprine arthritis encephalitis and enzootic abortion accreditation, scrapie genotyping and monitoring, and screening for caseous lymphadenitis (SAC, 2014).

The British Pig Health Scheme and Wholsome Pigs Scotland both monitor the occurrence of lesions relating to pleurisy, pneumonic conditions, hepatic milk spots, papular dermatitis, tail damage and papular stomatitis at abattoirs (Sanchez-Vasquez *et al.*, 2011). Reports are sent to participating pigs farmers, providing benchmark information; and regular periodic reports provide comparisons with other members of the schemes.

The UK's Poultry Health Scheme is based on a European Council Directive governing trade in various species of poultry and hatching eggs in the

European Union (DEFRA, 2013). There are regular farm inspections to ensure that the required standards for membership (e.g., adequate biosecurity) are met, and premises are monitored routinely for freedom from infection with *Salmonella pullorum*, *S. gallinarum*, *S. arizonae*, *Mycoplasma gallisepticum* and *M. meleagridis* (depending on the species of poultry).

Companion-animal schemes

Companion-animal health schemes are concerned with disease prevention in the individual animal, rather than in a group (although several individuals may be involved in kennels and stables). Production is not relevant to companion-animal schemes. (The track performance of racehorses and greyhounds is loosely described as 'production' but the animals are usually more commercial than companion.) Schemes involve:

- routine examination of animals (Jones, 2006);
- prophylaxis, such as vaccination (Greene and Hall, 2012);
- routine therapy, including dosing with anthelmintics (AVA, 2013);
- advice on diet and management (Fascetti *et al.*, 2010; Hand *et al.*, 2010).

Table 26.14 outlines a health scheme for dogs in the US. This may require modification in other countries (e.g., heartworm prevention and rabies vaccination would be unnecessary in areas where the diseases were absent). Nowadays, companion animals may also be implanted with identity microchips (see Chapter 4). Health schemes for 'stable' canine populations (e.g., breeding kennels and multiple-pet households) and 'transient' populations (e.g., veterinary hospitals, pet shops and boarding kennels) are given by Hurley and Baldwin (2012). They include nutritional recommendations, vaccination, ectoparasite and endoparasite control, and the management of reproduction, environment and common diseases. Systematic record keeping is advised, to provide a kennel profile, facilitating the detection of deviations from normal breeding performance and expected patterns of morbidity and mortality.

A health scheme for cats in the US is listed in Table 26.15. This also can be modified to suit local conditions (e.g., omission of routine rabies vaccination in countries free from the disease). There are also preventive medicine schemes for feline populations (Foley, 2012).

Table 26.14 General outline of a health scheme for dogs in the US. (Adapted from Hoskins *et al.*, 2006.)

I First office visit for health programme (usually at six weeks of age) A Conduct a general physical examination and record body weight B Check for external parasites and dermatophytes and initiate appropriate therapy: 1 Fleas, ticks and ear mites (<i>Otodectes cyanotis</i>) 2 Mange mites, especially <i>Demodex canis</i> and <i>Sarcoptes scabiei</i> 3 Dermatophytes, particularly <i>Microsporum</i> spp. and <i>Trichophyton mentagrophytes</i> C Conduct faecal examination including both direct smear and flotation D Initiate administration of heartworm preventive management E Administer an anthelmintic for hookworms and roundworms and, if tapeworms are present, administer praziquantel or epsiprantel F Vaccinate against canine distemper/infectious canine hepatitis/leptospirosis/canine parainfluenza virus/canine parvovirus/canine coronavirus, and, possibly, against kennel cough (<i>Bordetella bronchiseptica</i>), canine Lyme borreliosis and <i>Giardia</i> G Advise on nutrition and routine grooming H Provide owner with client education pamphlets on topics such as the following: 1 Identification, treatment and control of fleas, ticks and ear mites 2 Benefits of preventive management for canine heartworm disease 3 Management of normal and abnormal puppy behaviours 4 Skin, nail and ear care 5 'How to' on grooming and nutrition I Complete the dog's health record for the owner	III Third office visit for health programme (usually at 12 weeks of age) A Conduct a general physical examination and record body weight B Check for external parasites and dermatophytes and initiate appropriate therapy: 1 Fleas, ticks and ear mites (<i>Otodectes cyanotis</i>) 2 Mange mites, especially <i>Demodex canis</i> and <i>Sarcoptes scabiei</i> 3 Dermatophytes, particularly <i>Microsporum</i> spp. and <i>Trichophyton mentagrophytes</i> C Conduct faecal examination including both direct smear and flotation D Adjust dosage of heartworm preventive according to body weight E Administer an anthelmintic for hookworms and roundworms and, if tapeworms are present, administer praziquantel or epsiprantel F Vaccinate against canine distemper/infectious canine hepatitis/leptospirosis/canine parainfluenza virus/canine parvovirus/canine coronavirus and rabies, possibly, against kennel cough (<i>Bordetella bronchiseptica</i>), canine Lyme borreliosis and <i>Giardia</i> G Adjust nutrition according to health needs and, if needed, change the grooming procedures H Provide owner with client education pamphlets on topics such as the following: 1 Identification, treatment and control of fleas, ticks and ear mites 2 Dental, skin, nail and ear care 3 'How to' on grooming and nutrition 4 Management of normal and abnormal puppy behaviours 5 Recommendations for spaying and castration 6 Exercise and its importance I Complete the dog's health record for the owner
II Second office visit for health programme (usually at nine weeks of age) A Conduct a general physical examination and record body weight B Check for external parasites and dermatophytes and initiate appropriate therapy: 1 Fleas, ticks and ear mites (<i>Otodectes cyanotis</i>) 2 Mange mites, especially <i>Demodex canis</i> and <i>Sarcoptes scabiei</i> 3 Dermatophytes, particularly <i>Microsporum</i> spp. and <i>Trichophyton mentagrophytes</i> C Conduct faecal examination including both direct smear and flotation D Adjust dosage of heartworm preventive according to body weight E Administer an anthelmintic for hookworms and roundworms and, if tapeworms are present, administer praziquantel or epsiprantel F Vaccinate against canine distemper/infectious canine hepatitis/leptospirosis/canine parainfluenza virus/canine parvovirus/canine coronavirus, and, possibly, against kennel cough (<i>Bordetella bronchiseptica</i>), canine Lyme borreliosis and <i>Giardia</i> G Adjust nutrition according to health needs and, if needed, change the grooming procedures H Provide owner with client education pamphlets on topics such as the following: 1 Identification, treatment and control of fleas, ticks and ear mites 2 Benefits of preventive management for canine heartworm disease 3 Dental, skin, nail and ear care 4 'How to' on grooming and nutrition 5 Management of normal and abnormal puppy behaviours I Complete the dog's health record for the owner	IV Subsequent visits for health programme (usually annual visits) A Conduct a general physical examination and record body weight B Check for external parasites and dermatophytes and initiate appropriate therapy: 1 Fleas, ticks and ear mites (<i>Otodectes cyanotis</i>) 2 Mange mites, especially <i>Demodex canis</i> and <i>Sarcoptes scabiei</i> 3 Dermatophytes, particularly <i>Microsporum</i> spp. and <i>Trichophyton mentagrophytes</i> C Conduct faecal flotation and occult heartworm examination, or all tests, for intestinal and heartworm infection screen D Adjust dosage of heartworm preventive according to body weight E Administer an anthelmintic according to faecal examination findings F Vaccinate against canine distemper/infectious canine hepatitis/leptospirosis/canine parainfluenza virus/canine parvovirus/canine coronavirus/rabies, and, possibly, against kennel cough (<i>Bordetella bronchiseptica</i>), canine Lyme borreliosis and <i>Giardia</i> G Adjust nutrition according to health needs and, if needed, change the grooming procedures H Provide owner with client education pamphlets on topics such as the following: 1 Identification, treatment and control of fleas, ticks and ear mites 2 Dental, skin, nail and ear care 3 'How to' on grooming and nutrition 4 Management of normal and abnormal puppy behaviours 5 Exercise and its importance I Complete the dog's health record for the owner

Table 26.15 General outline of a health scheme for cats in the US. (Adapted from Hoskins *et al.*, 2006.)

I First office visit for health programme (usually at 8–10 weeks of age) A Perform a general physical examination and record body weight B Check for external parasites and dermatophytes and initiate appropriate therapy for: 1 Fleas and ear mites (<i>Otodectes cyanotis</i>) 2 Mange mites, especially <i>Notoedres cati</i>, <i>Demodex</i> spp. and <i>Cheyletiella</i> spp. 3 Dermatophytes, particularly <i>Microsporum</i> spp. and <i>Trichophyton mentagrophytes</i> C Perform faecal examination, including both direct smear and flotation D Administer anthelmintics, such as pyrantel pamoate for roundworms and hookworms and praziquantel or epsiprantel for tapeworms (if present) E Vaccinate against feline viral rhinotracheitis/feline calicivirus/feline panleucopenia/<i>Chlamydia</i>/feline leukaemia/feline infectious peritonitis/<i>Bordetella</i>/<i>Giardia</i> F Advise on nutrition and routine grooming G Provide owner with client education pamphlets on topics such as: 1 Identification, treatment and control of fleas, ticks and ear mites 2 Benefits of vaccination against feline leukaemia 3 Management of normal and abnormal cat behaviour 4 Grooming and nutrition H Complete the cat's health record for the owner	E Vaccinate against feline viral rhinotracheitis/feline calicivirus/feline panleucopenia/<i>Chlamydia</i>/feline leukaemia/feline infectious peritonitis/<i>Bordetella</i>/<i>Giardia</i>/rabies F Adjust nutrition and grooming procedures G Provide owner with client education pamphlets on topics such as: 1 Identification, treatment and control of fleas, ticks and ear mites 2 Benefits of vaccination against feline leukaemia 3 Dental, skin, nail and ear care 4 Management of normal and abnormal cat behaviours 5 Exercise and its importance 6 Recommendations for spaying and castration H Complete the cat's health record for owner
II Second office visit for health programme (usually at 12–14 weeks of age) A Perform a general physical examination and record body weight B Check for external parasites and dermatophytes and initiate appropriate therapy for: 1 Fleas and ear mites (<i>Otodectes cyanotis</i>) 2 Mange mites, especially <i>Notoedres cati</i>, <i>Demodex</i> spp. and <i>Cheyletiella</i> spp. 3 Dermatophytes, particularly <i>Microsporum</i> spp. and <i>Trichophyton mentagrophytes</i> C Perform faecal examination, including both direct smear and flotation D Administer anthelmintics, such as pyrantel pamoate for roundworms and hookworms and praziquantel or epsiprantel for tapeworms (if present)	III Subsequent visits for health programme (usually annual visits) A Perform a general physical examination and record body weight B Check for external parasites and dermatophytes and initiate appropriate therapy for: 1 Fleas and ear mites (<i>Otodectes cyanotis</i>) 2 Mange mites, especially <i>Notoedres cati</i>, <i>Demodex</i> spp. and <i>Cheyletiella</i> spp. 3 Dermatophytes, particularly <i>Microsporum</i> spp. and <i>Trichophyton mentagrophytes</i> C Perform faecal examination (faecal flotation) D Administer an anthelmintic according to faecal examination findings E Vaccinate against feline viral rhinotracheitis/feline calicivirus/feline panleucopenia/<i>Chlamydia</i>/feline leukaemia/feline infectious peritonitis/<i>Bordetella</i>/<i>Giardia</i>/rabies F Adjust nutrition and grooming procedures G Provide owner with client education pamphlets on topics such as: 1 Identification, treatment and control of fleas, ticks and ear mites 2 Benefits of vaccination against feline leukaemia 3 Dental, skin, nail and ear care 4 Management of normal and abnormal cat behaviours 5 Exercise and its importance 6 Recommendations for spaying and castration H Complete the cat's health record for owner

Equine health schemes focus on vaccination, parasite control, care of the teeth and feet, and optimum nutrition (Williamson, 1995; Anon., 2007; Frappe, 2010; Reinemeyer and Nielsen, 2013). Stable hygiene is also important because infections can be readily spread by fomites such as water buckets, bits, trailers and clothing (Timoney *et al.*, 1988). Table 26.16 is an example of a health scheme from the US. Fraser (1969), Owen (1985) and Loving and Johnston (1995) describe equine schedules in the UK, and

Verberne and Mirck (1976) list a programme for use in The Netherlands. Again, the programmes may need to be modified to suit local management practices and the preference of the veterinarian and owner.

Health schemes are also described for rabbits (Sayers, 2010), psittacine birds (Romagnano, 1999) and ferrets (Ivey and Morrissey, 1999).

Uptake of companion-animal schemes by owners can be poor, but can be improved by formal procedures such as 'compliance gap analysis' (Wilson, 2014).

Table 26.16 General outline of a health scheme for horses in the US. (Adapted from Hoskins *et al.*, 2006; and Hammer *et al.*, 2014.)

First quarter: January–March	
<i>All horses</i>	Deworm at least every eight weeks, or obtain faecal egg count and deworm horses with egg counts greater than 150 eggs/g. Exercise care in choice of anthelmintics for mares in the third trimester. Begin deworming foals at two months of age. Trim feet every six weeks, and more frequently in foals requiring limb correction. Dentistry: check twice yearly and float teeth as needed. Remove wolf teeth and retained caps in two-, three- and four-year-old thoroughbreds. Immunize for respiratory disease: influenza, strangles and rhinopneumonitis. In south-eastern US immunize for equine encephalitis
<i>Stallions</i>	Perform complete breeding examination. Maintain stallions under lights if being used for early breeding
<i>Pregnant mares</i>	Immunize with tetanus toxoid and open sutured mares 30 days prepartum. Develop a colostrum bank. Ninth-day breeding only for mares with normal foaling history and normal reproductive tract. Wash udders of foaling mares
<i>Non-pregnant (open) mares</i>	Maintain under lights if being used for early breeding. Perform daily teasing. Perform reproductive tract examination during oestrus. Mares should not be too fat but in gaining condition during breeding season
<i>Newborn foals</i>	Dip navel in disinfectant. Carefully, give a cleansing enema at birth. Administer tetanus prophylaxis if indicated by history. Perform immunoglobulin test at 12–24 hours
Second quarter: April–June	
<i>All horses</i>	Deworm at least every eight weeks, or obtain faecal egg count and deworm horses with egg counts greater than 150 eggs/g. Trim feet every six weeks. Do not forget the foals and yearlings. Dentistry: check teeth and remove or float teeth as needed. Immunize for equine encephalomyelitis. Administer appropriate vaccine boosters
<i>Stallions</i>	Maintain an exercise programme. Monitor the semen quality
<i>Broodmares</i>	Palpate at 21, 42 and 60 days after successful breeding
<i>Foals</i>	Creep-feed the foals, and provide free-choice minerals. Group foals by sex and size when weaned
Third quarter: July–September	
<i>All horses</i>	Deworm at least every eight weeks, or obtain faecal egg count and deworm horses with egg counts greater than 150 eggs/g. Clip and sweep the pastures. Trim feet every six weeks. Continue corrective trimming on foals. Dentistry: check teeth and remove or float teeth as needed
<i>Stallions</i>	Maintain an exercise programme
<i>Broodmares</i>	Administer rhinopneumonitis boosters to pregnant mares according to manufacturer's labelled directions. Administer appropriate vaccine boosters to foals and yearlings. Check condition of mare's udder at weaning and reduce amount of feed given until milk flow is reduced
<i>Foals</i>	Administer all appropriate immunizations. Provide free-choice minerals. Maintain a protein supplement in creep feeders
Fourth quarter: October–December	
<i>All horses</i>	Deworm at least every eight weeks. Deworm to treat tapeworms and bots. Trim feet every six weeks. Continue corrective trimming on foals. Dentistry: check teeth and remove or float teeth as needed
<i>Stallions</i>	Continue exercise programme. Check immunizations. Perform breeding examination
<i>Broodmares</i>	Confirm pregnancy. Begin treating open mares. Check immunizations

Further reading

- Abbott, K.A., Taylor, M. and Stubbings, L.A. (2012) *Sustainable Worm Control Strategies for Sheep*, 4th edn. SCOPS, Malvern
- Boersema, S.-J., Cannas da Silva, J., Mee, J. and Noordhuizen, J. (2010) *Farm Health and Productivity Management of Young Dairy Stock*. Wageningen Academic Publishers, Wageningen
- Borsberry, S., Barrett, D.C. and Boyd, H. (2004) Herd fertility management. In: *Bovine Medicine: Diseases and Husbandry of Cattle*, 2nd edn. Ed. Andrews, A.H., pp. 652–677. Blackwell Science, Oxford. (A discussion of fertility management in beef and dairy cattle)
- Brand, A., Noordhuizen, J.P.T.M. and Schukken, Y.H. (1996) *Herd Health and Production Management in Dairy Practice*. Wageningen Pers, Wageningen
- Burr, P. (2004) Health schemes: are they only for the minority? *Cattle Practice*, **12**, 255–257
- Ellis, A.K. and McCommon, G.W. (2018) Preventive health programs. In: *McCurnin's Clinical Textbook for Veterinary Technicians*, 9th edn. Eds Basset, J.M., Beal, A.D. and Samples, O.M., pp. 241–273. Elsevier, St Louis
- Esslemont, R.J., Bailie, J.H. and Cooper, M.J. (1985) *Fertility Management in Dairy Cattle*. Collins Professional and Technical Books, London
- Fetrow, J., Stewart, S., Eicker, S. and Rapnicki, P. (2007) Reproductive health programs for dairy herds: analysis of records for assessment of reproductive performance. In: *Current Therapy in Large Animal Theriogenology*, 2nd edn. Eds Youngquist, R.S. and Threlfall, W.R., pp. 473–489. Saunders-Elsevier, St Louis
- Gascoigne, E. (2016) Sheep flock health planning and the role of sheep veterinary surgeons. *Livestock*, **21**, 370–374
- Green, M. (Ed.) (2012) *Dairy Herd Health*. CABI, Wallingford
- Hand, M.S., Thatcher, C.D., Remillard, R.L., Roudebush, P. and Novotny, B.J. (Eds) (2010) *Small Animal Clinical Nutrition*, 5th edn. Mark Morris Institute, Topeka. (Includes dietary advice for small animals)
- Hill, F.W.G., Brand, A. and Schukken, Y.H. (1990) Protocols for herd health and productivity control in dairy herds: young dairy stock. In: *The Veterinary Annual*, Vol. **30**. Eds Grunsell, C.S.G. and Raw, M.-E., pp. 23–33. Wright, London
- Lovatt, F. (2004) Developing flock health plans. *In Practice*, **26**, 290–295
- Moss, R. (Ed.) (1992) *Livestock Health and Welfare*. Longman Scientific and Technical, Harlow
- OECD (2012) *Livestock Diseases: Prevention, Control and Compensation Schemes*. OECD Publications, Paris. (Includes examples of national disease control schemes throughout the world)
- Radostits, O.M. (Ed.) (2001) *Herd Health: Food Animal Production Medicine*, 3rd edn. W.B. Saunders, Philadelphia
- Robertson, J. (2006) Pig herd health plans – an active tool to monitor and manage UK pig herd health. *The Pig Journal*, **58**, 45–54
- Ruegg, P.M. (Ed.) (2006) Barnyard epidemiology and performance assessment. *Veterinary Clinics of North America: Food Animal Practice*, **22** (1), 1–270
- Sibley, R. (2000) Planning health care on dairy farms. *In Practice*, **22**, 405–407
- Sibley, R. (2006) Developing health plans for the dairy herd. *In Practice*, **28**, 114–121
- Smith, M.C. and Sherman, D.M. (2009) *Goat Medicine*, 2nd edn. Wiley-Blackwell, Ames

27

The control and eradication of disease

The preceding chapters have outlined the scope of veterinary epidemiology. In reiteration: epidemiology is the study of disease and factors affecting its distribution in populations. This study involves the **description** and **analysis** of disease in groups of animals using three related and overlapping techniques:

1. observation and recording of the natural occurrence of disease and its determinants, and presentation of the recorded observations;
2. statistical analysis (including statistical modelling) of the observations;
3. mathematical modelling.

These three techniques are used in conjunction with clinical, pathological and, sometimes, experimental investigations to:

- estimate disease morbidity and mortality;
- elucidate the cause of disease;
- understand the ecology of disease (transmission, maintenance and nidality);
- investigate the efficiency of different techniques of disease control.

This final chapter outlines the ways in which the results of epidemiological investigations are applied to the **control** and **eradication** of disease¹.

Definition of 'control' and 'eradication'

Control

'Control' is the reduction of the morbidity and mortality from disease, and is a general term embracing all measures intended to interfere with the unrestrained occurrence of disease, whatever its cause (Done, 1985). It is an ongoing process (Porta, 2014).

¹ Such results may be required to justify disease-control options in **evidence-based policy briefs**, which may be either **advocacy briefs** (arguing in favour of a particular course of action) or **objective briefs** (giving balanced information, enabling the policy maker to make up his or her mind). The construction of these briefs is detailed by *The Sure Collaboration* (2015).

Control can be achieved by **treating** diseased animals, which therefore reduces disease prevalence, and by **preventing**² disease, which therefore reduces both incidence and prevalence. Veterinary medicine, like human medicine, developed as a healing art concerned with the treatment of sick individuals. This approach continues, with the improvement of medical and surgical skills. However, prevention is an increasingly important part of disease control, being better than cure on humanitarian and, frequently, economic grounds.

Eradication

The term 'eradication' was first applied in the 19th century to the regional elimination of *infectious* diseases of animals; notably, Texas fever from cattle in the US, and pleuropneumonia, glanders and rabies from European animals. Since then, the term has been used in four different senses.

First, it has been used to mean the *extinction of an infectious agent* (Cockburn, 1963); eradication has not been completed if a single infectious agent survives anywhere in nature. According to this definition, very few diseases have been eradicated; smallpox and

² Three types of prevention sometimes are distinguished (Caplan and Grunebaum, 1967): **primary**, which modifies determinants to prevent or postpone new cases of disease, and therefore reduces incidence; **secondary**, which detects and treats disease promptly to shorten disease duration or to prolong life; and **tertiary**, which treats long-term cases to reduce dysfunction or to prolong life; (i.e., to make outcome less severe). The last type can relate closely to **palliative care**, which is an approach that improves the quality of life of patients and their families facing problems associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and assessment and treatment of pain and other problems (physical and – in humans – psychosocial and spiritual). It is therefore a component of, but is not restricted to, end-of-life care, and is relevant to both human and veterinary medicine (Lindley and Watson, 2010; Shearer, 2011). In this book, prevention is synonymous with primary prevention, whereas treatment encompasses secondary and tertiary prevention.

rinderpest are examples of those that have (Fenner *et al.*, 1988; FAO/OIE, 2011)³.

Secondly, eradication has been defined as the *reduction of infectious disease prevalence in a specified area to a level at which transmission does not occur* (Andrews and Langmuir, 1963). For instance, in local areas in northern Nigeria, trypanosomiasis can be 'eradicated' by clearing the vector (the tsetse fly) from riverine areas (Jordan, 1986).

Thirdly, eradication has been defined as the *reduction of infectious disease prevalence to a level at which the disease ceases to be a major health problem, although some transmission may still take place* (Maslakov, 1968).

Fourthly, and most commonly in veterinary medicine, eradication refers to the *regional extinction of an infectious agent*. For example, since the eradication of foot-and-mouth disease in the UK following the epidemic in 2001, no foot-and-mouth disease virus particles are believed to be present in the country (apart from laboratory stocks⁴). Disease may be accidentally reintroduced into regions from which it has been previously eradicated (e.g., foot-and-mouth disease into European countries in 2001: see *Table 1.1*), in which circumstance it is usually subjected rapidly to another eradication programme, to regain disease-free status. For further examples, see *Table 1.4*.

Eradication involves a *time-limited* campaign (Yekutieli, 1980).

Elimination The intermediate concept of elimination (Latin: *ex* = 'out of'; *limen, liminis* = 'threshold') also can be identified. This is reduction in the incidence of infectious disease in a defined area below the level achieved by control, so that either very few or no cases occur, although the infectious agent may be allowed to persist (Payne, 1963; Spinu and Biberi-Moroianu, 1969). It can refer to either a specified disease or a specified infection. A stricter definition requires that the incidence of the disease or infection is reduced to zero, although continued intervention measures are required (Dowdle, 1998). Human measles and poliomyelitis are examples.

Strategies of control and eradication

Doing nothing

In some circumstances, the natural history of a disease is such that the incidence of the disease is reduced by

doing nothing. This is not strictly a technique of control, but illustrates that the incidence of disease may be reduced by natural changes in host/parasite relationships without the intervention of humans. Thus, bluetongue does not commonly occur in Northern Europe in winter because the vector (*Culicoides* spp.) of the causal virus is inactive then (Wilson *et al.*, 2008). Similarly, the incidence of trypanosomiasis in the dry savannah regions of Nigeria is reduced during the dry season when the tsetse fly is absent.

Quarantine

Quarantine is the isolation of animals that are either infected or suspected of being so, or of non-infected animals that are at risk. Quarantine is an old method of disease control (see Chapter 1) that is still very valuable. It is used to isolate animals when they are imported from countries where transboundary diseases are endemic; for example, the compulsory quarantine of dogs, cats and zoo animals when they are imported to some countries to prevent the introduction of rabies⁵. It also is used to isolate animals suspected of being infected, until infection is either confirmed or discounted by clinical examination or laboratory testing (e.g., cows suspected of being infected with *Brucella abortus*). Similarly, when an infectious agent is not spreading within a herd or flock, quarantine may be adopted as part of a control campaign. Ultimately, eradication can be achieved by the gradual removal of affected animals; for example, enzootic bovine leucosis (MAFF, 1981) appeared in the UK in 1978 and was eradicated by 1996 using a policy that included within-herd quarantine of seropositive individuals, which were gradually culled when economically expedient. Quarantine is commonly applied in human medicine during epidemics to isolate infected from susceptible individuals⁶.

The period of quarantine depends on the incubation period of the agent, the time taken for the infection to be confirmed, and the time taken for an infected

³ A stricter definition of extinction also includes absence from laboratory stocks, as well as in nature (Dowdle, 1998); there are no such examples.

⁴ A much smaller outbreak in 2007 was the result of leakage from a laboratory facility (Anderson, 2008).

⁵ Some regions have abandoned quarantine in favour of a policy that allows freer movement of animals (although unnecessary journeys are eschewed). Thus, in the European Union, dogs and cats can travel between countries, subject to identity microchipping, vaccination against rabies, serological sampling to assess antibody status, and treatment against selected parasites to reduce the risk of transmission of zoonotic diseases (the 'PETS' travel scheme: Fooks *et al.*, 2002; Feeney, 2004).

⁶ It can be applied overzealously, witnessed by the isolation of symptomless health workers returning from regions in Africa affected by Ebola virus (Drazen *et al.*, 2014).

animal to become non-infectious (either with or without treatment)⁷.

Slaughter

The productivity of animals usually is decreased when they are chronically diseased. If a disease is infectious, affected animals can be a source of infection to others. In such circumstances it may be economically and technically expedient to slaughter the affected animals. For example, clinical bovine mastitis can produce a 15% drop in the milk yield of dairy cattle (Parsons, 1982); cows that have three or more bouts of mastitis per lactation therefore are often culled.

In eradication campaigns, infected animals may be slaughtered to remove sources of infection. Thus, in some countries, all cloven-hoofed animals in infected herds or flocks are slaughtered during foot-and-mouth disease epidemics. Wild animals also may be slaughtered to reduce the spread of infection; for example, by increasing the intensity of hunting to control the spread of African swine fever in wild boar (EFSA, 2015).

Eradication of specific diseases from herds often involves a **test-and-removal** strategy, in which all animals are tested, and only those testing positive are removed and slaughtered. Examples include bovine tuberculosis (see Chapter 20) and porcine reproductive and respiratory syndrome (Dee *et al.*, 2000).

Pre-emptive slaughter Culling of infected animals during epidemics often is accompanied by the slaughter of animals that may have been exposed to infection and therefore be at risk of developing disease; for example, those that have had direct contact with people or vehicles that have been in close proximity to infected animals. Although clinical signs of disease might not be present, these animals are removed to pre-empt the possible development of disease, and therefore reduce the risk of further outbreaks.

The **blanket culling** of animals on premises immediately surrounding an infected farm (**contiguous culling**), notably for the control of foot-and-mouth disease, is a particular category of pre-emptive culling that also aims to 'get ahead' of the disease before clinical signs occur. However, this is a contentious policy (Kitching *et al.*, 2005; Mansley *et al.*, 2011): although sometimes justified by mathematical models (Ferguson *et al.*, 2001), field studies have failed to identify a beneficial effect (Honhold *et al.*, 2004a; Thrusfield *et al.*, 2005a). It has been described as a 'blunt

instrument' (Anderson, 2002), involving the slaughter of a large proportion of healthy animals.

Slaughter of animals in epidemics is usually accompanied by other procedures designed to reduce the risk of transmission (e.g., disinfection, and destruction of carcasses by burning or burial); this is termed '**stamping out**' (see Appendix I and Geering *et al.*, 2001)⁸. These two procedures can be laborious (see Chapter 6, footnote 17, for the disinfection protocol used in the Scottish 2001 foot-and-mouth disease epidemic, for instance), fraught with practical problems (e.g., if widespread sub-surface aquifers supplying drinking water limit the areas over which carcasses can be buried: Thrusfield *et al.*, 2005b), and costly (e.g., approximately £710 000 000 in the 2001 UK foot-and-mouth disease epidemic: Thompson *et al.*, 2002).

Vaccination

Vaccines can confer immunity not only to many bacteria and viruses, but also to some helminths. They are used to *prevent* disease, frequently *routinely*, at regular intervals; for example, canine distemper vaccination.

Strategic vaccination Vaccination may be deployed **strategically** to prevent incursion of disease from endemic areas, and the spread of disease when epidemics occur; this is **emergency vaccination** (Barnett *et al.*, 2002). There are several strategies, dictated by different circumstances. First, animals in an area surrounding an infected region are vaccinated to provide a circumjacent barrier against spread of infection; this is **ring vaccination**. For instance, in the 1950s and 1960s, rinderpest was endemic in the Karamoja region of north-eastern Uganda but was absent from other parts of the country. A 20-mile-deep ring of cattle around the region was vaccinated to prevent transmission to disease-free areas. Similarly, ring vaccination is an option if an outbreak of foot-and-mouth disease occurs in the European Union (Donaldson and Doel, 1992; Toma *et al.*, 2002) or the US (Hagerman *et al.*, 2012). More recently, ring vaccination has been shown to be effective in controlling human Ebola virus infections (Henao-Restrepo *et al.*, 2015).

Secondly, a barrier, not completely circumscribing an infected region, may be created (**barrier vaccination**, **frontier vaccination**, **immune-belt vaccination**). Thus, in 1954, the *European Commission for the Control of Foot-and-Mouth Disease* established a buffer zone of vaccination in European Turkey (Thrace),

⁷ The word is derived from the Italian '*quaranta*', meaning 40: the number of days for which humans formerly were isolated.

⁸ The procedure dates back to Lancisi's control of rinderpest in 18th-century Rome (see Chapter 1).

Bulgaria and Greece to prevent entry into Europe of exotic strains of foot-and-mouth disease virus from Asiatic Turkey. The zone was maintained until 1991, when vaccination ceased in Europe (Kitching, 1998; Leforban and Gerbier, 2003).

Thirdly, vaccination can be practised both within and around a region where an outbreak occurs; this strategy (which includes ring vaccination) is **suppressive** or **dampening-down vaccination**. Examples were seen in the foot-and-mouth disease epidemic in The Netherlands in 2001 (Bouma *et al.*, 2003) and during outbreaks of bluetongue in the UK in 2008 (DEFRA, 2010).

These three strategies are applied in countries that do not routinely vaccinate. Additionally, vaccination may be undertaken in countries that do routinely vaccinate, either to boost immunity following an outbreak, or to provide immunity against novel serotypes of virus.

Emergency vaccines are generally manufactured to a higher potency than routine vaccines. The effectiveness of emergency vaccination programmes is inversely correlated with the time span between diagnosis of the index case and implementation of vaccination (Capua and Marangon, 2005). The vaccination strategy therefore is facilitated by preparatory stockpiling of vaccines in vaccine banks (Forman and Garland, 2002) and may need to be supplemented by post-

vaccination serological surveys to ensure that pockets of infection do not remain (Paton *et al.*, 2014).

Inactivated (killed) and live vaccines Vaccines may be **inactivated** (e.g., by alkylating agents, which render bacterial and virus nucleic acid incapable of replication: Dermer and Ham, 1969). Alternatively, they may be **live**, and the organisms usually attenuated. Each type of vaccine has advantages and disadvantages (Table 27.1). Inactivated vaccines are safer than live vaccines and can be produced more quickly when new agents or serotypes are discovered. However, they cost more than live vaccines, and stimulate mucosal and cell-mediated immunity less quickly and less effectively; therefore, 'booster' doses are frequently required. On the other hand, there is a danger that the live immunizing agent might revert to a virulent form. Also, it may be difficult to differentiate serologically between infections due to live vaccinal strains of agent and to natural 'field' strains (e.g., classical swine fever and avian influenza: Uttenthal *et al.*, 2010; also see 'Vaccinate or slaughter?' later in this chapter).

Natural immunization When animals are exposed to a low level of challenge from agents in the environment, natural immunization can occur. This may be widespread in the young when exposed to infections while still protected by maternal antibodies (Zinkernagel,

Table 27.1 Advantages and disadvantages of live versus killed conventional vaccines. (Adapted and expanded from Babiuk, 2002. Reproduced with permission from Elsevier.)

	<i>Advantages</i>	<i>Disadvantages</i>
Live vaccines	Mode of action similar to natural infection Multiply in host; induce range of immune responses Duration of immunity usually long-lasting No adverse side-effects to foreign protein Cell-mediated immunity good Promote cellular and humoral memory Single doses usually required	Possible reversion to virulence Possible contaminating viruses Interference by other agents and passive antibody Storage problems/shorter shelf-life Possible production of latency Possible induction of abortion Variable safety in immunocompromised individuals Higher development costs
Killed vaccines	Virulent microbes can be used Excellent safety in immunodeficient individuals Quite stable/longer shelf-life Easy to produce Lower development costs	Require large amounts of antigen/may not contain protective antigens if these are secreted proteins Reactions can develop to foreign protein or adjuvants Immunity is usually short-lived; multiple doses required Do not produce local immunity May not inactivate all of the agent Cell-mediated immunity poor Other agents that are resistant to inactivating agent may be present (e.g., prions) May induce aberrant disease

Table 27.2 The basic reproductive number, R_0 (effective reproductive number, $R_{(t)}$) and critical vaccination threshold, p_c , for some infectious diseases.

Infection	Species	R_0 ($R_{(t)}$)	Critical vaccination threshold	
			p_c (%): $1 - 1/R_0$ ($1 - 1/R_{(t)}$) $\times 100$	Source
Contagious bovine pleuropneumonia	Ox	3.2–4.6	69–78	Mariner <i>et al.</i> (2006)
Measles	Human	31	97	van Boven <i>et al.</i> (2010)
Mumps	Human	2.5	64*	van Boven <i>et al.</i> (2013)
Peste des petits ruminants	Sheep and goat	(3.3–6.5)	(70–85)	Kivara <i>et al.</i> (2013)
Rabies	Dog	1.1–1.7	9–41	Hampson <i>et al.</i> (2009)
Rinderpest (globally eradicated)	Ox	1.5–4.6	33–78	Roeder <i>et al.</i> (2013)
Rubella	Human	3.0–9.8	67–90	Sficas <i>et al.</i> (2007)
Smallpox (globally eradicated)	Human	3.5–6.0	71–83	Gani and Leach (2001)

* Corrected for imperfect vaccinal efficacy.

2003). This mechanism has been enhanced in pigs by feeding faeces back to pregnant animals, a technique called 'feedback'. Immunity to pathogens, for example porcine transmissible gastroenteritis virus and parvovirus (Stein *et al.*, 1982), thus may be increased and passed passively to the piglets via colostrum after birth. Although this may be of value when no other means of control is available, there are dangers (Porter, 1979): some agents, such as enteropathogenic *Escherichia coli* can increase in virulence when passaged *in vivo*.

New vaccine technology Modern biotechnology has facilitated new techniques for identifying immunologically-active antigens and presenting antigens to the host (Wray and Woodward, 1990; Biggs, 1993; Bourne, 1993; Pastoret *et al.*, 1997; Schultz, 1999; Brun *et al.*, 2011). The former include the cloning of T cells specific to small areas of stimulating antigens, and identification of parts of an infectious agent's proteins that are responsible for stimulation of the immune response (epitopes). The latter include attenuated mutants produced by deletion of genes responsible for pathogenicity (e.g., Aujeszky's disease vaccine produced by deletion of the thymidine kinase gene), and 'vectored vaccines': attenuated virus and bacterial vectors that also carry immunizing antigens derived from other infectious agents. Vectors under development include vaccinia virus, fowlpox virus, Newcastle disease virus, turkey herpesvirus, adenoviruses, *E. coli* and *Salmonella* spp., and plasmid DNA to encode *Mycobacterium tuberculosis* antigens (Vordermeier *et al.*, 2000; Buddle *et al.*, 2002; Draper and Heeney, 2010; Ertl, 2016), subsequently boosted by recombinant protein (Vordermeier *et al.*, 2003). There are also potential alternatives to conventional whole

inactivated vaccines, including synthetic epitopes (produced either chemically or in prokaryotic systems such as *E. coli*) and synthetic virus capsids (Ludi and Rodriguez, 2013).

Vaccine coverage A proportion of a population needs to be vaccinated to eradicate an infection. This is the **critical vaccination threshold (critical vaccination fraction: Halloran *et al.*, 2010)** (more generally, the **herd immunity threshold**), which is related to the basic reproductive number, R_0 , and the effective reproductive number, $R_{(t)}$ (see Chapter 8). Table 27.2 lists the critical vaccination threshold, p_c , (generally assuming that the vaccine is perfect and confers lifelong protection: Scherer and McLean, 2002) for some infectious diseases, and illustrates that these are specific to each pathogen⁹. Low values of R_0 therefore generally provide less of a challenge to eradication than high values.

Vaccination of dynamic populations, in which new susceptible animals are added by birth, and animals are lost by death, requires modification of vaccination coverage. Thus, for example, for annual vaccination coverage, p_a :

$$p_a = \frac{p_c}{e^{-(d+r)}}$$

⁹ If vaccination does not confer solid immunity against infection in all recipients then the threshold level of vaccination required to protect a population increases. If vaccination protects only a proportion, E , among those vaccinated (E standing for effectiveness against infection transmission in the field) then the critical vaccination coverage level should be $(1 - 1/R_0)/E$ (Fine *et al.*, 2011). Thus, if E is less than $1 - 1/R_0$, it would be impossible to eliminate an infection, even by vaccinating the whole population.

Table 27.3 Vaccination strategies, objectives, time frame, critical success factors and alternative/complementary control measures for highly-pathogenic avian influenza. (From Hinrichs *et al.*, 2010.)

Vaccination strategy	Objective	Time frame	Critical factor	Alternatives
Preventive	Protect individual/specific flocks/birds	Variable, depending on risk of exposure to infectious virus	Accuracy of the exposure risk assessment	Improve biosecurity, limit contact to secure sources
Emergency	Curtail potential of an acute epidemic after virus introduction	Short-term	Time to achieve immunity	Movement control and pre-emptive depopulation
Routine	Reduction in mortality/production losses in endemic situations; in the longer term may facilitate eradication of highly pathogenic avian influenza virus in domestic poultry	Medium-term to long-term	Effective immunization coverage (reduction of between-flock R_0 to <1)	Passive and active surveillance with rapid 'stamping out'
	Reduction of human health risk		Reduction in virus shedding by infected birds	Behavioural change reducing human exposure

where:

d = annual death rate;

r = annual birth rate;

e = base of natural logarithms.

Further modification also is required to incorporate duration of vaccine-induced immunity and the interval between vaccination (Scherer and McLean, 2002; Hampson *et al.*, 2009). Additionally, strategies for vaccination of wildlife may need to consider migration patterns and the distribution of natural barriers such as rivers (Russell *et al.*, 2006), and risks to endangered wildlife (Haydon *et al.*, 2006).

Table 27.3 lists vaccination strategies and alternative approaches to disease control, with particular reference to highly pathogenic avian influenza. This indicates that vaccination is only one of a range of strategies that can be combined to effect disease control.

Strategies are discussed in detail by Biggs (1982). The potential and application of vaccination for the control of several parasitic diseases, such as East Coast fever, fascioliasis, echinococcosis, haemonchosis and trypanosomiasis, are described by Urquhart (1980), Willetts and Cobon (1993), Lightowlers (1996), Meeusen and Maddox (1999), Smith (1999), Dalton and Mulcahy (2001) and Hewitson and Maizels (2014).

Therapeutic and prophylactic chemotherapy

Antibiotics, anthelmintics, other drugs and hyperimmune serum are used (therapeutically) to treat diseases, and are administered (prophylactically) at times of high risk to prevent disease and thus to

increase productivity¹⁰. Examples include the pre-operative and post-operative use of antibiotics to prevent bacterial infections, and the addition of antibiotics to livestock feed to promote growth (a declining practice)¹¹. These procedures can have undesirable consequences; notably, the selection of strains of bacteria that are resistant to the antibiotic to which they have been exposed. Furthermore, bacterial resistance can be transferred between bacteria (see Chapter 5).

Sometimes, chemotherapy can be used to eradicate disease. Thus, warble fly was eradicated from the UK through the use of 'warblecides' (MAFF, 1987)¹².

Movement of hosts

Animals can be removed from 'high-risk' areas where infections are endemic. This control strategy is implemented in tropical countries where hosts are seasonally migrated from areas in which biological vectors are active. The Fulani tribe in West Africa traditionally migrate, with their livestock, from the south to the north in the wet season to avoid the tsetse fly.

¹⁰ This approach is not always practicable, notably in some marine species (Richardson, 2016).

¹¹ Current concerns over antibiotic resistance justify the rational use of antibiotics, rather than their indiscriminate application (Jessen *et al.*, 2012), which is a particular problem in countries where pharmaceutical purveyors are poorly trained (e.g., Higham *et al.*, 2016). There are similar concerns over resistance to anthelmintics, which has led to treatment regimens aimed at reducing its development (Besier, 2011).

¹² Warbles, which have a marked impact on productivity, affected 40% of cattle in England and Wales, and 20% in Scotland, in the late 1970s, before eradication began.

Similarly, horses may be moved indoors at night, to prevent infection with African horse sickness virus, which is transmitted by night-flying vectors of the genus *Culicoides* (Chalmers, 1968).

Restriction of movement of hosts

The most effective measure for reducing the risk of transmission during epidemics and eradication campaigns is to stop the movement of livestock and animal products. Thus, during epidemics of foot-and-mouth disease in the UK, the movement of livestock and animal products is prohibited within a 3-km radius of infected holdings (the so-called 'Protection Zone'), and livestock and animal products can only be moved within a 10-km radius under licence (the so-called 'Surveillance Zone'). These measures are prescribed in law in the UK and European Union. Other measures aimed at reducing the risk of disease spread within zones, such as the control of slurry spreading (see Table 6.8) and hunting, are also applied.

Rarely, a national ban on animal movements may be enacted; for instance, during the 2001 epidemic of foot-and-mouth disease in the UK (Gibbens *et al.*, 2001b), the goal being to prevent the commonest form of spread of the disease: the movement of infected animals (Donaldson, 1987, 1993). However, such a step should not be taken lightly (HMSO, 1969) because it has far-reaching consequences on trade and welfare (e.g., by possibly depriving animals of access to food). Imposition of the ban in the UK epidemic was justified because the disease was not confined to a single focus (DEFRA, 2002b).

Restriction of international animal movements is important in ensuring that countries remain free from transboundary diseases (e.g., banning the importation of live animals and their products from countries in which foot-and-mouth disease is endemic).

Mixed, alternate, sequential and rotational grazing

The level of infection with some nematodes can be reduced by mixed, alternate and sequential grazing (Brunsdon, 1980) and rotational grazing (Buckingham *et al.*, 2013). This contrasts with **set stocking**, in which a group of animals is allocated to an area of pasture for a significant part of the growing season at a fixed stocking rate.

The **mixed grazing** of susceptible animals with stock that are genetically or immunologically resistant to helminths reduces pasture contamination to an acceptable level. Thus, adult cattle (immune) can be grazed with calves (susceptible). Similarly, cattle (resistant to *Ostertagia circumcincta*) can be grazed with sheep (susceptible).

The **alternate grazing** of a pasture with different species of livestock again reduces pasture contamination

(Waller, 1997; Barger, 1999). Thus, annual alternation of sheep and cattle in Norway has reduced to negligible levels the challenge to sheep from nematode species that overwinter (*Ostertagia* spp. and *Nematodirus* spp.: Helle, 1971).

The **sequential grazing** at different times of resistant and susceptible animals of the same species reduces pasture contamination. Thus, when clean grazing is not available in the late summer and autumn, trichostrongyle infections of calves (susceptible animals) can be reduced by transferring them to pastures that have been grazed by cows (resistant) (Burger, 1976).

Rotational grazing involves subdivision of pasture, in which each constituent paddock is grazed for a short time and then rested for a longer period during which nematode burdens are expected to decline. This strategy can be effective in temperate climates if an appropriate rest period is selected (Colvin *et al.*, 2008), but is more likely to be of value in tropical countries, where larval survival times are short and can be readily incorporated within a practicable rotation length (Barger, 1999).

Detailed discussions of grazing management strategies to control helminths are given by Barger (1996, 1997), Niezen and others (1996), Rahmann and Seip (2007) and Wheeler (2011). These strategies, and other approaches such as vaccination and biological control (e.g., the use of predaceous fungi: see Chapter 7), offer means of helminth control without the risk of resistance attendant on the use of anthelmintics.

Control of biological vectors

Infectious diseases transmitted by biological vectors can be controlled by removing the vectors. Insect vectors can be killed with insecticides. The habitat of the vectors can be destroyed; for example, by draining land to remove snails that are intermediate hosts of *Fasciola hepatica* (Torgerson and Claxton, 1999). Alternatively, an animal that competes with the vector can be introduced into the habitat (Okamoto and Amarasekare, 2012); for example, the exclusion of the molluscan vectors of schistosomiasis by a snail that is not a vector (see Chapter 7). Some infections of definitive hosts may be prevented by the elimination of infective material found at post-mortem meat inspection of intermediate hosts; for instance, the inspection of cattle to identify and condemn meat containing *Cysticercus bovis* cysts. Modern recombinant DNA technology offers the prospect of controlling trypanosomiasis in humans and animals by eliminating the ability of the tsetse vector to transmit the pathogen by introducing foreign genes with 'anti-pathogenic' activity (Weiss and Aksoy, 2014).

Control of mechanical vectors

Living organisms that mechanically transmit infectious agents can be controlled by destruction and disinfection. Biting fleas that transmit bacteria, for example, can be destroyed by insecticides. People also can act as mechanical vectors; thus, the veterinarian must impose strict procedures for personal disinfection when dealing with outbreaks of highly-contagious infectious diseases such as foot-and-mouth disease.

Disinfection of fomites

Fomites (see Chapter 6) can be disinfected to prevent the transmission of infectious agents. Fomites include farm equipment, vehicles, surgical instruments and sometimes drugs themselves, the last two being associated with iatrogenic transmission (see Chapter 6). Food is heat-treated (e.g., the pasteurization of milk) to destroy microbes and their heat-sensitive toxins, to prevent foodborne infection. Contaminated environments can pose considerable challenges to the effective removal of pathogens (Hawkins *et al.*, 2015).

Biosecurity

'Biosecurity' is a relatively new term for a relatively old concept. It is the application of management practices that reduce¹³ the opportunities for infectious agents to gain access to, or spread within, a food-animal production unit (Anderson, 1998; Toma *et al.*, 1999). Biosecurity also can be extended to the national level, with the aim of excluding pathogens and pests from a country (Wansbrough, 2004). During epidemics, biosecurity also is relevant to preventing infection being taken from animals on infected farms to healthy animals on 'clean' farms, and it is understandable that it may be heightened during such events (Dórea *et al.*, 2010).

Biosecurity encompasses cleanliness¹⁴, disinfection, reduction of exposure (e.g., maintenance of perimeter fencing¹⁵, testing of animals before inclusion into a herd, isolation of new additions and diseased animals, and waste management), management of personnel (limiting visitors, adequate training of staff), and ensuring that animals can be traced (England, 2002)¹⁶. Some infections are particularly likely to be introduced by the addition of animals (Barrett, 2001)

¹³ It is impossible to achieve zero risk in a farm environment (Pritchard *et al.*, 2005; Graham *et al.*, 2008).

¹⁴ The intuitive value of cleanliness is supported by experimental investigations (Alvarez *et al.*, 2001) and intervention trials (Gibbens *et al.*, 2001a).

¹⁵ Adequate methods for preventing mixing of animals are relevant not only to adjacent livestock but also to feral animals. Visits of badgers to farms to consume cattle feed, for instance, may be a source of transmission of tuberculosis to cattle (Roper *et al.*, 2003).

¹⁶ Collett (2013) classifies biosecurity into **conceptual** (location of premises), **structural** (farm layout, perimeter fencing, drainage

and so it is important that they are tested appropriately before inclusion (e.g., Table 20.30). Biosecurity therefore is essentially the application of 'informed common sense'. Van Schaik and others (2001) discuss the economic aspects of biosecurity measures. Such measures may be particularly challenging in the less-economically-developed countries; for example, in reducing the spread of Newcastle disease virus and highly-pathogenic avian influenza virus between backyard poultry flocks (Conan *et al.*, 2012). Gunn and others (2008), Heffernan and others (2008), Toma and others (2013), Ruston and others (2016) and Shorthall and others (2016) explore farmers' attitudes towards biosecurity, and Shorthall and Kaler (2016) address practical issues.

Niche filling

The presence of one organism within a niche can prevent its occupation by another organism. This is **epidemiological interference** (a particular case of *competitive exclusion*, see Chapter 7), and has been investigated experimentally in the poultry industry where suspensions of endogenous intestinal microbes have been fed to day-old chicks to prevent colonization of their digestive tract by virulent *Salmonella* spp. (Pivnick and Nurmi, 1982), *Campylobacter jejuni* and *E. coli* (Nurmi, 1988; Mead, 2000). This technique of control has the advantage over prophylactic antibiotic chemotherapy that antibiotic resistance is not encouraged. In Sweden, it has been successful in restricting colonization by *Salmonella* spp. nationwide (Wierup *et al.*, 1988, 1992) but has had limited success elsewhere (Humbert *et al.*, 1997; La Ragione and Mead, 2013). Its effect also may benefit from simultaneous vaccination (Braukmann *et al.*, 2016).

Improvement in environment, husbandry and feeding

The diseases of intensively produced animals, particularly cattle and pigs, are major contemporary problems (Webster, 2011), which can be controlled only when epidemiological investigations have identified the determinants associated with inadequate management. The relatively high average levels of mastitis in dairy herds housed in winter in straw-yards (Whitaker *et al.*, 2004), compared with cubicles, makes this point because a proportion of farms using straw-yards, nevertheless, have very little mastitis. This illustrates that it is not the housing system, *per se*, which is responsible, but differences in the way the system is managed (the undesirable provision of damp bedding in this example).

and housing design) and **procedural** (routine procedures to prevent the introduction and spread of infection).

Genetic improvement

Many diseases of both agricultural and companion animals have a variable heritable component (Foley *et al.*, 1979; Ackerman, 2011; HSVMA, 2011; see also Chapter 5). A disease may be determined predominantly genetically, as in canine cyclic neutropenia (the gray collie syndrome: Cheville, 1975), which is carried by a simple autosomal recessive lethal gene. Alternatively, a disease may be determined by several factors, only one of which is genetic, as in canine hip dysplasia (Morgan *et al.*, 2000) whose determinants also include growth rate, body type and pelvic muscle mass. The incidence of such diseases can be reduced by early detection, followed by voluntary agreement of owners of affected animals not to breed from the animals. Established techniques include, for example, radiography to identify hip dysplasia.

A valuable aid to the identification of genetic conditions and predispositions is **genetic screening** (Jolly *et al.*, 1981) to identify diseased animals by screening either the total population at risk or the part that is mainly responsible for the maintenance of a particular disease. The latter technique is commonly applied in veterinary medicine because animal populations tend to be very large, and most animals of 'superior' genetic potential are concentrated in pedigree 'nuclei' that are used for breeding. Human populations are screened to detect hereditary dietary deficiencies (e.g., aminoacidurias, so facilitating dietary control) and to identify serious genetic diseases prenatally so that elective abortion can be considered (Burke *et al.*, 2011). In veterinary medicine, the main value of screening is the reduction in defective gene frequency by identifying and removing normal and partially expressed carriers. The relatively short generation time of domestic animals, and artificial breeding techniques, help towards this objective. Thus, in the National Scrapie Plan in Great Britain, screening is used to identify resistant and susceptible sheep, according to the relative resistance of the various genotypes (Table 27.4). The Plan initially aims to concentrate on promoting the ARR gene (the most resistant), whilst excluding the VRQ gene (the most susceptible), and allowing the continued use of sheep with the AHQ, ARH and ARQ genes for a limited period. This has resulted in a positive, but limited, effect on breeding for resistance in the national sheep flock (Ortiz-Pelaez *et al.*, 2014).

Defects can be identified by clinical examination of animals; for example, canine progressive retinal atrophy (PRA) (Black, 1972). However, the main benefit of screening is in the detection of heterozygotes where disease is either subclinical or is transmitted by healthy carriers. Techniques include test matings, cytogenetic studies (e.g., bovine Robertsonian translocations:

Table 27.4 Ovine genotypes and their relative resistance to scrapie. (From DEFRA, 2006.)

Genotype*	Type	Degree of resistance/susceptibility
ARR/ARR	1	Sheep that are genetically most resistant to scrapie
ARR/AHQ	2	Sheep that are genetically resistant to scrapie, but will need careful selection when used for further breeding
ARR/ARH		
ARR/ARQ		
AHQ/AHQ	3	Sheep that genetically have little resistance to scrapie and will need careful selection when used for further breeding
AHQ/ARH		
AHQ/ARQ		
ARH/ARH	4	Sheep that are genetically susceptible to scrapie and should not be used for breeding unless in the context of an approved controlled breeding programme
ARH/ARQ		
ARQ/ARQ		
ARR/VRQ	5	Sheep that are highly susceptible to scrapie and should not be used for breeding
AHQ/VRQ		
ARH/VRQ		
ARQ/VRQ		
VRQ/VRQ		

* A, alanine; H, histidine; Q, glutamine; R, arginine; V, valine.

Gustavsson, 1969) and biochemical analyses (e.g., bovine mannosidosis: Jolly *et al.*, 1973). The current focus is on the identification of defective genes themselves using recombinant DNA techniques (Goldspink, 1993). Two methods are commonly employed: the **candidate gene** approach, and identification of **linked markers**. The candidate gene technique (Kwon and Goate, 2000) utilizes knowledge of genes in humans and the mouse (about which much is known), whose mutations result in diseases that are similar to ones affecting domestic animals. For example, PRA in the Irish setter is similar to retinitis pigmentosa in the mouse, which is caused by the *rde* mutant gene. This facilitated identification of the same gene as the cause of PRA. Linked markers (Traas *et al.*, 2006) are genes that are so closely associated on the chromosome that they are inherited together in 80% or more of cases. Thus, although causal genes may not be specifically identified, their approximate location between known markers allows their presence to be detected in animals (Langston *et al.*, 1999).

Comprehensive listing of markers is an essential component of mapping of the entire genome, such as that of the dog (Ostrander and Ruvinsky, 2010). A large range of DNA screening schemes is now available (e.g., Kennel Club, 2015).

The requirements for a genetic screening programme are listed by Jolly *et al.* (1981):

- the disease must occur in a defined population sufficiently frequently to make the disease of economic or social importance; the defined population may be a family, a herd or a breed of animal within certain geographical boundaries;
- a simple, relatively inexpensive test, capable of identifying heterozygotes with a high degree of accuracy, should be available; as most tests in biological systems do not have a sensitivity and specificity (see Chapter 20) of 100%, there should be provision for follow-up testing, either with a more specific supplementary test or by replication of the original method;
- control by culling of heterozygotes should neither have a deleterious effect on the overall genetic make-up of the population nor deplete breeding stock to an uneconomic or disadvantageous level;
- the logistics of the programme should be acceptable to the breeders and be preceded by adequate educational and public relations programmes;
- the logistics of the programme should satisfy the breeders but not encroach on other necessary disease-prevention programmes; where possible, the programme should be integrated with other disease-control schemes to simplify specimen collection;
- there should be provision for adequate genetic counselling and either breed society or kennel club rules or legislation to ensure that control is instigated on

the basis of information provided by the screening tests.

Table 27.5 illustrates the economic benefit of a screening programme for bovine mannosidosis. This disease causes a lethal nervous syndrome in Angus and Murray Gray cattle. It is inherited recessively; carriers can be detected biochemically because their blood α -mannosidase levels are approximately half of the normal levels. The cost–benefit analysis relates to New Zealand, where the prevalence of heterozygotes before the programme was 10%, and approximately one million Angus and Murray Gray calves are born each year, with a total estimated loss due to the disease of \$281 000. Different values for the prevalence, number and value of susceptible cattle, cost of screening test and discount rate affect the results of such an analysis and therefore the economic viability of the control programme, which has effectively reduced the α -mannosidosis gene prevalence to an insignificant level, with disease incidence reduced from an estimated 3000 cases per year to a negligible level (Jolly, 2002).

Other genetic diseases to which screening can be applied are bovine protoporphyria and canine inherited bleeding and eye defects (Jolly *et al.*, 1981), fucosidosis, a lysosomal storage disease of English springer spaniels (Barker *et al.*, 1988), and portosystemic shunts in Irish wolfhounds (van Steenbeek *et al.*, 2009). Benefit:cost ratios of greater than one have been demonstrated for screening and control programmes for canine hip dysplasia (Swenson *et al.*, 1997a) and elbow arthrosis (Swenson *et al.*, 1997b). Some examples of canine genetic disease certification schemes, based on screening, are given in Chapter 21.

The relationship between phenotype and heritable diseases can also be used to advise owners of an

Table 27.5 Cost–benefit analysis of a genetic screening programme for bovine mannosidosis in New Zealand, at different levels of heterozygote prevalence. (From Jolly and Townsley, 1980.)

Time horizon	Costs and benefits 10% discount rate	Prevalence of heterozygotes			
		10%	7.5%	5.0%	2.5%
Incurred first 8 years	Cumulative costs	\$500 000	\$500 000	\$500 000	\$500 000
	Discounted costs	\$333 433	\$333 433	\$333 433	\$333 433
20 years	Cumulative benefits	\$3 471 600	\$1 910 800	\$874 300	\$211 100
	Discounted benefits	\$963 838	\$530 850	\$243 588	\$58 576
	Benefit:cost ratio	2.89	1.59	0.73	0.18
Infinity	Discounted benefits	\$1 381 527	\$760 951	\$348 951	\$83 994
	Benefit:cost ratio	4.41	2.28	1.04	0.25
Break even time	Undiscounted benefits and costs	9 years	11 years	15 years	>20 years

appropriate **selective breeding** policy (Wood *et al.*, 2004). For example, ocular pectinate ligament dysplasia (PLD) is related to the development of adult-onset glaucoma in dogs (Read *et al.*, 1998) and so selective breeding to reduce the severity of PLD may be an appropriate strategy to reduce the incidence of glaucoma, which develops after bitches have reached their peak breeding age. Additionally, observational studies can be used to assess the degree of risk, for dichotomous disease traits (e.g., fragmented coronoid process: Guthrie and Pidduck, 1990), associated with the relatedness of ancestors of cases (Ubbink *et al.*, 1998a). The relative risks obtained by this procedure, which does not require the mode of inheritance to be known, can be used to select suitable breeding stock to reduce the incidence of disease.

The incidence of some infectious diseases also can be reduced by selective breeding (Gogolin-Ewens *et al.*, 1990). For example, certain cattle are known to be tolerant to trypanosomiasis (so called 'trypanotolerant' cattle: d'Ieteren *et al.*, 1998), and there is potential for breeding resistance to helminths (McManus *et al.*, 2014). Other infectious diseases for which genetic resistance has been reported include foot-and-mouth disease and tuberculosis in cattle; jaagsiekte and scrapie in sheep; brucellosis and leptospirosis in pigs; Newcastle disease, infectious bronchitis, Marek's disease and coccidiosis in poultry (Payne, 1982); and salmonellosis in pigs and poultry (Wigley, 2004).

Modern **transgenic biology**, which involves the introduction of specific genes into the genome, now offers a means of changing the genome of the animal more rapidly than it has ever been changed by traditional selective breeding. The techniques are being directed at improved productivity (Ward *et al.*, 1990; Rajoriya *et al.*, 2013) but also could be used to introduce artificial genes that block amplification of pathogens and to control non-infectious diseases (Wells and Wells, 2002; Kues and Niemann, 2011)¹⁷. Potential veterinary applications include cancer gene therapy, the treatment of inflammatory diseases (e.g., arthritis) and lentivirus infections (Argyle, 1999; Pavlin *et al.*, 2012)¹⁸, as well as manipulation of biological vectors described earlier in this chapter.

For a discussion of the improvement of disease-resistance traits using conventional breeding

programmes, markers and transgenic techniques, see OIE (1998) and Bishop and others (2010).

Minimal disease methods

Disease can be reduced in intensively reared livestock by disinfecting infected premises and by treating infected animals or removing them from the animal unit. Uninfected animals can be produced by caesarian section and by hatching uninfected eggs from poultry. These combined techniques are termed **minimal disease methods**. They have been applied commercially only in pig and poultry units (Deen *et al.*, 2001). Successes include the eradication of enzootic pneumonia from some pig herds in the UK.

Choosing appropriate techniques

Techniques of control and eradication have developed gradually. Until the germ theory of infectious disease was adequately supported in the 19th century, only those people who believed in contagion could attempt to control infectious disease. Thus, Lancisi controlled rinderpest in Italy by a slaughter policy (see Chapter 1). Those who accepted the miasmatic theory of cause occasionally succeeded in controlling disease by applying basic sanitary principles such as disinfection and fumigation.

A combination of the various techniques that have been described is applied to the control and eradication of disease. An example is the use of vaccination, followed by mass testing of animals and slaughter of infected animals to control contagious bovine pleuropneumonia in Nigeria (David-West, 1980) and brucellosis in the UK (MAFF, 1983). Various strategies to complement vaccination in the control of highly-pathogenic avian influenza have already been listed in Table 27.3. The choice of technique involves assessing, technically and economically, the most efficient strategy for a particular disease and system of management (Sellers, 1982), noting that the maximum economic benefits may be affected by agricultural (Andersson *et al.*, 1997) and trade (Junker *et al.*, 2009) policy (see also Chapter 25).

Table 27.6 lists some eradicable diseases in the UK and the methods chosen for eradication. Slaughter and quarantine campaigns against widespread epidemics and exotic (transboundary) diseases usually are conducted at the national level by government veterinary services. These campaigns frequently are supported by legislation.

Vaccination, treatment, alteration in environment and minimal disease techniques generally are carried out at the local level, and are often concerned with individual or herd problems, such as distemper in dogs (vaccination), and helminthiasis in herds of cattle and

¹⁷ Horizontal gene transfer is known to occur naturally, and may have a functional role (Crisp *et al.*, 2015).

¹⁸ In human medicine, attention has focussed on single-gene traits such as adenosine deaminase deficiency; but, in veterinary medicine, these can be tackled by selective breeding.

Table 27.6 Some eradicable diseases in the UK and chosen methods of eradication. (Updated from Sellers, 1982.)

Method of eradication	Cattle	Sheep	Pigs	Poultry
Slaughter of infected and in-contact animals	Exotic infections, e.g., foot-and-mouth disease	Exotic infections, e.g., foot-and-mouth disease	Exotic infections, e.g., foot-and-mouth disease, classical swine fever	Exotic infections, e.g., Newcastle disease
Identify infected or genetically-susceptible animals and either destroy, quarantine, treat or administer prophylactic vaccination or treatment	Tuberculosis, brucellosis, warble fly infection, leptospirosis, enzootic bovine leucosis, Johne's disease, infectious bovine rhinotracheitis (stud bulls)	Johne's disease, scrapie	Tuberculosis, Aujeszky's disease	Pullorum disease, fowl typhoid, egg drop syndrome, fowl pox, duck hepatitis
Improvement of environment and management, and treatment	Streptococcal mastitis, coliform mastitis	Footrot, liver fluke	Streptococcal meningitis, mange	Chronic respiratory disease, <i>Mycoplasma meleagridis</i> infection
Minimal disease methods		Maedi-visna	Enzootic pneumonia, atrophic rhinitis	

flocks of sheep (treatment and management practices). Vaccination sometimes may be carried out as part of a national government policy, for example against bovine brucellosis.

Vaccinate or slaughter? The need to choose an appropriate strategy is brought into sharp focus when considering either vaccination or slaughter (or a combination of the two techniques). This may arise in both long-term eradication campaigns and the short-term control of epidemics. The choice usually is unique to the disease, and depends on its natural history, characteristics of the available diagnostic tests, availability of vaccines, and economic constraints. Two current examples are the control of bovine tuberculosis and foot-and-mouth disease.

Bovine tuberculosis has been successfully eradicated in many countries and regions using the intradermal skin test, followed by slaughter of reactors. However, the existence of wildlife reservoirs (e.g., badgers in the UK and Ireland, opossums in New Zealand and the white-tailed deer in the US: Krebs, 1997; de Lisle *et al.*, 2001) has rendered the disease refractory in some areas. The bacille Calmette Guérin (BCG) vaccine is the only currently available vaccine, but its efficacy is unpredictable in cattle and humans¹⁹. Moreover, it compromises the specificity of the intradermal skin test (Francis, 1947), resulting in false-positive reactions. Thus, 'test-and-slaughter' currently

remains the method of choice, despite its lack of success in some areas. However, new vaccines, which do not interfere with the intradermal test, are being developed (Vordermeier *et al.*, 2000), and some may be applicable to wildlife (Buddle *et al.*, 2002), suggesting that vaccination, as well as slaughter, may be a viable option for control in problem areas (Conlan *et al.*, 2015).

Foot-and-mouth disease is commonly controlled by routine vaccination in countries in which it is endemic, using inactivated vaccine. Current vaccines usually require regular 'boosting' at least every six months (Doel, 2003). Epidemics in countries from which the disease is absent may be controlled either by 'stamping out' alone or by emergency suppressive vaccination (outlined earlier in this chapter)²⁰. Alternatively, both techniques can be used together by combining suppressive vaccination with slaughter of infected animals and their close contacts. However, this generates a problem because vaccination, whilst preventing clinical disease, does not prevent infection. Moreover, vaccinated ruminant animals that are subsequently infected may become carriers of the virus (see Chapter 5). Although it is possible to distinguish between infected and vaccinated animals (Mackay, 1996), this discrimination is lost if infected animals have been previously vaccinated. Consequently, in

¹⁹ See Suazo and others (2003) for a review of the use of BCG vaccine in animals.

²⁰ The major constraint on vaccination for a country normally free from the disease is the loss of export trade of livestock and animal products due to embargoes imposed by trading partners. (See Chapter 25 for a general discussion of the consequences of trade bans.)

the European epidemic in 2001, any animal displaying foot-and-mouth disease antibody was considered as potentially infected, and it and cloven-hoofed animals in contact were slaughtered. Therefore, all animals that were 'emergency vaccinated' needed to be slaughtered. In The Netherlands, emergency suppressive vaccination was used because the contingency plan allowed vaccination if there was a lack of culling capacity, with a consequent reduction in the urgency to slaughter livestock. Subsequently, over 200 000 vaccinated animals were slaughtered (Bouma *et al.*, 2003) and export status was regained more rapidly than if the animals had been allowed to live. The policy, however, was extremely controversial and provoked strong protests from the farming community; indeed, some farmers refused to have their animals slaughtered, resulting in a spate of legal cases (Plumiers *et al.*, 2002)²¹.

Diagnostic tests that can distinguish between natural infection and vaccination have been developed (Sørensen *et al.*, 1998). Natural infection induces the production of non-structural virus proteins (NSPs), which are not produced following vaccination with purified inactivated vaccines that have had NSP antigens removed (Barteling, 2002). The tests, which detect antibodies to NSPs, used in conjunction with appropriate vaccines, therefore offer the prospect of 'emergency vaccinating' animals during an epidemic, without the need for subsequent slaughter (so-called 'vaccination to live')²². However, there is variation in the magnitude of NSP response in infected animals. Therefore, in the absence of precise estimates of the tests' sensitivities, it is difficult to determine an appropriate sampling protocol for detecting infection (see Chapter 13), and all animals in each epidemiological unit may need to be sampled (Clavijo *et al.*, 2004). Moreover, there is evidence that some cattle, vaccinated and subsequently challenged with live virus and confirmed persistently infected, may not be detected in some NSP tests, causing false-negative results (Brocchi *et al.*, 2006). Other newer diagnostic methods (e.g., polymerase chain reaction techniques: Callens *et al.*, 1998) may provide the means of identifying virus, thus raising the economically viable

prospect of using emergency vaccination, without slaughtering vaccinated animals, and without the risk of continued infection and trade restrictions (Bates *et al.*, 2003). Additionally, new high-potency vaccines may effectively inhibit the carrier state, thereby removing one obstacle to vaccination (Barnett *et al.*, 2004). Suttmoller and others (2003) and Parida (2009) discuss this topic in broad reviews of foot-and-mouth disease.

Important factors in control and eradication programmes

Before either a control or an eradication campaign can be undertaken, several factors must be considered. These include:

- the level of knowledge about the cause of the disease and, if infectious, also about its transmission and maintenance, including host range and the nature of the host/parasite relationship;
- veterinary infrastructure;
- diagnostic feasibility;
- adequate surveillance;
- availability of replacement stock;
- producers' and society's views;
- the disease's public health significance;
- the possible ecological consequences;
- the existence of suitable legislation with provision for compensation;
- economic costs and the availability of funds for the programme.

Knowledge of the cause, maintenance and transmission of disease

A complete knowledge of the natural history of a disease, although not always necessary to control or eradicate the disease (recall Lancisi's eradication of rinderpest), is necessary to develop the *most effective* means of control. When the various disease determinants have been defined, often by epidemiological studies, a suitable control strategy can be selected; for example, improving ventilation to reduce respiratory disease in intensively reared pigs. If a disease is infectious, then a knowledge of its incubation period and method of transmission, including the life-cycle and habitat of any vectors, assists in control. If the incubation period is long (e.g., tuberculosis), tests may have to be repeated several times to identify all infected animals. If the generation time (see Chapter 6) is short (e.g., foot-and-mouth disease), diagnosis and removal of affected animals must be rapid. If an infectious agent is aeri ally transmitted (e.g., Aujeszky's disease or foot-and-mouth disease), control necessitates visiting all herds that may be

²¹ The judges ruled against the claims of the farmers in all cases. Massive resistance against culling necessitated special-duty police and armoured cars to protect culling teams, some of whom were molested or taken hostage. A minister of religion organized a non-stop church service on a farm where animals had to be culled, claiming that the law prohibited the culling team and police from interrupting the service.

²² Tests based on NSP antibody detection are one example of tests that enable 'differentiation of infected from vaccinated animals': so called 'DIVA' tests (Uttenthal *et al.*, 2010).

infected (see *EpiMAN*: Chapter 11). In contrast, strictly contagious diseases (e.g., contagious bovine pleuropneumonia) can be controlled by simple quarantine of infected livestock. Careful disinfection of fomites is required when agents can survive outside the host (e.g., African swine fever).

A knowledge of the host range of an infectious agent, the host/parasite relationship (both considered separately later) and the likelihood of transmission between different hosts also is desirable. Lack of such knowledge can pose major challenges to disease-control policy (e.g., the control of bovine spongiform encephalopathy in relation to variant Cruetzfeld–Jacob disease in humans: Hueston, 2013).

Host range

An infectious agent that infects or can be transmitted by only one species of host is easier to control than an agent with a wide host range. The global eradication of human smallpox was possible because the virus infected only humans; control, by quarantine and vaccination, therefore needed to be directed towards only humans. Similarly, the British bovine brucellosis eradication programme required control of infection only in cattle because only cattle can transmit *Brucella abortus* significantly. On the contrary, it already has been mentioned that a current obstacle to the control of bovine tuberculosis in England is considered to be the presence of the infection in badgers (Krebs, 1997). Agents that are transmitted by arthropod vectors may be particularly difficult to control because of the problems associated with controlling infection in the arthropod.

Table 27.7 tabulates, for several infectious diseases, the factors that have been discussed.

Nature of the host/parasite relationship

Exogenous agents (see Chapter 5) have been, and still are, the causes of the major animal plagues (see Table 1.1). Control is relatively straightforward and eradication is possible. Infected animals can be identified relatively easily using clinical and laboratory diagnosis, and can be removed by slaughter or quarantine.

The endogenous agents, by definition, however, are ubiquitous and their eradication is therefore impracticable because it would require elimination of the agent from most animals, including healthy ones in which no disease is present. Many infections of intensively reared animals are endogenous. Diseases in which endogenous agents are incriminated are best controlled by alteration of other determinants; for example, by improving hygiene to prevent mastitis involving *E. coli*.

Veterinary infrastructure

Veterinary services must be capable of implementing control and eradication campaigns. Moreover, there also should be contingency plans in place that clearly specify the roles, responsibilities and actions to be taken by veterinary and support staff at all levels in the event of an emergency. The ‘fitness for purpose’ of the contingency plans should be tested regularly by simulation exercises, and any deficiencies or gaps addressed and resolved (e.g., the ‘Silver Birch’ contingency plan for foot-and-mouth disease in the UK: Animal Health, 2011).

There are three main requirements for the infrastructure:

1. a mobile field service, comprising adequately trained veterinarians and veterinary auxiliaries;
2. adequate diagnostic facilities;
3. adequate research facilities.

The first two requirements are important when controlling infectious diseases such as the classic animal plagues whose causes are understood. The third requirement must be fulfilled to improve the techniques of controlling diseases whose causes are not known, and also is needed to elucidate the causes of new and emerging diseases, such as those of intensive animal production, so that the most appropriate control strategies can be selected.

Many more-economically-developed countries possess the first two requirements, mainly because their veterinary services evolved at the beginning of the 20th century to deal with the major plagues that were common then, such as foot-and-mouth disease, pleuropneumonia and rinderpest. However, fulminating epidemics usually put considerable strain on resources. The epidemic of foot-and-mouth disease in the UK in 2001, for example, required substantial supplementation of government veterinary staff by veterinarians from general practice, overseas, universities and research institutes, as well as involving veterinary students and additional ancillary staff (Figure 27.1).

The less-economically-developed countries often lack the first two requirements. These countries have over half of the world’s livestock units (see Table 1.8) but contain only 20% of the world’s veterinary force. Disease-control programmes in these countries therefore should include two stages (Mussman *et al.*, 1980):

1. a short-term programme that includes the development of diagnostic and field services, training of personnel to deal with the major exotic diseases, and associated control techniques, such as prevention of entry of diseases across borders;

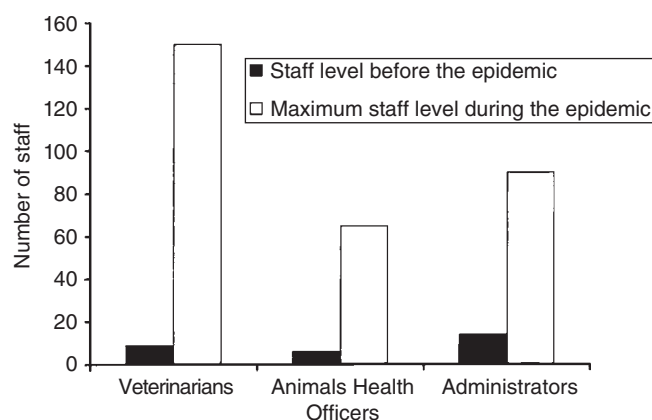
Table 27.7 Factors influencing the control of some infectious disease. (Updated from Rees and Davies, 1992.)

Disease	Incubation period		Host range			Method of transmission				
	Long*	Short [†]	Single	Limited	Wide	Contagious	Fomites	Vectors	Airborne	Vertical/ milk-borne
Foot-and-mouth disease		•		• ¹		•	•	?	•	
Classical swine fever		•	• ¹			• ²	• ²	?		• ³
African swine fever		•	• ¹			•	•	•		
Swine vesicular disease		•	•			•	•			
Peste des petits ruminants		•	• ¹			•				
Vesicular stomatitis		•		• ¹		•		•	?	
Lumpy skin disease		•	•			?		?		
Rift Valley fever		•		• ^{1,4}				•		
Bluetongue				• ¹				•		
Sheep/goat pox		•	•			•				
African horse sickness		•		•				•		
Teschen disease		•	•			•	•			
Tuberculosis	•				• ¹	•	•			
Brucellosis	•		• ¹			•	•			
Contagious bovine pleuropneumonia	•		•			•				
Maedi-visna	•		•							•
Scrapie	•		•							•

• Factor characteristic present.

? Factor characteristic suspected.

* More than four weeks (average).

[†] Less than four weeks (average).¹ The hosts include wildlife.² Postnatal infections.³ Prenatal infections.⁴ The hosts include humans.**Fig. 27.1** Staff levels in the Ministry of Agriculture, Fisheries and Food (renamed the Department for Environment, Food and Rural Affairs) in south-west Scotland, during the epidemic of foot-and-mouth disease in the UK in 2001. (Source: Thrusfield *et al.*, 2005b.)

2. a long-term programme, similar to those present in some more-economically-developed countries, that includes disease reporting systems, facilities for field surveys, and economic and epidemiological modelling.

Additionally, policies that protect the livelihoods of small-scale farmers (by controlling relevant diseases such as brucellosis) should be linked to policies that control diseases that have an impact on trade; for example, foot-and-mouth disease (Heath, 2006).

Diagnostic feasibility

Control and eradication can be carried out successfully only if a disease can be recognized. The main techniques of recognition are by:

- clinical signs;
- pathological changes;
- isolation of causal agents;
- demonstration of an immune, allergic or biochemical response, or novel nucleotide sequences;
- epidemiological identification of changes of a variable in a population.

Presenting signs may be observed either in the individual sick animal or in its offspring (e.g., congenital abnormalities). The value of signs varies because they can be either pathognomonic or indicative of general lesions and causes (see *Figure 10.3*).

Identification of pathological changes may substantiate clinical impressions and may be of value when clinical signs are absent, but again the changes may have several causes.

Isolation of causal agents is the most valuable means of identification of disease, but agents may be missed in a specimen (i.e., there may be false-negatives).

Identification of an immune reaction is frequently used in control programmes. It should be recalled that each serological test has its own inherent diagnostic sensitivity and specificity (see Chapter 20) whose acceptability for the control programme must be considered. Recall, too, that the predictive value of a test depends on the prevalence of the disease: the predictive value of a positive result decreases as control reduces prevalence because the proportion of false-positives increases, and so appropriate test strategies must be identified (see *Table 20.22*). Moreover, tests need to be applied with regard to a disease's natural history and the goals of the testing (e.g., screening herds versus testing animals before inclusion in a herd; see *Table 20.30*).

Epidemiological diagnosis includes the detection of changes in production trends in populations, for

example by constructing Shewhart charts and cusums (see Chapter 12).

Adequate surveillance

Control and eradication programmes require effective surveillance (see Chapter 21). Several surveillance and monitoring systems have been introduced in Chapter 11, where the principles of data collection also were described. They are therefore not discussed further here. When new national or international disease-control policies are formulated, the options for surveillance may need to be reassessed. Existing data-gathering procedures may be utilized; alternatively, new systems, based on active data collection (e.g., *NAHMS*: see Chapter 11), may be developed. Davies (1993) discusses the merits and disadvantages of these two approaches in the context of the European Union. Sudden outbreaks of infectious disease require prompt surveillance to identify potential sources and spread of infection; an example is given later in this chapter ('Outbreak investigation').

Availability of replacement stock

If a control or an eradication campaign involves the slaughter of many animals, sufficient replacement stock should be available in the livestock industry to minimize disruption to production. This consideration has not been critical hitherto, although it has been cited as a potential problem if a slaughter campaign were used to control or eradicate bovine tuberculosis in less-economically-developed countries (FAO/WHO, 1967).

Producers' opinions and cooperation

The attitude of animal producers can affect the success of control and eradication campaigns. The resistance of farmers to the slaughter of animals that had been suppressively vaccinated during the foot-and-mouth disease epidemic in The Netherlands in 2001 has already been mentioned. In Mexico in the 1940s, a radical slaughter campaign to eradicate foot-and-mouth disease had to be abandoned because of farmers unwillingness to comply (Shahan, 1953) – social tensions became so severe that some veterinarians were killed by farmers (Machado, 1969). In contrast, farmers in Ethiopia actively participated in the control of trypanosomiasis (Girmay *et al.*, 2016).

Producers' opinions and the degree of their cooperation are influenced by their understanding of the control campaign²³. Farmers in recently endemic areas of

²³ There also is an economic incentive for livestock owners not to disclose sick animals if buyers may use the information against them (Sheriff and Osgood, 2010).

bovine tuberculosis in the UK, for example, demonstrated a fatalistic approach to the disease, many believing that they could do little to halt the spread of disease (e.g., by better control of badgers) and that occurrence of the disease on their farms was just a matter of luck (Enticott *et al.*, 2015). This may be because the farmers perceived the disease to be a political problem, thus posing a challenge to policy makers. In contrast, farmers require little encouragement to control lameness in dairy cattle because of their awareness of the pain and loss of productivity that it causes, and their general pride in having healthy herds (Leach *et al.*, 2010b).

An important preliminary step therefore is a detailed explanation of a control campaign's rationale to farmers (e.g., Mabiane *et al.*, 2009). In the more-economically-developed countries, pamphlets and audio-visual presentations, such as posters at ports of entry warning of the risk of bringing rabies into the UK, are useful. In the less-economically-developed countries, especially where illiteracy is widespread, these techniques may not be satisfactory and may need to be replaced or supplemented by more direct contact with farmers (Chain, 1980). However, educational campaigns may not always be fruitful. For example, a campaign by the Dutch Ministry of Public Health to increase veterinarians', physicians' and the public's awareness of toxocariasis achieved little (Overgaauw, 1996).

Public opinion

The opinion of society may be an important consideration in a possible control or eradication scheme. Bovine brucellosis and canine distemper have a similar natural history. Both are transmitted by a single host species: cattle in the case of brucellosis, dogs in the case of distemper (ferrets and mink are excluded because their contribution is minor). In situations where bovine brucellosis can be eradicated on economically justifiable grounds, a vaccination and slaughter campaign is socially acceptable in most countries (excepting Hindu countries for religious reasons). A slaughter campaign to control distemper, even though technically feasible, would not be acceptable in many Western countries because of social attitudes towards dogs. In other countries, slaughter of companion animals may be acceptable to the public; for example, in China, slaughtering dogs to improve environmental hygiene (*The Times*, 1983) and control rabies (*Xinhua News Agency*, 2014), and culling cats to reduce the putative risk of transmission of severe acute respiratory syndrome (SARS) (*Daily Mail*, 2004). The epidemic of foot-and-mouth disease in the UK in 2001 revealed the public's concern over the mass slaughter of animals when supposedly effective vaccines were

available, and provides impetus for 'vaccination to live' strategies (Scudamore, 2004). Thus, effective communication by veterinary services is critical in influencing public reaction to disease-control strategies (Hueston, 2007).

Again, education of the public plays an important part in influencing attitudes²⁴. The virtual eradication of echinococcosis from Iceland over the last century was achieved by explaining to the public the dangers of keeping dogs unhygienically (Beard, 1973), beginning with the publication, in the 19th century, of a pamphlet that outlined precautions against infection (Krabbe, 1864). More recently, canine genetic disease-control programmes in Finland have received the support of dog owners (Leppänen *et al.*, 2000a) and breeders (Leppänen *et al.*, 2000b) as a result of their awareness of the benefits.

Knowledge of anthropology may be invaluable. In the border region of New Guinea, oestrous postponement drugs have been injected into village bitches to regulate the dog population in relation to the control of diseases, such as rabies, that might enter the country from Indonesia. Treated dogs were tagged with brightly coloured discs. However, villagers removed the discs to wear for personal adornment. Thus, treated bitches subsequently could not be identified.

Public health considerations

Over 70% of the known pathogens are infectious to both humans and other animals. Many of these – the zoonoses – are naturally transmissible between humans and animals. The control of zoonotic diseases is the main concern of veterinary public health authorities.

The public health significance of a disease may be a major factor in determining the need for control, usually when human infection is either fatal (e.g., rabies, tuberculosis, and, in the past, glanders) or when the infection can be clinically severe, such as occupationally-acquired leptospirosis, brucellosis or anthrax in farmers and abattoir workers.

In many cases, however, prevention of human infection is secondary to control of the infection in animals. Routine prophylactic administration of anthelmintics to dogs, for instance, is practised primarily to prevent

²⁴ Lack of education and backwardness, however, may not be the main causes of lack of cooperation. Reasons may be more complex, involving historical distrust of political and administrative structures, evidenced, for example, in Ebola virus eradication in West Africa (Dhillon and Kelly, 2015; Bardosh *et al.*, 2016). (There also may be the much wider issue of the degree of the public's trust in science: SteelFisher *et al.*, 2015.) Additionally, livestock owners may mistrust one another (e.g., during voluntary control schemes: Heffernan *et al.*, 2016).

clinical and subclinical disease in dogs, although animal owners sometimes are made aware of the potential risk of human infection (e.g., Woodruff, 1976). The control of infectious disease in livestock usually is undertaken because of the financial impact of diseases; a decrease in the incidence of human infection is an added bonus; for example, when controlling bovine brucellosis.

Ecological consequences

It has been argued that control and, particularly, eradication of an infectious agent may disturb the 'balance of nature' in an ecosystem (see Yekutieli (1980) for a detailed discussion). The elimination of an infectious agent may free a niche that could be occupied by a more virulent organism. This phenomenon is relatively poorly understood, and the consequences of alterations to the ecology of pathogens are highly variable (Fenton, 2013).

The use of insecticides to destroy arthropod vectors could kill other animals in the arthropods' ecosystem. These considerations have, so far, only been theoretical in relation to animal disease control, although the use of insecticides to control insect pests has resulted in the death of other animals, such as birds that have ingested the insecticides (Carson, 1963). More recently, oestrogens excreted by women taking oral contraceptive tablets have been found in parts of rivers close to waste treatment plants, and are associated with reduced fertility in fish (Bhandari *et al.*, 2015), and the veterinary use of the non-steroidal anti-inflammatory drug, diclofenac, in livestock has caused collapse of Asian vulture populations as they fed on carrion from treated animals (Cuthbert *et al.*, 2014).

Anthelmintic residues also might disturb the ecosystem. For example, concern has been expressed over the avermectin group of parasiticides, which can be excreted unchanged, and so, through their effect on insects that breed in dung, might affect the rate of decomposition of dung pats, with consequent local effects on soil composition and earthworm populations, and more widespread effects on pastureland ecology (Herd *et al.*, 1993; Steel and Wardhaugh, 2002). However, such adverse effects have not been unequivocally demonstrated (Herd *et al.*, 1993; Wratten *et al.*, 1993; Beynon, 2012).

The requirement for legislation and compensation

Control and eradication programmes are more effective when supported by legislation, sometimes accompanied by penalties when the legislation is contravened. For example, in Australia, New Zealand, Papua New Guinea and other areas in which rabies is absent, there is legislation forbidding the entry,

without quarantine, of animals from countries in which the disease is present. Severe fines are imposed (and the imported animals sometimes destroyed) if the legislation is ignored.

The benefits of disease control in agriculture are frequently realized by the consumer; thus, tuberculosis eradication results in uninfected milk. The culling of an infected cow, however, represents a financial loss to the farmer. An essential part of many control programmes therefore is the compensation of producers for the loss of infected animals as a result of the programme²⁵. Thus, from February 1990, farmers were awarded 100% of the value of affected cattle as part of the bovine spongiform encephalopathy control programme in the UK. In other cases, bonuses can be offered to increase cooperation of owners; for example, awarding a bonus to farmers whose herd bulk milk somatic cell count (which is an indirect measure of bovine mastitis) is below a defined level; and those whose herd bulk cell count is above the defined level, are penalized. Delays in compensation may result in farmers hiding animals in outbreaks that are controlled by slaughter of affected animals (e.g., classical swine fever: Madzimure *et al.*, 2012). There is a wide variety of compensation schemes, ranging from those offering 70% of an animal's market value (in Vietnam) to those paying its full value (in The Netherlands), with funding being federal or regional, sometimes supported by complementary insurance schemes (OECD, 2012).

Financial support

Control and eradication campaigns require financial support. The control of companion-animal diseases readily draws financial support from owners: canine, equine and feline vaccination programmes are examples.

Livestock disease-control campaigns are usually funded either totally or partially by the government or by non-governmental sources (Parsons, 1982). Total government support is often given to the control of transboundary infectious diseases of major economic importance. Diagnostic tests, vaccines, disinfection, compensation, quarantine facilities and veterinary staff are funded by the government. Examples of such diseases are foot-and-mouth disease and swine vesicular disease. There may be partial government support; for example, when a control scheme is initially voluntary and then compulsory (funded by the government). An example is the British bovine

²⁵ Compensation for all animals slaughtered to control foot-and-mouth disease began in the UK and Denmark in 1886 and 1893, respectively, and became the rule in the European Union in 1991.

brucellosis scheme which initially was financed by farmers, with incentives and compensation awarded for inclusion in the scheme, and which then became compulsory. Alternatively, costs may be shared by government and the livestock industry; for example, in Britain, before sheep scab was deregulated, the disease was controlled by dipping; the dips were provided by farmers; veterinary supervision was funded by the government. Occasionally, the programme may be funded entirely by the producer (e.g., bovine virus diarrhoea eradication in Sweden: Greiser-Wilke *et al.*, 2003).

State financial support also can be provided indirectly; for example, through the state laboratory diagnostic services and participation in herd health schemes. Non-governmental financial support is supplied indirectly by the pharmaceutical industry in the development of therapeutic and prophylactic drugs and vaccines. In parts of Germany some of the costs of bovine virus diarrhoea eradication are funded from public animal insurance (Greiser-Wilke *et al.*, 2003). The extent of government support is reflected by current political and economic attitudes. Support may be reduced in circumstances when the government becomes less 'paternalistic' and when it supports private enterprise rather than state control. Currently, in the more-economically-developed countries, there is a trend towards a joint solution between the public and private sectors. This is a complex issue, which is addressed in detail by Koontz and others (2006).

Control or eradication?

The factors discussed above are relevant to both disease control and eradication. However, in the livestock sector, governments usually need to decide on the final objective: control or eradication of a disease in national herds and flocks (Rees and Davies, 1992). Recall that the former is an ongoing process, requiring regular financial support, whereas the latter is time-limited. Eradication is therefore the more attractive option because its costs are limited to the duration of the programme, whereas its benefits are enduring.

However, before an eradication campaign is undertaken, a government must be certain that:

- all technical resources (e.g., manpower) are available;
- the agricultural community fully supports the policy to reduce the risk of illegal trade in infected animals;
- state borders can be adequately 'policed';
- adequate diagnostic facilities (see earlier) and other tools (e.g., effective vaccines) are available.

Moreover, eradication programmes should only begin when success is reasonably certain. During the initial and intermediate stages of a campaign, costs usually outweigh benefits; for example, in a programme to eradicate an infectious disease, based initially on vaccination and subsequently on a test-and-slaughter policy. If vaccination failed (e.g., because of movement of infected animals into cleared zones), there could be continuous high costs with few potential benefits.

National control schemes – without eradication – are becoming harder to justify because they need to demonstrate continuous long-term benefits in excess of costs. However, they have a role when the livestock industry is willing to support the control measures (e.g., tick-dipping to control East Coast fever) and when diseases are of public health significance (see earlier).

Diseases can be divided broadly into three major categories, each of which is generally associated with a particular, global, approach to managing disease (Figure 27.2). Categorization usually is based on economic importance, prevalence and a country's historical experience; therefore, membership of each category will vary country by country. The major epidemic diseases include those in the former OIE List A (e.g., foot-and-mouth disease) and are usually the subject of legislation (Figure 27.3a). These often are controlled with government funding; and compensation is often from the public purse. However, the prevention and control of endemic diseases, which represent the main global burden (Figure 27.3b), are the responsibility of producers. The control of

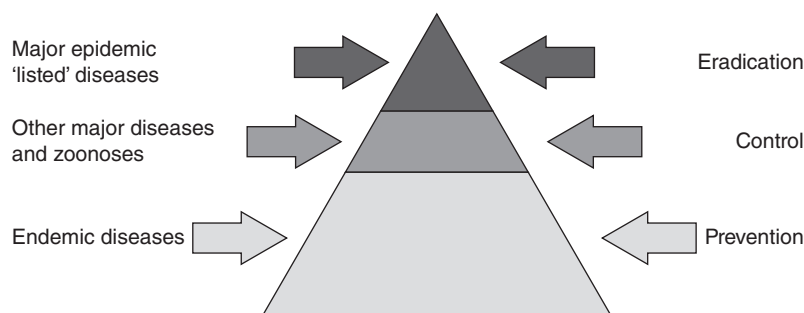
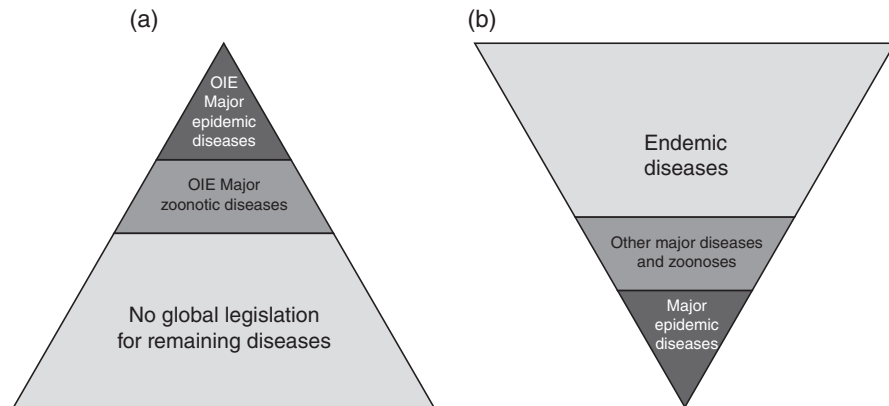


Fig. 27.2 Classification and major principles for the control of infectious disease of animals: the three areas of the pyramid indicate the relative number of diseases involved. (Adapted from Wierup, 2012.)

Fig. 27.3 Relative magnitudes of infectious diseases of animals: (a) according to legislation for control; (b) global burden (losses of production, and costs of control and medical care.) (Adapted from Wierup, 2012.)



foodborne zoonoses (e.g., salmonellosis and campylobacteriosis) also is usually the responsibility of producers, even though such diseases seldom cause significant clinical disease or economic losses in animals.

A further degree of complexity is added when regional policies are considered. For example, a potential Europe-wide strategy for the eradication of bovine virus diarrhoea would have to consider, and reconcile, different perceptions of its feasibility between those countries that currently practise compulsory control and those that undertake voluntary control (Heffernan *et al.*, 2009).

Outbreak investigation

Control of disease is a particularly acute challenge when disease occurs as an outbreak, either in isolation or as part of a wider epidemic. Outbreaks may occur when the cause of the disease is either infectious or non-infectious and is either initially known or unknown. In both circumstances, clinical and epidemiological expertise are required, the goals being to prevent further cases and, if applicable, to treat affected animals appropriately. Examples of an outbreak induced by a known infectious cause, and an outbreak ascribed to an initially unknown non-infectious cause, are now explored.

Cause known: foot-and-mouth disease

Foot-and-mouth disease is a highly contagious disease, which can be spread by animal contact, fomites and aerially (see Chapter 6). The actions taken after suspicion of a case of foot-and-mouth disease (or other transboundary, formerly *OIE* List A, diseases) are embodied in UK and European Union law. Following

confirmation of disease (usually based on clinical signs), animals on affected farms are slaughtered, animals within the neighbouring area are subject to movement restrictions, and enhanced biosecurity measures are introduced, as outlined earlier in this chapter; the goal of these procedures being to prevent further virus production and dissemination to other susceptible livestock. A detailed veterinary investigation is undertaken on each affected farm to find the source of virus (from which the virus already may have been disseminated to other farms) and to assess the likelihood of animals on other premises having been exposed to infection.

Dangerous contacts

The immediate task is to identify, and assess, susceptible livestock that may have been exposed to infection; for example, those on adjacent farms that may have had close contact with the infected animals and those that may have been infected by contaminated personnel. If the risk of exposure is considered to be high, the animals are classified as '**dangerous contacts**' and slaughtered to reduce the risk of potential disease occurrence and transmission. If not considered as dangerous contacts, they are subject to regular clinical examination (to identify the disease if it subsequently develops), movement restrictions, and increased biosecurity, to reduce the risk of potential transmission of infection.

Tracing

Simultaneously, the recent movements of animals, personnel and vehicles from, and to, the affected farm, are **traced**, to identify any potential spread of disease to other livestock premises and to detect a putative source of infection for the affected farm, respectively. This information is gathered by the veterinarian attending the affected farm, and by a team of dedicated field epidemiologists. Multiple vehicle and personnel

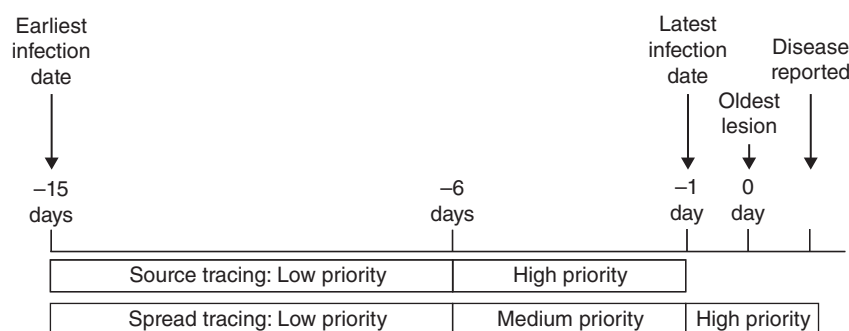


Fig. 27.4 Forward (spread) and backward (source) 'tracing windows' used in investigating an outbreak of foot-and-mouth disease. (Adapted from Thrusfield *et al.*, 2005b.)

tracings, such as milk-tanker and feed-lorry tracings, are conducted from information supplied by milk-collection and feed-supply companies. 'Tracing windows', based on the time during which virus may be excreted from infected animals, are then defined.

Forward (spread) tracings Forward (spread) tracings focus on potential spread of disease to other livestock premises. All movements of animals, personnel and vehicles from the affected farm to other premises from the beginning of the maximum incubation period (14 days: Garland and Donaldson, 1990) to the date when movement restrictions were imposed (when disease is reported and investigated) are considered. However, to take account of the accepted precision of ageing of lesions as being $\pm$ one day for lesions up to seven days old (MAFF, 1986), the actual 'tracing window' 'opens' 15 days prior to the estimated date of first (i.e., oldest) clinical lesion (the earliest time that infection could have arrived on the farm, and therefore be distributed to other farms) and is 'closed' on the date of disease reporting and investigation (when stringent controls are instituted, and therefore no infection can leave the farm) (Figure 27.4). Animals on premises thus identified are then assessed and either categorized as dangerous contacts (and therefore slaughtered) or placed under appropriate restrictions and subjected to regular clinical inspections, conservatively until 21 days after exposure.

Visits are prioritized within the 'tracing window'. Highest priority is given to the period from the date of the oldest lesion (minus one day, to account for the precision), from when virus is likely to be shed. Medium priority is accorded to the five-day period before the latest infection date, representing the average incubation period of the disease during which virus may have been present on the farm (Gibbens and Wilesmith, 2002).

Backward (source) tracings Backward (source) tracings address putative sources of infection. Animals

showing signs of disease are considered to have been exposed to infection at some time during the 2–14 days prior to the appearance of the first lesions (2–14 days being the accepted range of the incubation period for foot-and-mouth disease: Sellers and Forman, 1973; Garland and Donaldson, 1990). Thus, the source 'tracing window' opens 15 days before the estimated date of the first lesion; this comprises the 14 days maximum incubation period plus one day to allow for the accepted precision of lesion ageing (Figure 27.4). The window is 'closed' one day before the estimated date of the first lesion, again allowing for the accepted precision of lesion ageing and a minimum incubation period of two days; this is therefore the latest time that infection could have been introduced. Animals on any premises identified by the tracings are then clinically inspected. No further action is taken in the absence of clinical signs of disease.

Visits are again prioritized, with high priority being given to the five-day period before the latest infection date, consistent with the average incubation period.

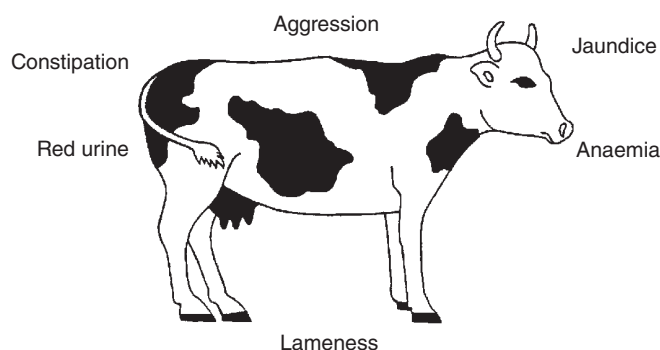
The tracing procedure, if implemented comprehensively, therefore should both identify potential sources of infection of the affected holding and 'capture' all farms potentially infected by it²⁶. If the outbreak is one of many, then tracing can be extensive. Thus, in the epidemic in the UK in 2001, with 2030 outbreaks (see Figure 4.1b), approximately 80 000 sheep flocks, alone, were inspected as part of the epidemiological inquiries linked to affected farms (DEFRA, 2002a).

Thrusfield and others (2005b) report this example in greater detail.

An example of forward and backward tracing in classical swine fever outbreaks is given by Elbers and others (2001).

²⁶ Airborne transmission is excluded from this assessment, but may be explored by meteorological virus-dispersion models (see Chapter 6).

Fig. 27.5 Clinical signs associated with mortality in cattle on a farm in South Africa, 1989.



Cause unknown: chronic copper poisoning

The following example relates to chronic copper poisoning in cattle (*Bos indicus*) on a farm close to the Kruger National Park in South Africa in the late 1980s (Gummow *et al.*, 1991).

Initially, a clinical history was taken. The farmer reported that 39 out of 189 cattle had died over three months in the current year (1989), demonstrating signs of a haemolytic crisis (Figure 27.5). The obvious differential diagnosis for the region, based on clinical signs, included vector-borne haemoparasitic disease (i.e., babesiosis or anaplasmosis), but examination of blood smears failed to demonstrate the parasites. The private veterinarians who were involved diagnosed leptospirosis, based on the gross pathology, and treated the herd with dihydrostreptomycin. However, the antibiotic treatment, followed by vaccination against leptospirosis, failed to alleviate the problem.

An epidemiological investigation involves recording of characteristics of the disease in the **population**, including *when* and *where* the disease occurs. This revealed that all of the deaths occurred in cattle over 18 months of age, although there were younger cattle on the holding. Moreover, there had been no deaths in the previous year (1988), but seven deaths in 1987, when there had also been a high mortality in sheep on the farm. An interesting meteorological finding was that there was above-average rainfall in 1988 and below-average rainfall in 1987 and 1989. The farm also was located in a copper-rich area of the country.

The accumulated findings:

- clinical signs of a haemolytic crisis²⁷;
- only cattle over 18 months of age dying (long accumulation period);
- high case fatality, but low morbidity;

- gross pathology and histopathological evidence of a haemolytic crisis;
- location in a copper-rich area;
- the particular susceptibility of sheep (high mortality in the year when there were only a few deaths in cattle);

are consistent with chronic copper poisoning (Gummow, 1996; Roder, 2001). Gross pathological and histopathological findings, and hepatic copper levels – 343–600 parts per million (ppm) wet matter – were consistent with this toxicity.

Thus, a diagnosis of copper poisoning was made, and surviving animals treated prophylactically with a 'lick' containing zinc sulphate and sulphur.

Additionally, further investigations were required to identify the precise source of the copper. A survey of the area revealed that a large open-cast copper mine was located to the south-east of the farm (Figure 27.6). Meteorological records indicated that the wind usually blew towards the north-west, in the direction of the farm; and occasionally towards the south-east (in the direction of the Kruger National Park). Thus, the source of the copper causing the poisoning could be either air pollution or the soil. Subsequent analysis of copper levels on the surface soil and grass were above the accepted maximum levels for grass (10 ppm) but the deep soil levels were generally low. This suggested that the source was air pollution. Moreover, low levels in washed-grass samples implied that the contamination lay in the dust covering the grass. This explained the temporal relationship between rainfall and mortality: in wet years the dust would be rinsed off the grass, reducing the chance of ingestion.

Examination of copper levels of culled buffalo and impala in the Kruger National Park revealed high organ copper levels only to the south-east of the mine (in which direction the wind sometimes blew²⁸),

²⁷ The aggression (Figure 27.5), reported by the farmer, was a coincidental behavioural finding in some cattle, and therefore constituted a 'red herring'.

²⁸ The relatively small home ranges (see Chapter 7) of these species maintained the concentration of affected animals under the plume.

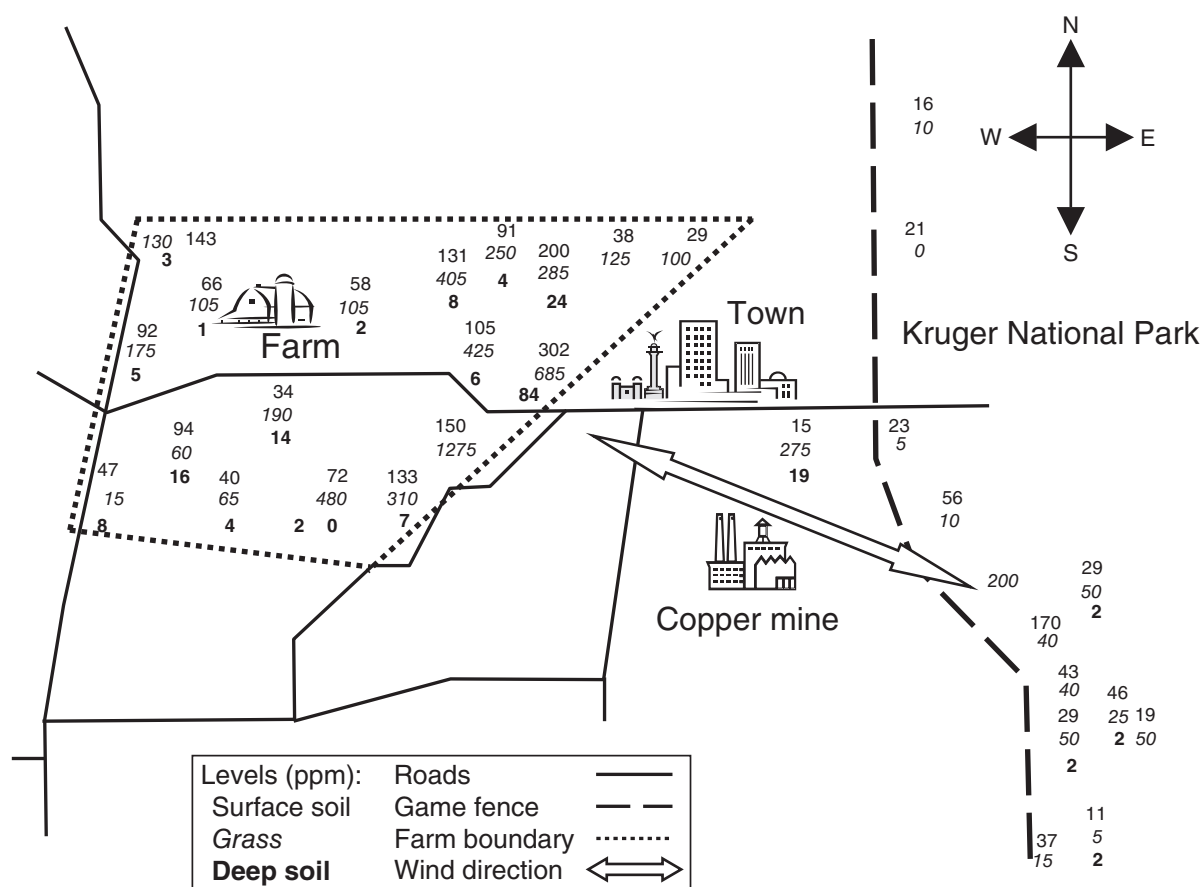


Fig. 27.6 The distribution of soil and grass copper levels (dry mass in parts per million, ppm) in an area close to the Kruger National Park, South Africa, 1989. (Adapted from Gummow *et al.*, 1991.)

adding credence to the hypothesis of air pollution. Subsequently, a more efficient electrostatic precipitator was fitted to the mine's furnace, reducing air pollution.

This investigation also highlights that epidemiological inquiries are as concerned with ruling out disease as with ruling it in. The link to low rainfall periods was inconsistent with vector-borne outbreaks and leptospirosis; the age profile of cases was inconsistent with a vector-borne or infectious disease, and the occurrence in *B. indicus* – a species relatively resistant to tick-borne diseases – also reduced the likelihood of the disease being vector-borne.

The epidemiological approach to investigation of outbreaks

Comparison of the two examples above reveals that outbreak investigations can take very different forms. Hence, a universal prescriptive approach is not possible. The component tasks of the 'epidemiological

approach' to investigation of outbreaks, however, can be generally identified. Not all of these activities may be necessary, or possible, for every investigation, and the order in which they are undertaken may vary (noting that they often overlap).

Establish a case definition

In some instances, it may be relatively straightforward to formulate a case definition. However, one that is too restrictive may exclude relevant cases from the investigation, whereas a definition that is too broad could increase the investigative work that is required and lead to erroneous conclusions. A case definition may include a number of criteria by which a case may be identified (e.g., *Table 4.1*). Even when the causal agent is known (as in the foot-and-mouth disease example), a case definition that can be applied in the field may be needed. Hence, in the 2001 foot-and-mouth outbreak in the UK, case identification initially relied on clinical signs. In other instances, definition of outbreaks, and therefore of cases, may be problematic (Ribble *et al.*,

1998). Indicators may be unexplained mortality or clinical disease (e.g., Waldner *et al.*, 1999), subclinical disease or productivity losses (e.g., Waldner *et al.*, 2001a). Scanning and syndromic surveillance (see Chapter 21) are useful means of identifying clinical outbreaks; and Shewhart and cusum charts (see Chapter 12) provide methods for identifying shortfalls in production.

Define the population at risk

In order to investigate an outbreak, it is necessary to delimit the population at risk. This includes identification of the species at risk and also the geographical range over which the outbreak occurs (or to which it may extend). Identification of *B. indicus* as at-risk animals – a species relatively resistant to tick-borne diseases – also reduced the likelihood of the disease being vector-borne in the copper poisoning example.

Case finding

Identification of cases is necessary not only to contain the outbreak but also to enable further epidemiological evaluation. Thus, during foot-and-mouth disease outbreaks, case finding will include forward tracing and identification of dangerous contacts (see also Figure 24.7) and serological surveillance (see Chapter 21 and Figure 11.11), the latter being necessary in countries aiming to maintain a disease-free status for the purpose of trading.

Calculate the frequency of disease

Depending on the methods of identification and the duration of risk, disease prevalence, incidence or attack rate (see Chapter 4) should be estimated. Comparison of the risk of disease between animal sub-populations can detect epidemiologically-important features. Thus, in the copper poisoning example, the cumulative nature of the poisoning resulted in cases only becoming clinically apparent in older animals. Identification of increased risk in older animals also provided evidence against alternative potential causes, such as vector-borne diseases and leptospirosis.

Observational studies Disease risk between sub-populations can be simply explored using observational studies (see Chapter 15). Retrospective cohort studies are feasible for outbreaks in small, well-defined populations in which all exposed and non-exposed individuals are identifiable. Table 27.8 classifies individuals in a small food-poisoning outbreak in which all individuals have been traced, focussing on individuals who ate Food A.

Table 27.8 Frequency of food consumption during an outbreak of food poisoning. (Hypothetical data.)

Exposure	Ill	Not ill	Totals	Attack rate (%)
Ate Food A	53	23	76	70
Did not eat Food A	2	97	99	2
Totals	55	120	175	31

Although the overall attack rate is 31%, most is attributable to cases in individuals who ate Food A, and the risk attributable to consumption of Food A is 68% (i.e., 70% – 2%). Alternatively, the relative risk is 70%/2% = 35, and most cases (53/55) were exposed to Food A. This provides strong evidence for Food A being the source of the food poisoning.

If clearly defined cohorts of exposed and unexposed individuals cannot be found then, if some cases have already been identified, a case-control design is appropriate, and odds ratios can be used to establish an increased risk associated with exposure to a particular factor. The controls must not have the disease in question, but should represent the population from which the cases come, and it is usual to calculate confidence intervals for the odds ratio.

Describe the temporal and spatial pattern of disease

The time and place of occurrence of an outbreak can be key factors in exploring causal hypotheses (see Chapter 3). Thus, in the copper poisoning example, examining the occurrence of cases over time identified a pattern consistent with cases occurring during dry years, again providing evidence against vector-borne diseases and leptospirosis as the causal agents, and exemplifying reasoning by the method of difference (see Chapter 3). Similarly, consideration of the location of the farm identified it as being in a copper-rich area. Epidemic curves (see Chapter 8) and geographical information systems (see Chapter 11) were used extensively to describe and investigate the 2001 foot-and-mouth disease epidemic in the UK.

Formulate and test hypotheses

Outbreaks may raise issues relating to causality (see Chapter 3), identification of the determinants of disease (see Chapter 5) and routes of transmission of infectious diseases (see Chapter 6). In the copper poisoning example, identification of copper toxicity as the cause of the mortality led to the hypothesis that the source may be the copper mine. Testing of hypotheses

may entail application of statistical tests of association (see Chapters 14 and 15) or use of statistical models (see Chapter 22)

Plan and implement preventive/control measures and surveillance strategies

The goal of outbreak investigation is to provide evidence to facilitate effective disease prevention, control and surveillance (see Chapter 21). These activities may prudently commence during initial outbreak investigation and therefore are informed by early epidemiological findings, and may be modified as new information becomes available. Thus, initial tracing during the 2001 outbreak of foot-and-mouth disease in the UK revealed that widespread dissemination of the virus had occurred before the index case was identified, necessitating early imposition of movement restrictions and the implementation of suitable surveillance strategies, including post-epidemic serological surveillance of sheep flocks (Mansley *et al.*, 2011).

Plan or modify strategies for future outbreaks

The information obtained during an outbreak can inform strategies to prevent or control future outbreaks of the same or different diseases. For example, epidemiological field investigations during the 2001 foot-and-mouth outbreak in the UK investigations (Honhold *et al.*, 2004a; Thrusfield *et al.*, 2005a) failed to identify a beneficial effect of the contiguous cull policy and therefore cast doubt on its future use (Mansley *et al.*, 2011).

Waldner and Campbell (2006) describe outbreak investigation in livestock production systems. Reingold (2000) and Dicker and others (2006) provide general overviews of outbreak investigation. Hugh-Jones (2006) presents a protocol for distinguishing between natural and intentionally-induced animal disease outbreaks.

Veterinary medicine in the 21st century

Chapter 1 described how veterinary medicine coped with various challenges during its development. This final chapter ends with some thoughts on the future direction of the veterinary profession, highlighting the contribution that epidemiology will make. The topic is discussed in greater detail by Henderson (1982), Hugh-Jones (1983), Pritchard (1986, 1989),

Michell (1993), IAEA (1998) and Catanzaro and Hall (2002).

Livestock medicine

Multifactorial diseases are the major problems in intensive livestock enterprises. Investigation of their cause does not involve the study of a simple infectious agent, but of several determinants associated with host, agent and environment. The environment is also recognized as important because of its significance to animal welfare (Ekesbo, 1992). Observational studies (see Chapter 15) provide a critical framework for identifying the many determinants of disease in intensive enterprises. Epidemiological principles and concepts are also applicable to welfare issues (McInerney, 1991; Willeberg, 1991).

Modelling of livestock units, using variables associated with disease and production, will continue to develop, facilitated by decision support systems (see Chapter 11). These also facilitate definition of the most suitable technical and economic production variables, and provide methods for assessing individual and herd performance (e.g., Huirne *et al.*, 1991, 1992; Huirne and Dijkhuizen, 1994).

In the less-economically-developed countries, there is a need for an improvement in the 'quality' of data. For example, the rinderpest eradication campaign highlighted the need for application of appropriate sampling techniques in its terminal stages (see Chapter 13). Participatory epidemiology (see Chapter 21) is expanding to fulfil this goal. Additionally, there is a move towards privatization of veterinary services in these countries, stemming from a decline in the efficiency of public services due to financial constraints (de Hann and Umali, 1992). This move is likely to expand, although not without some resistance (Turkson and Brownie, 1999).

Methods of identifying infectious diseases are becoming more analytically sensitive and refined. Smaller quantities of antigen can be detected, and subtle differences between strains can be identified using ELISA tests, monoclonal antibodies and the newer molecular techniques (see Chapter 2). All of these techniques are now being applied to the diagnosis of bacterial, virus and parasitic diseases of animals (Ambrosio and de Waal, 1990; Knowles and Gorham, 1990; OIE, 1993). However, they have some disadvantages, and older techniques therefore still have an important role (Wilson, 1993). For example, the analytical sensitivity and specificity of DNA probe technology – which involves a whole new level of

complexity – is similar to conventional microscopy in diagnosing human malaria. Similarly, the polymerase chain reaction can produce false-positive results due to contamination (Pang *et al.*, 1992) and is relatively slow.

New techniques are producing vaccines that are safer than formerly. For example, sub-unit vaccines comprise only virus capsid antigens and lack nucleic acid, and therefore cannot be pathogenic. Vaccines against helminths (e.g., *Dictyocaulus viviparus*) are few but they have a considerable advantage over the current anthelmintics, with the latter's associated risk of resistance and short period of action. The breeding of resistant stock may be a more useful technique because methods such as embryo cloning, superovulation and nuclear transplantation can accelerate this otherwise slow process. However, the fostering of genetically-engineered (transgenic) animals in agriculture to increase production is not without dangers; notably, reduced immune function (Greger, 2012).

Companion-animal medicine

In the more-economically-developed countries, the number of pet-owning households is increasing (e.g., Singleton, 1993), and there is a concomitant increase in the proportion of veterinarians engaged in companion-animal practice (e.g., *Figure 1.6*). The public's expectation of veterinary services will continue to rise and this will be reflected in improvements in the quality of patient care, involving better surgical techniques and medical therapy, and newer diagnostic methods (Leutenegger *et al.*, 2003). All of these are now subject to more critical evaluation than previously in properly-designed clinical trials (see Chapter 17) and diagnostic-test validation (see Chapter 20).

Advances in gene-targeted cancer therapy, evidenced in human medicine (Semizarov and Blomme, 2008; Al-Lazikani *et al.*, 2012; Sakharkar *et al.*, 2015) has given rise to **precision medicine**: an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment and lifestyle for each individual²⁹. Although

its application in veterinary medicine is more distant, companion-animal cancer therapy (Blomme and Spear, 2010) and vaccination in all species (Kernot, 2016) are clear candidates.

The collation of electronic patient records from veterinary practices is expanding (see Chapters 11 and 21), although they are still an underutilized resource in companion-animal practice (Krone *et al.*, 2014). Further expansion should increase the amount of data that are available for epidemiological and other veterinary research, resulting in large, but varied, data sets (so-called 'big data': Jacobs, 2009), thereby extending the knowledge base for evidence-based veterinary medicine.

The aim of medical epidemiologists is to ensure that each person enjoys a long life with morbidity confined to a short period before death³⁰. This goal can be shared by veterinarians, and its achievement requires research on improved preventive techniques, such as vaccination, and on the determinants associated with chronic and refractory diseases, such as canine heart disease and dermatoses. Observational studies again provide a critical framework for such research. This research is still hampered by a lack of basic demographic and morbidity data from a wide cross-section of the companion-animal population, although more data are becoming available (see *Table 4.2*). However, the availability of inexpensive microcomputers to veterinary practitioners, and the expansion of computer networks, notably the Internet, should facilitate the gathering and sharing of these data.

Epidemiology plays a central role in the continuing development and improvement of livestock and companion-animal veterinary medicine. Nevertheless, its contemporary objectives retain many similarities with those of ancient Greek medicine, described by Hippocrates, in the 'Second Constitution' of Book 1 of his *Epidemics*, as to:

'Declare the past, diagnose the present, foretell the future'

(Jones, 1923)

²⁹ There is a lot of overlap between the terms 'precision medicine' and 'personalized medicine'. The latter is an older term with a meaning similar to precision medicine. However, 'personalized' could be misinterpreted to imply that treatments and preventions are being developed uniquely for each individual; in precision medicine, the focus is on identifying which approaches will be effective for which patients based on genetic, environmental and

lifestyle factors. Precision medicine therefore is the preferred term, although the two terms sometimes are used interchangeably. Senn (2001) and Schork (2015) argue that conventional clinical trials – which assess average responses in groups – are not suitable for precision therapies, and therefore that trials of such treatments need to be refined appropriately.

³⁰ In some Western countries, increased life expectancy is an added goal (USDHHS, 2000).

Further reading

- Animal Health Australia (2008) *Australian Veterinary Emergency Plan (AUSVETPLAN)*, Edition 3. Primary Industries Ministerial Council, Canberra. (*National contingency planning framework for the management of animal disease emergencies in Australia*)
- Artois, M., Blancou, J., Dupeyron, O. and Gilot-Fromont, E. (2011) Sustainable control of zoonotic pathogens in wildlife: how to be fair to wild animals? *Revue Scientifique et Technique, Office International des Epizooties*, **30**, 733–743
- Artois, M., Delahay, R., Guberti, V. and Cheeseman, C. (2001) Control of infectious diseases of wildlife in Europe. *The Veterinary Journal*, **162**, 141–152
- Babiuk, L.A. (2002) Vaccination: a management tool in veterinary medicine. *The Veterinary Journal*, **164**, 188–201
- Bardosh, K. (Ed.) (2016) *One Health: Science, Politics and Zoonotic Disease in Africa*. Taylor and Francis, London. (*A discussion of intersectoral collaboration to control zoonotic diseases*)
- Biggs, P.M. (1985) Infectious disease and its control. *Philosophical Transactions of the Royal Society of London, Series B*, **310**, 259–274
- Blancou, J. (2003) *History of the Surveillance and Control of Transmissible Animal Diseases*. Office International des Epizooties, Paris
- Bourne, F.J. (1993) Biotechnology and farm animal medicine. In: *The Advancement of Veterinary Science. The Bicentenary Symposium Series. Volume 1: Veterinary Medicine beyond 2000*. Ed. Michell, A.R., pp. 41–57. CAB International, Wallingford. (*A discussion of the application of modern biotechnology to animal disease control*)
- Brander, G.C. and Ellis, P.R. (1976) *The Control of Disease*. Baillière Tindall, London. (*An old, but sound, general discussion of the prevention of infectious diseases in animals, including zoonoses*)
- Brückner, G. and Saraiva-Vieira, V.E. (2012) OIE strategy for the control and eradication of foot and mouth disease at regional and global levels. *Compendium of Technical Items Presented to the OIE World Assembly of Delegates and to OIE Regional Commissions*, 2010, pp. 187–198. Office International des Epizooties, Paris
- Charlier, J., Morgan, E.R., Rinaldi, L., van Dijk, J., Demeler, J. et al. (2014) Practices to optimise gastrointestinal nematode control on sheep, goat and cattle farms in Europe using targeted (selective) treatments. *Veterinary Record*, **175**, 250–255
- Correa Melo, E. and Gerster, F. (Coordinators) (2003) Veterinary services: organisation, quality assurance, evaluation. *Revue Scientifique et Technique, Office International des Epizooties*, **22**, 355–768. (*A global review of veterinary services*)
- Department for Environment, Food and Rural Affairs (2010) *Vaccination as a Control Tool for Exotic Animal Disease: Key Considerations*. Department for Environment, Food and Rural Affairs, London
- Dworkin, M.S. (Ed.) (2010) *Outbreak Investigations Around the World: Case Studies in Infectious Disease Field Epidemiology*. Jones and Bartlett, Sudbury. (*Interesting examples of human outbreak investigation and control, also including general principles*)
- Ellis-Iversen, J., Cook, A., Watson, E., Nielen, M. Larkin, L. et al. (2010) Perceptions, circumstances and motivators that influence implementation of zoonotic control programs on cattle farms. *Preventive Veterinary Medicine*, **93**, 276–285. (erratum: **94**, 318)
- England, J.J. (Ed.) (2002) Animal health emergency diseases. *Veterinary Clinics of North America, Food Animal Practice*, **18** (3), 1–583. (*A general description of some transboundary diseases, including their control*)
- FAO (1999) *New Technologies in the Fight Against Transboundary Animal Diseases*. FAO Animal Production and Health Paper No. 144. Food and Agriculture Organization of the United Nations, Rome. (*Includes descriptions of advances in epidemiological and diagnostic techniques, and vaccines, relevant to the control of infectious diseases*)
- Geering, W.A., Penrith, M.-L. and Nyakahuma (2001) *Manual on Procedures for Disease Eradication and Stamping Out*. FAO Animal Health Manual No. 12. Food and Agriculture Organization of the United Nations, Rome. (*A detailed consideration of the practical aspects of destruction and disposal of animals, and decontamination procedures*)
- Geering, W.A., Roeder, P.L. and Obi, T.U. (1999) *Manual on the Preparation of National Animal Disease Emergency Preparedness Plans*. FAO Animal Health Manual No. 6. Food and Agriculture Organization of the United Nations, Rome. (*A comprehensive discussion of the procedures and structures necessary for controlling outbreaks of infectious diseases; also containing a list of training aids, including books, interactive software and videos*)
- Gregg, M.B. (Ed.) (2008) *Field Epidemiology*, 3rd edn. Oxford University Press, Oxford. (*A medical text, which contains the basic principles of outbreak investigation*)
- Hanson, R.P. and Hanson, M.G. (1983) *Animal Disease Control*. Iowa State University Press, Ames. (*A description of principles and techniques relating to regional control programmes*)

- Horzinek, M.C. (2010) Vaccination protocols for companion animals: the veterinarian's perspective. *Journal of Comparative Pathology*, **142**, Suppl. 1, S129–S132
- House, J.A., Kocan, K.M. and Gibbs, E.P.J. (Eds) (2000) *Tropical Veterinary Diseases: Control and Prevention in the Context of the New World Order. Annals of the New York Academy of Sciences*, Vol. **916**. The New York Academy of Sciences, New York
- Inamura, M., Rushton, J. and Antón, J. (2015) *Risk Management of Outbreaks of Livestock Diseases*. OECD Publications, Paris
- Kahn, L.H. (2009) *Who's in Charge? Leadership during Epidemics, Bioterror Attacks, and other Public Health Crises*. ABD-CLIO, LLC, Santa Barbara. (A discussion of governance during disease emergencies, with case studies including bovine spongiform encephalopathy, foot-and-mouth disease, anthrax and swine influenza)
- Kaufmann, S.H.E. (Ed.) (2004) *Novel Vaccination Strategies*. Wiley-VCH, Weinheim
- Lütticken, D., Segers, R.P.A.M. and Visser, N. (2007) Veterinary vaccines for public health and prevention of viral and bacterial zoonotic diseases. *Revue Scientifique et Technique, Office International des Epizooties*, **26**, 165–177
- McNab, W.B. and Dubé, C. (2007) Simple models to assist in communicating key principles of animal disease control. *Veterinaria Italiana*, **43**, 317–326. (A guide to explaining to livestock owners and policy makers the role of the various components of infectious animal disease control in reducing the basic reproductive number, R_0)
- McVey, S. and Shi, J. (2010) Vaccines in veterinary medicine: a brief review of history and technology. *Veterinary Clinics of North America, Small Animal Practice*, **40**, 381–392
- Nielsen, T.G., Christensen, B. and Dantzer, V. (Eds) (1996) Decision of vaccination strategy in relation to increased trade of animals and animal products. *Acta Veterinaria Scandinavica, Suppl.* **90**, 1–118
- OECD (2012) *Livestock Diseases: Prevention, Control and Compensation Schemes*. OECD Publications, Paris. (Includes examples of national disease control schemes throughout the world)
- OIE (2014) *Guidelines for Animal Disease Control*. Office International des Epizooties, Paris
- Pastoret, P.-P., Blancou, J., Vannier, P. and Verschueren, C. (Eds) (1997) *Veterinary Vaccinology*. Elsevier, Amsterdam
- Patterson, D.F. (1993) Understanding and controlling inherited diseases in dogs and cats. *Tijdschrift voor Diegeneeskunde*, **118**, Suppl. 1, 23S–27S
- Perry, B., McDermott, J. and Randolph, T. (2001) Can epidemiology and economics make a meaningful contribution to national animal-disease control? *Preventive Veterinary Medicine*, **48**, 231–260
- Peters, A.R. (Ed.) (1994) *Vaccines for Veterinary Applications*. Butterworth Heinemann, Oxford
- Rees, W.G.H. and Davies, G. (1992) Legislation for health. In: *Livestock Health and Welfare*. Ed. Moss, R., pp. 118–159. Longman Scientific and Technical, Harlow. (A description of national and international control and eradication schemes)
- Rich, K.M. and Perry, B.D. (2012) Controlling animal disease in Africa. In: *Health and Animal Agriculture in Developing Countries*. Eds Zilberman, D., Otte, J., Roland-Holst, D. and Pfeiffer, D., pp. 305–325. Springer, New York
- Roth, J.A., Richt, J.A. and Morozov, I.A. (Eds) (2013) *Vaccines and Diagnostics for Transboundary Animal Diseases*. Karger, Basel
- Royal Society (2002) *Infectious Diseases in Livestock: Scientific Questions Relating to the Transmission, Prevention and Control of Epidemic Outbreaks of Infectious Diseases of Livestock in Great Britain*. Policy document 15/02. The Royal Society, London. (A broad discussion of the issues relating to epidemic control, focussing on the UK, but addressing general principles of wider relevance)
- Scherer, A. and McLean, A. (2002) Mathematical models of vaccination. *British Medical Bulletin*, **62**, 187–199
- Schnurrenberger, P.R., Sharman, R.S. and Wise, G.H. (1987) *Attacking Animal Diseases: Concepts and Strategies for Control and Eradication*. Iowa State University Press, Ames. (A general discussion of disease control, mainly with American examples)
- Schudel, A. and Lombard, M. (Eds) (2004) *Control of Infectious Animal Diseases by Vaccination*. Developments in Biologicals, Vol. 119. Karger, Basel
- Schultz, R.D. (Ed.) (1999) *Veterinary Vaccines and Diagnostics. Advances in Veterinary Medicine*, **41**, 1–815. Academic Press, San Diego
- Sutmoller, P., Barteling, S.S., Olascoaga, R.C. and Sumption, K.J. (2003) Control and eradication of foot-and-mouth disease. *Virus Research*, **91**, 101–144
- US Department of Health and Human Resources (1998) *Preventing Emerging Infectious Diseases. A Strategy for the 21st Century*. US Department of Health and Human Resources, Centres for Disease Control and Prevention, Atlanta. (A comprehensive discussion of the control of emerging human infections in the US, but also including some general principles and details of zoonotic and foodborne infections)

- Wobeser, G.A. (2007) *Disease in Wild Animals: Investigation and Management*, 2nd edn. Springer, Berlin and Heidelberg
- Woods, G.T. (Ed.) (1986) *Practices in Veterinary Public Health and Preventive Medicine in the United States*. Iowa State University Press, Ames. (A general discussion of veterinary public health, including environmental health, and animal disease control in the US)
- Yekutiel, P. (1980) *Eradication of Infectious Disease – A Critical Study*. Contributions to Epidemiology and Biostatistics, Vol. 2. Karger, Basel
- Zinsstag, J., Abakar, M.F., Ibrahim, M., Tschopp, R., Crump, L. *et al.* (2016) Cost-effective control strategies for animal and zoonotic diseases in pastoralist populations. *Revue Scientifique et Technique, Office International des Epizooties*, **35**, 673–681

General reading

Books

- Blaha, T. (Ed.) (1989) *Applied Veterinary Epidemiology*. Elsevier, Amsterdam
- Dohoo, I., Martin, M. and Stryhn, H. (2009) *Veterinary Epidemiologic Research*, 2nd edn. AVC, Charlottetown
- Halpin, B. (1975) *Patterns of Animal Disease*. Baillière Tindall, London. (*An old but concise digest of veterinary epidemiology*)
- Houe, H., Ersbøl, A.K. and Toft, N. (Eds) (2004) *Introduction to Veterinary Epidemiology*. Biofolia, Frederiksberg
- Hugh-Jones, M.E., Hubbert, W. and Hagstad, H.V. (1995) *Zoonoses: Recognition, Control and Prevention*. Iowa State University Press, Ames. (*Includes some general epidemiological principles and methods*)
- Law, G.R. and Pascoe, S.W. (2013) *Statistical Epidemiology*. CABI, Wallingford
- Leech, F.B. and Sellers, K.C. (1979) *Statistical Epidemiology in Veterinary Science*. Charles Griffin and Company, London and High Wycombe
- Martin, S.W., Meek, A.H. and Willeberg, P. (1987) *Veterinary Epidemiology: Principles and Methods*. Iowa State University Press, Ames
- Meek, A.H. and Martin, S.W. (1991) Epidemiology of infectious disease. In: *Microbiology of Animals and Animal Products*. World Animal Science, A6. Ed. Woolcock, J.B., pp. 141–180. Elsevier, Amsterdam. (*Abridged adaptation of Martin et al., 1987*)
- Noordhuizen, J.P.T.M., Frankena, K., Thrusfield, M.V. and Graat, E.A.M. (2001) *Application of Quantitative Methods in Veterinary Epidemiology*, revised reprint. Wageningen Pers, Wageningen
- Pfeiffer, D. (2010) *Veterinary Epidemiology: An Introduction*. Wiley-Blackwell, Chichester
- Porta, M. (2014) *A Dictionary of Epidemiology*, 6th edn. Oxford University Press, Oxford
- Putt, S.N.H., Shaw, A.P.M., Woods, A.J., Tyler, L. and James, A.D. (1987) *Veterinary Epidemiology and Economics in Africa*. ILCA Manual No. 3. International Livestock Centre for Africa, Addis Ababa. <http://www.fao.org/Wairdocs/ILRI/x5436E/x5436E00.htm>
- Schwabe, C.W. (1984) *Veterinary Medicine and Human Health*. 3rd edn. Williams and Wilkins, Baltimore and London
- Schwabe, C.W., Riemann, H.P. and Franti, C.E. (1977) *Epidemiology in Veterinary Practice*. Lea and Febiger, Philadelphia
- Sergeant, E. and Perkins, N. (2015) *Epidemiology for Field Veterinarians: An Introduction*. CABI, Wallingford
- Slater, M.R. (2003) *Veterinary Epidemiology*. Butterworth Heinemann, St Louis
- Smith, R.D. (2006) *Veterinary Clinical Epidemiology*, 3rd edn. CRC Press, Boca Raton
- Toma, B., Dufour, B., Sanaa, M., Bénét, J.J., Moutou, F. et al. (1999) *Applied Veterinary Epidemiology and the Control of Disease in Populations*. AEEMA, Paris
- Toma, B., Vaillancourt, J.-P., Dufour, B., Eloit, M., Moutou, F. et al., (Eds) (1999) *Dictionary of Veterinary Epidemiology*. Iowa State University Press, Ames
- Villarreal, A. (2015) *Practical Clinical Epidemiology for the Veterinarian*. John Wiley and Sons, Ames

Proceedings

- Epidemiology at Work* (1990) Refresher Course for Veterinarians. Proceedings 144, North Head, 5–7 October 1990. Post Graduate Committee in Veterinary Science, University of Sydney, Sydney
- Epidemiology in Animal Health* (1983) Proceedings of a symposium held at the British Veterinary Association's Centenary Congress, Reading, 22–25 September 1982. Society for Veterinary Epidemiology and Preventive Medicine
- Kennedy, D. (Ed.) (1990) *Epidemiological Skills in Animal Health*. Proceedings 143, Sydney, 1–5 October 1990. Post Graduate Committee in Veterinary Science, University of Sydney, Sydney

- Proceedings from the Veterinary Epidemiology Seminar, Palmerston North, 26–27 February 2007. VetLearn, Massey University, Palmerston North
- Proceedings of the Dutch Society for Veterinary Epidemiology and Economics. 1989– (1989–1990 in Dutch) (continuing) (annual)
- Proceedings of the International Society for Veterinary Epidemiology and Economics. 1976– (continuing) (triennial)
- Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine. 1984– (continuing) (annual)

Journals

Papers on veterinary epidemiology are published in a wide range of journals. *Preventive Veterinary Medicine* is the main journal devoted to the subject.¹ National veterinary journals such as *Acta Veterinaria Scandinavica*, the *American Journal of Veterinary Research*, the *Australian Veterinary Journal*, the *Canadian Veterinary Journal*, the *Journal of the American Veterinary Medical Association*, the *New Zealand Veterinary Journal*, *The Veterinary Journal* (formerly the *British Veterinary Journal*) and the *Veterinary Record*; disciplinary journals such as *Cancer Research*, the *International Journal of Parasitology*, the *Journal of National Cancer Institute* and the *Journal of Pathology*; and journals specializing in particular species, such as the *Equine Veterinary Journal*, the *Journal of Small Animal Practice* and the *Journal of the American*

Animal Hospitals Association, also publish epidemiological papers. *Epidemiology and Infection* (formerly the *Journal of Hygiene, Cambridge*) focusses on infectious diseases. The *Bulletin of the Pan American Health Organization* (now defunct), *Bulletin of the World Health Organization*, *Revue Scientifique et Technique Office International des Epizooties*, *Tropical Animal Health and Production* and the *World Animal Review* (now defunct) contain material relevant to less-economically-developed countries. *Emerging Infectious Diseases* and *Transboundary and Emerging Diseases* publish reports of new infections and infections that are increasing in importance. *Animal Health Research Reviews* provides broad descriptions of many diseases. *Infection Ecology & Epidemiology*, *International Journal of One Health*, *One Health* and *PLOS ONE* (formerly *PLoS ONE*) also have substantial epidemiological content.

The *American Journal of Epidemiology* (formerly the *American Journal of Hygiene*) is primarily medical, but occasionally publishes veterinary material. It, *Epidemiology*, and the *International Journal of Epidemiology*, also contain papers on quantitative methods. These methods are also published in statistical journals such as *Applied Statistics*, *Biometrics*, the *Journal of the Royal Statistical Society (Series A and B)*, *Mathematical Biosciences*, *Risk Analysis* and *Statistics in Medicine*. *Evidence-Based Medicine* reports clinical trials (including meta-analyses) and assessments of procedures; although primarily medical, the veterinary reader will find some of the methods that are reported in this journal to be of value.

¹ An overview of the contents of this journal, from 1982 to 1997, is given in volume 30 (1997), pp. 181–333; and, from 1997 to 2001, in volume 50 (2001), pp. 187–273.

Appendices

Appendices V, IX, XXI and XXII are taken from Tables III, IV, VII and V, respectively, in *Statistical Tables for Biological, Agricultural and Medical Research*, 6th edition (1974), edited by Fisher, R. and Yates, F. and published by Longman Group Limited

(previously published by Oliver and Boyd Limited, Edinburgh), and are reproduced with the permission of the authors and publishers.

Appendix I

Glossary of terms

This glossary provides brief definitions of some common epidemiological terms that are used in this book. More comprehensive guides are *Dictionary of Veterinary Epidemiology* (edited by B. Toma, J.-P. Vaillancourt, B. Dufour, M. Eloit, F. Moutou, W. Marsh, J.-J. Bénet, M. Sanaa and P. Michel, Iowa State University Press, Ames, 1999), *A Dictionary of Epidemiology*, 6th edition (edited by M. Porta, Oxford University Press, New York, 2014)¹, and *The Cambridge Dictionary of Statistics in the Medical Sciences* (B.S. Everitt, Cambridge University Press, Cambridge, 1995), from which some of the definitions below are derived.

Abiotic (components): non-living chemical and physical factors in an environment.

Accuracy: the degree to which an individual measurement represents the true value of the attribute that is being measured: the greater the accuracy, the greater the degree.

Adjustment: a summarizing procedure for a parameter (see Appendix II), for example, incidence or mortality, in which the effects of differences in the composition of populations compared (e.g., different age distributions) are minimized. Two common techniques are **direct** and **indirect standardization**.

Antibody: a protein produced by an animal's immunological system in response to exposure to a foreign substance (an antigen; q.v.). Sometimes antibodies are produced against the individual's own proteins, causing autoimmune disease. Antibodies display specificity (q.v.) to particular antigens.

Antigen: a substance (usually a protein) that induces a specific immune response (e.g., circulating antibody production).

Association: a general term to describe the relationship between two variables (see Appendix II). The

association is 'positive' when the variables occur together more frequently than is expected by chance; the association is 'negative' when they occur less frequently than is expected by chance.

Asymptotic method: any statistical method based on an approximation to a Normal distribution (q.v.) or other probability distribution that becomes more accurate as sample size increases.

Bias: systematic (as opposed to random) departure from true values.

Big data: the capability to manage a huge volume of disparate data, at the right speed and within the right time frame, to allow real-time analysis and reaction. Big data has three characteristics: 1. volume (how much data); 2. velocity (how fast that data is processed); and 3. variety (the various types of data).

Binomial distribution: a probability distribution relating to two mutually exclusive and exhaustive outcomes (e.g., the birth of either male or female animals), where successive outcomes (e.g., births) are independent and occur with constant probability.

Biosecurity: management-practice activities that reduce the opportunities for infectious agents to gain access to, or spread within, a food-animal production unit.

Carrier:

1. an animal that is infected with an infectious agent without displaying clinical signs, and that can be a source of infection to other animals
2. (**of foot-and-mouth disease virus**) an animal in which virus persists in the pharyngeal region for more than four weeks after infection
3. (**genetic**) an animal that is heterozygous for a normal and an abnormal gene, the latter of which is not expressed but may be detected by tests.

Case: an animal in a population or study group identified as having a particular disease or other health-related event that is being investigated.

Case-control study: an observational study (q.v.) in which a group of diseased animals (cases) is

¹ An English–French and French–English dictionary, based on the 1st edition (1983), is also published (Fabia *et al.*, 1988).

compared with a group of non-diseased animals (controls) with respect to exposure to a hypothesized cause.

Causality: the relating of causes to the effects that they produce.

Clinical trial: a systematic study in the species for which a prophylactic or therapeutic procedure is intended in order to establish the procedure's prophylactic or therapeutic effects. A 'field trial' is a clinical trial undertaken in the field; that is, under husbandry and management practices typical of those under which the procedure is intended to be used.

Cohort study: an observational study (q.v.) in which a group of animals exposed to a hypothesized cause is compared with a group not so exposed, with respect to development of a disease.

Commensals: microbes found on the skin or within the body that do not usually cause disease (cf. Pathogen).

Confidence interval: a range of values within which the value of a parameter (see Appendix II) lies with a specified level of confidence.

Confounding: the inseparability from a given data set of the effects of two possible causes of an observed result, because both occur together.

Continuous variable: a variable (see Appendix II) that may take any value in an interval; the interval may be finite or infinite.

Correlation: see Association.

Cost-benefit analysis: see Social cost-benefit analysis.

Cross-product ratio: see Odds ratio.

Cross-sectional study: an observational study (q.v.) in which animals are classified according to presence or absence of disease, and presence or absence of exposure to a hypothesized causal factor, at a particular point in time.

Cross-sectional survey: a survey (q.v.) undertaken at a particular point in time.

Database: a structured collection of data, organized so that it can be accessed easily by a range of computer software.

Determinant: a factor that affects the health of a population.

Discrete variable: a variable (see Appendix II) for which there is a definite distance from one value of the variable to the next possible value (e.g., numbers of cases of disease: 1, 2, 3 ... where the distance is one).

Ecosystem: mutually interrelated communities of species and abiotic components (q.v.), existing as a system with specific interactions and exchange of matter, energy and information.

Endemic:

1. the predictable level of occurrence of disease, infection, antibody, etc.
2. the usual presence of disease, infection, antibody, etc.

Endogenous:

1. normally from within an animal
2. (**characteristic**) an innate characteristic of an animal (e.g., breed).

Epidemic: an occurrence of disease in excess of its anticipated frequency (also used adjectivally).

Epidemic curve: a graph plotting the number of new cases against time of onset of disease; thus, an epidemic curve plots incidence.

Epidemiology (veterinary): the investigation of disease, other health-related events, and production in animal populations and the making of inferences from the investigation in an attempt to improve the health and productivity of the populations.

Evidence-based medicine: the process of finding relevant information in the veterinary literature to address a specific clinical problem; the application of simple rules of science and common sense to determine the validity of information; the application of the information to a clinical question; that is, patient care based on the best available studies.

Exogenous:

1. normally from outside an animal
2. (**characteristic**) a characteristic that is not innate, to which an animal is exposed (e.g., climate, toxic substances and microbes).

Experimental study: a study (q.v.) in which the investigator can allocate animals to different categories; thus, the conditions of the study are controlled by the investigator.

Extrinsic factor: see Exogenous (2).

Extrinsic incubation period: the time between the entry of an infectious agent into an arthropod vector and the time at which the arthropod becomes infectious.

Field trial: see Clinical trial.

Fomites (singular **fomes**): inanimate communicators of infection (cf. Vector).

Health and productivity schemes: systems for recording disease and productivity in groups of animals (usually herds and flocks), their aim being to improve health and productivity of the groups.

Horizontal (lateral) transmission: transmission of an infection from an individual to any other individual in a population, but excluding vertical transmission (q.v.).

Humanosis: see Zoonosis.

Hypothesis: a proposition that can be tested formally; after which the hypothesis may be either 'supported' or 'rejected'.

Inapparent infection: an infection that does not produce clinical signs.

Incidence: the number of new cases that occur over a specified period of time. It is usually expressed in relation to the population at risk and the time during which the population is observed.

Informatics: the supply of information through the medium of the computer.

Interaction:

1. **(biological)** the interdependent operation of two or more causes to produce an effect
2. **(statistical)** in an epidemiological context, a quantitative interdependence between two or more factors, such that the frequency of disease when two or more factors are present is either in excess of that expected from the combined effects of each factor (positive interaction) or less than the combined effect (negative interaction).

Intrinsic factor see Endogenous (2).

Likelihood: the probability of a set of observations, given the value of a parameter (see Appendix II) or set of parameters.

Longitudinal study:

1. a cohort study (q.v.)
2. a general description of both cohort and case-control studies (q.v.), so called because these studies investigate exposure to a hypothesized cause and development of disease (the effect) when cause and effect are separated temporally.

Longitudinal survey: a survey (q.v.) that records events over a period of time.

Migration: the regular, usually seasonal, movement of all or part of an animal population to and from a given area (cf. Transhumance).

Misclassification: the incorrect allocation of individuals or features to categories to which they do not belong (e.g., the classification of a diseased animal as non-diseased).

Model:

1. **(biological)** a system that uses animals to study diseases, pathological conditions and impaired function; the model may be induced experimentally or may be constructed using naturally-occurring conditions
2. **(economic)** a simplified representation of reality showing the interrelationships between selected economic variables
3. **(mathematical)** a representation of a system, process or relationship in mathematical form in which equations are used to simulate the behaviour of the system or process under study
4. **(statistical)** a theoretical model of distributions of samples of data; it embodies a set of assumptions concerning the generation of the observed data, and similar data from a larger population; the

assumptions describe a set of probability distributions, some of which are assumed to adequately approximate the distribution from which a particular data set is sampled; more generally, statistical models are part of the foundation of statistical inference.

Monitoring: the routine collection of information on disease, productivity and other characteristics possibly related to them in a population.

Morbidity: the amount of disease in a population (commonly defined in terms of incidence or prevalence; q.v.).

Mortality: a measure of the number of deaths in a population.

Multifactorial disease: a disease that depends on the presence of several factors for its induction. Most diseases are multifactorial, although some may have one major component cause (e.g., foot-and-mouth disease virus is the cause of foot-and-mouth disease), in which case they are commonly termed 'unifactorial'.

Multivariate analysis: a set of statistical techniques used to study the variation in several variables simultaneously; more specifically, multivariate analysis refers to investigation of several *response* variables, whereas **multivariable analysis** explores the effects of several *explanatory* variables.

Necessary cause: a cause that must always be present for a disease to occur (e.g., *Mycobacterium tuberculosis* is the necessary cause of tuberculosis).

Nidality: the characteristic of an infectious agent to occur in distinct nidi (q.v.) associated with particular geographical, climatic and ecological conditions.

Nidus (plural **nidi**): a focus of infection.

nm (nanometre): 10^{-9} m; equivalent to the obsolescent millimicron (μm).

Normal distribution: a probability distribution relating to continuous data and characterized by a symmetrical bell-shaped distribution with 'tails' extending to infinity.

Observational study: an epidemiological study (q.v.) in which the investigator has no freedom, or does not exercise his or her freedom, to allocate animals to different categories; disease is studied as it occurs 'naturally'.

Odds: the ratio (q.v.) of the probability of an event occurring to that of it not occurring.

Odds ratio: the ratio of two odds: a measure of association commonly used in observational studies (q.v.). The odds are defined differently, depending on the type of study. Thus, in a cohort study (q.v.), a *disease odds ratio* is estimated: this is the odds in favour of disease among exposed individuals divided by the odds in favour of disease among unexposed individuals (cf. Relative risk). In diagnostic testing, the

diagnostic odds ratio is the ratio of the odds of test positivity in diseased individuals relative to the odds of test positivity in healthy individuals.

One Health:

1. the promotion of health through interdisciplinary study and action, across all animal species
2. detailedly, the collaborative effort of multiple health science professions, together with their related disciplines and institutions – working locally, nationally and globally – to attain optimal health for people, domestic animals, wildlife, plants and our environment.

One-tailed test: a statistical significance test based on the assumption that the data have only one possible direction of variability.

Outbreak: an identified occurrence of disease involving one or more animals. In more-economically-developed countries, an outbreak is frequently synonymous with disease occurrence on individual farms or holdings. The term sometimes refers to a bout of disease occurrence stemming from a single source, irrespective of the number of premises involved.

Pandemic: a geographically widespread (sometimes global) epidemic (also used adjectivally).

Parameter: see Appendix II.

Pathogen: an organism that produces disease.

Pathogenicity: the ability of an infectious agent to cause disease.

Point (common) source epidemic: an epidemic resulting from exposure of animals to a single common cause.

Poisson distribution: a probability distribution relating to the distribution of events, independently, either throughout space (an area) or over time.

Population at risk: the population that is naturally susceptible to a disease.

Precision:

1. the reciprocal of the variance of an estimate
2. the quality of being lucidly and clearly defined.

Predictive value:

1. **(of a positive test result)** the probability that an animal with a 'positive' test is a true 'positive'
2. **(of a negative test result)** the probability that an animal with a 'negative' test result is a true 'negative'.

Prevalence: the number of occurrences of disease, infection, antibody presence and so on in a population, usually relating to a particular point in time; it is commonly expressed as the proportion of the population at risk.

Probability interval: an interval that contains a parameter (see Appendix II) with a specified probability.

Proportion: a ratio (q.v.) in which the numerator is part of the denominator.

Prospective study: a cohort study.

Rate: a ratio (q.v.) that indicates the change in one quantity with respect to one or more others over time. Thus, incidence rate is the number of new cases of disease occurring in a population observed for a defined period of time (e.g. 10 cases per 100 animal-years at risk).

Ratio: a value obtained by dividing one quantity (the numerator) by another (the denominator); for example, the number of males born per female birth. Proportions and rates are ratios.

Refinement: the quality of being sharply defined. Thus, a refined serological test will detect subtle antigenic differences between microbes, whereas a less refined test will only identify major antigenic groups.

Relative odds: see Odds ratio.

Relative risk: the ratio of disease incidence in individuals exposed to a hypothesized cause, to the incidence in those not so exposed. It is a measure of association commonly used in cohort studies (q.v.). (cf. Odds ratio.)

Reliability: the degree of stability exhibited when a measurement is repeated under identical conditions; reliability therefore may be demonstrated by repeating a measurement.

Reservoir: an animate or inanimate object on or in which an infectious agent usually lives, and which therefore is often a source of infection by the agent.

Retrospective study:

1. a case-control study (so-called because the study looks back from effect to cause)
2. any study that collects and utilizes historical data.

Risk: the probability that an event will occur.

Risk ratio: see Relative risk.

Sample: a selected part of a population.

Sampling error:

1. the difference between a sample's result and the population characteristic that is being estimated
2. that part of the overall estimation error of a parameter (see Appendix II) caused by the random nature of the sample.

Sampling variation: the variation shown by different samples of the same size caused by the chance inclusion of individuals in the samples.

Screening: the identification of unrecognized disease or defect in an apparently healthy population.

Sensitivity (of a surveillance system; q.v.):

1. **(of case detection)** the proportion of individual animals or herds in the population of interest that have the health-related condition of interest and that the surveillance system is able to detect
2. **(of outbreak detection)** the probability that the surveillance system will detect a significant increase (outbreak) of disease

3. **(presence)** the probability that disease will be detected if present at a certain level.

Sensitivity (of a test):

1. **(diagnostic)** the proportion of diseased animals that are detected by a test
2. **(analytical)** the ability of a test to detect amounts of antigen, enzyme, nucleic acid and so on; a sensitive test will detect small amounts.

Social cost–benefit analysis: an economic technique used in epidemiology to assess the costs of disease and impaired productivity in relation to the benefits that accrue from their control.

Specificity:

1. degree of refinement; the greater the specificity, the greater the degree
2. **(diagnostic (of a test))** the proportion of non-diseased animals that are detected by a test
3. **(analytical (of a test))** degree of refinement in an infectious agent (e.g., antigenic type or nucleic acid composition) that can be detected by a test; the greater the specificity, the greater the degree.

Sporadic: the irregular, unpredictable occurrence of disease or infection.

Spreadsheet: a computer software package providing a representation of a large rectangular area upon which data tabulation may be displayed and a variety of calculations performed.

'Stamping-out policy' the *World Organisation for Animal Health (Office International des Epizooties)* defines stamping out as the carrying out under the authority of the veterinary administration, on confirmation of a disease, of zoo-sanitary prophylactic measures, consisting of killing the animals that are affected and those suspected of being affected in the herd and, where appropriate, those in other herds that have been exposed to infection by direct animal-to-animal contact, or by indirect contact of any kind likely to cause transmission of the causal pathogen. All susceptible animals, vaccinated or unvaccinated, on an infected premises should be killed and the carcasses destroyed by burning or burial, or by any other method which will eliminate the spread of infection through the carcasses or products of the animals killed. This policy should be accompanied by approved cleansing and disinfection procedures.

A 'modified stamping-out policy' is any policy where the above zoo-sanitary measures are not implemented in full.

Study: an investigation that involves the testing of a causal hypothesis. A study may be either experimental (q.v.) or observational (q.v.).

Sufficient cause: the complex of component causes that induces a disease. Several different sufficient causes may induce the same disease.

Surveillance (veterinary): the ongoing systematic collection and collation of useful information about disease, infection, intoxication or welfare in a defined animal population, closely integrated with timely analysis and interpretation of this information, and dissemination of relevant results to those requiring them, including those responsible for control measures.

Surveillance system: an integrated collection of one or more surveillance networks, designed to undertake surveillance (q.v.); each network comprises the institutional infrastructure, personnel and agencies assembled to conduct surveillance of one or more diseases in a given region.

Survey: an investigation involving the collection of information and in which a causal hypothesis usually is not tested (cf. Study). It may suggest aspects worthy of study.

Synergism: a positive statistical interaction (q.v.) where a causal pathway can be inferred (other authors may use the term differently).

Threshold level:

1. the spatial density of susceptible animals required to initiate an epidemic
2. the minimum concentration of an infectious agent in a vertebrate host's circulation that allows successful transmission to an arthropod vector
3. a critical level for the number and combination of genes above which a genetically-determined disease occurs.

Two-tailed test: a statistical significance test based on the assumption that the data are distributed in both directions from some central value(s).

Transhumance: the seasonal migration of livestock to suitable grazing grounds (cf. Migration).

Validity: a term with a variety of meanings; in this book it is the degree to which a diagnostic test or survey produces, *on average*, an accurate result. It is therefore a long-run property of the test or survey.

Variable: see Appendix II.

Vector: a living organism (frequently an arthropod) that communicates an infectious agent from an infected to a susceptible animal.

Vertical transmission: transmission of an infection from one individual to its offspring.

Virulence: the disease-evoking power of an infectious agent in a particular host.

Zoonosis: an infection shared in nature by humans and other vertebrates; it also is used, more specifically, to refer to a disease that normally exists in non-human animals (i.e., can be perpetuated in them) but that can infect humans, thus contrasting with a **humanosis**, which is an infection normally present in humans that can spread to other animals.

Appendix II

Basic mathematical notation and terms

Variables

There are properties of members of a population that vary between the members, for example, weights of cows or breed of pig; these properties are **variables**. Their values are denoted by letters of the Roman alphabet, which usually take the lower case, x , y , z , etc. The letters often have subscripts (small-sized numbers to the right of, and slightly below, the letter), for example:

the weights x_1 , x_2 , x_3 of three calves,
 $x_1 = 230$ kg, $x_2 = 221$ kg, $x_3 = 155$ kg.

Constants

There are two types of constant:

1. **universal constants** have a single value, for example, $\pi = 3.141 \dots$
2. **parameters** are constants that are fixed for a particular study, but which may change from one study to another. Greek letters, λ , μ and so on, usually denote parameters. For example, the infectivity rate of a particular parasite could be denoted by λ , where λ may change from one isolate to another.

Logarithms

Logarithms are a class of arithmetical functions distinguished by a characteristic known as the **base**, such that the logarithm of a number is the power to which the base of the logarithm must be raised to give the number. Two bases are frequently used: base 10 and base e , where $e =$ the universal exponential constant, 2.718 281 ... Logarithms to base 10 are also called 'common' logarithms, whereas logarithms to the base e (abbreviated to 'log_{*e*}' or 'ln') are called 'natural' or

Napierian logarithms. Additionally, logarithms to the base 2 sometimes are used.

Thus, $\log_{10} 100 = 2$ (i.e., $10^2 = 100$),

$$\log_e 5 = 1.609 \text{ (i.e., } 2.7181^{1.609} = 5),$$

$$\log_2 8 = 3 \text{ (i.e., } 2^3 = 8).$$

(Note that the logarithm of one to any base is zero, and the logarithm of zero to any base is 'minus infinity'.)

The inverse of logarithmic transformation is antilogarithmic transformation. Thus, $\text{antilog}_{10} 2 = 10^2 = 100$.

Exponential function: $\exp(x)$

The exponential function, $\exp(x)$, alternatively written e^x , is the particular case of antilogarithmic transformation that relates to natural logarithms. For example, if $x = 4$, $\exp(x) = 2.718^4 = 54.6$.

Summation notation: Σ

Σ is used to denote the sum of a set of data. For example:

$$\sum_{i=1}^6 = x_1 + x_2 + x_3 + x_4 + x_5 + x_6.$$

This means 'the sum of all the values of x from x_1 to x_6 , inclusive'. This notation is of value if, for instance, one wanted to add x_3 , x_4 and x_5 from the series of values, in which case one would write:

$$\sum_{i=3}^5 x_i.$$

In biological calculations it is usually necessary to add *all* of the values of x from x_1 to the last value of x , omitting none of the values in the series. It is

therefore sufficient to write Σx , in which case it is assumed that all of the x values in the series are being added.

This system of notation also can be applied to powers. For example, Σx^2 means 'square all the individual values of x in the series and then add these square values together'. For example, if:

$$x_1 = 2, x_2 = 3, x_3 = 2,$$

$$\text{then } \Sigma x^2 = x_1^2 + x_2^2 + x_3^2 = 4 + 9 + 4 = 17.$$

$(\Sigma x)^2$ means 'add together all the values of x in the series and then square the result'. For example, if:

$$x_1 = 2, x_2 = 3, x_3 = 2,$$

$$\text{then } \Sigma(x)^2 = (x_1 + x_2 + x_3)^2 = (2 + 3 + 2)^2 = 49.$$

Order of calculation

Multiplication and division are conducted before addition and subtraction. Thus:

$$\begin{aligned} 6 \times 3 + 1 &= 18 + 1 \\ &= 19. \end{aligned}$$

Brackets are used to indicate the order of calculation, taking precedence over multiplication and division when calculations would otherwise be ambiguous. Three types of brackets are commonly used: parentheses (), braces {} and square brackets [], usually, but not always, in that order.

Thus: $3 [3 + \{6(4 + 2)\}]$

is calculated as $4 + 2 = 6$,

$$\text{then } 6 \times 6 = 36,$$

$$\text{then } 3 + 36 = 39,$$

$$\text{then } 3 \times 39 = 117.$$

Similarly: $1 + 6 \times 3 = 19$,

but $(1 + 6) \times 3 = 21$.

Pocket calculators and computer software packages compute values following the order of calculation described above. Therefore, in circumstances in which brackets are required to avoid ambiguity, calculations must be undertaken in unambiguous stages, or by inclusion of brackets within the calculations.

Magnitude notation

$>$ = greater than (e.g., $6 > 5$);

$<$ = less than (e.g., $5 < 6$);

$\geq$ = greater than or equal to;

$\leq$ = less than or equal to.

A line through any of these symbols means 'not', for example, $\nlessgtr$ means 'not greater than'.

Approximation notation

The symbol $\approx$, read as 'approximately equal to', is used to indicate approximation. For example, the base of natural logarithms $e = 2.718\ 281 \dots$ and may be written as $e \approx 2.72$.

Estimation notation

Parameters are frequently estimated from a **sample** drawn from a population. The sample produces an **estimate** of the population parameter. An estimate is indicated either by $\wedge$ (a 'hat') or by a single asterisk, *. Thus, a sample estimate of disease prevalence is presented as either $\hat{P}$ (P 'hat') or P^* . The 'hat' notation is used in this book.

Factorial notation: $x!$

$x!$ is used to denote the successive multiplication of all positive integers (whole numbers) between x and 1. For example:

$$6! = 6 \times 5 \times 4 \times 3 \times 2 \times 1 = 720$$

$$2! = 2 \times 1 = 2$$

$$1! = 1.$$

(Note that $0!$ conventionally equals 1.)

Modulus notation: $|x|$

Vertical lines on each side of a numerical quantity, x , mean that the positive sign of the value of x should be used. The value thus obtained is known as the **absolute value** of x . For example, $|-2|$ is read as +2. Similarly, $|-6 + 1|$ will simplify to $|-5|$, which is read as +5. Also, $-6 + 1 = -5$, but $|-6| + 1 = 7$.

Appendix III

Some computer software

This directory lists some computer software packages that are of value to veterinary epidemiology. The list is not exhaustive. The descriptions of the various packages differ in the degree of detail and, for some packages, the list is hardly more than enumerative, but should be sufficient to allow potential users to identify appropriate software.

Relevant references are in square brackets, following the names of the packages. (Package manuals are not included.)

Most of the packages run in the MS Windows environment.

The main addresses of suppliers are listed. Some suppliers also have local offices in various countries. Note that the Internet Uniform Resource Locators (URLs) of suppliers may change. If this occurs, most Internet 'search engines' (e.g., Google™; <http://google.com>), specifying either the package name or its supplier, should locate the new URLs.

Internet searches also can locate software for specific statistical procedures and calculations.

Package/ reference	Functions	Further information/Supplier/URL
CIA [Altman <i>et al.</i> , 2000]	Confidence interval estimation means and their differences medians and their differences proportions and their differences regression and correlation relative risks and odds ratios standardized rates and ratios survival analyses sensitivity and specificity <i>kappa</i> likelihood ratio ROC curves clinical trials and meta-analyses	Supplied with <i>Statistics with Confidence</i> (Altman <i>et al.</i> , 2000) http://www.som.soton.ac.uk/cia/
ClinTools	Contingency tables (number needed to treat, relative risks and odds ratios) Effect sizes Meta-analyses Random numbers	http://www.clintools.com/
CSurvey	Sample size determination for cluster sampling, using probability proportional to size (PPS) sampling	http://www.ph.ucla.edu/epi/csury.html
Epi Info	Questionnaire design Descriptive statistics and graphics Surveys: simple random, stratified and cluster sampling Contingency tables (relative risks and odds ratios) Logistic regression Sample size determination: surveys case-control, cohort and cross-sectional studies Survival analysis Mapping	Epi Info™ Help Desk Centers for Disease Control and Prevention 1600 Clifton Rd Mail Stop E-91 Atlanta, GA 30333, USA http://www.cdc.gov/epiinfo
Epi Tools [Sergeant, 2015]	Surveillance utilities: one-stage representative freedom surveys two-stage representative freedom surveys risk-based freedom surveys random sampling from a population estimating true prevalence pooled prevalence calculator <i>Survey Toolbox</i> for livestock diseases and freedom in finite populations herd-sensitivity and freedom in finite populations Epidemiological studies: sample size calculations summarizing of categorical or continuous data statistical significance testing probability distributions bioequivalence analysis Diagnostic tests: compare two tests tests in parallel or series positive and negative predictive values for a test probabilities of numbers of false-positives probability of infection in a test-negative sample likelihood ratios and probability of infection in a tested individual estimated true prevalence and predictive values from survey testing test evaluation against a gold standard ROC analysis for test with continuous outcome repeatability analysis for tests with continuous outcome	AusVet Animal Health Services PO Box 1278 1a/109 Herries Street Toowoomba, QLD 4350, Australia, and AusVet Animal Health Services 46 Boulevard de la Croix Rousse 69001 Lyon, France http://epitools.ausvet.com.au

<i>Package/ reference</i>	<i>Functions</i>	<i>Further information/Supplier/URL</i>
<i>GENSTAT</i> [McConway <i>et al.</i> , 2009]	Comprehensive statistical analyses	VSN International Ltd 2 Amberside House Wood Lane Hemel Hempstead HP2 4TP, UK http://www.vсни.co.uk
<i>LogXact</i>	Binary logistic regression and multinomial regression	Cytel Software Corporation 675 Massachusetts Avenue Cambridge MA 02139, USA http://www.cytel.com
<i>Minitab</i>	Comprehensive statistical analyses	Minitab Inc. Quality Plaza, 1829 Pine Hall Road State College PA 16801-3008, USA http://www.minitab.com
<i>MLPowSim</i>	Sample size calculation for mixed-effects regression models	Centre for Multilevel Modelling Graduate School of Education University of Bristol 35 Berkeley Square Bristol BS8 1JA, UK http://www.bristol.ac.uk/cmm/software/mlpowsim/
<i>MLwiN</i>	Mixed-effects regression	Centre for Multilevel Modelling Graduate School of Education University of Bristol 35 Berkeley Square Bristol BS8 1JA, UK http://www.bristol.ac.uk/cmm/software/mlwin/
<i>ModelRisk</i>	Risk analysis	Vose Software Franklin Rooseveltlaan 348 Ghent, 9000, Belgium http://www.vosesoftware.com
<i>NCSS</i>	Comprehensive statistical analyses	NCSS 329 North 1000 East Kaysville UT 84037, USA http://www.ncss.com
<i>nQuery Advisor + nTerim</i>	Sample size and power: means, proportions, non-parametric methods, agreement, superiority/equivalence/non-inferiority trials, regression, survival	Statistical Solutions One International Place 100 Oliver Street, Suite 1400 Boston, MA 02110, USA http://www.statsols.com
<i>OpenEpi</i>	Counts and measurements in descriptive and analytical studies Stratified analysis with exact confidence limits Matched pair and person–time analysis sample Size and power calculations Random numbers Sensitivity, specificity and other evaluation statistics $r \times c$ tables χ^2 test for trend	http://www.openepi.com
<i>PASS</i>	Sample size and power: correlation, diagnostic tests, cross-over/superiority/ equivalence/non-inferiority trials, incidence rates, means, proportions, regression, survival Confidence interval estimation Experimental design	NCSS 329 North 1000 East Kaysville UT 84037, USA http://www.ncss.com

(Continued)

Package/ reference	Functions	Further information/Supplier/URL
<i>Power and Precision</i>	Power analyses and confidence interval estimation	Biostat 14 North Dean Street Englewood, NJ 07631, USA http://www.power-analysis.com
<i>Powersim Studio</i>	General simulation modelling	Powersim Software AS Litleåsvegen 79 N-5132 Nyborg Norway http://www.powersim.com
<i>PSPP</i>	Statistical analyses (Free alternative to <i>SPSS</i>)	https://www.gnu.org/software/pspp/
<i>QuickCalcs</i>	Statistical calculations	http://www.graphpad.com/quickcalcs/
<i>R</i>	Comprehensive statistical analyses	The R Foundation for Statistical Computing c/o Institute for Statistics and Mathematics Wirtschaftsuniversität Wien Welthandelsplatz 1 1020 Vienna, Austria https://www.r-project.org/
<i>Random.org</i>	Online random number generator	http://www.random.org
<i>RCalc</i> [Tryfos, 1996]	Sampling: simple random, stratified, cluster	Replaces <i>SCALC</i> , which was supplied with <i>Sampling Methods for Applied Research</i> (Tryfos, 1996) http://www.yorku.ca/ptryfos/compprog.htm
<i>Review Manager (RevMan)</i>	Preparation and maintenance of systematic reviews	Nordic Cochrane Centre Rigshospitalet, 7811 Blegdamsvej 9 2100 København Ø Denmark http://ims.cochrane.org/revman
<i>@RISK</i>	Quantitative risk analysis	Palisade EMEA & India 1 The Green, West Drayton Middlesex UB7 7PN, UK http://www.palisade.com
<i>Risk Matrix</i>	Construction of risk matrices	The MITRE Corporation 202 Burlington Road Bedford MA 01730-1420, USA http://www.mitre.org/research/technology-transfer/open-source-software/risk-matrix
<i>SampleCalc</i> [Tryfos, 1996]	Calculation of point estimates and confidence intervals of population characteristics for simple and complex sampling strategies	http://www.yorku.ca/ptryfos/compprog.htm
<i>SAS</i>	Comprehensive statistical analyses	SAS Institute Inc. 100 SAS Campus Drive Cary NC 27513-2414, USA http://www.sas.com
<i>SatScan</i>	Analysis of spatial, temporal and spatio-temporal data	Department of Population Medicine Harvard Medical School and Harvard Pilgrim Health Care 133 Brookline Avenue, 6th Floor Boston MA 02215, USA http://www.satscan.org

<i>Package/ reference</i>	<i>Functions</i>	<i>Further information/Supplier/URL</i>
<i>SiZ</i>	Design, simulation and analysis of fixed-sample clinical trials	Cytel Software Corporation 675 Massachusetts Avenue Cambridge MA 02139, USA http://www.cytel.com
<i>SPSS</i>	Comprehensive statistical analyses	SPSS Inc 23 S Wacker Drive Chicago IL 60606, USA http://www.spss.com
<i>Stata</i>	Comprehensive statistical analyses	StataCorp LP 4905 Lakeway Drive College Station TX 77845, USA http://www.stata.com
<i>StaTable</i>	Tail areas and percentage points of statistical distributions	Cytel Software Corporation 675 Massachusetts Avenue Cambridge MA 02139, USA http://www.cytel.com
<i>Statistics Calculators</i>	Statistical calculations	http://www.danielsoper.com/statcalc3/
<i>StatXact</i>	Exact <i>P</i> values for contingency tables and non-parametric tests	Cytel Software Corporation 675 Massachusetts Avenue Cambridge MA 02139, USA http://www.cytel.com
<i>TAGS</i> [Pouillot <i>et al.</i> , 2002]	Estimation of the sensitivity and specificity of diagnostic tests in the absence of a gold standard	Epidemiological Support to Risk Analysis Unit French Agency for Food Safety, BP 19 F-94701 Maisons-Alfort Cedex France, and Department of Medicine and Epidemiology School of Veterinary Medicine University of California, Davis CA 95616, USA http://faculty.vetmed.ucdavis.edu/faculty/iagardner/diagnostictests/module01.htm#download
<i>WinBUGS</i>	Bayesian statistical modelling	MRC Biostatistics Unit Cambridge Biomedical Campus Cambridge Institute of Public Health Forvie Site Robinson Way Cambridge CB2 0SR, UK https://www.mrc-bsu.cam.ac.uk/software/bugs/the-bugs-project-winbugs/
<i>WinPepi</i> [Abramson, 2011]	Contingency tables: relative risk, odds ratio Power and sample size calculations Diagnostic tests: sensitivity and specificity ROC curves optimum cut-off points Random sampling Agreement Survival analysis Life tables Logistic regression Poisson regression	http://www.brixtonhealth.com

Appendix IV

Veterinary epidemiology on the Internet

This appendix lists the Uniform Resource Locators (URLs) of some veterinary and related Internet sites of relevance to veterinary epidemiology. Site URLs may change, in which case users may be redirected

to alternative locations. If this does not occur, an Internet search on the title, using an appropriate search engine (e.g., Google™: <http://google.com>) should locate any new URL.

<i>Title (subject)</i>	<i>URL</i>
<i>ActivEpi Web</i> (Electronic text book for teaching epidemiology. Companion textbook: Kleinbaum <i>et al.</i> , 2013)	http://www.activepi.com/
<i>African Wildlife Disease Database</i>	http://www.waza.org/en/site/conservation/waza-conservation-projects/overview/african-wildlife-disease-database
<i>AGRICOLA (National Agriculture Library Catalog)</i> (General agricultural bibliographic database)	http://agricola.nal.usda.gov/
<i>American Association of Public Health Veterinarians</i>	http://www.aaphv.org
<i>Animal Health in Australia</i>	http://www.animalhealthaustralia.com.au
<i>Association for Veterinary Epidemiology and Preventive Medicine</i>	http://www.avepm.org/
<i>CABI</i> (Animal and veterinary science bibliographic database)	http://www.cabi.org/publishing-products/online-information-resources/cab-abstracts/
<i>Canadian Wildlife Health Coopertative</i>	http://www.cwhc-rcsf.ca/
<i>Canary Database</i> (Compilation of research on animals as sentinels of human environmental health hazards)	http://canarydatabase.org/
<i>Center for Epidemiology and Animal Health</i>	https://www.aphis.usda.gov/wps/portal/?1dmy&urile=wcm:path:/APHIS_Content_Library/SA_Our_Focus/SA_Animal_Health/SA_Program_Overview/SA_CEAH
<i>Centre for Veterinary Epidemiology and Risk Analysis (CVERA)</i>	http://www.ucd.ie/cvera/
<i>Centres for Disease Control and Prevention</i>	http://www.cdc.gov
<i>Cochrane Collaboration</i> (Reviews of clinical trials and other interventions)	http://www.cochrane.org
Epidemiology and related 'supercourses'	http://www.pitt.edu/~super1/index1.htm
<i>Epidemiology Monitor</i> (Developments and resources for epidemiology)	http://www.epimonitor.net
<i>EpiVet</i> (Repository of information related to veterinary epidemiology, and electronic mailing list)	http://lists.upei.ca/mailman/listinfo/epivet

Title (subject)	URL
<i>Equator Network</i> (Multilingual reporting guidelines for main study types: diagnostic/prognostic studies, observational studies, randomized controlled trials, systematic reviews, etc.)	http://www.equator-network.org/
<i>European College of Veterinary Public Health</i>	http://www.ecvph.org
<i>EXCITE (Excellence in Curriculum Innovation through Teaching Epidemiology)</i>	http://www.cdc.gov/excite/
<i>FOCUS in Field Epidemiology</i>	http://sph.unc.edu/nciph/focus/
<i>Food and Agriculture Organization of the United Nations (FAO)</i>	http://www.fao.org/
<i>International Society for Veterinary Epidemiology and Economics (ISVEE)</i>	http://isvee.net/
<i>International Veterinary Information Service</i>	http://www.ivis.org
<i>National Animal Health Monitoring System (NAHMS)</i>	http://www.aphis.usda.gov/animal_health/nahms/
<i>National Veterinary Cancer Registry</i>	http://nationalveterinarycancerregistry.org/
<i>Net-Epi (InterNet-enabled Epidemiology)</i> (Free tools for epidemiology and public health)	https://code.google.com/p/netepi/
<i>OIE (World Organisation for Animal Health)</i>	http://www.oie.int
<i>Pan American Health Organization</i>	http://www.paho.org/
<i>Participatory Epidemiology</i>	http://www.participatoryepidemiology.info/
<i>ProMED-mail</i> (Monitoring of new and emerging diseases)	http://www.promedmail.org/
<i>PubMed</i> (Medical bibliographic database)	http://www.ncbi.nlm.nih.gov/pubmed
<i>Society for Veterinary Epidemiology and Preventive Medicine</i>	http://www.svepm.org.uk
<i>Veterinary Medical Databases</i>	http://www.vmdb.org
<i>Wildlife Data Integration Network</i>	http://www.wdin.org
<i>Wildlife Disease Database</i>	http://www.cwhc-rscf.ca/wildlife_disease_database.php
<i>World Health Organization (WHO)</i>	http://www.who.int/en/
<i>WWWeb Epidemiology & Evidence-based Medicine Sources for Veterinarians</i> (Epidemiology, evidence-based medicine and biostatistics academic resources)	http://www.vetmed.wsu.edu/courses-jmgay/Epilinks.htm

Appendix V

Student's *t*-distribution

Degrees of freedom	Probability												
	.9	.8	.7	.6	.5	.4	.3	.2	.1	.05	.02	.01	.001
1	.158	.325	.510	.727	1.000	1.376	1.963	3.078	6.314	12.706	31.821	63.657	636.619
2	.142	.289	.445	.617	.816	1.061	1.386	1.886	2.920	4.303	6.965	9.925	31.598
3	.137	.277	.424	.584	.765	.978	1.250	1.638	2.353	3.182	4.541	5.841	12.924
4	.134	.271	.414	.569	.741	.941	1.190	1.533	2.132	2.776	3.747	4.604	8.610
5	.132	.267	.408	.559	.727	.920	1.156	1.476	2.015	2.571	3.365	4.032	6.869
6	.131	.265	.404	.553	.718	.906	1.134	1.440	1.943	2.447	3.143	3.707	5.959
7	.130	.263	.402	.549	.711	.896	1.119	1.415	1.895	2.365	2.998	3.499	5.408
8	.130	.262	.399	.546	.706	.889	1.108	1.397	1.860	2.306	2.896	3.355	5.041
9	.129	.261	.398	.543	.703	.883	1.100	1.383	1.833	2.262	2.821	3.250	4.781
10	.129	.260	.397	.542	.700	.879	1.093	1.372	1.812	2.228	2.764	3.169	4.587
11	.129	.260	.396	.540	.697	.876	1.088	1.363	1.796	2.201	2.718	3.106	4.437
12	.128	.259	.395	.539	.695	.873	1.083	1.356	1.782	2.179	2.681	3.055	4.318
13	.128	.259	.394	.538	.694	.870	1.079	1.350	1.771	2.160	2.650	3.012	4.221
14	.128	.258	.393	.537	.692	.868	1.076	1.345	1.761	2.145	2.624	2.977	4.140
15	.128	.258	.393	.536	.691	.866	1.074	1.341	1.753	2.131	2.602	2.947	4.073
16	.128	.258	.392	.535	.690	.865	1.071	1.337	1.746	2.120	2.583	2.921	4.015
17	.128	.257	.392	.534	.689	.863	1.069	1.333	1.740	2.110	2.567	2.898	3.965
18	.127	.257	.392	.534	.688	.862	1.067	1.330	1.734	2.101	2.552	2.878	3.922
19	.127	.257	.391	.533	.688	.861	1.066	1.328	1.729	2.093	2.539	2.861	3.883
20	.127	.257	.391	.533	.687	.860	1.064	1.325	1.725	2.086	2.528	2.845	3.850
21	.127	.257	.391	.532	.686	.859	1.063	1.323	1.721	2.080	2.518	2.831	3.819
22	.127	.256	.390	.532	.686	.858	1.061	1.321	1.717	2.074	2.508	2.819	3.792
23	.127	.256	.390	.532	.685	.858	1.060	1.319	1.714	2.069	2.500	2.807	3.767
24	.127	.256	.390	.531	.685	.857	1.059	1.318	1.711	2.064	2.492	2.797	3.745
25	.127	.256	.390	.531	.684	.856	1.058	1.316	1.708	2.060	2.485	2.787	3.725
26	.127	.256	.390	.531	.684	.856	1.058	1.315	1.706	2.056	2.479	2.779	3.707
27	.127	.256	.389	.531	.684	.855	1.057	1.314	1.703	2.052	2.473	2.771	3.690
28	.127	.256	.389	.530	.683	.855	1.056	1.313	1.701	2.048	2.467	2.763	3.674
29	.127	.256	.389	.530	.683	.854	1.055	1.311	1.699	2.045	2.462	2.756	3.659
30	.127	.256	.389	.530	.683	.854	1.055	1.310	1.697	2.042	2.457	2.750	3.646
40	.126	.255	.388	.529	.681	.851	1.050	1.303	1.684	2.021	2.423	2.704	3.551
60	.126	.254	.387	.527	.679	.848	1.046	1.296	1.671	2.000	2.390	2.660	3.460
120	.126	.254	.386	.526	.677	.845	1.041	1.289	1.658	1.980	2.358	2.617	3.373
∞	.126	.253	.385	.524	.674	.842	1.036	1.282	1.645	1.960	2.326	2.576	3.291

The table gives the percentage points most frequently required for significance tests and confidence limits based on Student's *t*-distribution. Thus the probability of observing a value of *t*, with 10 degrees of freedom, greater in **absolute value** than 3.169 (i.e., < -3.169 or > +3.169) is exactly 0.01 or 1%.

Appendix VI

Multipliers used in the construction of confidence intervals based on the Normal distribution, for selected levels of confidence

These multipliers are based on two-tailed probabilities for critical significance levels, extracted from Appendix XV.

Confidence interval	80%	90%	95%	99%	99.9%
Multiplier	1.282	1.645	1.960	2.576	3.291

Appendix VII

Values of exact 95% confidence limits for proportions

(From Beyer, 1968)

These tables give exact confidence limits for a proportion, based on the binomial distribution. The first (x) column indicates the numerator in the proportion; the first ($n - x$) row indicates the sample size; n , minus the numerator, x . For example, if 14 animals were

sampled, of which six were diseased, then $x = 6$, $n = 14$, and $n - x = 8$. Thus, the point estimate of prevalence is $6/14 = 0.428$ (42.8%) and, from the table, the interval estimate = 0.177, 0.711 (17.7%, 71.1%).

Denominator minus numerator (n – x)										
	1	2	3	4	5	6	7	8	9	
Numerator of the proportion (x)	0	975	842	708	602	522	459	410	369	336
		000	000	000	000	000	000	000	000	000
	1	987	906	806	716	641	579	527	483	445
		013	008	006	005	004	004	003	003	003
	2	992	932	853	777	710	651	600	556	518
		094	068	053	043	037	032	028	025	023
	3	994	947	882	816	755	701	652	610	572
		194	147	118	099	085	075	067	060	055
	4	995	957	901	843	788	738	692	651	614
		284	223	184	157	137	122	109	099	091
	5	996	968	915	863	813	766	723	684	649
		359	290	245	212	187	167	151	139	128
	6	996	968	925	878	833	789	749	711	677
		421	349	299	262	234	211	192	177	163
	7	997	972	933	891	849	808	770	734	701
		473	400	348	308	277	251	230	213	198
	8	997	975	940	901	861	823	787	753	722
		517	444	390	349	316	289	266	247	230
	9	997	977	945	909	872	837	802	770	740
		555	482	428	386	351	323	299	278	260
	10	998	979	950	916	882	848	816	785	756
		587	516	462	419	384	354	329	308	289
	11	998	981	953	922	890	858	827	797	769
		615	546	492	449	413	383	357	335	315
	12	998	982	957	927	897	867	837	809	782
		640	572	519	476	440	410	384	361	340
	13	998	983	960	932	903	874	846	819	793
		661	595	544	501	465	435	408	384	364
	14	998	984	962	936	909	881	854	828	803
		681	617	566	524	488	457	430	407	385
	15	998	985	964	939	913	887	861	836	812
		698	636	586	544	509	478	451	427	406
	16	999	986	966	943	918	893	868	844	820
		713	653	604	563	529	498	471	447	425
	17	999	987	968	946	922	898	874	851	828
		727	669	621	581	547	516	488	465	443

Denominator minus numerator (n – x)										
	1	2	3	4	5	6	7	8	9	
Numerator of the proportion (x)	18	999	988	970	948	925	902	879	857	835
		740	683	637	597	564	533	506	482	460
	19	999	988	971	950	929	906	884	862	841
		751	696	651	612	579	549	522	498	476
	20	999	989	972	953	932	910	889	868	847
		762	708	664	626	593	564	537	513	492
	22	999	990	975	956	937	917	897	877	858
		781	730	688	651	619	590	565	541	519
	24	999	991	976	960	942	923	904	885	867
		797	749	708	673	642	614	589	566	545
	26	999	991	978	962	945	928	910	893	875
		810	765	726	693	663	636	611	588	567
	28	999	992	980	965	949	932	916	899	882
		822	779	743	710	681	655	631	609	588
	30	999	992	981	967	952	936	920	904	889
		833	792	757	725	697	672	649	627	607
	35	999	993	983	971	958	944	930	916	902
		855	818	786	758	732	708	686	666	647
	40	999	994	985	975	963	951	938	925	912
		871	838	809	783	759	737	717	698	679
	45	999	995	987	977	967	956	944	933	921
		885	855	828	804	782	761	742	724	707
	50	1000	995	988	979	970	960	949	939	928
		896	868	843	821	800	781	763	746	730
	60	1000	996	990	983	975	966	957	948	939
		912	888	867	848	830	813	797	782	767
	80	1000	997	992	987	981	974	967	960	953
		933	915	898	882	868	855	842	820	816
	100	1000	998	994	989	984	979	973	967	962
		946	931	917	904	892	881	870	859	849
	200	1000	999	997	995	992	989	986	983	980
		973	965	957	951	944	938	932	926	920
	500	1000	1000	999	998	997	996	995	993	992
		989	986	983	980	977	974	972	969	967
	∞	1000	1000	1000	1000	1000	1000	1000	1000	1000
		1000	1000	1000	1000	1000	1000	1000	1000	1000

Denominator minus numerator ($n - x$)										
		10	11	12	13	14	15	16	17	18
Numerator of the proportion (x)	0	308 000	285 000	265 000	247 000	232 000	218 000	206 000	195 000	185 000
	1	413 002	385 002	360 002	339 002	319 002	302 002	287 001	273 001	260 001
	2	484 021	454 019	428 018	405 017	383 016	364 015	347 014	331 013	317 012
	3	538 050	508 047	481 043	456 040	434 038	414 036	396 034	379 032	363 030
	4	581 084	551 078	524 073	499 068	476 064	456 061	437 057	419 054	403 052
	5	616 118	587 110	560 103	535 097	512 091	491 087	471 082	453 078	436 075
	6	646 152	617 142	590 133	565 126	543 119	522 113	502 107	484 102	467 098

(Continued)

Denominator minus numerator (n – x)										
	10	11	12	13	14	15	16	17	18	
Numerator of the proportion (x)	7	671	643	616	592	570	549	529	512	494
		184	173	163	154	146	139	132	126	121
	8	692	665	639	616	593	573	553	535	518
		215	203	191	181	172	164	156	149	143
	9	711	685	660	636	615	594	575	557	540
		244	231	218	207	197	188	180	172	165
	10	728	702	678	655	634	614	595	577	560
		272	257	244	232	221	211	202	194	186
	11	743	718	694	672	651	631	612	594	578
		298	282	268	256	244	234	224	215	207
	12	756	732	709	687	666	647	628	611	594
		322	306	291	278	266	255	245	235	227
	13	768	744	722	701	680	661	643	626	609
		345	328	313	299	287	275	264	255	245
	14	779	756	734	713	694	675	657	640	624
		366	349	334	320	306	295	283	273	264
	15	789	766	745	725	705	687	669	653	637
		386	369	353	339	325	313	302	291	281
	16	798	776	755	736	717	698	681	665	649
		405	388	372	357	343	331	319	308	298
	17	806	785	765	745	727	709	692	676	660
		423	406	389	374	360	347	335	324	314
	18	814	793	773	755	736	719	702	686	671
		440	422	406	391	376	363	351	340	329
	19	821	801	782	763	745	728	712	696	681
		456	439	422	406	392	379	366	355	344
	20	827	808	789	771	753	737	720	705	690
		472	454	437	421	407	393	381	369	358
	22	839	820	803	785	768	752	737	722	707
		500	481	465	449	434	421	408	396	385
	24	849	831	814	798	782	766	751	737	723
		525	507	490	475	460	446	433	421	410
	26	858	841	825	809	794	779	764	750	736
		548	530	513	497	483	469	456	444	432
	28	866	850	834	819	804	790	776	762	749
		569	551	535	519	504	491	478	465	453
	30	873	858	843	828	814	800	786	773	760
		588	571	554	539	524	510	498	485	473
	35	888	874	860	847	834	821	809	797	785
		628	612	596	581	567	554	541	529	517
	40	900	887	875	862	850	838	827	815	804
		662	646	631	616	602	590	578	566	555
	45	909	898	886	875	864	853	842	831	821
		690	675	661	647	633	621	609	598	587
	50	917	906	896	885	875	865	854	844	835
		714	700	686	673	660	648	636	625	614
	60	929	920	911	902	893	884	874	866	857
		752	740	727	715	703	692	681	670	660
	80	945	938	931	923	916	909	901	894	887
		804	793	783	773	763	753	744	734	726
	100	955	949	943	937	931	925	919	913	907
		838	829	820	811	802	794	786	778	770
	200	977	974	970	967	964	961	957	954	950
		914	909	903	898	893	888	883	878	873
	500	991	989	988	986	985	984	982	981	979
		964	962	960	957	955	953	950	948	946
	∞	1000	1000	1000	1000	1000	1000	1000	1000	1000
		1000	1000	1000	1000	1000	1000	1000	1000	1000

Denominator minus numerator (n – x)										
	19	20	22	24	26	28	30	35	40	
Numerator of the proportion (x)	0	176	168	154	142	132	123	116	100	088
		000	000	000	000	000	000	000	000	000
	1	249	238	219	203	190	178	167	145	129
		001	001	001	001	001	001	001	001	001
	2	304	292	270	251	235	221	208	182	162
		012	011	010	009	009	008	008	007	006
	3	349	336	312	292	274	257	243	214	191
		029	028	025	024	022	020	019	017	015
	4	388	374	349	327	307	290	275	242	217
		050	047	044	040	038	035	033	029	025
	5	421	407	381	358	337	319	303	268	241
		071	068	063	058	055	051	048	042	037
	6	451	436	410	386	364	345	328	292	263
		094	090	083	077	072	068	064	056	049
	7	478	463	435	411	389	369	351	314	283
		116	111	103	096	090	084	080	070	062
	8	502	487	459	434	412	391	373	334	302
		138	132	123	115	107	101	096	084	075
	9	524	508	481	455	433	412	393	353	321
		159	153	142	133	125	118	111	098	088
	10	544	528	500	475	452	431	412	372	338
		179	173	161	151	142	134	127	112	100
	11	561	546	519	493	470	449	429	388	354
		199	192	180	160	159	150	142	126	113
	12	578	563	535	510	487	465	446	404	369
		218	211	197	186	175	166	157	140	125
	13	594	579	551	525	503	481	461	419	384
		237	229	215	202	191	181	172	153	138
	14	608	593	566	540	517	496	476	433	398
		255	247	232	218	206	196	186	166	150
	15	621	607	579	554	531	509	490	446	410
		272	263	248	234	221	210	200	179	162
	16	634	619	592	567	544	522	502	459	422
		288	280	263	249	236	224	214	191	173
	17	645	631	604	579	556	535	515	471	434
		304	295	278	263	250	238	227	203	185
	18	656	642	615	590	568	547	527	483	445
		319	310	293	277	264	251	240	215	196
	19	666	652	626	601	578	557	538	494	456
		334	324	307	291	277	264	252	227	207
	20	676	662	636	612	589	568	548	504	467
		348	338	320	304	289	276	264	238	217
	22	693	680	654	631	614	588	568	524	487
		374	364	346	329	314	300	287	260	237
	24	709	696	671	648	626	605	586	543	505
		399	388	369	352	337	322	309	281	257
	26	723	711	686	663	642	622	603	559	522
		422	411	386	374	358	343	330	300	276
	28	736	724	700	678	657	637	618	575	538
		443	432	412	395	378	363	349	319	294
	30	748	736	713	691	670	651	632	590	552
		462	452	432	414	397	382	368	337	311
	35	773	762	740	719	700	681	663	622	586
		506	496	476	457	441	425	410	378	351
	40	793	783	763	743	724	706	689	649	614
		544	533	513	495	478	462	448	414	386
	45	811	801	781	763	745	728	711	673	639
		576	566	548	528	511	495	480	447	419

(Continued)

		<i>Denominator minus numerator (n – x)</i>								
		19	20	22	24	26	28	30	35	40
<i>Numerator of the proportion (x)</i>	50	825	816	797	780	763	746	731	694	660
		604	594	575	557	540	525	510	476	447
	60	848	840	823	807	792	777	763	728	697
		650	641	622	605	589	574	559	526	497
	80	880	874	860	846	833	820	808	778	750
		717	708	692	676	662	647	634	603	575
	100	901	895	883	872	860	847	838	812	787
		762	755	740	726	713	700	687	658	632
	200	947	943	937	930	923	917	910	894	878
		868	863	854	845	836	828	819	799	780
	500	978	976	973	970	967	964	961	954	947
		944	941	937	933	928	924	920	910	901
	∞	1000	1000	1000	1000	1000	1000	1000	1000	1000
		1000	1000	1000	1000	1000	1000	1000	1000	1000

		<i>Denominator minus numerator (n – x)</i>							
		45	50	60	80	100	200	500	∞
<i>Numerator of the proportion (x)</i>	0	079	071	060	045	036	018	007	000
		000	000	000	000	000	000	000	000
	1	115	104	088	067	054	027	011	000
		001	001	000	000	000	000	000	000
	2	145	132	112	085	069	035	014	000
		005	005	004	003	002	001	000	000
	3	172	157	133	102	083	043	017	000
		013	012	010	008	006	003	001	000
	4	196	179	152	118	096	049	020	000
		023	021	017	013	011	005	002	000
	5	218	200	170	132	108	056	023	000
		033	030	025	019	016	008	003	000
	6	239	219	187	145	119	062	026	000
		044	040	034	026	021	011	004	000
	7	258	237	203	158	130	068	028	000
		056	051	043	033	027	014	005	000
	8	276	254	218	171	141	074	031	000
		067	061	052	040	033	017	007	000
	9	293	270	233	184	151	080	033	000
		079	072	061	047	038	020	008	000
	10	310	286	248	196	162	086	036	000
		091	083	071	055	045	023	009	000
	11	325	300	260	207	171	091	038	000
		102	094	080	062	051	026	011	000
	12	339	314	273	217	180	097	040	000
		114	104	089	069	057	030	012	000
	13	353	327	285	227	189	102	043	000
		125	115	098	077	063	033	014	000
	14	367	340	297	237	198	107	045	000
		136	125	107	084	069	036	015	000
	15	379	352	308	247	206	112	047	000
		147	135	116	091	075	039	016	000
	16	391	364	319	256	214	117	050	000
		158	146	126	099	081	043	018	000
	17	402	375	330	266	222	122	052	000
		169	156	134	106	087	046	019	000
	18	413	386	340	274	230	127	054	000
		179	165	143	113	093	050	021	000

		<i>Denominator minus numerator (n – x)</i>							
		<i>45</i>	<i>50</i>	<i>60</i>	<i>80</i>	<i>100</i>	<i>200</i>	<i>500</i>	∞
<i>Numerator of the proportion (x)</i>	19	424	396	350	283	238	132	056	000
		189	175	152	120	099	053	022	000
	20	434	406	359	292	245	137	059	000
		199	184	160	126	105	057	024	000
	22	454	425	378	308	260	146	063	000
		219	203	177	140	117	063	027	000
	24	472	443	395	324	274	155	067	000
		237	220	193	154	128	070	030	000
	26	489	460	411	338	287	164	072	000
		255	237	208	167	140	077	033	000
	28	505	475	426	353	300	172	076	000
		272	254	223	180	153	083	036	000
	30	520	490	441	366	313	181	080	000
		289	269	237	192	162	090	039	000
	35	553	524	474	397	342	201	090	000
		327	306	272	222	188	106	046	000
	40	581	553	503	425	368	220	099	000
		361	340	303	250	213	122	053	000
	45	607	579	529	451	392	238	109	000
		393	370	332	276	236	137	061	000
	50	630	602	552	474	415	255	118	000
		421	398	359	301	259	152	068	000
	60	668	641	593	515	455	287	136	000
		471	448	407	345	300	181	083	000
	80	724	699	655	580	520	342	169	000
		549	526	485	420	370	234	111	000
	100	764	741	700	630	571	395	199	000
		608	585	545	480	429	280	138	000
	200	863	848	819	766	720	550	319	000
		762	745	713	658	605	450	253	000
	500	939	932	917	889	862	747	531	000
		891	882	864	831	801	681	469	000
	∞	1000	1000	1000	1000	1000	1000	1000	–
		1000	1000	1000	1000	1000	1000	1000	–

Appendix VIII

Values from the Poisson distribution for calculating 90%, 95% and 99% confidence intervals for observed numbers from 0 to 100

(From Altman *et al.*, 2000)

If x is the observed number in a study, x_L and x_U give the lower and upper confidence limits for the population mean, assuming that the observed number is from a Poisson distribution.

x	90%		Level of confidence 95%		99%	
	x_L	x_U	x_L	x_U	x_L	x_U
0		2.996	0	3.689	0	5.298
1	0.051	4.744	0.025	5.572	0.005	7.430
2	0.35	6.296	0.242	7.225	0.103	9.274
3	0.81	7.754	0.619	8.767	0.338	10.977
4	1.366	9.154	1.090	10.242	0.672	12.594
5	1.970	10.513	1.623	11.668	1.078	14.150
6	2.613	11.842	2.202	13.059	1.537	15.660
7	3.285	13.148	2.814	14.423	2.037	17.134
8	3.981	14.435	3.454	15.763	2.571	18.578
9	4.695	15.705	4.115	17.085	3.132	19.998
10	5.425	16.962	4.795	18.390	3.717	21.398
11	6.169	18.208	5.491	19.682	4.321	22.779
12	6.924	19.443	6.201	20.962	4.943	24.145
13	7.690	20.669	6.922	22.230	5.580	25.497
14	8.464	21.886	7.654	23.490	6.231	26.836
15	9.246	23.097	8.395	24.740	6.893	28.164
16	10.036	24.301	9.145	25.983	7.567	29.482
17	10.832	25.499	9.903	27.219	8.251	30.791
18	11.634	26.692	10.668	28.448	8.943	32.091
19	12.442	27.879	11.439	29.671	9.644	33.383
20	13.255	29.062	12.217	30.888	10.353	34.668
21	14.072	30.240	12.999	32.101	11.069	35.946
22	14.894	31.415	13.787	33.308	11.792	37.218
23	15.719	32.585	14.580	34.511	12.521	38.484
24	16.549	33.752	15.377	35.710	13.255	39.745
25	17.382	34.916	16.179	36.905	13.995	41.000
26	18.219	36.077	16.984	38.096	14.741	42.251
27	19.058	37.234	17.793	39.284	15.491	43.497
28	19.901	38.389	18.606	40.468	16.245	44.738
29	20.746	39.541	19.422	41.649	17.004	45.976
30	21.594	40.691	20.241	42.827	17.767	47.209
31	22.445	41.838	21.063	44.002	18.534	48.439
32	23.297	42.982	21.888	45.174	19.305	49.665
33	24.153	44.125	22.716	46.344	20.079	50.888
34	25.010	45.266	23.546	47.512	20.857	52.107
35	25.870	46.404	24.379	48.677	21.638	53.324
36	26.731	47.541	25.214	49.839	22.422	54.537
37	27.595	48.675	26.051	51.000	23.208	55.748
38	28.460	49.808	26.891	52.158	23.998	56.955

x	90%		Level of confidence 95%		99%	
	x _L	x _U	x _L	x _U	x _L	x _U
39	29.327	50.940	27.733	53.314	24.791	58.161
40	30.196	52.069	28.577	54.469	25.586	59.363
41	31.066	53.197	29.422	55.621	26.384	60.563
42	31.938	54.324	30.270	56.772	27.184	61.761
43	32.812	55.449	31.119	57.921	27.986	62.956
44	33.687	56.573	31.970	59.068	28.791	64.149
45	34.563	57.695	32.823	60.214	29.598	65.341
46	35.441	58.816	33.678	61.358	30.407	66.530
47	36.320	59.935	34.534	62.500	31.218	67.717
48	37.200	61.054	35.391	63.641	32.032	68.902
49	38.082	62.171	36.250	64.781	32.847	70.085
50	38.965	63.287	37.111	65.919	33.664	71.266
51	39.849	64.402	37.973	67.056	34.483	72.446
52	40.734	65.516	38.836	68.191	35.303	73.624
53	41.620	66.628	39.701	69.325	36.125	74.800
54	42.507	67.740	40.566	70.458	36.949	75.974
55	43.396	68.851	41.434	71.590	37.775	77.147
56	44.285	69.960	42.302	72.721	38.602	78.319
57	45.176	71.069	43.171	73.850	39.431	79.489
58	46.067	72.177	44.042	74.978	40.261	80.657
59	46.959	73.284	44.914	76.106	41.093	81.824
60	47.852	74.390	45.786	77.232	41.926	82.990
61	48.746	75.495	46.660	78.357	42.760	84.154
62	49.641	76.599	47.535	79.481	43.596	85.317
63	50.537	77.702	48.411	80.604	44.433	86.479
64	51.434	78.805	49.288	81.727	45.272	87.639
65	52.331	79.907	50.166	82.848	46.111	88.798
66	53.229	81.008	51.044	83.968	46.952	89.956
67	54.128	82.108	51.924	85.088	47.794	91.112
68	55.028	83.208	52.805	86.206	48.637	92.269
69	55.928	84.306	53.686	87.324	49.482	93.423
70	56.830	85.405	54.568	88.441	50.327	94.577
71	57.732	86.502	55.452	89.557	51.174	95.729
72	58.634	87.599	56.336	90.672	52.022	96.881
73	59.537	88.695	57.220	91.787	52.871	98.031
74	60.441	89.790	58.106	92.900	53.720	99.180
75	61.346	90.885	58.992	94.013	54.571	100.328
76	62.251	91.979	59.879	95.125	55.423	101.476
77	63.157	93.073	60.767	96.237	56.276	102.622
78	64.063	94.166	61.656	97.348	57.129	103.767
79	64.970	95.258	62.545	98.458	57.984	104.912
80	65.878	96.350	63.435	99.567	58.840	106.056
81	66.786	97.441	64.326	100.676	59.696	107.198
82	67.695	98.532	65.217	101.784	60.553	108.340
83	68.604	99.622	66.109	102.891	61.412	109.481
84	69.514	100.712	67.002	103.998	62.271	110.621
85	70.425	101.801	67.895	105.104	63.131	111.761
86	71.336	102.889	68.789	106.209	63.991	112.899
87	72.247	103.977	69.683	107.314	64.853	114.037
88	73.159	105.065	70.579	108.418	65.715	115.174
89	74.071	106.152	71.474	109.522	66.578	116.310
90	74.984	107.239	72.371	110.625	67.442	117.445
91	75.898	108.325	73.268	111.728	68.307	118.580
92	76.812	109.410	74.165	112.830	69.172	119.714
93	77.726	110.495	75.063	113.931	70.038	120.847
94	78.641	111.580	75.962	115.032	70.905	121.980
95	79.556	112.664	76.861	116.133	71.773	123.112
96	80.472	113.748	77.760	117.232	72.641	124.243
97	81.388	114.832	78.660	118.332	73.510	125.373
98	82.305	115.915	79.561	119.431	74.379	126.503
99	83.222	116.997	80.462	120.529	75.250	127.632
100	84.139	118.079	81.364	121.627	76.120	128.761

Appendix IX

The χ^2 distribution

Degrees of freedom	Value of P				
	0.99	0.95	0.05	0.01	0.001
1	0.000 157	0.00 393	3.841	6.635	10.83
2	0.0201	0.103	5.991	9.210	13.82
3	0.115	0.352	7.815	11.34	16.27
4	0.297	0.711	9.488	13.28	18.47
5	0.554	1.145	11.07	15.09	20.51
6	0.872	1.635	12.59	16.81	22.46
7	1.239	2.167	14.07	18.48	24.32
8	1.646	2.733	15.51	20.09	26.13
9	2.088	3.325	16.92	21.67	27.88
10	2.558	3.940	18.31	23.21	29.59
11	3.053	4.575	19.68	24.72	31.26
12	3.571	5.226	21.03	26.22	32.91
13	4.107	5.892	22.36	27.69	34.53
14	4.660	6.571	23.68	29.14	36.12
15	5.229	7.261	25.00	30.58	37.70
16	5.812	7.962	26.30	32.00	39.25
17	6.408	8.672	27.59	33.41	40.79
18	7.015	9.390	28.87	34.81	42.31
19	7.633	10.12	30.14	36.19	43.82
20	8.260	10.85	31.41	37.57	45.31
21	8.897	11.59	32.67	38.93	46.80
22	9.542	12.34	33.92	40.29	48.27
23	10.20	13.09	35.17	41.64	49.73
24	10.86	13.85	36.42	42.98	51.18
25	11.52	14.61	37.65	44.31	52.62
26	12.20	15.38	38.89	45.64	54.05
27	12.88	16.15	40.11	46.96	55.48
28	13.56	16.93	41.34	48.28	56.89
29	14.26	17.71	42.56	49.59	58.30
30	14.95	18.49	43.77	50.89	59.70

The table gives the percentage points most frequently required for significance tests based on χ^2 . Thus the probability of observing a χ^2 with five degrees of freedom **greater** in value than 11.07 is 0.05 or 5%. Again, the probability of observing a χ^2 with five degrees of freedom **smaller** in value than 0.554 is $1 - 0.99 = 0.01$ or 1%.

Appendix X

Technique for selecting a simple random sample

Example An investigator requires a random sample of 10 animals from a population of 90.

Construct a sampling frame of all animals and label them consecutively from one to 90. The random numbers in the following table (page 662) are arranged in groups of two columns for visual convenience (other tables may have different numbers of columns grouped together).

1. Select two columns arbitrarily, to correspond to tens and units – use columns 27 and 28 for visual convenience.
2. Select a row arbitrarily – say row 7: number 89.

3. Move down the columns: 89, 97, 32, 21, 60, 48, 10, 98, 23, 89, 08, 15, 44, 68....All numbers greater than 90 are ignored. The first 10 numbers are then: 89, 32, 21, 60, 48, 10, 23, 89, 08, 15.
4. Number 89 has been selected twice. The second 89 should be rejected* and the next available number chosen: 44.

The sample size of 10 is then made up of animals labelled 89, 32, 21, 60, 48, 10, 23, 8, 15, 44.

If the table is used repeatedly, then the row and column starting point should be changed.

* Rejection of numbers that occur more than once is performed if animals are not returned to the selection pool after having been selected once: sampling 'without replacement'. If animals are returned to the pool after having been selected once, so that they can be selected again – sampling 'with replacement' – then a number corresponding to an animal can be selected more than once (number 89 in this example).

Table of random numbers. (Extracted from Lindley and Scott, 1984.)

84 42	56 53	87 75	18 91	76 66	64 83	97 11	69 41	80 92	38 75
28 87	77 03	57 09	85 86	46 86	40 15	31 81	78 91	30 22	88 58
64 12	39 65	37 93	76 46	11 09	56 28	94 54	10 14	30 73	80 30
49 41	73 76	49 64	06 70	99 37	72 60	39 16	02 26	91 90	16 54
06 46	69 31	24 33	52 67	85 07	01 33	16 33	43 98	17 62	52 52
75 56	96 97	65 20	68 68	60 97	90 46	63 37	10 34	41 64	85 01
09 35	89 97	97 10	00 76	39 82	49 94	15 89	60 65	57 03	91 68
73 81	11 08	52 73	64 85	22 72	85 16	15 97	76 28	41 95	00 33
49 69	80 41	46 62	26 32	58 16	88 76	54 32	06 37	46 45	28 95
64 60	49 70	33 73	71 57	83 26	19 25	86 21	64 60	11 01	86 70
93 05	36 44	59 19	99 51	54 21	37 48	18 60	22 92	68 34	39 02
39 88	11 26	68 92	81 14	12 16	37 64	61 48	21 69	77 76	33 00
89 34	19 12	83 76	35 11	96 53	04 76	63 10	93 68	52 42	73 20
77 29	03 26	45 36	15 17	27 28	79 58	38 98	73 52	63 72	48 41
86 75	51 29	70 78	24 78	94 78	64 17	32 23	95 52	87 79	14 30
95 98	77 51	14 65	76 49	42 36	11 33	23 89	32 01	60 48	91 44
22 09	01 14	04 96	97 56	92 52	83 44	45 08	72 78	10 36	26 70
30 49	36 23	36 81	11 76	91 08	67 60	01 15	64 77	21 33	72 29
77 59	88 92	17 75	04 47	18 02	94 84	71 44	87 63	06 04	49 33
03 50	80 26	74 74	18 85	92 20	64 39	98 68	29 26	90 14	77 36
46 32	79 69	41 06	26 04	47 24	67 10	66 69	21 55	66 63	48 47
65 73	98 08	05 96	92 27	22 86	54 87	95 87	40 27	09 97	47 21
68 82	77 73	08 37	28 47	73 49	10 65	53 48	87 74	02 99	52 86
93 98	12 19	82 69	61 08	00 42	88 83	70 85	08 48	74 94	88 61
61 27	39 16	42 17	89 81	27 44	12 33	43 24	92 41	55 13	45 01
54 74	04 79	72 61	21 87	23 83	96 56	97 63	67 02	67 30	36 89
28 00	40 86	92 97	06 22	37 37	83 00	97 17	08 06	43 95	76 84
61 78	71 16	41 01	69 63	35 96	60 65	09 44	93 42	72 11	22 85
68 60	92 99	60 97	53 55	34 61	43 40	77 96	19 87	63 49	22 47
21 76	13 39	25 89	91 38	25 19	44 33	11 36	72 21	40 90	76 95
73 59	53 04	35 13	12 31	88 70	05 40	43 42	47 17	03 86	14 10
85 68	66 48	05 24	28 97	84 84	91 65	62 83	89 68	07 51	01 02
60 30	10 46	44 34	19 56	00 83	20 53	53 05	29 03	47 55	23 26
44 63	80 62	80 80	99 43	33 87	70 52	51 62	02 12	02 90	44 44
89 38	13 68	31 31	97 15	35 67	23 74	76 96	62 82	62 19	65 58
55 20	77 12	79 81	42 15	30 67	88 83	69 08	99 82	20 39	92 40
67 40	42 16	46 06	60 74	61 22	95 47	24 62	81 06	19 67	15 06
57 19	76 98	65 64	55 28	34 03	58 62	35 22	67 40	04 88	17 59
21 72	97 04	82 62	09 54	35 17	22 73	35 72	53 65	95 48	55 12
46 89	95 61	31 77	14 14	24 14	91 58	76 56	19 33	98 67	09 04
99 73	85 64	96 58	61 65	60 83	62 10	87 00	82 63	39 90	83 17
85 52	98 27	40 33	09 59	80 17	22 06	84 03	41 48	76 07	26 69
50 12	17 86	50 57	91 28	42 29	83 87	00 87	93 52	53 47	08 65
92 84	02 93	44 36	93 19	08 54	76 62	31 65	94 68	38 04	62 31
69 74	30 25	68 65	19 77	57 05	71 56	91 30	16 66	70 48	78 65
51 69	76 00	20 92	58 21	24 33	74 08	66 90	61 89	56 83	39 58
27 25	81 29	75 02	85 09	58 89	77 83	03 40	21 14	45 90	54 01
44 03	62 96	68 65	24 57	44 43	07 72	59 16	04 94	23 36	55 85
40 59	49 20	48 63	35 74	33 12	96 25	59 35	07 45	80 97	19 90
92 91	07 14	82 22	50 70	75 15	69 71	31 20	60 06	99 56	57 74

Appendix XI

Sample sizes

This appendix comprises sample sizes required to attain a desired confidence interval around expected prevalence values of 5%, 10%, 20%, 30%, 40% and 50% (from WHO, 1973). For example, if the expected

prevalence is 30%, consult *Figure 4*. A sample size of 200 will produce a 95% confidence interval of 24% to 36%. A sample size of 800 will produce a 95% confidence interval of 27% to 33%.

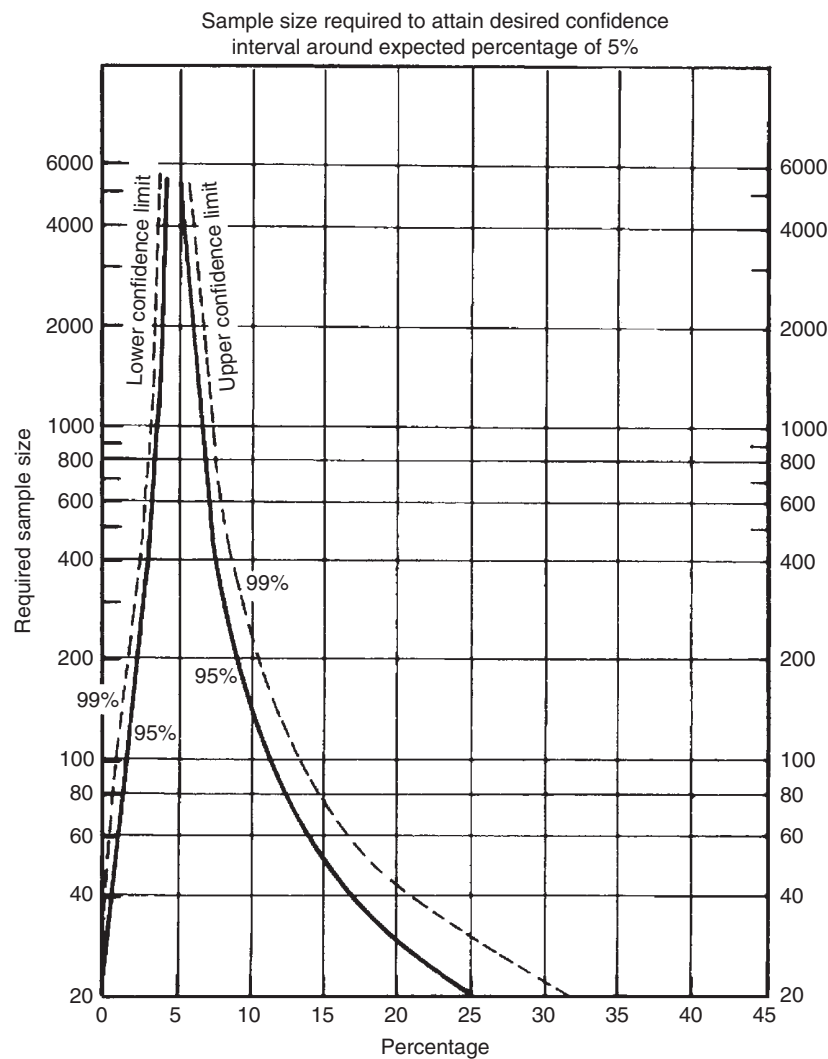


Fig. 1

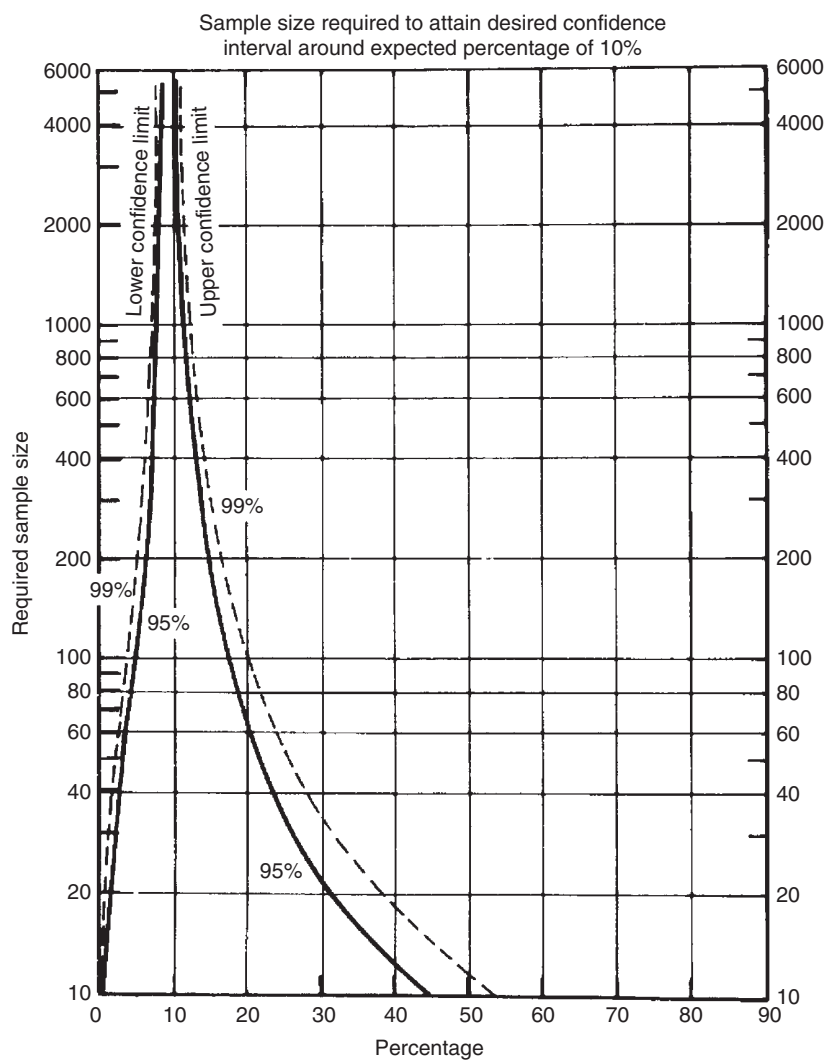


Fig. 2

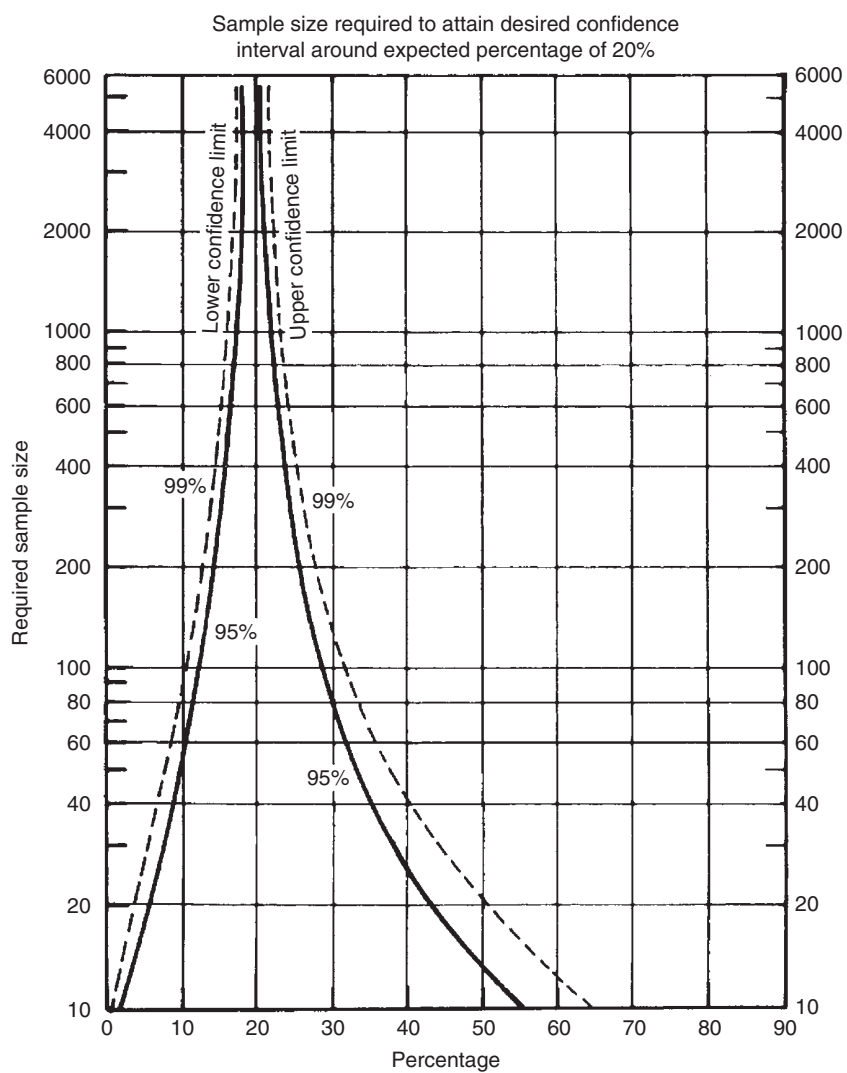


Fig. 3

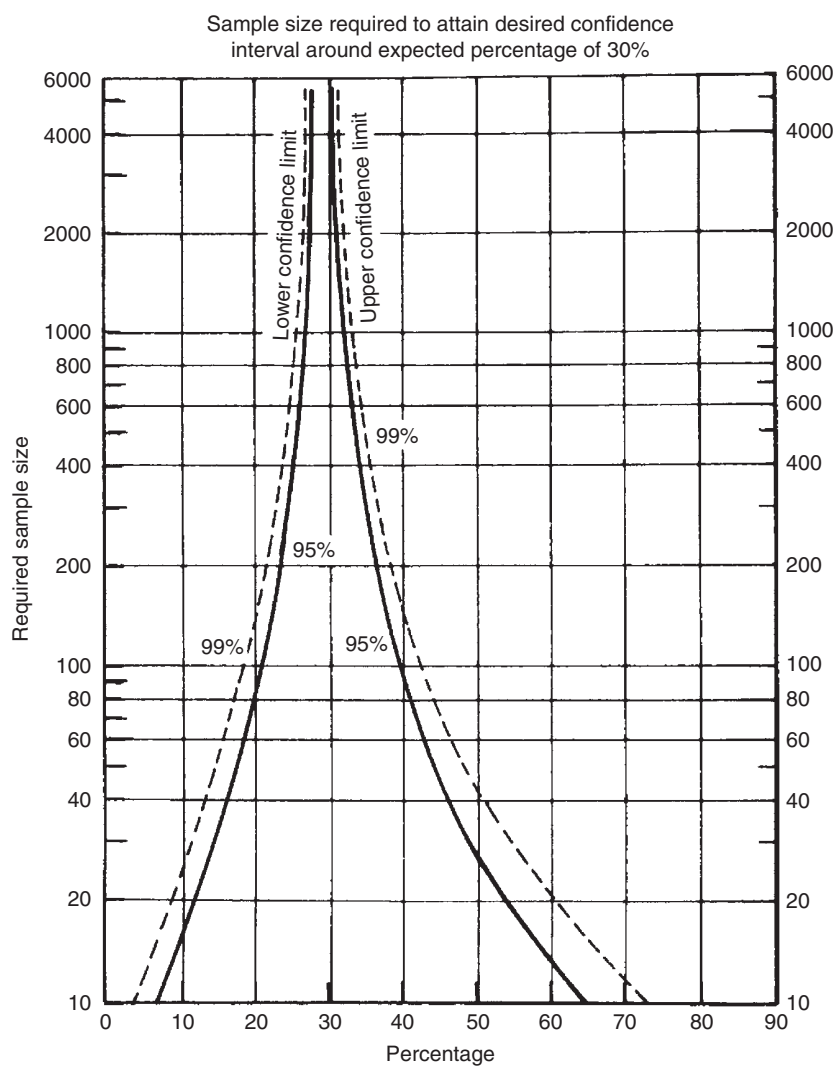


Fig. 4

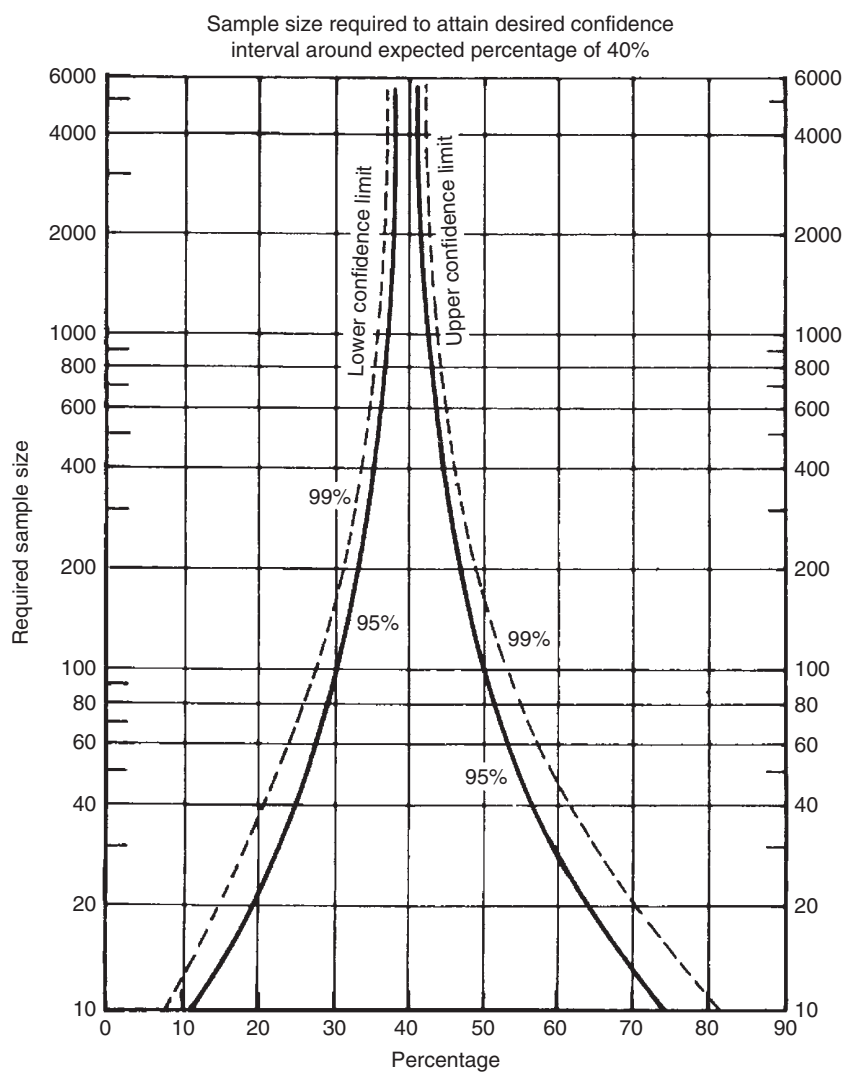


Fig. 5

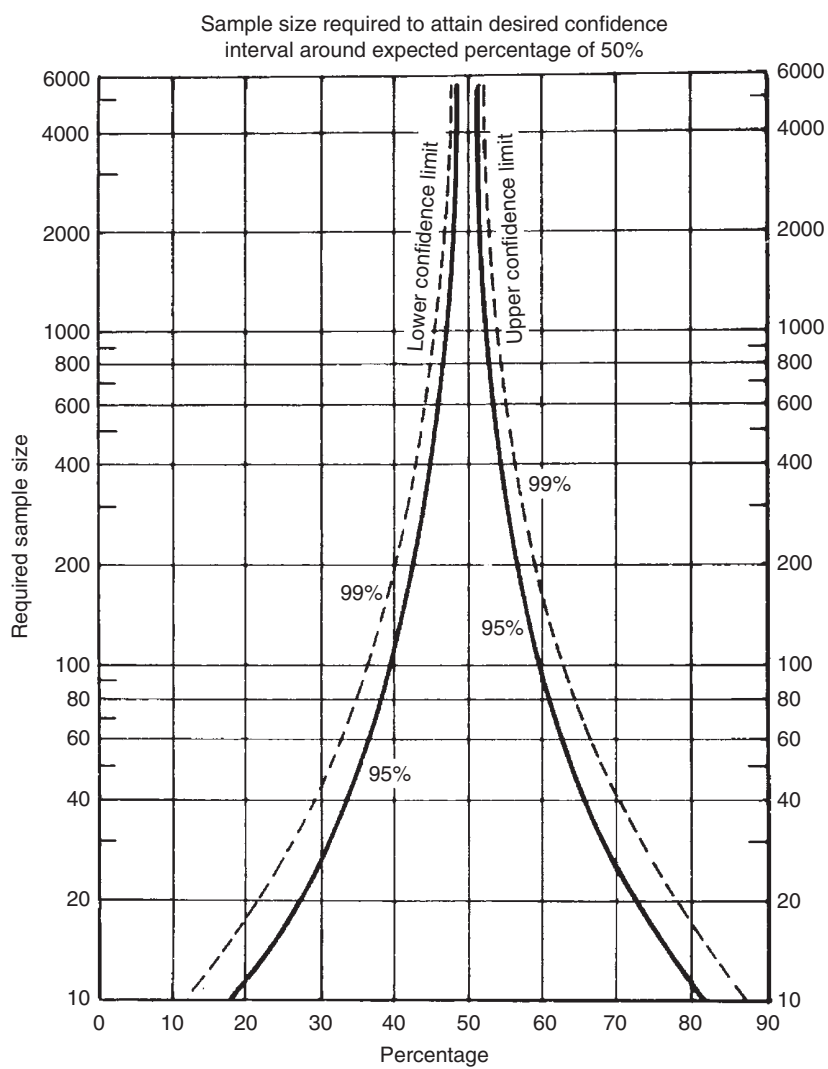


Fig. 6

Appendix XII

The probability of detecting a small number of cases in a population

(Adapted from Cannon and Roe, 1982)

These tables give the probability of detecting at least one case for different sampling fractions and numbers of cases in the population.

Example A 40% sample from a herd of 20 animals would have a 97.6% chance of including at least one positive if six were present in the herd.

20% Sampling

Population size	Number sampled	Number of positives in the population							
		1	2	3	4	5	6	7	8
10	2	0.200	0.378	0.533	0.667	0.778	0.867	0.933	0.978
20	4	0.200	0.368	0.509	0.624	0.718	0.793	0.852	0.898
30	6	0.200	0.366	0.501	0.612	0.702	0.773	0.830	0.874
40	8	0.200	0.364	0.498	0.607	0.694	0.764	0.819	0.863
50	10	0.200	0.363	0.498	0.603	0.689	0.758	0.813	0.857
60	12	0.200	0.363	0.495	0.601	0.686	0.755	0.809	0.853
70	14	0.200	0.362	0.494	0.599	0.684	0.752	0.807	0.850
80	16	0.200	0.362	0.493	0.598	0.683	0.751	0.804	0.847
90	18	0.200	0.362	0.492	0.597	0.682	0.749	0.803	0.846
100	20	0.200	0.362	0.492	0.597	0.681	0.748	0.802	0.844
∞	∞	0.200	0.360	0.486	0.590	0.672	0.738	0.790	0.832

30% Sampling

Population size	Number sampled	Number of positives in the population							
		1	2	3	4	5	6	7	8
10	3	0.300	0.533	0.708	0.833	0.917	0.967	0.992	1.000
20	6	0.300	0.521	0.681	0.793	0.871	0.923	0.956	0.976
30	9	0.300	0.517	0.672	0.782	0.857	0.909	0.943	0.965
40	12	0.300	0.515	0.668	0.776	0.851	0.902	0.936	0.960
50	15	0.300	0.514	0.666	0.773	0.847	0.898	0.933	0.956
60	18	0.300	0.514	0.665	0.770	0.844	0.895	0.930	0.954
70	21	0.300	0.513	0.663	0.769	0.842	0.893	0.928	0.952
80	24	0.300	0.513	0.663	0.768	0.841	0.892	0.927	0.951
90	27	0.300	0.512	0.662	0.767	0.840	0.891	0.926	0.950
100	30	0.300	0.512	0.661	0.766	0.839	0.890	0.925	0.949
∞	∞	0.300	0.510	0.657	0.760	0.832	0.882	0.918	0.942

40% Sampling

Population size	Number sampled	Number of positives in the population							
		1	2	3	4	5	6	7	8
10	4	0.400	0.667	0.833	0.929	0.976	0.995	1.000	1.000
20	8	0.400	0.653	0.807	0.898	0.949	0.976	0.990	0.996
30	12	0.400	0.648	0.799	0.888	0.940	0.969	0.984	0.993
40	16	0.400	0.646	0.795	0.884	0.935	0.965	0.981	0.990
50	20	0.400	0.645	0.793	0.881	0.933	0.963	0.980	0.989
60	24	0.400	0.644	0.791	0.879	0.931	0.961	0.978	0.988
70	28	0.400	0.643	0.790	0.878	0.930	0.960	0.977	0.987
80	32	0.400	0.643	0.789	0.877	0.929	0.959	0.977	0.987
90	36	0.400	0.643	0.789	0.876	0.928	0.959	0.976	0.987
100	40	0.400	0.642	0.788	0.876	0.927	0.958	0.976	0.986
∞	∞	0.400	0.640	0.784	0.870	0.922	0.953	0.972	0.983

50% Sampling

Population size	Number sampled	Number of positives in the population							
		1	2	3	4	5	6	7	8
10	5	0.500	0.778	0.917	0.976	0.996	1.000	1.000	1.000
20	10	0.500	0.763	0.895	0.957	0.984	0.995	0.998	0.994
30	15	0.500	0.759	0.888	0.950	0.979	0.992	0.997	0.999
40	20	0.500	0.756	0.885	0.947	0.976	0.990	0.996	0.998
50	25	0.500	0.755	0.883	0.945	0.975	0.989	0.995	0.998
60	30	0.500	0.754	0.881	0.944	0.974	0.988	0.995	0.998
70	35	0.500	0.754	0.880	0.943	0.973	0.988	0.994	0.998
80	40	0.500	0.753	0.880	0.942	0.973	0.987	0.994	0.997
90	45	0.500	0.753	0.879	0.942	0.972	0.987	0.994	0.997
100	50	0.500	0.753	0.879	0.941	0.972	0.987	0.994	0.997
∞	∞	0.500	0.750	0.875	0.937	0.969	0.984	0.992	0.996

60% Sampling

Population size	Number sampled	Number of positives in the population							
		1	2	3	4	5	6	7	8
10	6	0.600	0.867	0.967	0.994	1.000	1.000	1.000	1.000
20	12	0.600	0.853	0.951	0.986	0.994	0.997	1.000	1.000
30	18	0.600	0.848	0.946	0.982	0.994	0.997	1.000	1.000
40	24	0.600	0.846	0.943	0.980	0.993	0.997	0.999	1.000
50	30	0.600	0.845	0.942	0.979	0.993	0.997	0.999	1.000
60	36	0.600	0.844	0.941	0.978	0.992	0.997	0.999	1.000
70	42	0.600	0.843	0.940	0.978	0.992	0.997	0.999	1.000
80	48	0.600	0.843	0.940	0.977	0.992	0.997	0.999	1.000
90	54	0.600	0.843	0.939	0.977	0.991	0.997	0.999	1.000
100	60	0.600	0.842	0.939	0.977	0.991	0.997	0.999	1.000
∞	∞	0.600	0.840	0.936	0.974	0.990	0.996	0.998	1.000

Appendix XIII

The probability of failure to detect cases in a population

(From Cannon and Roe, 1982)

This table gives the probability of failure to detect diseased animals from an 'infinite' population with the specified proportion of positives in the population.

Example Tests of a series of random samples of 25 animals from a large population in which 10% of animals are positive would fail to detect any positives in 7.2% of such sample groups.

Prevalence	Number of animals in sample tested									
	5	10	25	50	75	100	200	250	500	1000
1%	0.951	0.904	0.778	0.605	0.471	0.366	0.134	0.081	0.007	0.000
2%	0.904	0.817	0.603	0.364	0.220	0.133	0.018	0.006	0.000	
3%	0.859	0.737	0.467	0.218	0.102	0.048	0.002	0.000		
4%	0.815	0.665	0.360	0.130	0.047	0.017	0.000			
5%	0.774	0.599	0.277	0.077	0.021	0.006	0.000			
6%	0.734	0.539	0.213	0.045	0.010	0.002	0.000			
7%	0.696	0.484	0.163	0.027	0.004	0.001	0.000			
8%	0.659	0.434	0.124	0.015	0.002	0.000				
9%	0.624	0.389	0.095	0.009	0.001	0.000				
10%	0.590	0.349	0.072	0.005	0.000					
12%	0.528	0.279	0.041	0.002	0.000					
14%	0.470	0.221	0.023	0.001	0.000					
16%	0.418	0.175	0.013	0.000						
18%	0.371	0.137	0.007	0.000						
20%	0.328	0.107	0.004	0.000						
24%	0.254	0.064	0.001	0.000						
28%	0.193	0.037	0.000							
32%	0.145	0.021	0.000							
36%	0.107	0.012	0.000							
40%	0.078	0.006	0.000							
50%	0.031	0.001	0.000							
60%	0.010	0.000								

Appendix XIV

Sample sizes required for detecting disease with probability, p_1 , and threshold number of positives

These tables give the number of animals that need to be sampled, and the upper limit of the number of test-positive animals that can be identified in the sample, while still concluding, with probability p_1 , that disease is absent.

The threshold number of positives is given in brackets; the probability of incorrectly concluding that a healthy population is diseased is given in square brackets.

Example (consult the first table) A diagnostic test has a sensitivity of 99% and specificity of 99%. If 173 animals are sampled from a population of 500 in which,

if disease is present, the minimum prevalence is 5%, up to four test-positive animals can be identified in the sample, while still concluding, with a probability, p_1 , of 0.99, that disease is absent. The probability of observing more than four test-positive animals in a disease-free population is no greater than 0.05. Thus, if more than four test-positive animals are identified in the sample, the probability of incorrectly concluding that a healthy population is diseased is no greater than 0.05 (i.e., it can be concluded that the population is diseased, with at least 95% 'confidence').

$p_1 = 0.99$; sensitivity = 99%; specificity = 99%

Population size	Prevalence														
	50%			20%			10%			5%			1%		
	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]
30	6 (0)	9 (1)	9 (1)	15 (0)	20 (1)	24 (2)	23 (0)	28 (1)	*	27 (0)	*	*	*	*	*
50	7 (0)	10 (1)	10 (1)	17 (0)	24 (1)	29 (2)	29 (0)	44 (2)	44 (0)	*	*	*	*	*	*
100	7 (0)	10 (1)	10 (1)	19 (0)	26 (1)	33 (2)	34 (0)	58 (2)	67 (3)	55 (0)	96 (3)	*	99 (0)	*	*
150	7 (0)	10 (1)	10 (1)	19 (0)	27 (1)	35 (2)	36 (0)	63 (2)	74 (3)	59 (0)	113 (3)	126 (4)	123 (0)	*	*
200	7 (0)	10 (1)	10 (1)	19 (0)	28 (1)	35 (2)	37 (0)	66 (2)	78 (3)	65 (0)	129 (3)	160 (5)	157 (0)	*	*
300	7 (0)	10 (1)	10 (1)	20 (0)	29 (1)	36 (2)	38 (0)	69 (2)	82 (3)	68 (0)	161 (4)	179 (5)	182 (0)	*	*
500	7 (0)	11 (1)	11 (1)	20 (0)	29 (1)	37 (2)	39 (0)	71 (2)	98 (4)	71 (0)	173 (4)	215 (6)	202 (0)	*	*
1 000	7 (0)	11 (1)	11 (1)	20 (0)	29 (1)	37 (2)	40 (0)	73 (2)	101 (4)	74 (0)	183 (4)	229 (6)	217 (0)	*	*
5 000	7 (0)	11 (1)	11 (1)	20 (0)	30 (1)	38 (2)	41 (0)	75 (2)	103 (4)	76 (0)	191 (4)	264 (7)	228 (0)	2 078 (28)	2 754 (40)
10 000	7 (0)	11 (1)	11 (1)	20 (0)	30 (1)	38 (2)	41 (0)	75 (2)	104 (4)	76 (0)	193 (4)	267 (7)	230 (0)	2 406 (32)	?

* = Required accuracy cannot be achieved, even by sampling all animals in the population.

? = Unable to compute (population size too large).

 $p_1 = 0.99$; sensitivity = 95%; specificity = 99%

Population size	Prevalence														
	50%			20%			10%			5%			1%		
	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]
30	7 (0)	10 (1)	10 (1)	16 (0)	21 (1)	25 (2)	24 (0)	30 (1)	*	28 (0)	*	*	*	*	*
50	7 (0)	10 (1)	10 (1)	18 (0)	25 (1)	30 (2)	30 (0)	46 (2)	46 (2)	46 (0)	*	*	*	*	*
100	7 (0)	11 (1)	11 (1)	19 (0)	28 (1)	35 (2)	35 (0)	60 (2)	70 (3)	58 (0)	100 (3)	*	*	*	*
150	7 (0)	11 (1)	11 (1)	20 (0)	29 (1)	36 (2)	38 (0)	66 (2)	77 (3)	61 (0)	118 (3)	143 (5)	128 (0)	*	*
200	7 (0)	11 (1)	11 (1)	20 (0)	29 (1)	37 (2)	39 (0)	69 (2)	81 (3)	67 (0)	134 (3)	167 (5)	163 (0)	*	*
300	7 (0)	11 (1)	11 (1)	21 (0)	30 (1)	38 (2)	40 (0)	72 (2)	98 (4)	71 (0)	167 (4)	204 (6)	188 (0)	*	*
500	7 (0)	11 (1)	11 (1)	21 (0)	30 (1)	39 (2)	41 (0)	74 (2)	102 (4)	74 (0)	180 (4)	223 (6)	208 (0)	*	*
1 000	8 (0)	11 (1)	11 (1)	21 (0)	31 (1)	39 (2)	42 (0)	76 (2)	105 (4)	76 (0)	190 (4)	260 (7)	222 (0)	*	*
5 000	8 (0)	11 (1)	11 (1)	21 (0)	31 (1)	39 (2)	42 (0)	78 (2)	107 (4)	78 (0)	198 (4)	273 (7)	232 (0)	2 242 (30)	2 929 (42)
10 000	8 (0)	11 (1)	11 (1)	21 (0)	31 (1)	40 (2)	42 (0)	78 (2)	108 (4)	79 (0)	226 (5)	276 (7)	235 (0)	2 579 (34)	?

 $p_1 = 0.99$; sensitivity = 95%; specificity = 95%

Population size	Prevalence														
	50%			20%			10%			5%			1%		
	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]
30	6 (0)	12 (2)	14 (3)	14 (0)	*	*	22 (0)	*	*	26 (0)	*	*	*	*	*
50	7 (0)	13 (2)	15 (3)	16 (0)	37 (4)	45 (6)	25 (0)	48 (3)	*	39 (0)	*	*	*	*	*
100	7 (0)	13 (2)	16 (3)	17 (0)	47 (5)	57 (7)	28 (0)	94 (8)	*	41 (0)	*	*	69 (0)	*	*
150	7 (0)	13 (2)	16 (3)	17 (0)	49 (5)	60 (7)	29 (0)	118 (10)	145 (14)	42 (0)	*	*	68 (0)	*	*
200	7 (0)	13 (2)	16 (3)	17 (0)	50 (5)	66 (8)	30 (0)	124 (10)	161 (15)	44 (0)	*	*	73 (0)	*	*
300	7 (0)	14 (2)	16 (3)	18 (0)	51 (5)	68 (8)	30 (0)	137 (11)	178 (16)	45 (0)	*	*	74 (0)	*	*
500	7 (0)	14 (2)	16 (3)	18 (0)	52 (5)	69 (8)	30 (0)	151 (12)	193 (17)	46 (0)	400 (27)	*	75 (0)	*	*
1 000	7 (0)	14 (2)	17 (3)	18 (0)	53 (5)	70 (8)	31 (0)	154 (12)	107 (18)	46 (0)	465 (31)	604 (43)	76 (0)	*	*
5 000	7 (0)	14 (2)	17 (3)	18 (0)	53 (5)	71 (8)	31 (0)	166 (13)	220 (19)	46 (0)	504 (33)	687 (48)	76 (0)	*	*
10 000	7 (0)	14 (2)	17 (3)	18 (0)	53 (5)	71 (8)	31 (0)	167 (13)	221 (19)	47 (0)	520 (34)	705 (49)	76 (0)	*	*

$p_1 = 0.95$; sensitivity = 99%; specificity = 99%

Population size	Prevalence														
	50%			20%			10%			5%			1%		
	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]
30	5 (0)	5 (0)	7 (1)	11 (0)	17 (1)	21 (2)	18 (0)	26 (1)	30 (2)	23 (0)	30 (1)	*	*	*	*
50	5 (0)	5 (0)	7 (1)	12 (0)	19 (1)	24 (2)	21 (0)	32 (1)	40 (2)	37 (0)	49 (1)	*	*	*	*
100	5 (0)	5 (0)	8 (1)	13 (0)	20 (1)	26 (2)	24 (0)	48 (2)	57 (3)	41 (0)	77 (2)	99 (4)	89 (0)	*	*
150	5 (0)	5 (0)	8 (1)	13 (0)	20 (1)	27 (2)	25 (0)	50 (2)	61 (3)	41 (0)	82 (2)	113 (4)	97 (0)	*	*
200	5 (0)	5 (0)	8 (1)	13 (0)	21 (1)	28 (2)	26 (0)	52 (2)	63 (3)	45 (0)	109 (3)	127 (4)	120 (0)	*	*
300	5 (0)	5 (0)	8 (1)	13 (0)	21 (1)	28 (2)	26 (0)	53 (2)	66 (3)	46 (0)	116 (3)	154 (5)	130 (0)	*	*
500	5 (0)	5 (0)	8 (1)	13 (0)	21 (1)	28 (2)	26 (0)	55 (2)	67 (3)	48 (0)	121 (3)	163 (5)	139 (0)	*	*
1 000	5 (0)	5 (0)	8 (1)	13 (0)	21 (1)	29 (2)	26 (0)	56 (2)	69 (3)	49 (0)	125 (3)	169 (5)	145 (0)	*	*
5 000	5 (0)	5 (0)	8 (1)	13 (0)	22 (1)	29 (2)	27 (0)	56 (2)	70 (3)	50 (0)	129 (3)	175 (5)	149 (0)	1 487 (21)	2 104 (32)
10 000	5 (0)	5 (0)	8 (1)	13 (0)	22 (1)	29 (2)	27 (0)	57 (2)	70 (3)	50 (0)	129 (3)	176 (5)	150 (0)	1 641 (23)	2 338 (35)

 $p_1 = 0.95$; sensitivity = 95%; specificity = 99%

Population size	Prevalence														
	50%			1%			20%			10%			5%		
	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]
30	5 (0)	5 (0)	8 (1)	12 (0)	18 (1)	22 (2)	19 (0)	27 (1)	*	24 (0)	*	*	*	*	*
50	5 (0)	5 (0)	8 (1)	12 (0)	19 (1)	25 (2)	22 (0)	33 (1)	41 (2)	39 (0)	*	*	*	*	*
100	5 (0)	5 (0)	8 (1)	13 (0)	21 (1)	28 (2)	25 (0)	50 (2)	60 (3)	42 (0)	80 (2)	*	93 (0)	*	*
150	5 (0)	5 (0)	8 (1)	14 (0)	21 (1)	28 (2)	26 (0)	53 (2)	64 (3)	43 (0)	102 (3)	118 (4)	100 (0)	*	*
200	5 (0)	5 (0)	8 (1)	14 (0)	22 (1)	29 (2)	26 (0)	54 (2)	66 (3)	47 (0)	113 (3)	148 (5)	123 (0)	*	*
300	5 (0)	5 (0)	8 (1)	14 (0)	22 (1)	29 (2)	27 (0)	56 (2)	68 (3)	48 (0)	120 (3)	160 (5)	134 (0)	*	*
500	5 (0)	5 (0)	8 (1)	14 (0)	22 (1)	30 (2)	27 (0)	57 (2)	70 (3)	49 (0)	126 (3)	169 (5)	142 (0)	*	*
1 000	5 (0)	5 (0)	8 (1)	14 (0)	22 (1)	30 (2)	27 (0)	58 (2)	71 (3)	50 (0)	130 (3)	175 (5)	148 (0)	*	*
5 000	5 (0)	5 (0)	8 (1)	14 (0)	22 (1)	30 (2)	28 (0)	59 (2)	72 (3)	51 (0)	133 (3)	204 (6)	152 (0)	1 636 (23)	2 263 (34)
10 000	5 (0)	5 (0)	8 (1)	14 (0)	23 (1)	30 (2)	28 (0)	59 (2)	73 (3)	52 (0)	134 (3)	205 (6)	153 (0)	1 735 (24)	2 503 (37)

 $p_1 = 0.95$; sensitivity = 95%; specificity = 95%

Population size	Prevalence														
	50%			20%			10%			5%			1%		
	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]
30	5 (0)	7 (1)	12 (3)	10 (0)	25 (3)	*	16 (0)	*	*	20 (0)	*	*	*	*	*
50	5 (0)	10 (2)	13 (3)	11 (0)	28 (3)	36 (5)	18 (0)	49 (5)	*	29 (0)	*	*	*	*	*
100	5 (0)	10 (2)	13 (3)	11 (0)	35 (4)	45 (6)	19 (0)	79 (7)	*	28 (0)	*	*	47 (0)	*	*
150	5 (0)	10 (2)	13 (3)	12 (0)	36 (4)	46 (6)	20 (0)	91 (8)	121 (12)	28 (0)	*	*	46 (0)	*	*
200	5 (0)	11 (2)	13 (3)	12 (0)	36 (4)	47 (6)	20 (0)	94 (8)	132 (13)	29 (0)	*	*	49 (0)	*	*
300	5 (0)	11 (2)	13 (3)	12 (0)	37 (4)	48 (6)	20 (0)	104 (9)	136 (13)	30 (0)	268 (19)	*	49 (0)	*	*
500	5 (0)	11 (2)	13 (3)	12 (0)	37 (4)	48 (6)	20 (0)	106 (9)	148 (14)	30 (0)	300 (21)	423 (32)	49 (0)	*	*
1 000	5 (0)	11 (2)	13 (3)	12 (0)	37 (4)	54 (7)	20 (0)	108 (9)	150 (14)	30 (0)	331 (23)	459 (34)	50 (0)	*	*
5 000	5 (0)	11 (2)	13 (3)	12 (0)	38 (4)	54 (7)	20 (0)	109 (9)	152 (14)	30 (0)	349 (24)	492 (36)	50 (0)	*	*
10 000	5 (0)	11 (2)	13 (3)	12 (0)	38 (4)	54 (7)	20 (0)	109 (9)	161 (15)	30 (0)	351 (24)	507 (37)	50 (0)	*	*

$p_1 = 0.90$; sensitivity = 99%; specificity = 99%

Population size	Prevalence														
	50%			20%			10%			5%			1%		
	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]
30	4 (0)	4 (0)	6 (1)	9 (0)	15 (1)	15 (1)	15 (0)	24 (1)	29 (2)	20 (0)	29 (1)	*	*	*	*
50	4 (0)	4 (0)	6 (1)	10 (0)	16 (1)	21 (2)	17 (0)	28 (1)	37 (2)	32 (0)	47 (1)	47 (1)	*	*	*
100	4 (0)	4 (0)	7 (1)	10 (0)	17 (1)	23 (2)	19 (0)	31 (1)	42 (2)	33 (0)	70 (2)	96 (4)	79 (0)	*	*
150	4 (0)	4 (0)	7 (1)	10 (0)	17 (1)	24 (2)	19 (0)	32 (1)	42 (2)	33 (0)	73 (2)	105 (4)	80 (0)	*	*
200	4 (0)	4 (0)	7 (1)	10 (0)	17 (1)	24 (2)	20 (0)	33 (1)	56 (3)	35 (0)	79 (2)	116 (4)	98 (0)	*	*
300	4 (0)	4 (0)	7 (1)	10 (0)	18 (1)	24 (2)	20 (0)	34 (1)	58 (3)	36 (0)	82 (2)	122 (4)	104 (0)	*	*
500	4 (0)	4 (0)	7 (1)	10 (0)	18 (1)	24 (2)	20 (0)	34 (1)	59 (3)	37 (0)	106 (3)	127 (4)	109 (0)	*	*
1 000	4 (0)	4 (0)	7 (1)	10 (0)	18 (1)	24 (2)	20 (0)	35 (1)	60 (3)	38 (0)	109 (3)	151 (5)	112 (0)	981 (15)	*
5 000	4 (0)	4 (0)	7 (1)	10 (0)	18 (1)	25 (2)	21 (0)	35 (1)	60 (3)	38 (0)	111 (3)	155 (5)	115 (0)	1 226 (18)	1 781 (28)
10 000	4 (0)	4 (0)	7 (1)	10 (0)	18 (1)	25 (2)	21 (0)	35 (1)	60 (3)	38 (0)	112 (3)	156 (5)	116 (0)	1 305 (19)	1 875 (29)

 $p_1 = 0.90$; sensitivity = 95%; specificity = 99%

Population size	Prevalence														
	50%			20%			10%			5%			1%		
	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]
30	4 (0)	4 (0)	7 (1)	9 (0)	15 (1)	15 (1)	16 (0)	25 (1)	*	21 (0)	30 (1)	*	*	*	*
50	4 (0)	4 (0)	7 (1)	10 (0)	17 (1)	22 (2)	18 (0)	29 (1)	38 (2)	33 (0)	49 (1)	49 (1)	*	*	*
100	4 (0)	4 (0)	7 (1)	10 (0)	18 (1)	24 (2)	20 (0)	33 (1)	44 (2)	34 (0)	73 (2)	100 (4)	82 (0)	*	*
150	4 (0)	4 (0)	7 (1)	11 (0)	18 (1)	25 (2)	20 (0)	34 (1)	57 (3)	37 (0)	75 (2)	109 (4)	83 (0)	*	*
200	4 (0)	4 (0)	7 (1)	11 (0)	18 (1)	25 (2)	21 (0)	34 (1)	58 (3)	38 (0)	82 (2)	120 (4)	101 (0)	*	*
300	4 (0)	4 (0)	7 (1)	11 (0)	18 (1)	25 (2)	21 (0)	35 (1)	60 (3)	39 (0)	106 (3)	126 (4)	107 (0)	*	*
500	4 (0)	4 (0)	7 (1)	11 (0)	18 (1)	25 (2)	21 (0)	49 (2)	61 (3)	39 (0)	110 (3)	152 (5)	111 (0)	*	*
1 000	4 (0)	4 (0)	7 (1)	11 (0)	19 (1)	25 (2)	21 (0)	49 (2)	62 (3)	39 (0)	113 (3)	156 (5)	115 (0)	*	*
5 000	4 (0)	4 (0)	7 (1)	11 (0)	19 (1)	26 (2)	21 (0)	50 (2)	63 (3)	40 (0)	115 (3)	160 (5)	117 (0)	1 310 (19)	1 875 (29)
10 000	4 (0)	4 (0)	7 (1)	11 (0)	19 (1)	26 (2)	21 (0)	50 (2)	63 (3)	40 (0)	116 (3)	161 (5)	118 (0)	1 391 (20)	2 029 (31)

 $p_1 = 0.90$; sensitivity = 95%; specificity = 95%

Population size	Prevalence														
	50%			20%			10%			5%			1%		
	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]	[1.0]	[0.05]	[0.01]
30	4 (0)	6 (1)	9 (2)	8 (0)	23 (3)	30 (5)	13 (0)	*	*	17 (0)	*	*	*	*	*
50	4 (0)	6 (1)	9 (2)	9 (0)	25 (3)	34 (5)	14 (0)	46 (4)	*	23 (0)	*	*	45 (0)	*	*
100	4 (0)	7 (1)	9 (2)	9 (0)	26 (3)	36 (5)	15 (0)	66 (6)	*	22 (0)	*	*	37 (0)	*	*
150	4 (0)	7 (1)	9 (2)	9 (0)	27 (3)	37 (5)	15 (0)	77 (7)	107 (11)	22 (0)	*	*	36 (0)	*	*
200	4 (0)	7 (1)	9 (2)	9 (0)	27 (3)	37 (5)	15 (0)	78 (7)	109 (11)	23 (0)	*	*	38 (0)	*	*
300	4 (0)	7 (1)	9 (2)	9 (0)	27 (3)	43 (6)	16 (0)	79 (7)	120 (12)	23 (0)	233 (17)	*	38 (0)	*	*
500	4 (0)	7 (1)	9 (2)	9 (0)	27 (3)	43 (6)	16 (0)	81 (7)	122 (12)	23 (0)	249 (18)	361 (28)	38 (0)	*	*
1 000	4 (0)	7 (1)	9 (2)	9 (0)	27 (3)	44 (6)	16 (0)	81 (7)	123 (12)	23 (0)	265 (19)	380 (29)	38 (0)	*	*
5 000	4 (0)	7 (1)	9 (2)	9 (0)	28 (3)	44 (6)	16 (0)	91 (8)	124 (12)	24 (0)	281 (20)	410 (31)	38 (0)	*	*
10 000	4 (0)	7 (1)	9 (2)	9 (0)	28 (3)	44 (6)	16 (0)	91 (8)	133 (13)	24 (0)	282 (20)	411 (31)	38 (0)	*	*

Appendix XV

Probabilities associated with the upper tail of the Normal distribution

(Derived from Beyer, 1981)

The body of the table gives the one-tailed probability, P , under the null hypothesis, of a random value of the standardized Normal deviate, $z[(x - \mu)/\sigma]$, being greater than the value in the margin. For example, the one-tailed probability of $z \geq 0.22$ or ≤ -0.22 is 0.4129. The probability is doubled for a two-tailed test.

The table can also be used to calculate multipliers for confidence intervals based on the Normal distribution. For example, the multiplier for a 95% confidence interval is derived from a two-tailed significance level of 0.05, corresponding to a one-tailed level of 0.0250, for which $z = 1.96$: the value of the multiplier.

z	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09
.0	.5000	.4960	.4920	.4880	.4840	.4801	.4761	.4721	.4681	.4641
.1	.4602	.4562	.4522	.4483	.4443	.4404	.4364	.4325	.4286	.4247
.2	.4207	.4168	.4129	.4090	.4052	.4013	.3974	.3936	.3897	.3859
.3	.3821	.3783	.3745	.3707	.3669	.3632	.3594	.3557	.3520	.3483
.4	.3446	.3409	.3372	.3336	.3300	.3264	.3228	.3192	.3156	.3121
.5	.3085	.3050	.3015	.2981	.2956	.2912	.2877	.2843	.2810	.2776
.6	.2743	.2709	.2676	.2643	.2611	.2578	.2546	.2514	.2483	.2451
.7	.2420	.2389	.2358	.2327	.2296	.2266	.2236	.2206	.2177	.2148
.8	.2119	.2090	.2061	.2033	.2005	.1977	.1949	.1922	.1894	.1867
.9	.1841	.1814	.1788	.1762	.1736	.1711	.1685	.1660	.1635	.1611
1.0	.1587	.1562	.1539	.1515	.1492	.1469	.1446	.1423	.1401	.1379
1.1	.1357	.1335	.1314	.1292	.1271	.1251	.1230	.1210	.1190	.1170
1.2	.1151	.1131	.1112	.1093	.1075	.1056	.1038	.1020	.1003	.0985
1.3	.0968	.0951	.0934	.0918	.0901	.0885	.0869	.0853	.0838	.0823
1.4	.0808	.0793	.0778	.0764	.0749	.0735	.0721	.0708	.0694	.0681
1.5	.0668	.0655	.0643	.0630	.0618	.0606	.0594	.0582	.0571	.0559
1.6	.0548	.0537	.0526	.0516	.0505	.0495	.0485	.0475	.0465	.0455
1.7	.0446	.0436	.0427	.0418	.0409	.0401	.0392	.0384	.0375	.0367
1.8	.0359	.0351	.0344	.0336	.0329	.0322	.0314	.0307	.0301	.0294
1.9	.0287	.0281	.0274	.0268	.0262	.0256	.0250	.0244	.0239	.0233
2.0	.0228	.0222	.0217	.0212	.0207	.0202	.0197	.0192	.0188	.0183
2.1	.0179	.0174	.0170	.0166	.0162	.0158	.0154	.0150	.0146	.0143
2.2	.0139	.0136	.0132	.0129	.0125	.0122	.0119	.0116	.0113	.0110
2.3	.0107	.0104	.0102	.0099	.0096	.0094	.0091	.0089	.0087	.0084
2.4	.0082	.0080	.0078	.0075	.0073	.0071	.0069	.0068	.0066	.0064
2.5	.0062	.0060	.0059	.0057	.0055	.0054	.0052	.0051	.0049	.0048
2.6	.0047	.0045	.0044	.0043	.0041	.0040	.0039	.0038	.0037	.0036
2.7	.0035	.0034	.0033	.0032	.0031	.0030	.0029	.0028	.0027	.0026
2.8	.0026	.0025	.0024	.0023	.0023	.0022	.0021	.0021	.0020	.0019
2.9	.0019	.0018	.0018	.0017	.0016	.0016	.0015	.0015	.0014	.0014
3.0	.0013	.0013	.0013	.0012	.0012	.0011	.0011	.0011	.0010	.0010
3.1	.0010	.0009	.0009	.0009	.0008	.0008	.0008	.0008	.0007	.0007
3.2	.0007									
3.3	.0005									
3.4	.0003									
3.5	.00023									
3.6	.00016									
3.7	.00011									
3.8	.00007									
3.9	.00005									
4.0	.00003									

Appendix XVI

Lower- and upper-tail probabilities for W_x , the Wilcoxon–Mann–Whitney rank-sum statistic (From Siegel and Castellan, 1988)

The body of the table gives the one-tailed probability, P , of obtaining a value of $W_x \leq c_L$, and $W_s \geq c_U$ under the null hypothesis; W_x is the rank sum for the smaller group.

$m = 3$																				
c_L	$n = 3$	c_U	$n = 4$	c_U	$n = 5$	c_U	$n = 6$	c_U	$n = 7$	c_U	$n = 8$	c_U	$n = 9$	c_U	$n = 10$	c_U	$n = 11$	c_U	$n = 12$	c_U
6	.0500	15	.0286	18	.0179	21	.0119	24	.0083	27	.0061	30	.0045	33	.0035	36	.0027	39	.0022	42
7	.1000	14	.0571	17	.0357	20	.0238	23	.0167	26	.0121	29	.0091	32	.0070	35	.0055	38	.0044	41
8	.2000	13	.1143	16	.0714	19	.0476	22	.0333	25	.0242	28	.0182	31	.0140	34	.0110	37	.0088	40
9	.3500	12	.2000	15	.1250	18	.0833	21	.0583	24	.0424	27	.0318	30	.0245	33	.0192	36	.0154	39
10	.5000	11	.3143	14	.1964	17	.1310	20	.0917	23	.0667	26	.0500	29	.0385	32	.0302	35	.0242	38
11	.6500	10	.4286	13	.2857	16	.1905	19	.1333	22	.0970	25	.0727	28	.0559	31	.0440	34	.0352	37
12	.8000	9	.5714	12	.3929	15	.2738	18	.1917	21	.1394	24	.1045	27	.0804	30	.0632	33	.0505	36
13	.9000	8	.6857	11	.5000	14	.3571	17	.2583	20	.1879	23	.1409	26	.1084	29	.0852	32	.0681	35
14	.9500	7	.8000	10	.6071	13	.4524	16	.3333	19	.2485	22	.1864	25	.1434	28	.1126	31	.0901	34
15	1.0000	6	.8857	9	.7143	12	.5476	15	.4167	18	.3152	21	.2409	24	.1853	27	.1456	30	.1165	33
.9429			8	.8036	11	.6429	14	.5000	17	.3879	20	.3000	23	.2343	26	.1841	29	.1473	32	
.9714			7	.8750	10	.7262	13	.5833	16	.4606	19	.3636	22	.2867	25	.2280	28	.1824	31	
1.0000			6	.9286	9	.8095	12	.6667	15	.5394	18	.4318	21	.3462	24	.2775	27	.2242	30	
	.9643	8		.8690	11	.7417	14	.6121	17	.5000	20	.4056	23	.3297	26	.2681	29			
	.9821	7		.9167	10	.8083	13	.6848	16	.5682	19	.4685	22	.3846	25	.3165	28			
	1.0000	6		.9524	9	.8667	12	.7515	15	.6364	18	.5315	21	.4423	24	.3670	27			
.9762			8	.9083	11	.8121	14	.7000	17	.5944	20	.5000	23	.4198	26					
.9881			7	.9417	10	.8606	13	.7591	16	.6538	19	.5577	22	.4725	25					
1.0000			6	.9667	9	.9030	12	.8136	15	.7133	18	.6154	21	.5275	24					

<i>m = 4</i>																		
<i>c_L</i>	<i>n = 4</i>	<i>c_U</i>	<i>n = 5</i>	<i>c_U</i>	<i>n = 6</i>	<i>c_U</i>	<i>n = 7</i>	<i>c_U</i>	<i>n = 8</i>	<i>c_U</i>	<i>n = 9</i>	<i>c_U</i>	<i>n = 10</i>	<i>c_U</i>	<i>n = 11</i>	<i>c_U</i>	<i>n = 12</i>	<i>c_U</i>
10	.0143	26	.0079	30	.0048	34	.0030	38	.0020	42	.0014	46	.0010	50	.0007	54	.0005	58
11	.0286	25	.0159	29	.0095	33	.0061	37	.0040	41	.0028	45	.0020	49	.0015	53	.0011	57
12	.0571	24	.0317	28	.0190	32	.0121	36	.0081	40	.0056	44	.0040	48	.0029	52	.0022	56
13	.1000	23	.0556	27	.0333	31	.0212	35	.0141	39	.0098	43	.0070	47	.0051	51	.0038	55
14	.1714	22	.0952	26	.0571	30	.0364	34	.0242	38	.0168	42	.0120	46	.0088	50	.0066	54
15	.2429	21	.1429	25	.0857	29	.0545	33	.0364	37	.0252	41	.0180	45	.0132	49	.0099	53
16	.3429	20	.2063	24	.1286	28	.0818	32	.0545	36	.0378	40	.0270	44	.0198	48	.0148	52
17	.4429	19	.2778	23	.1762	27	.1152	31	.0768	35	.0531	39	.0380	43	.0278	47	.0209	51
18	.5571	18	.3651	22	.2381	26	.1576	30	.1071	34	.0741	38	.0529	42	.0388	46	.0291	50
19	.6571	17	.4524	21	.3048	25	.2061	29	.1414	33	.0993	37	.0709	41	.0520	45	.0390	49
20	.7571	16	.5476	20	.3810	24	.2636	28	.1838	32	.1301	36	.0939	40	.0689	44	.0516	48
21	.8286	15	.6349	19	.4571	23	.3242	27	.2303	31	.1650	35	.1199	39	.0886	43	.0665	47
22	.9000	14	.7222	18	.5429	22	.3939	26	.2848	30	.2070	34	.1518	38	.1128	42	.0852	46
23	.9429	13	.7937	17	.6190	21	.4636	25	.3414	29	.2517	33	.1868	37	.1399	41	.1060	45
24	.9714	12	.8571	16	.6952	20	.5364	24	.4040	28	.3021	32	.2268	36	.1714	40	.1308	44
25	.9857	11	.9048	15	.7619	19	.6061	23	.4667	27	.3552	31	.2697	35	.2059	39	.1582	43
26	1.0000	10	.9444	14	.8238	18	.6758	22	.5333	26	.4126	30	.3177	34	.2447	38	.1896	42
27			.9683	13	.8714	17	.7364	21	.5960	25	.4699	29	.3666	33	.2857	37	.2231	41
28			.9841	12	.9143	16	.7939	20	.6586	24	.5301	28	.4196	32	.3304	36	.2604	40
29			.9921	11	.9429	15	.8424	19	.7152	23	.5874	27	.4725	31	.3766	35	.2995	39
30			1.0000	10	.9667	14	.8848	18	.7697	22	.6448	26	.5275	30	.4256	34	.3418	38
31					.9810	13	.9182	17	.8162	21	.6979	25	.5804	29	.4747	33	.3852	37
32					.9905	12	.9455	16	.8586	20	.7483	24	.6334	28	.5253	32	.4308	36
33					.9952	11	.9636	15	.8929	19	.7930	23	.6823	27	.5744	31	.4764	35
34					1.0000	10	.9788	14	.9232	18	.8350	22	.7303	26	.6234	30	.5236	34

$m = 5$												
c_L	$n = 5$	c_U	$n = 6$	c_U	$n = 7$	c_U	$n = 8$	c_U	$n = 9$	c_U	$n = 10$	c_U
15	.0040	40	.0022	45	.0013	50	.0008	55	.0005	60	.0003	65
16	.0079	39	.0043	44	.0025	49	.0016	54	.0010	59	.0007	64
17	.0159	38	.0087	43	.0051	48	.0031	53	.0020	58	.0013	63
18	.0278	37	.0152	42	.0088	47	.0054	52	.0035	57	.0023	62
19	.0476	36	.0260	41	.0152	46	.0093	51	.0060	56	.0040	61
20	.0754	35	.0411	40	.0240	45	.0148	50	.0095	55	.0063	60
21	.1111	34	.0628	39	.0366	44	.0225	49	.0145	54	.0097	59
22	.1548	33	.0887	38	.0530	43	.0326	48	.0210	53	.0140	58
23	.2103	32	.1234	37	.0745	42	.0466	47	.0300	52	.0200	57
24	.2738	31	.1645	36	.1010	41	.0637	46	.0415	51	.0276	56
25	.3452	30	.2143	35	.1338	40	.0855	45	.0559	50	.0376	55
26	.4206	29	.2684	34	.1717	39	.1111	44	.0734	49	.0496	54
27	.5000	28	.3312	33	.2159	38	.1422	43	.0949	48	.0646	53
28	.5794	27	.3961	32	.2652	37	.1772	42	.1199	47	.0823	52
29	.6548	26	.4654	31	.3194	36	.2176	41	.1489	46	.1032	51
30	.7262	25	.5346	30	.3775	35	.2618	40	.1818	45	.1272	50
31	.7897	24	.6039	29	.4381	34	.3108	39	.2188	44	.1548	49
32	.8452	23	.6688	28	.5000	33	.3621	38	.2592	43	.1855	48
33	.8889	22	.7316	27	.5619	32	.4165	37	.3032	42	.2198	47
34	.9246	21	.7857	26	.6225	31	.4716	36	.3497	41	.2567	46
35	.9524	20	.8355	25	.6806	30	.5284	35	.3986	40	.2970	45
36	.9722	19	.8766	24	.7348	29	.5835	34	.4491	39	.3393	44
37	.9841	18	.9113	23	.7841	28	.6379	33	.5000	38	.3839	43
38	.9921	17	.9372	22	.8283	27	.6892	32	.5509	37	.4296	42
39	.9960	16	.9589	21	.8662	26	.7382	31	.6014	36	.4765	41
40	1.0000	15	.9740	20	.8990	25	.7824	30	.6503	35	.5235	40

<i>m</i> = 6										
<i>c_L</i>	<i>n</i> = 6	<i>c_U</i>	<i>n</i> = 7	<i>c_U</i>	<i>n</i> = 8	<i>c_U</i>	<i>n</i> = 9	<i>c_U</i>	<i>n</i> = 10	<i>c_U</i>
21	.0011	57	.0006	63	.0003	69	.0002	75	.0001	81
22	.0022	56	.0012	62	.0007	68	.0004	74	.0002	80
23	.0043	55	.0023	61	.0013	67	.0008	73	.0005	79
24	.0076	54	.0041	60	.0023	66	.0014	72	.0009	78
25	.0130	53	.0070	59	.0040	65	.0024	71	.0015	77
26	.0206	52	.0111	58	.0063	64	.0038	70	.0024	76
27	.0325	51	.0175	57	.0100	63	.0060	69	.0037	75
28	.0465	50	.0256	56	.0147	62	.0088	68	.0055	74
29	.0660	49	.0367	55	.0213	61	.0128	67	.0080	73
30	.0898	48	.0507	54	.0296	60	.0180	66	.0112	72
31	.1201	47	.0688	53	.0406	59	.0248	65	.0156	71
32	.1548	46	.0903	52	.0539	58	.0332	64	.0210	70
33	.1970	45	.1171	51	.0709	57	.0440	63	.0280	69
34	.2424	44	.1474	50	.0906	56	.0567	62	.0363	68
35	.2944	43	.1830	49	.1142	55	.0723	61	.0467	67
36	.3496	42	.2226	48	.1412	54	.0905	60	.0589	66
37	.4091	41	.2669	47	.1725	53	.1119	59	.0736	65
38	.4686	40	.3141	46	.2068	52	.1361	58	.0903	64
39	.5314	39	.3654	45	.2454	51	.1638	57	.1099	63
40	.5909	38	.4178	44	.2864	50	.1942	56	.1317	62
41	.6504	37	.4726	43	.3310	49	.2280	55	.1566	61
42	.7056	36	.5274	42	.3773	48	.2643	54	.1838	60
43	.7576	35	.5822	41	.4259	47	.3035	53	.2139	59
44	.8030	34	.6346	40	.4749	46	.3445	52	.2461	58
45	.8452	33	.6859	39	.5251	45	.3878	51	.2811	57
46	.8799	32	.7331	38	.5741	44	.4320	50	.3177	56
47	.9102	31	.7774	37	.6227	43	.4773	49	.3564	55
48	.9340	30	.8170	36	.6690	42	.5227	48	.3962	54
49	.9535	29	.8526	35	.7136	41	.5680	47	.4374	53
50	.9675	28	.8829	34	.7546	40	.6122	46	.4789	52
51	.9794	27	.9097	33	.7932	39	.6555	45	.5211	51

$m = 7$								
c_L	$n = 7$	c_U	$n = 8$	c_U	$n = 9$	c_U	$n = 10$	c_U
28	.0003	77	.0002	84	.0001	91	.0001	98
29	.0006	76	.0003	83	.0002	90	.0001	97
30	.0012	75	.0006	82	.0003	89	.0002	96
31	.0020	74	.0011	81	.0006	88	.0004	95
32	.0035	73	.0019	80	.0010	87	.0006	94
33	.0055	72	.0030	79	.0017	86	.0010	93
34	.0087	71	.0047	78	.0026	85	.0015	92
35	.0131	70	.0070	77	.0039	84	.0023	91
36	.0189	69	.0103	76	.0058	83	.0034	90
37	.0265	68	.0145	75	.0082	82	.0048	89
38	.0364	67	.0200	74	.0115	81	.0068	88
39	.0487	66	.0270	73	.0156	80	.0093	87
40	.0641	65	.0361	72	.0209	79	.0125	86
41	.0825	64	.0469	71	.0274	78	.0165	85
42	.1043	63	.0603	70	.0356	77	.0215	84
43	.1297	62	.0760	69	.0454	76	.0277	83
44	.1588	61	.0946	68	.0571	75	.0351	82
45	.1914	60	.1159	67	.0708	74	.0439	81
46	.2279	59	.1405	66	.0869	73	.0544	80
47	.2675	58	.1678	65	.1052	72	.0665	79
48	.3100	57	.1984	64	.1261	71	.0806	78
49	.3552	56	.2317	63	.1496	70	.0966	77
50	.4024	55	.2679	62	.1755	69	.1148	76
51	.4508	54	.3063	61	.2039	68	.1349	75
52	.5000	53	.3472	60	.2349	67	.1574	74
53	.5492	52	.3894	59	.2680	66	.1819	73
54	.5976	51	.4333	58	.3032	65	.2087	72
55	.6448	50	.4775	57	.3403	64	.2374	71
56	.6900	49	.5225	56	.3788	63	.2681	70
57	.7325	48	.5667	55	.4185	62	.3004	69
58	.7721	47	.6106	54	.4591	61	.3345	68
59	.8086	46	.6528	53	.5000	60	.3698	67
60	.8412	45	.6937	52	.5409	59	.4063	66
61	.8703	44	.7321	51	.5815	58	.4434	65
62	.8957	43	.7683	50	.6212	57	.4811	64
63	.9175	42	.8016	49	.6597	56	.5189	63

<i>m</i> = 8						
<i>c_L</i>	<i>n</i> = 8	<i>c_U</i>	<i>n</i> = 9	<i>c_U</i>	<i>n</i> = 10	<i>c_U</i>
36	.0001	100	.0000	108	.0000	116
37	.0002	99	.0001	107	.0000	115
38	.0003	98	.0002	106	.0001	114
39	.0005	97	.0003	105	.0002	113
40	.0009	96	.0005	104	.0003	112
41	.0015	95	.0008	103	.0004	111
42	.0023	94	.0012	102	.0007	110
43	.0035	93	.0019	101	.0010	109
44	.0052	92	.0028	100	.0015	108
45	.0074	91	.0039	99	.0022	107
46	.0103	90	.0056	98	.0031	106
47	.0141	89	.0076	97	.0043	105
48	.0190	88	.0103	96	.0658	104
49	.0249	87	.0137	95	.0078	103
50	.0325	86	.0180	94	.0103	102
51	.0415	85	.0232	93	.0133	101
52	.0524	84	.0296	92	.0171	100
53	.0652	83	.0372	91	.0217	99
54	.0803	82	.0464	90	.0273	98
55	.0974	81	.0570	89	.0338	97
56	.1172	80	.0694	88	.0416	96
57	.1393	79	.0836	87	.0506	95
58	.1641	78	.0998	86	.0610	94
59	.1911	77	.1179	85	.0729	93
60	.2209	76	.1383	84	.0864	92
61	.2527	75	.1606	83	.1015	91
62	.2869	74	.1852	82	.1185	90
63	.3227	73	.2117	81	.1371	89
64	.3605	72	.2404	80	.1577	88
65	.3992	71	.2707	79	.1800	87
66	.4392	70	.3029	78	.2041	86
67	.4796	69	.3365	77	.2299	85
68	.5204	68	.3715	76	.2574	84
69	.5608	67	.4074	75	.2863	83
70	.6008	66	.4442	74	.3167	82
71	.6395	65	.4813	73	.3482	81
72	.6773	64	.5187	72	.3809	80
73	.7131	63	.5558	71	.4143	79
74	.7473	62	.5926	70	.4484	78
75	.7791	61	.6285	69	.4827	77
76	.8089	60	.6635	68	.5173	76

<i>m = 9</i>									
<i>c_L</i>	<i>n = 9</i>	<i>c_U</i>	<i>n = 10</i>	<i>c_U</i>	<i>c_L</i>	<i>n = 9 (cont.)</i>	<i>n = 10 (cont.)</i>	<i>c_U</i>	
45	.0000	126	.0000	135	68	.0680	103	.0394	112
46	.0000	125	.0000	134	69	.0807	102	.0474	111
47	.0001	124	.0000	133	70	.0951	101	.0564	110
48	.0001	123	.0001	132	71	.1112	100	.0667	109
49	.0002	122	.0001	131	72	.1290	99	.0782	103
50	.0004	121	.0002	130	73	.1487	98	.0912	107
51	.0006	120	.0003	129	74	.1701	97	.1055	106
52	.0009	119	.0005	128	75	.1933	96	.1214	105
53	.0014	118	.0007	127	76	.2181	95	.1388	104
54	.0020	117	.0011	126	77	.2447	94	.1577	103
55	.0028	116	.0015	125	78	.2729	93	.1781	102
56	.0039	115	.0021	124	79	.3024	92	.2001	101
57	.0053	114	.0028	123	80	.3332	91	.2235	100
58	.0071	113	.0038	122	81	.3652	90	.2483	99
59	.0094	112	.0051	121	82	.3981	89	.2745	98
60	.0122	111	.0066	120	83	.4317	88	.3019	97
61	.0157	110	.0086	119	84	.4657	87	.3304	96
62	.0200	109	.0110	118	85	.5000	86	.3598	95
63	.0252	108	.0140	117	86	.5343	85	.3901	94
64	.0313	107	.0175	116	87	.5683	84	.4211	93
65	.0385	106	.0217	115	88	.6019	83	.4524	92
66	.0470	105	.0267	114	89	.6348	82	.4841	91
67	.0567	104	.0326	113	90	.6668	81	.6668	90

<i>m = 10</i>					
<i>c_L</i>	<i>n = 10</i>	<i>c_U</i>	<i>c_L</i>	<i>n = 10 (cont.)</i>	<i>c_U</i>
55	.0000	155	81	.0376	129
56	.0000	154	82	.0446	128
57	.0000	153	83	.0526	127
58	.0000	152	84	.0615	126
59	.0001	151	85	.0716	125
60	.0001	150	86	.0827	124
61	.0002	149	87	.0952	123
62	.0002	148	88	.1088	122
63	.0004	147	89	.1237	121
64	.0005	145	90	.1399	120
65	.0008	145	91	.1575	119
66	.0010	144	92	.1763	118
67	.0014	143	93	.1965	117
68	.0019	142	94	.2179	116
69	.0026	141	95	.2406	115
70	.0034	140	96	.2644	114
71	.0045	139	97	.2894	113
72	.0057	138	98	.3153	112
73	.0073	137	99	.3421	111
74	.0093	136	100	.3697	110
75	.0116	135	101	.3980	109
76	.0144	134	102	.4267	108
77	.0177	133	103	.4559	107
78	.0216	132	104	.4853	106
79	.0262	131	105	.5147	105
80	.0315	130			

Appendix XVII

Critical values of T^+ for the Wilcoxon signed ranks test

(From Siegel and Castellan, 1988)

The body of the table gives the one-tailed probability, P , under the null hypothesis, that $T^+ \geq c$, for N pairs of observations with non-zero differences.

N							
c	3	4	5	6	7	8	9
3	.6250						
4	.3750						
5	.2500	.5625					
6	.1250	.4375					
7		.3125					
8		.1875	.5000				
9		.1250	.4063				
10		.0623	.3125				
11			.2188	.5000			
12			.1563	.4219			
13			.0938	.3438			
14			.0625	.2813	.5313		
15			.0313	.2188	.4688		
16				.1563	.4063		
17				.1094	.3438		
18				.0781	.2891	.5273	
19				.0469	.2344	.4727	
20				.0313	.1875	.4219	
21				.0156	.1484	.3711	
22					.1094	.3203	
23					.0781	.2734	.5000

N							
c	3	4	5	6	7	8	9
24					.0547	.2305	.4551
25					.0391	.1914	.4102
26					.0234	.1563	.3672
27					.0156	.1250	.3262
28					.0078	.0977	.2852
29						.0742	.2480
30						.0547	.2129
31						.0391	.1797
32						.0273	.1504
33						.0195	.1250
34						.0117	.1016
35						.0078	.0820
36						.0039	.0645
37							.0488
38							.0371
39							.0273
40							.0195
41							.0137
42							.0098
43							.0059
44							.0039
45							.0020

(Continued)

c	N					
	10	11	12	13	14	15
28	.5000					
29	.4609					
30	.4229					
31	.3848					
32	.3477					
33	.3125	.5171				
34	.2783	.4829				
35	.2461	.4492				
36	.2158	.4155				
37	.1875	.3823				
38	.1611	.3501				
39	.1377	.3188	.5151			
40	.1162	.2886	.4849			
41	.0967	.2598	.4548			
42	.0801	.2324	.4250			
43	.0654	.2065	.3955			
44	.0527	.1826	.3667			
45	.0420	.1602	.3386			
46	.0322	.1392	.3110	.5000		
47	.0244	.1201	.2847	.4730		
48	.0186	.1030	.2593	.4463		
49	.0137	.0874	.2349	.4197		
50	.0098	.0737	.2119	.3934		
51	.0068	.0615	.1902	.3677		
52	.0049	.0508	.1697	.3424		
53	.0029	.0415	.1506	.3177	.5000	
54	.0020	.0337	.1331	.2939	.4758	
55	.0010	.0269	.1167	.2709	.4516	
56		.0210	.1018	.2487	.4276	
57		.0161	.0881	.2274	.4039	
58		.0122	.0757	.2072	.3804	
59		.0093	.0647	.1879	.3574	
60		.0068	.0549	.1698	.3349	.5110
61		.0049	.0461	.1527	.3129	.4890
62		.0034	.0386	.1367	.2915	.4670
63		.0024	.0320	.1219	.2708	.4452
64		.0015	.0261	.1082	.2508	.4235
65		.0010	.0212	.0955	.2316	.4020
66		.0005	.0171	.0839	.2131	.3808
67			.0134	.0732	.1955	.3599
68			.0105	.0636	.1788	.3394
69			.0081	.0549	.1629	.3193
70			.0061	.0471	.1479	.2997
71			.0046	.0402	.1338	.2807
72			.0034	.0341	.1206	.2622
73			.0024	.0287	.1083	.2444
74			.0017	.0239	.0969	.2271

c	N			
	12	13	14	15
75	.0012	.0199	.0863	.2106
76	.0007	.0164	.0765	.1947
77	.0005	.0133	.0676	.1796
78	.0002	.0107	.0594	.1651
79		.0085	.0520	.1514
80		.0067	.0453	.1384
81		.0052	.0392	.1262
82		.0040	.0338	.1147
83		.0031	.0290	.1039
84		.0023	.0247	.0938
85		.0017	.0209	.0844
86		.0612	.0176	.0757
87		.0009	.0148	.0677
88		.0006	.0123	.0603
89		.0004	.0101	.0535
90		.0002	.0083	.0473
91		.0001	.0067	.0416
92			.0054	.0365
93			.0043	.0319
94			.0034	.0277
95			.0026	.0240
96			.0020	.0206
97			.0015	.0177
98			.0012	.0151
99			.0009	.0128
100			.0006	.0108
101			.0004	.0090
102			.0003	.0075
103			.0002	.0062
104			.0001	.0051
105			.0001	.0042
106				.0034
107				.0027
108				.0021
109				.0017
110				.0013
111				.0010
112				.0008
113				.0006
114				.0004
115				.0003
116				.0002
117				.0002
118				.0001
119				.0001
120				.0000

Appendix XVIII

Values of K for calculating 95% confidence intervals for the difference between population medians for two independent samples

(From Altman *et al.*, 2000)

Sample sizes (n_1, n_2)		Level of confidence 95% (approx.)		Sample sizes (n_1, n_2)		Level of confidence 95% (approx.)	
Smaller	Larger	K	Exact level (%)	Smaller	Larger	K	Exact level (%)
5	5	3	96.8	6	24	34	95.6
5	6	4	97.0	6	25	36	95.4
5	7	6	95.2	7	7	9	96.2
5	8	7	95.5	7	8	11	96.0
5	9	8	95.8	7	9	13	95.8
5	10	9	96.0	7	10	15	95.7
5	11	10	96.2	7	11	17	95.6
5	12	12	95.2	7	12	19	95.5
5	13	13	95.4	7	13	21	95.4
5	14	14	95.6	7	14	23	95.4
5	15	15	95.8	7	15	25	95.3
5	16	16	96.0	7	16	27	95.3
5	17	18	95.2	7	17	29	95.3
5	18	19	95.4	7	18	31	95.3
5	19	20	95.6	7	19	33	95.2
5	20	21	95.8	7	20	35	95.2
5	21	23	95.1	7	21	37	95.2
5	22	24	95.3	7	22	39	95.2
5	23	25	95.5	7	23	41	95.2
5	24	26	95.6	7	24	43	95.2
5	25	28	95.1	7	25	45	95.2
6	6	6	95.9	8	8	14	95.0
6	7	7	96.5	8	9	16	95.4
6	8	9	95.7	8	10	18	95.7
6	9	11	95.0	8	11	20	95.9
6	10	12	95.8	8	12	23	95.3
6	11	14	95.2	8	13	25	95.5
6	12	15	95.9	8	14	27	95.8
6	13	17	95.4	8	15	30	95.3
6	14	18	95.9	8	16	32	95.5
6	15	20	95.5	8	17	35	95.1
6	16	22	95.1	8	18	37	95.3
6	17	23	95.6	8	19	39	95.5
6	18	25	95.3	8	20	42	95.1
6	19	26	95.7	8	21	44	95.3
6	20	28	95.4	8	22	46	95.5
6	21	30	95.1	8	23	49	95.2
6	22	31	95.5	8	24	51	95.4
6	23	33	95.3	8	25	54	95.1

(Continued)

Sample sizes (n_1, n_2)		Level of confidence 95% (approx.)	
Smaller	Larger	K	Exact level (%)
9	9	18	96.0
9	10	21	95.7
9	11	24	95.4
9	12	27	95.1
9	13	29	95.7
9	14	32	95.4
9	15	35	95.2
9	16	38	95.1
9	17	40	95.5
9	18	43	95.4
9	19	46	95.2
9	20	49	95.1
9	21	51	95.5
9	22	54	95.4
9	23	57	95.3
9	24	60	95.1
9	25	63	95.0
10	10	24	95.7
10	11	27	95.7
10	12	30	95.7
10	13	34	95.1
10	14	37	95.2
10	15	40	95.2
10	16	43	95.3
10	17	46	95.4
10	18	49	95.5
10	19	53	95.0
10	20	56	95.1
10	21	59	95.2
10	22	62	95.3
10	23	65	95.3
10	24	68	95.4
10	25	72	95.0
11	11	31	95.3
11	12	34	95.6
11	13	38	95.3
11	14	41	95.6
11	15	45	95.3
11	16	48	95.6
11	17	52	95.3
11	18	56	95.1
11	19	59	95.3
11	20	63	95.1
11	21	66	95.4
11	22	70	95.2
11	23	74	95.0
11	24	77	95.3
11	25	81	95.1
12	12	38	95.5
12	13	42	95.4
12	14	46	95.4
12	15	50	95.3
12	16	54	95.3
12	17	58	95.2
12	18	62	95.2
12	19	66	95.2
12	20	70	95.2
12	21	74	95.2
12	22	78	95.2
12	23	82	95.1
12	24	86	95.1
12	25	90	95.1
13	13	46	95.6
13	14	51	95.2
13	15	55	95.4
13	16	60	95.0
13	17	64	95.2
13	18	68	95.4
13	19	73	95.1
13	20	77	95.2
13	21	81	95.4
13	22	86	95.1
13	23	90	95.3
13	24	95	95.1
13	25	99	95.2
14	14	56	95.0
14	15	60	95.4
14	16	65	95.3
14	17	70	95.2
14	18	75	95.1
14	19	79	95.4
14	20	84	95.3
14	21	89	95.2
14	22	94	95.1
14	23	99	95.1
14	24	103	95.3
14	25	108	95.3
15	15	65	95.5
15	16	71	95.1
15	17	76	95.1
15	18	81	95.2
15	19	86	95.3
15	20	91	95.4
15	21	97	95.1
15	22	102	95.1
15	23	107	95.2
15	24	112	95.3
15	25	118	95.0
16	16	76	95.3
16	17	82	95.1
16	18	87	95.4
16	19	93	95.2
16	20	99	95.1
16	21	104	95.3
16	22	110	95.2
16	23	116	95.0
16	24	121	95.2
16	25	127	95.1
17	17	88	95.1
17	18	94	95.1
17	19	100	95.1
17	20	106	95.2
17	21	112	95.2

<i>Sample sizes (n_1, n_2)</i>		<i>Level of confidence 95% (approx.)</i>	
<i>Smaller</i>	<i>Larger</i>	<i>K</i>	<i>Exact level (%)</i>
17	22	118	95.2
17	23	124	95.2
17	24	130	95.2
17	25	136	95.2
18	18	100	95.3
18	19	107	95.1
18	20	113	95.2
18	21	120	95.1
18	22	126	95.2
18	23	133	95.0
18	24	139	95.2
18	25	146	95.0
19	19	114	95.0
19	20	120	95.3
19	21	127	95.3
19	22	134	95.2
19	23	141	95.2
19	24	148	95.2
19	25	155	95.1
20	20	128	95.1
20	21	135	95.2

<i>Sample sizes (n_1, n_2)</i>		<i>Level of confidence 95% (approx.)</i>	
<i>Smaller</i>	<i>Larger</i>	<i>K</i>	<i>Exact level (%)</i>
20	22	142	95.3
20	23	150	95.1
20	24	157	95.2
20	25	164	95.3
21	21	143	95.1
21	22	151	95.0
21	23	158	95.2
21	24	166	95.2
21	25	174	95.1
22	22	159	95.1
22	23	167	95.1
22	24	175	95.2
22	25	183	95.2
23	23	176	95.0
23	24	184	95.2
23	25	193	95.1
24	24	193	95.2
24	25	202	95.2
25	25	212	95.1

Appendix XIX

Values of K^* for calculating 95% confidence intervals for the difference between population medians for two related samples

(From Altman *et al.*, 2000)

Sample size (n)	Level of confidence 95% (approx.)		Sample size (n)	Level of confidence 95% (approx.)	
	K^*	Exact level (%)		K^*	Exact level (%)
6	1	96.9	29	127	95.2
7	3	95.3	30	138	95.0
8	4	96.1	31	148	95.2
9	6	96.1	32	160	95.0
10	9	95.1	33	171	95.2
11	11	95.8	34	183	95.2
12	14	95.8	35	196	95.1
13	18	95.2	36	209	95.0
14	22	95.1	37	222	95.1
15	26	95.2	38	236	95.1
16	30	95.6	39	250	95.1
17	35	95.5	40	265	95.0
18	41	95.2	41	280	95.0
19	47	95.1	42	295	95.1
20	53	95.2	43	311	95.1
21	59	95.4	44	328	95.0
22	66	95.4	45	344	95.1
23	74	95.2	46	362	95.0
24	82	95.1	47	379	95.1
25	90	95.2	48	397	95.1
26	99	95.1	49	416	95.1
27	108	95.1	50	435	95.1
28	117	95.2			

Appendix XX

Common logarithms ($\log_{10}$) of factorials of the integers 1–999

(From Lentner, 1982)

<i>n</i>	0	1	2	3	4	5	6	7	8	9
0	0.000 00	0.000 00	0.301 03	0.778 15	1.380 21	2.079 18	2.857 33	3.702 43	4.605 52	5.559 76
10	6.559 76	7.601 16	8.680 34	9.794 28	10.940 41	12.116 50	13.320 62	14.551 07	15.806 34	17.085 09
20	18.386 12	19.708 34	21.050 77	22.412 49	23.792 71	25.190 65	26.605 62	28.036 98	29.484 14	30.946 54
30	32.423 66	33.915 02	35.420 17	36.938 69	38.470 16	40.014 23	41.570 54	43.138 74	44.718 52	46.309 59
40	47.911 65	49.524 43	51.147 68	52.781 15	54.424 60	56.077 81	57.740 57	59.412 67	61.093 91	62.784 10
50	64.483 07	66.190 64	67.906 65	69.630 92	71.363 32	73.103 68	74.851 87	76.607 74	78.371 17	80.142 02
60	81.920 17	83.705 50	85.497 90	87.297 24	89.103 42	90.916 33	92.735 87	94.561 95	96.394 46	98.233 31
70	100.078 41	101.929 66	103.787 00	105.650 32	107.519 55	109.394 61	111.275 43	113.161 92	115.054 01	116.951 64
80	118.854 73	120.763 21	122.677 03	124.596 10	126.520 38	128.449 80	130.384 30	132.323 82	134.268 30	136.217 69
90	138.171 94	140.130 98	142.094 76	144.063 25	146.036 38	148.014 10	149.996 37	151.983 14	153.974 37	155.970 00
100	157.970 00	159.974 33	161.982 93	163.995 76	166.012 80	168.033 98	170.059 29	172.088 67	174.122 10	176.159 52
110	178.200 92	180.246 24	182.295 46	184.348 54	186.405 44	188.466 14	190.530 60	192.598 78	194.670 67	196.746 21
120	198.825 39	200.908 18	202.994 54	205.084 44	207.177 87	209.274 78	211.375 15	213.478 95	215.586 16	217.696 75
130	219.810 69	221.927 97	224.048 54	226.172 39	228.299 50	230.429 83	232.563 37	234.700 09	236.839 97	238.982 98
140	241.129 11	243.278 33	245.430 62	247.585 95	249.744 32	251.905 68	254.070 04	256.237 35	258.407 62	260.580 80
150	262.756 89	264.935 87	267.117 71	269.302 40	271.489 93	273.680 26	275.873 38	278.069 28	280.267 94	282.469 33
160	284.673 45	286.880 28	289.089 80	291.301 98	293.516 83	295.734 31	297.954 42	300.177 13	302.402 44	304.630 33
170	306.860 78	309.093 78	311.329 30	313.567 35	315.807 90	318.050 94	320.296 45	322.544 42	324.794 84	327.047 70
180	329.302 97	331.560 65	333.820 72	336.083 17	338.347 99	340.615 16	342.884 67	345.156 51	347.430 67	349.707 13
190	351.985 89	354.266 92	356.550 22	358.835 78	361.123 58	363.413 62	365.705 87	368.000 34	370.297 00	372.595 86
200	374.896 89	377.200 08	379.505 44	381.812 93	384.122 56	386.434 32	388.748 18	391.064 15	393.382 22	395.702 36
210	398.024 58	400.348 87	402.675 20	405.003 58	407.334 00	409.666 43	412.000 89	414.337 35	416.675 80	419.016 25
220	421.358 67	423.703 06	426.049 42	428.397 72	430.747 97	433.100 15	435.454 26	437.810 29	440.168 22	442.528 06
230	444.889 78	447.253 40	449.618 88	451.986 24	454.355 46	456.726 52	459.099 44	461.474 18	463.850 76	466.229 16
240	468.609 37	470.991 39	473.375 20	475.760 81	478.148 20	480.537 37	482.928 30	485.321 00	487.715 45	490.111 65
250	492.509 59	494.909 26	497.310 66	499.713 78	502.118 62	504.525 16	506.933 40	509.343 33	511.754 95	514.168 25
260	516.583 22	518.999 86	521.418 16	523.838 12	526.259 72	528.682 97	531.107 85	533.534 36	535.962 50	538.392 25
270	540.823 61	543.256 58	545.691 15	548.127 31	550.565 06	553.004 39	555.445 30	557.887 78	560.331 83	562.777 43
280	565.224 59	567.673 30	570.123 54	572.575 33	575.028 65	577.483 49	579.939 86	582.397 74	584.857 13	587.318 03
290	589.780 43	592.244 32	594.709 71	597.176 57	599.644 92	602.114 74	604.586 03	607.058 79	609.533 01	612.008 68
300	614.485 80	616.964 36	619.444 37	621.925 81	624.408 69	626.892 99	629.378 71	631.865 85	634.354 40	636.844 36
310	639.335 72	641.828 48	644.322 63	646.818 18	649.315 11	651.813 42	654.313 10	656.814 16	659.316 59	661.820 38
320	664.325 53	666.832 04	669.339 89	671.849 10	674.359 64	676.871 52	679.384 74	681.899 29	684.415 16	686.932 36
330	689.450 87	691.970 70	694.491 84	697.014 28	699.538 03	702.063 07	704.589 41	707.117 04	709.645 96	712.176 16
340	714.707 64	717.240 39	719.774 42	722.309 71	724.846 27	727.384 09	729.923 17	732.463 50	735.005 08	737.547 90
350	740.091 97	742.637 28	745.183 82	747.731 60	750.280 60	752.830 83	755.382 28	757.934 95	760.488 83	763.043 92
360	765.600 23	768.157 73	770.716 44	773.276 35	775.837 45	778.399 74	780.963 23	783.527 89	786.093 74	788.660 77
370	791.228 97	793.798 34	796.368 88	798.940 59	801.513 47	804.087 50	806.662 68	809.239 03	811.816 52	814.395 16
380	816.974 94	819.555 87	822.137 93	824.721 13	827.305 46	829.890 92	832.477 51	835.065 22	837.654 05	840.244 00
390	842.835 07	845.427 24	848.020 53	850.614 92	853.210 42	855.807 01	858.404 71	861.003 50	863.603 38	866.204 36
400	868.806 42	871.409 56	874.013 79	876.619 09	879.225 47	881.832 93	884.441 46	887.051 05	889.661 71	892.273 43
410	894.886 22	897.500 06	900.114 96	902.730 91	905.347 91	907.965 95	910.585 05	913.205 18	915.826 36	918.448 57

(Continued)

<i>n</i>	<i>0</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	<i>7</i>	<i>8</i>	<i>9</i>
420	921.071 82	923.696 11	926.321 42	928.947 76	931.575 12	934.203 51	936.832 92	939.463 35	942.094 80	944.727 25
430	947.360 72	949.995 20	952.630 68	955.267 17	957.904 66	960.543 15	963.182 63	965.823 12	968.464 59	971.107 05
440	973.750 51	976.394 95	979.040 37	981.686 77	984.334 15	986.982 51	989.631 85	992.282 16	994.933 44	997.585 68
450	1000.238 89	1002.893 07	1005.548 21	1008.204 31	1010.861 36	1013.519 37	1016.178 34	1018.838 25	1021.499 12	1024.160 93
460	1026.823 69	1029.487 39	1032.152 03	1034.817 61	1037.484 13	1040.151 58	1042.819 97	1045.489 29	1048.159 53	1050.830 71
470	1053.502 80	1056.175 82	1058.849 77	1061.524 63	1064.200 41	1066.877 10	1069.554 71	1072.233 22	1074.912 65	1077.592 99
480	1080.274 23	1082.956 37	1085.639 42	1088.323 37	1091.008 21	1093.693 95	1096.380 59	1099.068 12	1101.756 54	1104.445 85
490	1107.136 04	1109.827 12	1112.519 09	1115.211 94	1117.905 66	1120.600 27	1123.295 75	1125.992 11	1128.689 34	1131.387 44
500	1134.086 41	1136.786 24	1139.486 95	1142.188 51	1144.890 94	1147.594 24	1150.298 39	1153.003 39	1155.709 26	1158.415 98
510	1161.123 55	1163.831 97	1166.541 24	1169.251 35	1171.962 32	1174.674 12	1177.386 77	1180.100 26	1182.814 59	1185.529 76
520	1188.245 76	1190.962 60	1193.680 27	1196.398 77	1199.118 10	1201.838 26	1204.559 25	1207.281 06	1210.003 69	1212.727 15
530	1215.451 42	1218.176 52	1220.902 43	1223.629 16	1226.356 70	1229.085 05	1231.814 22	1234.544 19	1237.274 97	1240.006 56
540	1242.738 96	1245.472 15	1248.206 15	1250.940 95	1253.676 55	1256.412 95	1259.150 14	1261.888 13	1264.626 91	1267.366 48
550	1270.106 84	1272.847 99	1275.589 93	1278.332 66	1281.076 17	1283.820 46	1286.565 54	1289.311 39	1292.058 03	1294.805 44
560	1297.553 63	1300.302 59	1303.052 32	1305.802 83	1308.554 11	1311.306 16	1314.058 98	1316.812 56	1319.566 91	1322.322 02
570	1325.077 90	1327.834 53	1330.591 93	1333.350 08	1336.108 99	1338.868 66	1341.629 08	1344.390 26	1347.152 19	1349.914 87
580	1352.678 29	1355.442 47	1358.207 39	1360.973 06	1363.739 48	1366.506 63	1369.274 53	1372.043 17	1374.812 54	1377.582 66
590	1380.353 51	1383.125 10	1385.897 42	1388.670 48	1391.444 26	1394.218 78	1396.994 03	1399.770 00	1402.546 70	1405.324 13
600	1408.102 28	1410.881 15	1413.660 75	1416.441 07	1419.222 10	1421.003 86	1424.786 33	1427.569 52	1430.353 43	1433.138 04
610	1435.923 37	1438.709 41	1441.496 17	1444.283 63	1447.071 79	1449.860 67	1452.650 25	1455.440 54	1458.231 52	1461.023 22
620	1463.815 61	1466.608 70	1469.402 49	1472.196 98	1474.992 16	1477.788 04	1480.584 62	1483.381 88	1486.179 84	1488.978 49
630	1491.777 84	1494.577 87	1497.378 58	1500.179 99	1502.982 08	1505.784 85	1508.588 31	1511.392 45	1514.197 27	1517.002 77
640	1519.808 95	1522.615 81	1525.423 34	1528.231 55	1531.040 44	1533.850 00	1536.660 23	1539.471 14	1542.282 71	1545.094 96
650	1547.907 87	1550.721 45	1553.535 70	1556.350 61	1559.166 19	1561.982 43	1564.799 33	1567.616 90	1570.435 13	1573.254 01
660	1576.073 56	1578.893 76	1581.714 61	1584.536 13	1587.358 30	1590.181 12	1593.004 59	1595.828 72	1598.653 50	1601.478 92
670	1604.305 00	1607.131 72	1609.959 09	1612.787 10	1615.615 76	1618.445 07	1621.275 01	1624.105 60	1626.936 83	1629.768 70
680	1632.601 21	1635.434 36	1638.268 14	1641.102 56	1643.937 62	1646.773 31	1649.609 64	1652.446 59	1655.284 18	1658.122 40
690	1660.961 25	1663.800 73	1666.640 83	1669.481 57	1672.322 93	1675.164 91	1678.007 52	1680.850 75	1683.694 61	1686.539 09
700	1689.384 18	1692.229 90	1695.076 24	1697.923 20	1700.770 77	1703.618 96	1706.467 76	1709.317 18	1712.167 21	1715.017 86
710	1717.869 12	1720.720 99	1723.573 47	1726.426 56	1729.280 26	1732.134 56	1734.989 48	1737.845 00	1740.701 12	1743.557 85
720	1746.415 18	1749.273 12	1752.131 65	1754.990 79	1757.850 53	1760.710 87	1763.571 81	1766.433 34	1769.295 47	1772.158 20
730	1775.021 52	1777.885 44	1780.749 95	1783.615 05	1786.480 75	1789.347 04	1792.213 91	1795.081 38	1797.949 44	1800.818 08
740	1803.687 31	1806.557 13	1809.427 54	1812.298 53	1815.170 10	1818.042 25	1820.914 99	1823.788 31	1826.662 22	1829.536 70
750	1832.411 76	1835.287 40	1838.163 62	1841.040 41	1843.917 78	1846.795 73	1849.674 25	1852.553 35	1855.433 02	1858.313 26
760	1861.194 07	1864.075 46	1866.957 41	1869.839 94	1872.723 03	1875.606 69	1878.490 92	1881.375 71	1884.261 08	1887.147 00
770	1890.033 49	1892.920 55	1895.808 16	1898.696 34	1901.585 08	1904.474 39	1907.364 25	1910.254 67	1913.145 65	1916.037 18
780	1918.929 28	1921.821 93	1924.715 14	1927.608 90	1930.503 21	1933.398 08	1936.293 51	1939.189 48	1942.086 01	1944.983 08
790	1947.880 71	1950.778 89	1953.677 61	1956.576 89	1959.476 71	1962.377 07	1965.277 99	1968.179 44	1971.081 45	1973.983 99
800	1976.887 08	1979.790 72	1982.694 89	1985.599 61	1988.504 86	1991.410 66	1994.316 99	1997.223 87	2000.131 28	2003.039 22
810	2005.947 71	2008.856 73	2011.766 29	2014.676 38	2017.587 00	2020.498 16	2023.409 85	2026.322 07	2029.234 82	2032.148 11
820	2035.061 92	2037.976 26	2040.891 14	2043.806 54	2046.722 46	2049.638 92	2052.555 90	2055.473 40	2058.391 43	2061.309 99
830	2064.229 06	2067.148 67	2070.068 79	2072.989 43	2075.910 60	2078.832 29	2081.754 49	2084.677 22	2087.600 46	2090.524 22
840	2093.448 50	2096.373 30	2099.298 61	2102.224 44	2105.150 78	2108.077 64	2111.005 01	2113.932 89	2116.861 29	2119.790 19
850	2122.719 61	2125.649 54	2128.579 98	2131.510 93	2134.442 39	2137.374 35	2140.306 83	2143.239 81	2146.173 30	2149.107 29
860	2152.041 79	2154.976 79	2157.912 30	2160.848 31	2163.784 82	2166.721 84	2169.659 36	2172.597 37	2175.535 89	2178.474 91
870	2181.414 43	2184.354 45	2187.294 97	2190.235 98	2193.177 49	2196.119 50	2199.062 00	2202.005 00	2204.948 50	2207.892 49
880	2210.836 97	2213.781 95	2216.727 41	2219.673 38	2222.619 83	2225.566 77	2228.514 20	2231.462 13	2234.410 54	2237.359 44
890	2240.308 83	2243.258 71	2246.209 08	2249.159 93	2252.111 26	2255.063 09	2258.015 40	2260.968 19	2263.921 46	2266.875 22
900	2269.829 47	2272.784 19	2275.739 40	2278.695 09	2281.651 25	2284.607 90	2287.565 03	2290.522 64	2293.480 72	2296.439 29
910	2299.398 33	2302.357 85	2305.317 84	2308.278 31	2311.239 26	2314.200 68	2317.162 58	2320.124 95	2323.087 79	2326.051 11
920	2329.014 89	2331.979 15	2334.943 88	2337.909 09	2340.874 76	2343.840 90	2346.807 51	2349.774 59	2352.742 14	2355.710 15
930	2358.678 64	2361.647 59	2364.617 00	2367.586 88	2370.557 23	2373.528 04	2376.499 32	2379.471 06	2382.443 26	2385.415 93
940	2388.389 06	2391.362 65	2394.336 70	2397.311 21	2400.286 18	2403.261 61	2406.237 50	2409.213 85	2412.190 66	2415.167 93
950	2418.145 65	2421.123 83	2424.102 47	2427.081 56	2430.061 11	2433.041 12	2436.021 57	2439.002 49	2441.983 85	2444.965 67
960	2447.947 94	2450.930 66	2453.913 84	2456.897 47	2459.881 54	2462.866 07	2465.851 05	2468.836 47	2471.822 35	2474.808 67
970	2477.795 45	2480.782 66	2483.770 33	2486.758 44	2489.747 00	2492.736 01	2495.725 46	2498.715 35	2501.705 69	2504.696 47
980	2507.687 70	2510.679 37	2513.671 48	2516.664 03	2519.657 03	2522.650 47	2525.644 34	2528.638 66	2531.633 42	2534.628 61
990	2537.624 25	2540.620 32	2543.616 83	2546.613 78	2549.611 17	2552.608 99	2555.607 25	2558.605 95	2561.605 08	2564.604 64

Appendix XXI

The correlation coefficient

Degrees of freedom	Value of <i>P</i>				
	0.10	0.05	0.02	0.01	0.001
1	0.987 7	0.996 92	0.999 51	0.999 88	0.999 998 8
2	0.900 0	0.950 0	0.980 0	0.990 0	0.999 0
3	0.805	0.878	0.934 3	0.958 7	0.991 1
4	0.729	0.811	0.882	0.917 2	0.974 1
5	0.669	0.754	0.833	0.875	0.950 9
6	0.621	0.707	0.789	0.834	0.924 9
7	0.582	0.666	0.750	0.798	0.898
8	0.549	0.632	0.715	0.765	0.872
9	0.521	0.602	0.685	0.735	0.847
10	0.497	0.576	0.658	0.708	0.823
11	0.476	0.553	0.634	0.684	0.801
12	0.457	0.532	0.612	0.661	0.780
13	0.441	0.514	0.592	0.641	0.760
14	0.426	0.497	0.574	0.623	0.742
15	0.412	0.482	0.558	0.606	0.725
16	0.400	0.468	0.543	0.590	0.708
17	0.389	0.456	0.529	0.575	0.693
18	0.378	0.444	0.516	0.561	0.679
19	0.369	0.433	0.503	0.549	0.665
20	0.360	0.423	0.492	0.537	0.652
25	0.323	0.381	0.445	0.487	0.597
30	0.296	0.349	0.409	0.449	0.554
35	0.275	0.325	0.381	0.418	0.519
40	0.257	0.304	0.358	0.393	0.490
45	0.243	0.288	0.338	0.372	0.465
50	0.231	0.273	0.322	0.354	0.443
60	0.211	0.250	0.295	0.325	0.408
70	0.195	0.232	0.274	0.302	0.380
80	0.183	0.217	0.257	0.283	0.357
90	0.173	0.205	0.242	0.267	0.338
100	0.164	0.195	0.230	0.254	0.321

The table gives percentage points for the distribution of the estimated correlation coefficient r when the true value of ρ is zero. Thus when there are 10 degrees of freedom (i.e., in samples of 12) the probability of observing an r greater in **absolute value** than 0.576 (i.e., < -0.576 or $> +0.576$) is 0.05 or 5%.

Appendix XXII

The variance-ratio (F) distribution

5 per cent points															
f_2	f_1														
	1	2	3	4	5	6	7	8	9	10	12	15	20	30	∞
1	161.4	199.5	215.7	224.6	230.2	234.0	236.8	238.9	240.5	241.9	143.9	245.9	248.0	250.1	254.3
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.41	19.43	19.45	19.46	19.50
3	10.13	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81	8.79	8.74	8.70	8.66	8.62	8.53
4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.91	5.86	5.80	5.75	5.63
5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.68	4.62	4.56	4.50	4.36
6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.00	3.94	3.87	3.81	3.67
7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.57	3.51	3.44	3.38	3.23
8	5.32	4.45	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.28	3.22	3.15	3.08	2.93
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	3.07	3.01	2.94	2.86	2.71
10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.91	2.85	2.77	2.70	2.54
11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.85	2.79	2.72	2.65	2.57	2.40
12	4.75	3.89	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75	2.69	2.62	2.54	2.47	2.30
13	4.67	3.81	3.41	3.18	3.03	2.92	2.83	2.77	2.71	2.67	2.60	2.53	2.46	2.38	2.21
14	4.60	3.74	3.34	3.11	2.96	2.85	2.76	2.70	2.65	2.60	2.53	2.46	2.39	2.31	2.13
15	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.48	2.40	2.33	2.25	2.07
16	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49	2.42	2.35	2.28	2.19	2.01
17	4.45	3.59	3.20	2.96	2.81	2.70	2.61	2.55	2.49	2.45	2.38	2.31	2.23	2.15	1.96
18	4.41	3.55	3.16	2.93	2.77	2.66	2.58	2.51	2.46	2.41	2.34	2.27	2.19	2.11	1.92
19	4.38	3.52	3.13	2.90	2.74	2.63	2.54	2.48	2.42	2.38	2.31	2.23	2.16	2.07	1.88
20	4.35	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35	2.28	2.20	2.12	2.04	1.84
21	4.32	3.47	3.07	2.84	2.68	2.57	2.49	2.42	2.37	2.32	2.25	2.18	2.10	2.01	1.81
22	4.30	3.44	3.05	2.82	2.66	2.55	2.46	2.40	2.34	2.30	2.23	2.15	2.07	1.98	1.78
23	4.28	3.42	3.03	2.80	2.64	2.53	2.44	2.37	2.32	2.27	2.20	2.13	2.05	1.96	1.76
24	4.26	3.40	3.01	2.78	2.62	2.51	2.42	2.36	2.30	2.25	2.18	2.11	2.03	1.94	1.73
25	4.24	3.39	2.99	2.76	2.60	2.49	2.40	2.34	2.28	2.24	2.16	2.09	2.01	1.92	1.71
26	4.23	3.37	2.98	2.74	2.59	2.47	2.39	2.32	2.27	2.22	2.15	2.07	1.99	1.90	1.69
27	4.21	3.35	2.96	2.73	2.57	2.46	2.37	2.31	2.25	2.20	2.13	2.06	1.97	1.88	1.67
28	4.20	3.34	2.95	2.71	2.56	2.45	2.36	2.29	2.24	2.19	2.12	2.04	1.96	1.87	1.65
29	4.18	3.33	2.93	2.70	2.55	2.43	2.35	2.28	2.22	2.18	2.10	2.03	1.94	1.85	1.64
30	4.17	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21	2.16	2.09	2.01	1.93	1.84	1.62
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	1.12	2.08	2.00	1.92	1.84	1.74	1.51
60	4.00	3.15	2.76	2.53	2.37	2.25	2.17	2.10	2.04	1.99	1.92	1.84	1.75	1.65	1.39
120	3.92	3.07	2.68	2.45	2.29	2.17	2.09	2.02	1.96	1.91	1.83	1.75	1.66	1.55	1.25
∞	3.84	3.00	2.60	2.37	2.21	2.10	2.01	1.94	1.88	1.83	1.75	1.67	1.57	1.46	1.00

The table gives the 5% points of the distribution of the variance-ratio, $F = s_1^2/s_2^2$, where the numerator and denominator have f_1 and f_2 degrees of freedom, respectively. Thus if $f_1 = 7$ and $f_2 = 15$, the probability that the observed value of F is **greater** than 2.71 is exactly 0.05 or 5%.

1 per cent points

f_2	f_1														
	1	2	3	4	5	6	7	8	9	10	12	15	20	30	∞
1	4052	4999	5403	5625	5764	5859	5928	5982	6022	6056	6106	6157	6209	6261	6366
2	98.50	99.00	99.17	99.25	99.30	99.33	99.36	99.37	99.39	99.40	99.42	99.43	99.45	99.47	99.50
3	34.12	30.82	29.46	28.71	28.24	27.91	27.67	27.49	27.35	27.23	27.05	26.87	26.69	26.50	26.13
4	21.20	18.00	16.69	15.98	15.52	15.21	14.98	14.80	14.66	14.55	14.37	14.20	14.02	13.84	13.46
5	16.26	13.27	12.06	11.39	10.97	10.67	10.46	10.29	10.16	10.05	9.89	9.72	9.55	9.38	9.02
6	13.75	10.92	9.78	9.15	8.75	8.47	8.26	8.10	7.98	7.87	7.72	7.56	7.40	7.23	6.88
7	12.25	9.55	8.45	7.85	7.46	7.19	6.99	6.84	6.72	6.62	6.47	6.31	6.16	5.99	5.65
8	11.26	8.65	7.59	7.01	6.63	6.37	6.18	6.03	5.91	5.81	5.67	5.52	5.36	5.20	4.86
9	10.56	8.02	6.99	6.42	6.06	5.80	5.61	5.47	5.35	5.26	5.11	4.96	4.81	4.65	4.31
10	10.04	7.56	6.55	5.99	5.64	5.39	5.20	5.06	4.94	4.85	4.71	4.56	4.41	4.25	3.91
11	9.65	7.21	6.22	5.67	5.32	5.07	4.89	4.74	4.63	4.54	4.40	4.25	4.10	3.94	3.60
12	9.33	6.93	5.95	5.41	5.06	4.82	4.64	4.50	4.39	4.30	4.16	4.01	3.86	3.70	3.36
13	9.07	6.70	5.74	5.21	4.86	4.62	4.44	4.30	4.19	4.10	3.96	3.82	3.66	3.51	3.17
14	8.86	6.51	5.56	5.04	4.69	4.46	4.28	4.14	4.03	3.94	3.80	3.66	3.51	3.35	3.00
15	8.68	6.36	5.42	4.89	4.56	4.32	4.14	4.00	3.89	3.80	3.67	3.52	3.37	3.21	2.87
16	8.53	6.23	5.29	4.77	4.44	4.20	4.03	3.89	3.78	3.69	3.55	3.41	3.26	3.10	2.75
17	8.40	6.11	5.18	4.67	4.34	4.10	3.93	3.79	3.68	3.59	3.46	3.31	3.16	3.00	2.65
18	8.29	6.01	5.09	4.58	4.25	4.01	3.84	3.71	3.60	3.51	3.37	3.23	3.08	2.92	2.57
19	8.18	5.93	5.01	4.50	4.17	3.94	3.77	3.63	3.52	3.43	3.30	3.15	3.00	2.84	2.49
20	8.10	5.85	4.94	4.43	4.10	3.87	3.70	3.56	3.46	3.37	3.23	3.09	2.94	2.78	2.42
21	8.02	5.78	4.87	4.37	4.04	3.81	3.64	3.51	3.40	3.31	3.17	3.03	2.88	2.72	2.36
22	7.95	5.72	4.82	4.31	3.99	3.76	3.59	3.45	3.35	3.26	3.12	2.98	2.83	2.67	2.31
23	7.88	5.66	4.76	4.26	3.94	3.71	3.54	3.41	3.30	3.21	3.07	2.93	2.78	2.62	2.26
24	7.82	5.61	4.72	4.22	3.90	3.67	3.50	3.36	3.26	3.17	3.03	2.89	2.74	2.58	2.21
25	7.77	5.57	4.68	4.18	3.85	3.63	3.46	3.32	3.22	3.13	2.99	2.85	2.70	2.54	2.17
26	7.72	5.53	4.64	4.14	3.82	3.59	3.42	3.29	3.18	3.09	2.96	2.81	2.66	2.50	2.13
27	7.68	5.49	4.60	4.11	3.78	3.56	3.39	3.26	3.15	3.06	2.93	2.78	2.63	2.47	2.10
28	7.64	5.45	4.57	4.07	3.75	3.53	3.36	3.23	3.12	3.03	2.90	2.75	2.60	2.44	2.06
29	7.60	5.42	4.54	4.04	3.73	3.50	3.33	3.20	3.09	3.00	2.87	2.73	2.57	2.41	2.03
30	7.56	5.39	4.51	4.02	3.70	3.47	3.30	3.17	3.07	2.98	2.84	2.70	2.55	2.39	2.01
40	7.31	5.18	4.31	3.83	3.51	3.29	3.12	2.99	2.89	2.80	2.66	2.52	2.37	2.20	1.80
60	7.08	4.98	4.13	3.65	3.34	3.12	2.95	2.82	2.72	2.63	2.50	2.35	2.20	2.03	1.60
120	6.85	4.79	3.95	3.48	3.17	2.96	2.79	2.66	2.56	2.47	2.34	2.19	2.03	1.86	1.38
∞	6.63	4.61	3.78	3.32	3.02	2.80	2.64	2.51	2.41	2.32	2.18	2.04	1.88	1.70	1.00

The table gives the 1% points of the distribution of the variance-ratio, $F = s_1^2/s_2^2$, where the numerator and denominator have f_1 and f_2 degrees of freedom, respectively. Thus if $f_1 = 7$ and $f_2 = 15$, the probability that the observed value of F is **greater** than 4.14 is exactly 0.01 or 1%.

References

- Aalund, O., Willeberg, P., Mandrup, M. and Riemann, H. (1976) Lung lesions at slaughter: association to factors in the pig herd. *Nordisk Veterinærmedicin*, **28**, 481–486
- Abbas, S.S., Venkataramanan, V., Pathak, G. and Kakkar, M. (2011) Rabies control initiative in Tamil Nadu, India: a test case for the 'One Health' approach. *International Health*, **3**, 231–239
- Abbey, H. (1952) An examination of the Reed-Frost theory of epidemics. *Human Biology*, **24**, 201–233
- Abernethy, D.A., Upton, P., Higgins, I.M., McGrath, G., Goodchild, A.V. *et al.* (2013) Bovine tuberculosis trends in the UK and the Republic of Ireland, 1995–2010. *Veterinary Record*, **172**, 312; doi 10.1136/vr.100969
- Abramson, J.H. (1991) Meta-analysis: a review of pros and cons. *Public Health Reviews*, **18**, 1–47
- Abramson, J.H. (2011) WINPEPI updated: computer programs for epidemiologists, and their teaching potential. *Epidemiologic Perspectives & Innovations*, **8**, 1; doi 10.1186/1742-5573-8-1
- Abramson, J.H. and Abramson, Z.H. (1999) *Survey Methods in Community Medicine*, 5th edn. Churchill Livingstone, Edinburgh
- Abramson, J.H. and Abramson, Z.H. (2008) *Research Methods in Community Medicine*, 6th edn. John Wiley and Sons, Chichester
- Abusalab, S.M., Hamid, M.E. and Ahmed, S.M. (2003) Haemorrhagic septicaemia: a review. *Sudan Journal of Veterinary Research*, **18**, 1–14
- Abutarbush, S.M., Naylor, J.M., Parchoma, G., D'Eon, M., Petrie, L. *et al.* (2006) Evaluation of traditional instruction versus a self-learning computer module in teaching veterinary students how to pass a nasogastric tube in the horse. *Journal of Veterinary Medical Education*, **33**, 447–454
- ACAC (2010) *Contribution of the Pet Care Industry to the Australian Economy*, 7th edn. Australian Companion Animal Council, St Leonards. http://www.acac.org.au/pdf/ACAC%20Report%200810_sm.pdf (accessed 18 May 2015)
- Acheson, R.M., Hall, D.J. and Aird, L. (Eds) (1976) *Health Information, Planning and Monitoring. Seminars in Community Medicine*, Vol. 2. Oxford University Press, Oxford
- Ackerman, L. (2011) *The Genetic Connection: A Guide to Health Problems in Purebred Dogs*, 2nd edn. American Animal Hospital Association Press, Lakewood
- Ackoff, R.L. (1971) Towards a system of systems concepts. *Management Science*, **17**, 661–671
- Acock, A.C. (2005) Working with missing values. *Journal of Marriage and Family*, **67**, 1012–1028
- Acosta-Jamett, G., Cleaveland, S., Cunningham, A.A. and Bronsvoort, B.M. deC. (2010) Demography of domestic dogs in rural and urban areas of the Coquimbo region of Chile and its implications for disease transmission. *Preventive Veterinary Medicine*, **94**, 272–281
- Adams, V., Mobius-Winkler, S. and Schuler, G. (2009) Basic and translational research: from molecule, to mouse, to man. *European Journal of Cardiovascular Prevention & Rehabilitation*, **16**, S48–S52
- Adamson, M.L. (1986) Modes of transmission and evolution of life histories in zooparasitic nematodes. *Canadian Journal of Zoology*, **64**, 1375–1384
- ADAS (1989) *Deer Health Scheme*. Ministry of Agriculture, Fisheries and Food, State Veterinary Service, PB 0052. ADAS Publications, London
- Adler, N.E., Boyce, T., Chesney, M.A., Cohen, S., Folkman, S. *et al.* (1994) Socioeconomic status and health: the challenge of the gradient. *American Psychologist*, **49**, 15–24
- Advisory Committee (1964) *Smoking and Health: Report of the Advisory Committee to the Surgeon General of the Public Health Service*. Publication No. 1103. US Department of Health, Education, and Welfare, Public Health Service, Washington DC

- AGDA (2015) *Aquatic Animal Health: Disease Surveillance and Reporting*. Australian Government, Department of Agriculture, Canberra. <http://www.agriculture.gov.au/animal/aquatic/reporting> (accessed 20 August 2015)
- Agostini, A., Franco, A., Sommerfelt, I., de Lema, J.A. and Kistermann, J.C. (1986) Demographic study of the dog and cat populations in the General San Martin urban district. *Revista de Medicina Veterinaria*, **67**, 32–34, 36–37
- Agostini, A., Trinidad, J., Cornero, F., Tellechea, D. and Lopez, C. (2000) Animal population in a community with unsatisfied needs. Buenos Aires, 1999. *Revista de Medicina Veterinaria*, **81**, 47–50 (in Spanish; English abstract)
- Agrawal, M.C. and Shah, H.L. (1986) Stephanofilarial dermatitis in India. *Veterinary Research Communications*, **8**, 93–102
- Agresti, A. and Coull, B. (1998) Approximate is better than 'exact' for interval estimation of binomial proportions. *The American Statistician*, **52**, 119–126
- Agriculture Canada (1984) *Enzyme immunoassay: application to the detection of bovine antibody to Brucella abortus*. Agriculture Canada, Animal Disease Research Institute, Nepean
- Aguirre, A.A. (2009) Wild canids as sentinels of ecological health: a conservation medicine perspective. *Parasites & Vectors*, **2**, Suppl. 1, S7; doi 10.1186/1756-3305-2-S1-S7
- Aguirre, A.A., Ostfeld, R.S., Tabor, G.M., House, C. and Pearl, M.C. (2002) *Conservation Medicine: Ecological Health in Practice*. Oxford University Press, Oxford
- Ahl, V. and Allen, T.F.H. (1996) *Hierarchy Theory: A Vision, Vocabulary and Epistemology*. Columbia University Press, New York
- Ahlbom, A. and Alfredsson, L. (2005) Interaction: a word with two meanings creates confusion. *European Journal of Epidemiology*, **20**, 563–564
- Ahlers, C., Alders, R.G., Bagnol, B., Cambaza, A.B., Harun, M. *et al.* (2009) *Improving Village Chicken Production: A Manual for Field Workers and Trainers*. ACIAR Monograph No. 139. Australian Centre for International Agricultural Research, Canberra
- Ahmed, I., Sutton, A.J. and Riley, R.D. (2011) Assessment of publication bias, selection bias, and unavailable data in meta-analyses using individual participant data: a database survey. *British Medical Journal*, **344**, d7762; doi 10.1136/bmj.7762
- AHT (2015) *Pilot Field Vaccine Trial for Equine Grass Sickness*. Animal Health Trust, Newmarket. http://www.aht.org.uk/skins/Default/pdfs/egs_finalps.pdf (accessed 7 October 2015)
- Ahuja, V. (2004) The economic rationale of public and private sector roles in the provision of animal health services. *Revue Scientifique et Technique, Office International des Epizooties*, **23**, 33–45
- Ahuja, V. and Redmond, E. (2004) Livestock services and the poor. *Tropical Animal Health and Production*, **36**, 247–268
- AHVL (2012) *Pet Travel Scheme*. Animal Health and Veterinary Laboratories Agency. <http://www.defra.gov.uk/ahvla-en/imports-exports/pets/> (accessed 14 April 2012)
- Aikman, H. (1997) The association between arthritis and the weather. *International Journal of Biometeorology*, **40**, 192–199
- AIM (2014) *DairyCHAMP*. Agricultural Information Management, Ellensburg. <http://www.aginfoman.com/info/gp.asp?GID=6> (accessed 27 October 2014)
- Aitken, I.D. (1993) Environmental change and animal disease. In: *The Advancement of Veterinary Science. The Bicentenary Symposium Series. Volume 1: Veterinary Medicine Beyond 2000*. Ed. Michell, A.R., pp. 179–193. CAB International, Wallingford
- Aitken, I.D. (Ed.) (2007) *Diseases of Sheep*, 4th edn. Blackwell Publishing, Oxford
- Akaike, H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, **19**, 716–723
- Alba, A., Casal, J. and Domingo, M. (2004) Possible introduction of bluetongue into the Balearic Islands, Spain, in 2000, via air streams. *Veterinary Record*, **155**, 460–461
- Alban, L., Andersen, M.M., Asferg, T., Boklund, A., Fernández, N. *et al.* (2005) Risk assessment for introduction of wild boar (*Sus scrofa*) to Denmark. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Nairn, 30 March–1 April 2004*. Eds Reid, S.J.W., Menzies, F.D. and Russell, A.M., pp. 79–90
- Alban, L., Boes, J., Kreiner, H., Petertsen, J.V. and Willeberg, P. (2008) Towards risk-based surveillance for *Trichinella* spp. in Danish pig production. *Preventive Veterinary Medicine*, **17**, 340–357
- Albert, R.E., Alessandro, D., Lippmann, M. and Berger, J. (1971) Long-term smoking in the donkey. Effect on tracheobronchial particle clearance. *Archives of Environmental Health*, **22**, 12–19
- Albrow, M. and King, E. (Eds) (1990) *Globalization, Knowledge and Society*. Sage Publications, London
- Alexander, D.J. (1988) Newcastle disease: methods of spread. *Developments in Veterinary Virology*, **8**, 256–272
- Alexander, D.J. (2007) An overview of the epidemiology of avian influenza. *Vaccine*, **25**, 5637–5644
- Algers, B. (2012) Animal welfare – recent developments in the field. In: *Animal Science Reviews 2011*. Ed. Hemming, D., pp. 19–28. CABI, Wallingford

- Algers, B., Ekesbo, I and Stromberg, S. (1978) The impact of continuous noise on animal health. *Acta Veterinaria Scandinavica*, Suppl. **67**, 1–26
- Alhaji, N.B. and Babalobi, O.O. (2015) Participatory epidemiology of ethnoveterinary practices Fulani pastoralists used to manage contagious bovine pleuropneumonia and other cattle ailments in Niger State, Nigeria. *Journal of Veterinary Medicine*, Article ID 460408; doi 10.1155/2015/460408
- Alirol, E., Getaz, L., Stoll, B., Chappuis, F. and Loutan, L. (2011) Urbanisation and infectious diseases in a globalised world. *The Lancet Infectious Diseases*, **11**, 131–141
- Al-Lazikani, B., Banerji, U. and Workman, P. (2012) Combinatorial drug therapy for cancer in the post-genomic era. *Nature Biotechnology*, **30**, 7; doi 10.1038/nbt.2284
- Allen, A., Golden, B., Taylor, M., Patterson, D., Henriksen, D. *et al.* (2008) Evaluation of retinal imaging technology for the biometric identification of bovine animals in Northern Ireland. *Livestock Science*, **116**, 42–52
- Allen, L.J.S. (1994) Some discrete-time *SI*, *SIR*, and *SIS* epidemic models. *Mathematical Biosciences*, **124**, 83–105
- Allison, P.D. (2002) *Missing Data*. Sage Publications, Thousand Oaks
- Allport, R., Mosha, R., Bahari, M., Swai, E. and Catley, A. (2005) The use of community-based animal health workers to strengthen disease surveillance systems in Tanzania. *Revue Scientifique et Technique, Office International des Epizooties*, **24**, 921–932
- Allsopp, R. (1998) Geographic information systems (GIS) and remote sensing aid tsetse control in Botswana. *Pesticide Outlook*, **9** (4), 9–12
- Alm, E. and Arkin, A.P. (2003) Biological networks. *Current Opinion in Structural Biology*, **13**, 193–202
- Almada, A.A., Golden, C.D., Osofsky, S.A. and Myers, S.S. (2017) A case for Planetary Health/GeoHealth. *GeoHealth*, **1**, 75–78
- Alonzo, T.A. and Pepe, M.S. (1999) Using a combination of reference tests to assess the accuracy of a new diagnostic test. *Statistics in Medicine*, **18**, 2987–3003
- Alter, S. (2008) Defining information systems as work systems: implications for the IS field. *European Journal of Information Systems*, **17**, 448–469
- Altman, D.G. (1991a) *Practical Statistics for Medical Research*. Chapman and Hall, London
- Altman, D.G. (1991b) Randomisation – essential for reducing bias. *British Medical Journal*, **302**, 1481–1482
- Altman, D.G. (1998) Confidence intervals for the number needed to treat. *British Medical Journal*, **317**, 1309–1312
- Altman, D.G., Machin, D., Bryant, T.N. and Gardner, M.J. (Eds) (2000) *Statistics with Confidence*, 2nd edn. BMJ Books, London
- Alvarez, R.M., Amass, S.F., Anderson, C.D., Ragland, D., Grote, L.A. *et al.* (2001) Evaluation of biosecurity protocols to prevent mechanical transmission of transmissible gastro-enteritis virus of swine by pork production unit personnel. *The Pig Journal*, **48**, 22–33
- Amana, I.J., Carlson, N.E. and Slifka, M.A. (2007) Duration of humoral immunity to common viral and vaccine antigens. *The New England Journal of Medicine*, **357**, 1903–1915
- Ambrosio, R.E. and de Waal, D.T. (1990) Diagnosis of parasitic disease. *Revue Scientifique et Technique, Office International des Epizooties*, **9**, 759–788
- Ameri, A.A., Hendrickx, S., Jones, B., Mariner, J., Mehta, P. *et al.* (2009) *Introduction to Participatory Epidemiology and its Application to Highly Pathogenic Avian Influenza Participatory Disease Surveillance: A Manual for Participatory Disease Surveillance Practitioners*. International Livestock Research Institute, Nairobi
- Amezcu, M. del R., Pearl, D.L., Friendship, R.M. and McNab, W.B. (2010) Evaluation of a veterinary-based syndromic surveillance system implemented for swine. *Canadian Journal of Veterinary Research*, **74**, 241–251
- Amin, A., Shah, K.A. and Andrabi, A. (2012) Use of geoinformatics in livestock disease management. *VetScan*, **7** (1), Article **100**, 1–6
- Anand, K.J. and Craig, K.D. (1996) New perspectives in the definition of pain. *Pain*, **67**, 3–6
- Anderson, D.E., Lush, J.L. and Chambers, C. (1957) Studies on bovine ocular squamous carcinoma ('cancer eye'). II. Relationships between eyelid pigmentation and occurrence of cancer eye lesions. *Journal of Animal Science*, **16**, 739–746
- Anderson, E.C., Doughty, W.J. and Anderson, J. (1976) The role of sheep and goats in the epizootiology of foot-and-mouth disease in Kenya. *Journal of Hygiene, Cambridge*, **76**, 35–402
- Anderson, H., Henricson, B., Lundquist, P.-G., Wendenberg, H. and Wesall, J. (1968) Genetic hearing impairment in the Dalmatian dog. *Acta-otolaryngologica*, Suppl. 232, 1–34
- Anderson, I. (2002) *Foot and Mouth Disease 2001: Lessons to be Learned Inquiry*. The Stationery Office, London
- Anderson, I. (2008) *Foot and Mouth Disease 2007: A Review and Lessons Learned*. The Stationery Office, London
- Anderson, J.F. (1988) Mammalian and avian hosts of *Borrelia burgdorferi*. *Annals of the New York Academy of Sciences*, **539**, 180–191

- Anderson, J.F. (1998) Biosecurity – a new term for an old concept: how to apply it. *The Bovine Practitioner*, **32**, 61–70
- Anderson, R.C. (1984) The origins of zooparasitic nematodes. *Canadian Journal of Zoology*, **62**, 317–328
- Anderson, R.M. (1979) The influence of parasitic infection on the dynamics of host population growth. In: *Population Dynamics*. Eds Anderson, R.M., Turner, B.D. and Taylor, L.R., pp. 245–281. Blackwell Scientific Publications, Oxford
- Anderson, R.M. (1981) Population ecology of infectious disease agents. In: *Theoretical Ecology*. Ed. May, R.M., pp. 318–355. Blackwell Scientific Publications, Oxford
- Anderson, R.M. and May, R.M. (1986) The invasion, persistence and spread of infectious diseases within animal and plant communities. *Philosophical Transactions of the Royal Society of London, Series B*, **314**, 533–570
- Anderson, R.M. and May, R.M. (1988) Epidemiological parameters of HIV infection. *Nature*, **333**, 514–518
- Anderson, R.M. and May, R.M. (1991) *Infectious Diseases of Humans: Dynamics and Control*. Oxford University Press, Oxford
- Anderson, R.M., Jackson, H.C., May, R.M. and Smith, A. D.M. (1981) Population dynamics of fox rabies in Europe. *Nature*, **289**, 765–770
- Anderson, R.S. (1983) Trends in pet populations. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings*, Southampton, 12–13 April 1983. Ed. Thrusfield, M.V., pp. 94–97
- Anderson, T.W. (1958) *An Introduction to Multivariate Statistical Analysis*. John Wiley, New York
- Andersson, H., Lexmon, Å., Robertsson, J.-Å., Lundeheim, N. and Wierup, M. (1997) Agricultural policy and social returns to eradication programs: the case of Aujeszky's disease in Sweden. *Preventive Veterinary Medicine*, **29**, 311–328
- Andersson, M.G., Sundström, A. and Lindström, A. (2013) Bayesian networks for evaluation of evidence in forensic entomology. *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science*, **11**, Suppl. 1, S64–S77
- Andrewartha, H.G. and Birch, L.C. (1954) *The Distribution and Abundance of Animals*. University of Chicago Press, Chicago
- Andrewes, C. and Pereira, H.G. (1964) *Viruses of Vertebrates*. Baillière, Tindall and Cassell, London
- Andrews, E.J., Ward, B.C. and Altman, N.H. (1979) *Spontaneous Animal Models of Human Disease*. 2 vols. Academic Press, New York
- Andrews, J.M. and Langmuir, A.D. (1963) The philosophy of disease eradication. *American Journal of Public Health*, **53**, 1–6
- Andrews, L. (1988) Animal health information systems in Australia. *Revue Scientifique et Technique, Office International des Epizooties*, **7**, 569–575
- Ånestad, G., Vainio, K. and Hungnes, O. (2009) Interference between outbreaks of epidemic viruses: additional Norwegian observations (Letter). *Scandinavian Journal of Infectious Diseases*, **41**, 381–382
- Angell, J.W., Cripps, P.J., Grove-White, D.H. and Duncan, J.S. (2015) Practical tool for locomotion scoring in sheep: reliability when used by veterinary surgeons. *Veterinary Record*, **176**, 521; doi 10.1136/vr.102882
- Anholt, R.M., Berezowski, J., Ribble, C.S., Russell, M.L. and Stephen, C. (2014) Using informatics and the electronic medical record to describe antimicrobial use in clinical management of diarrhea cases at 12 companion animal practices. *PLoS ONE*, **9**, 7, e103190; doi 10.1371/journal.pone.0103190
- Animal Health (2011) *Exercise Silver Birch 2010, National Foot and Mouth Disease Exercise, Evaluation and Lessons Identified Report, March 2011*. Animal Health, Contingency Planning Division, London
- Animal Health Australia (2016) *Disease Strategy: Rift Valley fever (Version 4.0). Australian Veterinary Emergency Plan (AUSVETPLAN), Edition 4*. National Biosecurity Committee, Canberra
- Anon. (1816) Rural economy, agriculture, and husbandry. *Encyclopaedia Perthensis*, **19**, 391–497
- Anon. (1954) Age comparison between man and dog. *Journal of the American Veterinary Medical Association*, **124**, 236
- Anon. (1984) Tattoos to help keep track of pets. *Veterinary Practice*, **16** (20), 8
- Anon. (1985) Turkey rhinotracheitis of unknown aetiology in England and Wales. *Veterinary Record*, **117**, 653–654
- Anon. (1991) *Animals as Sentinels of Environmental Health Hazards*. Committee on Animals as Monitors of Environmental Hazards, Board of Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council. National Academy Press, Washington DC
- Anon. (1992) *Completing the Internal Market. Current Status, 1 January 1992*. *Veterinary and Plant Health Controls*, Vol. 5. Commission of the European Communities, Office for Official Publications of the European Communities, Luxembourg
- Anon. (1995) FECAVA to accept ISO standards on microchips. *Veterinary Record*, **136**, 306–308
- Anon. (1997) Wobbly possum disease virus thought to have been isolated. *Sentinel*, **62**, 1

- Anon. (2000) Microchipping: survey shows extent of veterinary support. *Veterinary Practice*, **32** (5), 1
- Anon. (2004) *Building Interdisciplinary Bridges to Health in a Globalized World: One World – One Health*. Wildlife Conservation Society/Rockefeller University Symposium, New York, 29 September 2004. http://www.oneworldonehealth.org/sept2004/owoh_sept04.html (accessed 17 August 2016)
- Anon. (2007) *Nutrient Requirements of Horses*, 6th revised edn. National Academies Press, Washington DC
- Anon. (2010) *The FAO-OIE-WHO Collaboration: Sharing Responsibilities and Coordinating Global Activities to Address Health Risks at the Animal-Human-Ecosystems Interface. A Tripartite Concept Note*. http://www.who.int/influenza/resources/documents/tripartite_concept_note_hanoi/en/ (accessed 16 August 2013)
- Anscombe, F.J. (1973) Graphs in statistical analysis. *The American Statistician*, **27**, 17–21
- Ansell, J. (1992) Reliability: industrial risk assessment. In: *Risk Analysis, Assessment and Management*. Eds Ansell, J. and Wharton, F., pp. 105–122. John Wiley, Chichester
- Anvik, J.O., Hague, A.E. and Rahaman, A. (1974) A method of estimating urban dog populations and its application to the assessment of canine fecal pollution and endoparasitism in Saskatchewan. *Canadian Veterinary Journal*, **15**, 219–223
- Anyamba, A., Chretien, J.-P., Small, J., Tucker, C.J., Formenty, P.B. *et al.* (2008) Prediction of a Rift Valley fever outbreak. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 955–959
- APHA (2015) APHA Animal Disease Surveillance Reports. Department for Environment, Food and Rural Affairs, London. <https://www.gov.uk/government/collections/animal-disease-surveillance-reports> (accessed 24 July 2015)
- APHIN (2014) *Animal Productivity & Health Information Network*. Atlantic Veterinary College, University of Prince Edward Island. <http://www.aphin.uepei.ca/> (accessed 27 October 2014)
- APPA (2013) *2013–2014 APPA National Pet Owners Survey*. American Pet Products Association, Greenwich
- APPA (2015) *American Pet Products Association, APPA 2015–2015 National Pet Owners Survey*. http://www.americanpetproducts.org/pubs_survey.asp (accessed 11 August 2015)
- Appleby, M.C. and Hughes, B.O. (Eds) (1997) *Animal Welfare*. CABI Publishing, Wallingford
- Appleby, M.C. and Sandøe, P. (2002) Philosophical debate on the nature of well-being: implications for animal welfare. *Animal Welfare*, **11**, 283–294
- Arámbulo, P.V. and Astudillo, V. (1991) Perspectives on the application of remote sensing and geographic information system to disease control and health management. *Preventive Veterinary Medicine*, **11**, 345–352
- Archer, D.C., Pinchbeck, G.L., Proudman, C.J. and Clough, H.E. (2006) Is equine colic seasonal? Novel applications of a model based approach. *BMC Veterinary Research*, **2**, 27
- Archer, S., Bell, N. and Huxley, J. (2010) Lameness in UK dairy cows: a review of the current status. *In Practice*, **32**, 492–504
- Archibald, A.L. and Imlah, P. (1985) The halothane sensitivity locus and its linkage relationships. *Animal Blood Groups and Biochemical Genetics*, **16**, 253–263
- Argyle, D.J. (1999) Gene therapy in veterinary medicine. *Veterinary Record*, **144**, 369–376
- Arimitsu, Y., Fukumura, K. and Shingaki, Y. (1989) Distribution of leptospirosis among stray dogs in the Okinawa Islands, Japan: comparison of the microcapsule and microscopic agglutination tests. *British Veterinary Journal*, **145**, 473–477
- Arino, J., Jordan, R. and van den Driessche, P. (2007) Quarantine in a multi-species epidemic model with spatial dynamics. *Mathematical Biosciences*, **206**, 46–60
- Arkin, C.F. and Wachtel, M.S. (1990) How many patients are necessary to assess test performance? *Journal of the American Medical Association*, **263**, 275–278
- Armenian, H.K. (1987) Incubation periods of cancer: old and new. *Journal of Chronic Diseases*, **40**, Suppl. 2, 9S–15S
- Armenian, H.K. and Lilienfeld, A.M. (1974) The distribution of incubation periods of neoplastic disease. *American Journal of Epidemiology*, **99**, 92–100
- Armenian, H.K. and Lilienfeld, A.M. (1983) Incubation periods of disease. *Epidemiologic Reviews*, **5**, 1–15
- Armitage, P. (1975) *Sequential Medical Trials*, 2nd edn. John Wiley and Sons, New York
- Armitage, P. and Berry, G. (1994) *Statistics in Medical Research*, 3rd edn. Blackwell Scientific Publishing, London
- Armitage, P., Berry, G. and Matthews, J.N.S. (2002) *Statistical Methods in Medical Research*, 4th edn. Blackwell Science, Oxford
- Armitage, P., McPherson, C.K. and Rowe, B.C. (1969) Repeated significance tests on accumulating data. *Journal of the Royal Statistical Society, Series A*, **132**, 235–244
- Armour, J. (1980) The epidemiology of helminth disease in farm animals. *Veterinary Parasitology*, **6**, 7–46
- Arneberg, P., Skorping, A., Grenfell, B. and Read, A.F. (1998) Host densities as determinants of abundance in

- parasite communities. *Proceedings of the Royal Society of London, Series B*, **265**, 1283–1289
- Arnesen, K., Gamlem, H., Glatte, E., Grøndalen, J., Moe, L. *et al.* (2001) The Norwegian Canine Cancer Register 1990–1998. Report from the project 'Cancer in the Dog'. *The European Journal of Companion Animal Practice*, **11**, 159–169
- Arnold, S., Arnold, P., Hubler, M., Casal, M. and Rüsch, P. (1989) Incontinentia urinae bei der kastrierten hunden: häufigkeit und rassedisposition. *Schweizer Archiv für Tierheilkunde*, **131**, 259–263 (in German; English translation: Urinary incontinence in spayed bitches: prevalence and breed predisposition. *The European Journal of Companion Animal Practice* (1992) **2**, 65–68)
- Arnott, R., Finger, S. and Smith, C.U.M. (Eds) (2003) *Trepanation: History, Discovery, Theory*. Swets and Zeitlinger, Lisse
- Arthur, C.P. and Louzis, C. (1988) A review of myxomatosis among rabbits in France. *Revue Scientifique et Technique, Office International des Epizooties*, **7**, 959–976
- Arvanitoyannis, I.S. (2009) HACCP and ISO 22000: Applications to Foods of Animal Origin. Blackwell Publishing, Oxford
- Arzul, I., Renault, T., Thebault, A. and Gerard, A. (2002) Detection of oyster herpesvirus DNA and proteins in asymptomatic *Crassostrea gigas* adults. *Virus Research*, **84**, 151–160
- Ascione, F.R. and Arkow, P. (Eds) (1999) *Child Abuse, Domestic Violence and Animal Abuse*. Purdue University Press, West Lafayette
- Ascione, F.R., Weber, C.V., Thompson, T.M., Heath, J., Maruyama, M. *et al.* (2006) Battered pets and domestic violence: animal abuse reported by women experiencing intimate violence and by nonabused women. *Violence Against Women*, **13**, 354–373
- Ashley, F.H., Waterman-Pearson, A.E. and Whay, H.R. (2005) Behavioural assessment of pain in horses and donkeys: application to clinical practice and future studies. *Equine Veterinary Journal*, **37**, 565–575
- Ashton, J. (Ed.) (1994) *The Epidemiological Imagination*. Open University Press, Buckingham
- Asplin, F.D. (1965) Duck hepatitis: vaccination against two serological types. *Veterinary Record*, **77**, 1529–1530
- Assmann, S.F., Pocock, S.J., Enos, L.E. and Kasten, L.E. (2000) Subgroup analysis and other (mis)uses of baseline data in clinical trials. *The Lancet*, **355**, 1064–1069
- Astudillo, V., Suttmoller, P., Saraiva, V. and Lopéz, A. (1997) Risks of introducing foot and mouth disease through the importation of beef from South America. *Revue Scientifique et Technique, Office International des Epizooties*, **16**, 33–44
- Atkins, P.J. and Robinson, P.A. (2013) Bovine tuberculosis and badgers in Britain: relevance of the past. *Epidemiology and Infection*, **141**, 1437–1444
- Atkinson, A.C. (1985) *Plots, Transformations, and Regression*. Clarendon Press, Oxford
- Atkinson, O. (2009) Guide to the rumen health visit. *In Practice*, **31**, 314–325
- Atkinson, O. (2013) Practical and effective management of foot lameness in dairy herds. *In Practice*, **35**, 171–182
- Atlas, R.M. and Bartha, R. (1998) *Microbial Ecology: Fundamentals and Applications*, 4th edn. Benjamin/Cummings, Menlo Park
- Atlas, R.M. and Maloy, S. (2014) *One Health: People, Animals, and the Environment*. ASM Press, Washington DC
- Aubert, M. (1994) Control of rabies in foxes: what are the appropriate measures? *Veterinary Record*, **134**, 55–59
- Aubert, M.F.A. (1999) Costs and benefits of rabies control in wildlife in France. *Revue Scientifique et Technique, Office International des Epizooties*, **18**, 533–543
- Audigé, L., Doherr, M.G., Hauser, R. and Salman, M.D. (2001) Stochastic modelling as a tool for planning animal-health surveys and interpreting screening-test results. *Preventive Veterinary Medicine*, **49**, 1–17
- Audy, J.R. (1958) The localisation of disease with special reference to the zoonoses. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **52**, 308–328
- Audy, J.R. (1960) *Relation of Foci of Zoonoses to Interspersed or Mosaic Vegetation*. Papers of the first WHO course on natural foci of infections, USSR
- Audy, J.R. (1961) The ecology of scrub typhus. In: *Studies in Disease Ecology*. Ed. May, J.M., pp. 389–432. Hafner Publishing Company, New York
- Audy, J.R. (1962) *Behavioral and Sociocultural Aspects of Natural Foci of Infections*. Papers of the second WHO course on natural foci of infections, Leningrad
- AVA (2013) *Standards of Care: Regular Health Check Standards for Dogs and Cats*. Australian Veterinary Association, St Leonards
- Avgerinou, M. and Ericson, J. (1997) A review of the concept of visual literacy. *British Journal of Educational Technology*, **28**, 280–291
- AVMA (2012) US Pet Ownership & Demographics Sourcebook 2012 Edition. American Veterinary Medical Association, Schaumburg
- AVMA (2013) Market Research Statistics: U.S. Veterinarians 2013. American Veterinary Medical Association, Schaumburg. <http://www.avma.org/KB/Resources/Statistics/Pages/Market-research-statistics-US-veterinarians.aspx> (Accessed 23 December 2013).

- Ayling, R.D., Bashiruddin, S.E. and Nicholas, R.A.J. (2004) *Mycoplasma* species and related organisms found from ruminants in Britain between 1990 and 2000. *Veterinary Record*, **155**, 413–416
- Ayres, E., Cameron, E., Kemp, R., Lietch, H., Mollison, A. *et al.* (2001) Oral lesions in sheep and cattle in Dumfries and Galloway. *Veterinary Record*, **148**, 720–723
- Azhar, M., Lubis, A.S., Siregar, E.S., Alders, R.G., Brum, E. *et al.* (2010) Participatory disease surveillance and response in Indonesia: strengthening veterinary services and empowering communities to prevent and control highly pathogenic avian influenza. *Avian Diseases*, **54**, 749–753
- Baba, A.I. and Cătoi, C. (2007) *Comparative Oncology*. Publishing House of the Romanian Academy, Bucharest
- Babiuk, L.A. (2002) Vaccination: a management tool in veterinary medicine. *The Veterinary Journal*, **164**, 188–201
- Babudieri, B. (1958) Animal reservoirs of leptospirosis. *Annals of the New York Academy of Science*, **70**, 393–413
- Backer, L.C., Grindem, C.B., Corbett, W.T., Cullins, L. and Hunter, J.L. (2001) Pets dogs as sentinels for environmental contamination. *The Science of the Total Environment*, **274**, 161–169
- Backeus, I. (1993) Ecotone versus ecocline: vegetation zonation and dynamics around a small reservoir in Tanzania. *Journal of Biogeography*, **20**, 209–218
- Baghbaninaghadehi, F., Armijo-Olivo, S. and Woodhouse, L. (2016) Fundamentals of randomization in clinical trial. *International Journal of Advanced Nutritional Health Science*, **4**, 174–187
- Bagnol, B. (2008) *Participatory Tools for Assessment and Monitoring of Poultry Raising Activities and Animal Disease Control*. FAO HPAI Communication Workshop 22 January 2007, Bangkok, Thailand. Version March 2008. http://www.participatoryepidemiology.info/userfiles/Participatory%20Tools_9_03_08.pdf (accessed 3 July 2015)
- Bailey, B.J.R. (1980) Large sample simultaneous confidence intervals for the multinomial probabilities based on transformation of the cell frequencies. *Technometrics*, **22**, 583–589
- Bailey, K.R. (1987) Inter-study differences: how should they influence the interpretation and analysis of results? *Statistics in Medicine*, **6**, 351–358
- Bailey, N.T.J. (1975) *The Mathematical Theory of Infectious Diseases*, 2nd edn. Charles Griffin, London and High Wycombe
- Bailey, N.T.J. (1995) *Statistical Methods in Biology*, 3rd edn. Cambridge University Press, Cambridge
- Bailey, T.A., Sleigh, I.M., Anderson, S.J., Tarr, D.B. and Naldo, J.L. (2003) Development of CAPTIVE, a computerised record system for the bustard captive-breeding programme at the national Avian Research Center, Abu Dhabi. *International Zoo Yearbook*, **38**, 219–229
- Baker, A. (1973) Scientific method. In: *A Random Walk in Science*. Ed. Mendoza, E., p. 202. Institute of Physics, London and Bristol
- Baker, D.M., MacAvoy, S.E. and Kim, K. (2007) Relationship between water quality, $\delta^{15}\text{N}$, and aspergillosis of Caribbean sea fan corals. *Marine Ecology Progress Series*, **343**, 123–130
- Baker, M. (1974) *Folklore and Customs of Rural England*. David and Charles, Newton Abbot
- Bakker, K.M., Marinez-Bakker, M.E., Helm, B. and Stevenson, T.J. (2016) Digital epidemiology reveals global childhood disease seasonality and the effects of immunization. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 6689–6694
- Balashov, Y.S. (2010) Academician E.N. Pavlovsky as an entomologist and President of the All-Union Entomological Society. *Entomological Review*, **90**, 144–148
- Baldock, F.C., Alexander, L. and More, S.J. (2003) Estimated and predicted changes in the cat population of Australian households from 1979 to 2005. *Australian Veterinary Journal*, **81**, 289–292
- Balkhy, H.H. and Memish, Z.A. (2003) Rift Valley fever: an uninvited zoonosis in the Arabian peninsula. *International Journal of Antimicrobial Agents*, **21**, 153–157
- Ballard, G.M. (1992) Industrial risk: safety by design. In: *Risk Analysis, Assessment and Management*. Eds Ansell, J. and Wharton, F., pp. 95–104. John Wiley, Chichester
- Balmer, O., Stearns, S.C., Schötzau, A. and Brun, R. (2009) Intraspecific competition between co-infecting parasite strains enhances host survival in African trypanosomes. *Ecology*, **90**, 3367–3378
- Balshem, H., Helfand, M., Schünemann, H.J., Oxman, A. D., Kunz, R. *et al.* (2011) GRADE guidelines: 3. Rating the quality of evidence. *Journal of Clinical Epidemiology*, **64**, 401–406
- Baltussen, W.H.M., Altena, H., Bakker, C.M. and Van Rijnberk, D. (1988) *Results on Dutch Swine Breeding Herds in 1987*. Swine Extension Service and Agricultural Economics Research Institute, Rosmalen
- Baltzell, P., Newton, H. and O'Connor, A.M. (2013) A critical review and meta-analysis of the efficacy of whole-cell killed *Trichomonas foetus* vaccines in beef cattle. *Journal of Veterinary Internal Medicine*, **27**, 760–770
- Bang, F.B. (1975) Epidemiological interference. *International Journal of Epidemiology*, **4**, 337–342
- Baraldi, A.N. and Enders, C.K. (2013) Missing data methods. In: *The Oxford Handbook of Quantitative Methods. Volume 2: Statistical Analysis*. Ed. Little, T. D., pp. 635–664. Oxford University Press, New York

- Barber, D., Rodríguez, R. and Salcedo, G. (2008) Molecular profiles: a new tool to substantiate serum banks for evaluation of potential allergenicity of GMO. *Food and Chemical Toxicology*, **46**, S35–S40
- Bardosh, K. (Ed.) (2016) *One Health: Science, Politics and Zoonotic Disease in Africa*. Routledge, Abingdon
- Bardosh, K., Leach, M. and Wilkinson, A. (2016) The limits of rapid response. In: *One Health: Science, Politics and Zoonotic Disease in Africa*. Ed. Bardosh, K., pp. 74–94. Routledge, Abingdon
- Bardosh, K., Waiswa, C. and Welburn, S.C. (2013) Conflict of interest: use of pyrethroids and amidines against tsetse and ticks in zoonotic sleeping sickness endemic areas of Uganda. *Parasites & Vectors*, **6**, 204; doi 10.1186/1756-3305-6-204
- Bardsley, E.L. and Thrusfield, M.V. (2014) Animal health studies using participatory epidemiology in the Mandrare Valley, Madagascar. *Tropical Animal Health and Production*, **46**, 133–137
- Barger, I.A. (1996) Prospects for integration of novel parasite control options into grazing systems. *International Journal for Parasitology*, **26**, 1001–1007
- Barger, I. (1997) Control by management. *Veterinary Parasitology*, **72**, 493–506
- Barger, I.A. (1999) The role of epidemiological knowledge and grazing management for helminth control in small ruminants. *International Journal of Parasitology*, **29**, 41–47
- Barger, I.A., Dash, K.M. and Southcott, W.H. (1978) Epidemiology and control of liver fluke in sheep. In: *The Epidemiology and Control of Gastrointestinal Parasites of Sheep in Australia*. Eds Donald, A.D., Southcott, W.H. and Dineen, J.K., pp. 65–74. Division of Animal Health, Commonwealth Scientific and Industrial Research Organisation, Australia
- Barkema, H.W., Schukken Y.H., Lam, T.J.G.M., Galligan, D.T., Beiboer, M.L. *et al.* (1997) Estimation of interdependence among quarters of the bovine udder with subclinical mastitis and implications for analysis. *Journal of Dairy Science*, **80**, 1592–1599
- Barker, C.G., Herrtage, M.E., Shanahan, F. and Winchester, B.G. (1988) Fucosidosis in English springer spaniels: results of a trial screening programme. *Journal of Small Animal Practice*, **29**, 623–630
- Barker, Z.E., Leach, K.A., Whay, H.R., Bell, N.J. and Main, D.C.J. (2010) Assessment of lameness prevalence and associated risk factors in dairy herds in England and Wales. *Journal of Dairy Science*, **93**, 932–941
- Barnard, B.J.H., Van Der Lugt, J.J. and Mushi, E.Z. (1994) Malignant catarrhal fever. In: *Infectious Diseases of Livestock: with Special Reference to Southern Africa*, Vol. 2. Eds Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C., pp. 946–957. Oxford University Press, Cape Town and Oxford
- Barnard C.S. and Nix, J.S. (1973) *Farm Planning and Control*. Cambridge University Press, Cambridge
- Barnard, G.A. (1959) Control charts for stochastic processes. *Journal of the Royal Statistical Society, Series B*, **21**, 239–271
- Barnes, E.M., Mead, G.C. and Griffiths, N.M. (1973) The microbiology and sensory evaluation of pheasants hung at 5, 10 and 15 C. *British Poultry Science*, **14**, 229–240
- Barnes, J. (Ed.) (1984) *The Complete Works of Aristotle*. 2 vols. Princeton University Press, Princeton
- Barnett, P., Garland, A.J.M., Kitching, R.P. and Schermbrucker, C.G. (2002) Aspects of emergency vaccination against foot-and-mouth disease. *Comparative Immunology, Microbiology and Infectious Diseases*, **25**, 345–364
- Barnett, P.V., Keel, P., Reid, S., Armstrong, R.M., Staham, R.J. *et al.* (2004) Evidence that high potency foot-and-mouth disease vaccine inhibits local virus replication and prevents the ‘carrier’ state in sheep. *Vaccine*, **22**, 1221–1232
- Barnett, T. and Whiteside, A. (2002) AIDS in the Twenty-First Century. Palgrave MacMillan, Basingstoke
- Barnett, V. (2003) *Sample Survey Principles and Methods*, 3rd edn.. Oxford University Press, New York
- Barone, G. (2012) Neurology. In: *The Cat: Clinical Medicine and Management*. Ed. Little, S., pp.734–767. Elsevier-Saunders, St Louis
- Barongo, M.B., Ståhl, K., Bett, B., Bishop, R.P., Fèvre, E. *et al.* (2015) Estimating the basic reproductive number (R_0) for African swine fever virus (ASFV) transmission between pig herds in Uganda. *PLoS ONE*, **10**, 5, e0125842; doi 10.1371/journal.pone.0125842
- Barr, C., Benefield, C., Bunce, B., Ridsdale, H. and Whittaker, M. (1986) *Landscape Changes in Britain*. Institute for Terrestrial Ecology, Huntingdon
- Barracough, G. (Ed.) (1984) *The Times Atlas of World History*, revised edn. Times Books, London
- Barrasa, M., Lamosa, S., Fernandez, M.D. and Fernandez, E. (2012) Occupational exposure to carbon dioxide, ammonia and hydrogen sulphide on livestock farms in north-west Spain. *Annals of Agricultural and Environmental Medicine*, **19**, 17–24
- Barrett, D.C. (2001) Biosecurity and herd health – a challenge for the 21st century. *Cattle Practice*, **9**, 97–103
- Barrett, M.A. and Bouley, T.A. (2014) Need for enhanced environmental representation in the implementation of One Health. *EcoHealth*, **12**, 212; doi 10.1007/s10393-014-0964-5

- Barriball, K.L., Christian, S.L., White, A.E. and Bergen, A. (1996) The telephone survey method: a discussion paper. *Journal of Advanced Nursing*, **24**, 115–121
- Barron, B.A. (1977) The effects of misclassification on the estimation of relative risk. *Biometrics*, **33**, 414–418
- Barry, B., Gonzales-Barron, U., McDonnell, K., Butler, F. and Ward, S. (2007) Using muzzle pattern recognition as a biometric approach for cattle identification. *Transactions of the American Society of Agricultural and Biological Engineers*, **50**, 1073–1080
- Barry, D. and Carrington, M. (2004) Antigenic variation. In: *The Trypanosomiasis*. Eds Maudlin, I., Holmes, P. H. and Miles, M.A., pp. 25–37. CAB Publishing, Wallingford
- Barry, J.D. and McCulloch, R. (2001) Antigenic variation in trypanosomes: enhanced phenotypic variation in a eukaryotic parasite. *Advances in Parasitology*, **49**, 1–70
- Barteling, S.J. (2002) Development and performance of inactivated vaccines against foot and mouth disease. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 577–588
- Bartholomew, M.J., Vose, D.J., Tollefson, L.R. and Travis, C.C. (2005) A linear model for managing the risk of antimicrobial resistance originating in food animals. *Risk Analysis*, **25**, 99–108
- Bartlett, J.W. and Frost, C. (2008) Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables. *Ultrasound in Obstetrics & Gynecology*, **31**, 466–475
- Bartlett, M.S. (1957) Measles periodicity and community size. *Journal of the Royal Statistical Society, Series A*, **120**, 48–60 (discussion: 60–70)
- Bartlett, M.S. (1960) The critical community size for measles in the United States. *Journal of the Royal Statistical Society, Series A*, **123**, 37–44
- Bartlett, P.C., Kaneene, J., Gibson, C.D., Erickson, R. and Mather, E.C. (1982) Development of a computerised dairy management and disease surveillance system: FAHRMX. In: *Proceedings of the First Symposium on Computer Applications in Veterinary Medicine*. College of Veterinary Medicine, Mississippi State University, 13–15 October 1982, pp. 21–29
- Bartlett, P.C., Kaneene, J.B., Kirk, J.H., Wilke, M.A. and Martenuik, J.V. (1986) Development of a computerized dairy herd health data base for epidemiological research. *Preventive Veterinary Medicine*, **4**, 3–14
- Bartlett, P.C., Kirk, J.H., Mather, E.C., Gibson, C. and Kaneene, J.B. (1985) FAHRMX: a computerized dairy herd health management network. *Compendium on Continuing Education for the Practising Veterinarian*, **7**, S124–S133
- Bartlett, P.C., Van Buren, J.W., Neterer, M. and Zhou, C. (2010) Disease surveillance and referral bias in the veterinary medical database. *Preventive Veterinary Medicine*, **94**, 264–271
- Bartley, L.M., Donnelly, C.A. and Anderson, R.M. (2002) Review of foot-and-mouth disease virus survival in animal excretions and on fomites. *Veterinary Record*, **151**, 667–669
- Bartsoca, C.S. (1966) *Two fourteenth century Greek descriptions of the 'Black Death'*. *Journal of the History of Medicine and Allied Sciences*, **XXI**, 394–400
- Basagoudanavar, S.H. and Hosamani, M. (2013) Transboundary diseases of animals: mounting concerns. *VetScan*, **7** (2), Article 119, 1–5
- Bass, P.D., Pendell, D.L., Morris, D.L., Scanga, J.A., Belk, K.E. *et al.* (2008) Sheep traceability systems in selected countries outside of North America. *The Professional Animal Scientist*, **24**, 302–307
- Basson, P.A. and Hofmeyer, J.M. (1973) Mortalities associated with wildlife capture operations. In: *The Capture and Care of Wild Animals*. Ed. Young, E., pp. 151–160. Human and Rousseau, Cape Town and Pretoria
- Bastos, A.D.S., Boshoff, C.I., Keet, D.F., Bengis, R.G. and Thomson, G.R. (2000) Natural transmission of foot-and-mouth disease virus between African buffalo (*Syncerus caffer*) and impala (*Aepyceros melampus*) in the Kruger National Park, South Africa. *Epidemiology and Infection*, **124**, 591–598
- Basu, S. and Basu, A. (1995) Comparison of several goodness-of-fit tests for the kappa statistic based on exact power and coverage probability. *Statistics in Medicine*, **14**, 347–356
- Bates, T. (1717–1719) A brief account of the contagious disease which raged among the milch cows near London, in the year 1714, and of the methods that were taken for suppressing it. *Philosophical Transactions of the Royal Society*, **30**, 872–885
- Bates, T.W., Carpenter, T.E. and Thurmond, M.C. (2003) Benefit-cost analysis of vaccination and preemptive slaughter as a means of eradicating foot-and-mouth disease. *American Journal of Veterinary Research*, **64**, 805–812
- Bateson, P. (2010) *Independent Inquiry into Dog Breeding*. University of Cambridge, Cambridge
- Baudet, A.E.R.F. (1923) Mortality in ducks in the Netherlands caused by a filterable virus; fowl plague. *Tijdschrift Diergeneesk*, **50**, 455–459 (in Dutch)
- Bauer, T.R., Adler, R.L. and Hickstein, D.D. (2009) Potential large animal models for gene therapy of human genetic diseases of the immune and blood cell systems. *ILAR Journal*, **50**, 168–186
- Baumann, M.P.O. (1990) The Nomadic Animal Health System (NAHA-System) in pastoral areas of central Somalia and its usefulness in epidemiological

- surveillance. MPVM Thesis, University of California-Davis
- Baxby, D. (1972) Smallpox-like virus from camels in Iran. *The Lancet*, **300**, 1063–1065
- Bayes, T. (1763) An essay towards solving a problem in the doctrine of chance. *Philosophical Transactions of the Royal Society*, **53**, 370–418
- Baylis, M. and Morse, A.P. (2012) Disease, human and animal health and environmental change. In: *The Sage Handbook of Environmental Change*, Vol. 2. Eds Matthews, J.A., Bartlein, P.J., Briffa, K.R., Dawson, A.G., de Vernal, A. *et al.*, pp. 387–405. Sage Publications, London
- Baylis, M. and Rawlings, P. (1998) Modelling the distribution and abundance of *Culicoides imicola* in Morocco and Iberia using climatic data and satellite imagery. In: *African Horse Sickness*. Eds Mellor, P.S., Baylis, M., Hamblin, C., Callisher, C.H. and Mertens, P. P.C., pp. 137–153. Springer, Vienna
- Baylis, M., Meiswinkel, R., and Venter, G.J. (1999) A preliminary attempt to use climate data and satellite imagery to model the abundance and distribution of *Culicoides imicola* (Diptera: Ceratopogonidae) in southern Africa. *Journal of the South African Veterinary Association*, **70**, 80–89
- Baylis, M., Mellor, P.S., Wittmann, E.J. and Rogers, D.J. (2001) Prediction of areas around the Mediterranean at risk of bluetongue by modelling the distribution of its vector using satellite imaging. *Veterinary Record*, **149**, 639–643
- Bazin, H. (2003) A brief history of the prevention of infectious diseases by immunisations. *Comparative Immunology, Microbiology and Infectious Diseases*, **26**, 293–308
- Beal, V.C. and McCallon, W.R. (1983) The use of mathematical models in animal disease program evaluation. In: *Proceedings of the Third International Symposium on Veterinary Epidemiology and Economics*. Arlington, Virginia, 6–10 September 1982, pp. 400–407. Veterinary Medical Publishing Company, Edwardsville
- Beam, S., Correa, M.T. and Davisdon, M.G. (1999) A retrospective-cohort study on the development of cataracts in dogs with diabetes mellitus: 200 cases. *Veterinary Ophthalmology*, **2**, 169–172
- Beard, T.C. (1973) The elimination of echinococcosis from Iceland. *Bulletin of the World Health Organization*, **48**, 653–660
- Beaudeau, F. and Fourichon, C. (1998) Estimating relative risk of disease from outputs of logistic regression when the disease is not rare. *Preventive Veterinary Medicine*, **36**, 243–256
- Beauvais, W., Cardwell, J.M. and Brodbelt, D.C. (2012) The effect of neutering on the risk of mammary tumours in dogs – a systematic review. *Journal of Small Animal Practice*, **53**, 314–322
- Beck, A.M. (1973) *The Ecology of Stray Dogs*. York Press, Baltimore
- Beck, E. and Strohmaier, K. (1987) Subtyping of European foot-and-mouth disease virus strains by nucleotide sequence determination. *Journal of Virology*, **61**, 1621–1629
- Becker, B.A. (1987) The phenomenon of stress: concepts and mechanisms associated with stress-induced response of the neuroendocrine system. *Veterinary Research Communications*, **11**, 443–456
- Bedford, T. and Cooke, R. (2001) *Probabilistic Risk Analysis: Foundations and Methods*. Cambridge University Press, Cambridge
- Beech, M.W.H. (1911) *The Suk, their Language and Folklore*. Clarendon Press, Oxford
- Beechinor, G. (1993) Regulatory and administrative implications of clinical trials. In: *Field Trial and Error*. Proceedings of the International Seminar with Workshops on the Design, Conduct and Interpretation of Field Trials, Berg en Dal, Netherlands, 27–28 April 1993. Eds Noordhuizen, J.P.T.M., Frankena, K., Ploeger, H. and Nell, T., pp. 29–35. Epidecon, Wageningen
- Beekhuis-Gibbon, L., Devitt, C., Whyte, P., O'Grady, L., More, S. *et al.* (2011a) A HACCP-based approach to mastitis control in dairy herds. Part 2: Implementation and evaluation. *Irish Veterinary Journal*, **64**, 7; doi 10.1186/2046-0481-64-7
- Beekhuis-Gibbon, L., Whyte, P., O'Grady, L., More, S. and Doherty, M.L. (2011b) A HACCP-based approach to mastitis control in dairy herds. Part 1: Development. *Irish Veterinary Journal*, **64**, 2; doi 10.1186/2046-0481-64-2
- Beers, M.H. and Berkow, R. (Eds) (1999) *The Merk Manual of Diagnosis and Therapy*, 17th edn. Merk Research Laboratories, Whitehouse Station
- Begg, C.B. and Greenes, R.A. (1983) Assessment of diagnostic tests when disease verification is subject to bias. *Biometrics*, **39**, 207–215
- Begg, D., Vernasca, G., Fischer, S. and Dornbusch, R. (2014) *Economics*, 11th edn. McGraw-Hill Education, Maidenhead
- Beilage, G. B., Salge, H.-J., Krabbe, H. and Blaha, T. (1991) Untersuchungen zur epidemiologie des seuchenhaften spätabortes der zuchtsauen (PRRS). *Collegium Veterinarium*, **22**, 62–64 (in German)
- Beinart, W. and Brown, K. (2013) *African Local Knowledge & Livestock Health: Diseases & Treatments in South Africa*. James Currey, Oxford
- Belaganahalli, M., Maan, S., Maan, N.S., Brownlie, J., Teash, R. *et al.* (2015) Genetic characterization of the tick-borne orbiviruses. *Viruses*, **7**, 2185–2209

- Belák, S. and Ballagi-Pordány, A. (1993) Application of the polymerase chain reaction (PCR) in veterinary diagnostic virology. *Veterinary Research Communications*, **17**, 55–72
- Belák, S., Ballagi-Pordány, A., Flensburg, J. and Virtanen, A. (1989) Detection of pseudorabies virus DNA sequences by the polymerase chain reaction. *Archives of Virology*, **108**, 279–286
- Bell, K.J. (1980) A study of productivity in sheep flocks. The application and evaluation of health and productivity programmes in south west Australia. In: Proceedings of the 10th Seminar of the Sheep and Beef Cattle Society of the New Zealand Veterinary Association. Lincoln College, Canterbury 11–12 July 1980, pp. 108–114
- Bell, W.G. (2001) *The Great Plague in London*. The Folio Society, London
- Bellet, C., Vergne, T., Grosbois, V., Holl, D., Roger, F. *et al.* (2012) Evaluating the efficiency of participatory epidemiology to estimate the incidence and impacts of foot-and-mouth disease among livestock owners in Cambodia. *Acta Tropica*, **123**, 31–38
- Bellet, C., Woodnutt, J., Green, L.E. and Kaler, J. (2015) Preventive services offered by veterinarians on sheep farms in the UK: opinions and drivers of proactiveness. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Ghent, 25–27 March 2015*. Eds Thulke, H.-H. and Verheyen, K., pp. 189–198
- Bellwood, D.R., Hughes, T.P., Folke, C. and Nyström, M. (2004) Confronting the coral reef crisis. *Nature*, **429**, 827–833
- Belshaw, Z. and Dean, R. (2015) Calling time on survival times? *Journal of Small Animal Practice*, **56**, 635–636
- Belshaw, Z., Aasher, L., Harvey, N.D. and Dean, R.S. (2015) Quality of life assessment in domestic dogs: an evidence-based rapid review. *The Veterinary Journal*, **206**, 203–212
- Bemis, D.A., Carmichael, L.E. and Appel, M.J. (1977) Naturally occurring respiratory disease in a kennel caused by *Bordetella bronchiseptica*. *Cornell Veterinarian*, **67**, 282–293
- Benavides, J., Walsh, P.D., Meyers, L.A., Raymond, M. and Caillaud, D. (2012) Transmission of infectious diseases en route to habitat hotspots. *PLoS ONE* **7**, 2, e31290; doi 10.1371/journal.pone.0031290
- Bender, R. and Lange, S. (2001) Adjusting for multiple testing – when and how? *Journal of Clinical Epidemiology*, **54**, 343–349
- Bendinelli, M., Pistello, M., Matteucci, D., Lombardi, S., Baldinotti, F. *et al.* (1993) Small animal model of AIDS and the feline immunodeficiency virus. *Advances in Experimental Medicine and Biology*, **335**, 189–202
- Bendixen, P.H. (1987) Notes about incidence calculations in observational studies (Letter). *Preventive Veterinary Medicine*, **5**, 151–156
- Benedictus, A., Hogeveen, H. and Berends, B.R. (2009) The price of the precautionary principle: cost-effectiveness of BSE intervention strategies in the Netherlands. *Preventive Veterinary Medicine*, **89**, 212–222
- Bengis, R.G. (1997) Animal health risks associated with the transportation and utilisation of wildlife products. *Revue Scientifique et Technique, Office International des Epizooties*, **16**, 104–110
- Bengis, R.G., Kock, R.A. and Fischer, J. (2002) Infectious animal diseases: the wildlife/livestock interface. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 53–65
- Benham, P.J.F. and Broom, D.M. (1989) Interactions between cattle and badgers at pasture with reference to bovine tuberculosis transmission. *British Veterinary Journal*, **145**, 226–241
- Benjamin, B. (1959) *Elements of Vital Statistics*. George Allen and Unwin, London
- Benjamini, Y. (1988) Opening the box of a boxplot. *The American Statistician*, **42**, 257–262
- Bennett, M. (2001) The LANSS Pain Scale: the Leeds assessment of neuropathic symptoms and signs. *Pain*, **92**, 147–157
- Bennett, N. (2008) Murray Valley encephalitis: indeed a ‘mysterious disease’. *Victorian Infectious Diseases Bulletin*, **11**, 94–107
- Bennett, N.M. (2009) Peculiarities of Murray Valley encephalitis (MVE) epidemics in south-eastern Australia: the Indian Ocean dipole (IOD) as a predictor of epidemics. *Victorian Infectious Diseases Bulletin*, **12** (4), 112–115
- Bennett, R.M. (1996) Willingness-to-pay measures of public support for farm animal welfare legislation. *Veterinary Record*, **139**, 320–321
- Bennett, R.M. (2003) The ‘direct costs’ of livestock disease: the development of a system of models for the analysis of 30 endemic livestock diseases in Great Britain. *Journal of Agricultural Economics*, **54**, 55–71
- Bennett, R.M. and Blaney, R.J.P. (2003) Estimating the benefits of farm animal welfare legislation using the contingent valuation method. *Agricultural Economics*, **29**, 85–98
- Bennett, R.M., and Ijpelaar, J. (2005) Updated estimates of the costs associated with thirty four endemic livestock diseases in Great Britain: a note. *Journal of Agricultural Economics*, **56**, 135–144
- Bennett, R. and Larson, D. (1996) Contingent valuation of the perceived benefits of farm animal welfare legislation: an exploratory survey. *Journal of Agricultural Economics*, **47**, 224–235
- Bennett, R.M. and Willis, K.G. (2008) Public values for badgers, bovine TB reduction and management

- strategies. *Journal of Environmental Planning and Management*, **51**, 511–523
- Bennett, R.M., Barker, Z.E., Main, D.C.J., Whay, H.R. and Leach K.A. (2014) Investigating the value dairy farmers place on a reduction of lameness in their herds using a willingness to pay approach. *The Veterinary Journal*, **199**, 72–75
- Bennett, R.M., McClement, I. and McFarlane, I. (2010) An economic decision support tool for simulating paratuberculosis control strategies in a UK suckler beef herd. *Preventive Veterinary Medicine*, **93**, 286–293
- Bennett, S., Woods, T., Liyanage, W.M. and Smith, D.L. (1991) A simplified general method for cluster sampling surveys of health in developing countries. *World Health Statistics Quarterly*, **44**, 98–106
- Berencsi, G., Kapusinszky, B., Rigó, Z. and Szomor, K. (2010) Interference among viruses circulating and administered in Hungary from 1931–2008. *Acta Microbiologica et Immunologica Hungarica*, **57**, 73–86
- Berentsen, P.B.M., Dijkhuizen A.A. and Oskam, A.J. (1992a) A critique of published cost-benefit analyses of foot-and-mouth disease. *Preventive Veterinary Medicine*, **12**, 217–227
- Berentsen, P.B.M., Dijkhuizen, A.A. and Oskam, A.J. (1992b) A dynamic model for cost-benefit analyses of foot-and-mouth disease. *Preventive Veterinary Medicine*, **12**, 229–243
- Berger, L., Speare, R. and Hyatt, A. (1999) Chytrid fungi and amphibian declines: overview, implications and further directions. In: *Declines and Disappearances of Australian Frogs*. Ed. Campbell, A., pp. 23–33. Environment Australia, Canberra
- Berger, V.W. and Antsygina, O. (2015) A review of randomization methods in clinical trials. *Clinical Investigation*, **5** (12), 1–7
- Bergquist, R. (2011) New tools for epidemiology: a space odyssey. *Mémoires do Instituto Oswaldo Cruz*, **106**, 892–900
- Bergsma, D.R. and Brown, K.S. (1971) White fur, blue eyes, and deafness in the domestic cat. *Journal of Heredity*, **62**, 171–185
- Berke, O. (2001) Choropleth mapping of regional count data of *Echinococcus multilocularis* among red foxes in Lower Saxony, Germany. *Preventive Veterinary Medicine*, **52**, 119–131
- Berkelmans, R. and van Oppen, M. (2006) The role of zooxanthellae in the thermal tolerance of corals: a ‘nugget of hope’ for coral reefs in an era of climate change. *Proceedings of the Royal Society of London, Series B*, **272**, 29–30
- Berkman, L.F. and Kawachi, I. (Eds) (2000) *Social Epidemiology*. Oxford University Press, New York
- Berkson, J. (1960) Smoking and cancer of the lung. *Proceedings of Staff Meeting of the Mayo Clinic*, **35**, 367–385
- Berkson, J. (1962) Mortality and marital status. *American Journal of Public Health*, **52**, 1318–1329
- Berlin, J.A. and Golub, R.M. (2014) Meta-analysis as evidence: building a better pyramid. *Journal of the American Medical Association*, **312**, 603–605
- Bernardo, T.M., Schotman, C.Y.L. and Chillaud, T. (1994) The future of international animal health reporting. In: *Proceedings of the 7th International Symposium on Veterinary Epidemiology and Economics*, Nairobi, 15–19 August 1994. Eds Rowlands, G.J., Kyule, M.N. and Perry, B.D. *The Kenya Veterinarian*, **18** (2), 44–46
- Bernoulli, M.D. (1766) Essai d’une nouvelle analyse de la mortalité causée par la petite vérole et des avantages de l’inoculation pour la prévenir. In: *Histoire de L’Académie Royale des Sciences, 1760, Avec les Mémoires de Mathématique et de Physique*. L’Imprimerie Royale, Paris. Translated in Bradley, L. (1971) *Smallpox Inoculation: An Eighteenth Century Mathematical Controversy*. University of Nottingham Adult Education Department, Nottingham
- Berns, K.I. and Bohenzky, R.A. (1987) Adeno-associated viruses: an update. *Advances in Virus Research*, **32**, 243–306
- Bertherat, E., Thullier, P., Shako, J.C., England, K., Koné, M.L. *et al.* (2011) Lessons learned about pneumonic plague diagnosis from two outbreaks, Democratic Republic of the Congo. *Emerging Infectious Diseases*, **17**, 778–784
- Besier, R.B. (2011) Refugia-based strategies for sustainable worm control: factors affecting the acceptability to sheep and goat owners. *Veterinary Parasitology*, **186**, 2–9
- Bessell, P.R., Orton, R., O’Hare, A., Mellor, D.J., Logue, D. *et al.* (2013) Developing a framework for risk-based surveillance of tuberculosis in cattle: a case study of its application in Scotland. *Epidemiology and Infection*, **141**, 314–323
- Bessell, P.R., Robinson, R.A., Golding, N., Searle, K.R., Handel, I.G. *et al.* (2014) Quantifying the risk of introduction of West Nile virus into Great Britain by migrating passerine birds. *Transboundary and Emerging Diseases*, **63**, e347–e359; doi 10.1111/tbed.12310
- Best, S.J. and Harrison, C.H. (2009) Internet survey methods. In: *The SAGE Handbook of Applied Social Research Methods*, 2nd edn. Eds Bickman, L. and Rog, D.J., pp. 413–434. Sage Publications, Thousand Oaks
- BETA (2011) *The National Equestrian Survey 2011*. British Equestrian Trade Association, Wetherby
- Bett, B., Jost, C., Allport, R. and Mariner, J. (2009) Using participatory epidemiological techniques to estimate the relative incidence and impact on livelihoods of livestock diseases amongst nomadic pastoralists in Turkana South District, Kenya. *Preventive Veterinary Medicine*, **90**, 195–203

- Beugnet, F., Chalvet-Monfray, K. and Loukos, H. (2009) FleaTickRisk: a meteorological model developed to monitor and predict the activity and density of three tick species and the cat flea in Europe. *Geospatial Health*, **4**, 97–113
- Beyer, W.H. (Ed.) (1968) CRC Handbook of Probability and Statistics, 2nd edn. Chemical Rubber Company, Cleveland
- Beyer, W.H. (Ed.) (1981) CRC Statistical and Mathematical Tables, 26th edn. CRC Press, Boca Raton
- Beynon, S.A. (2012) Potential environmental consequences of administration of anthelmintics to sheep. *Veterinary Parasitology*, **189**, 113–124
- Bhandari, R.K., vom Saal, F.S. and Tillitt, D.E. (2015) Transgenerational effects from early developmental exposures to bisphenol A or 17 α -ethinylestradiol in medaka, *Oryzias latipes*. *Scientific Reports*, **5**, 9303; doi 10.1038/srep09303
- Bibikova, V.A. (1977) Contemporary views on the interrelationships between fleas and the pathogens of human and animal diseases. *Annual Review of Entomology*, **22**, 23–32
- Biemer, P.P. and Lyberg, L.E. (2003) *Introduction to Survey Quality*. John Wiley and Sons, Hoboken
- Biggs, H.C. and Hare, K.M. (1994) Essential features of an integrated veterinary disease information system for Namibia. In: Proceedings of the 7th International Symposium on Veterinary Epidemiology and Economics, Nairobi, 15–19 August 1994. Eds Rowlands, G.J., Kyule, M.N. and Perry, B.D. *The Kenya Veterinarian*, **18** (2), 29–31
- Biggs, P.M. (1982) Vaccination. In: *The Control of Infectious Diseases in Farm Animals*, pp. 21–27. British Veterinary Association Trust Project on the Future of Animal Health Control, London
- Biggs, P.M. (1993) Developments in vaccines. In: *The Advancement of Veterinary Science. The Bicentenary Symposium Series. Volume 4: Veterinary Medicine – Growth Points and Comparative Medicine*. Ed. Michell, A.R., pp. 13–27. CAB International, Wallingford
- Biggs, P.M., Milne, B.S., Frazier, J.A., McDougall, J.S. and Stuart, J.C. (1974) Lymphoproliferative disease in turkeys. *Proceedings of the World's Poultry Congress*, **15**, 55–56
- Billar, S.D. and Oser, S.M. (2015) Another look at confidence intervals: proposal for a more relevant and transparent approach. *Nuclear Instruments and Methods in Physics Research A*, **774**, 103–119
- Binois, A. (2015) Excavating the history of ancient veterinary practices. *Veterinary Record*, **176**, 564–569
- Bird, R.B., Bird, D.W., Codding, B.F., Parker, C.H. and Jones, J.H. (2008) The 'fire stick' farming hypothesis: Australian Aboriginal foraging strategies, biodiversity, and anthropogenic fire mosaics. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 14796–14801
- Bishop, S.C., Axford, R.F.E., Nicholas, F.W. and Owen, J. B. (Eds) (2010) *Breeding for Disease Resistance in Farm Animals*, 3rd edn. CAB International, Wallingford
- Bisson, I.-A., Ssebide, B.J. and Marra, P.P. (2015) Early detection of emerging zoonotic diseases with animal morbidity and mortality monitoring. *EcoHealth*, **12**, 98–103
- Black, A. (1972) Progressive retinal atrophy: a review of the genetics and an appraisal of the eradication scheme. *Journal of Small Animal Practice*, **13**, 295–314
- Black, F.L. (1966) Measles endemicity in insular populations: critical community size and its evolutionary implication. *Journal of Theoretical Biology*, **11**, 207–211
- Black, F.L. (1975) Infectious diseases in primitive societies. *Science*, **187**, 515–518
- Black, M.A. and Craig, B.A. (2002) Estimating disease prevalence in the absence of a gold standard. *Statistics in Medicine*, **21**, 2653–2669
- Black, R.E., Levine, M.M., Clements, M.L., Hughes, T.P. and Blaser, M.J. (1988) Experimental *Campylobacter jejuni* infection in humans. *Journal of Infectious Diseases*, **157**, 472–479
- Blackburn, H. and Labarthe, D. (2012) Stories from the evolution of guidelines for causal inference in epidemiologic associations: 1953–1965. *American Journal of Epidemiology*, **176**, 1071–1077
- Blackmore, D.K. (1963) The incidence and aetiology of thyroid dysplasia in budgerigars (*Melopsittacus undulatus*). *Veterinary Record*, **75**, 1068–1072
- Blackmore, D.K. (1964) A survey of disease in British wild foxes (*Vulpes vulpes*). *Veterinary Record*, **76**, 527–533
- Blackmore, D.K. (1983) Practical problems associated with the slaughter of stock. In: *Stunning of Animals for Slaughter*. Ed. Eikelenboom, G., pp. 167–178. Martin Nijhoff Publishers, The Hague
- Blackmore, D.K. and Hathaway, S.C. (1980) The nidality of zoonoses. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 7–11 May 1979. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 207–213. Australian Government Publishing Service, Canberra
- Blackmore, D.K. and Schollum, L.M. (1983) The serological investigation of occupational exposure to leptospirosis. In: *Proceedings of the Third International Symposium on Veterinary Epidemiology and Economics, Arlington, 6–10 September, 1982*, pp. 544–551. Veterinary Medical Publishing Company, Edwardsville
- Blackmore, D.K., Hill, A. and Jackson, O.F. (1971) The incidence of mycoplasma in pet and colony maintained cats. *Journal of Small Animal Practice*, **12**, 207–216

- Blaha, T. (Ed.) (1989) *Applied Veterinary Epidemiology*. Elsevier, Amsterdam
- Blaha, T. (1992) Regional eradication of swine dysentery by gradual elimination of the pathogen from infected herds. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Edinburgh, 1–3 April 1992*. Ed. Thrusfield, M.V., pp. 186–189
- Blair, P.H. (1963) *Roman Britain and Early England, 55 BC–AD 871*. Thoman Nelson and Sons, London and Edinburgh
- Blaisdell, J.D. (1994) The Curse of the Pharaohs: anthrax in Ancient Egypt. *Bulletin van het Veterinari Historisch Genootschap*, **10**, 311–314
- Blajan, L. (1979) The systems for veterinary information at the national and worldwide levels. *Bulletin Office International des Epizooties*, **91**, 795–821
- Blajan, L. and Chillaud, T. (1991) The OIE world animal disease information system. *Revue Scientifique et Technique, Office International des Epizooties*, **10**, 71–87
- Blajan, L. and Welte, V.R. (1988) Information, a prerequisite for veterinary activities. *Review Scientifique et Technique, Office International des Epizooties*, **7**, 463–478
- Blake, A., Sinclair, M.T. and Sugiyarto, G. (2003) Quantifying the impact of foot and mouth disease on tourism and the UK economy. *Tourism Economics*, **9**, 449–465
- Blaker, H. (2000) Confidence curves and improved exact confidence intervals for discrete distributions. *Canadian Journal of Statistics*, **28**, 783–798
- Blamire, R.V., Crowley, A.J. and Goodhand, R.H. (1970) A review of some animal diseases encountered at meat inspection 1960–1968. *Veterinary Record*, **87**, 234–238
- Blamire, R.V., Goodhand, R.H. and Taylor, K.C. (1980) A review of some animal diseases encountered at meat inspections in England and Wales, 1969 to 1978. *Veterinary Record*, **106**, 195–199
- Blancou, J. (2001) A history of the traceability of animals and animal products. *Revue Scientifique et Technique, Office International des Epizooties*, **20**, 420–425
- Bland, M. (2015) *An Introduction to Medical Statistics*, 4th edn. Oxford University Press, Oxford
- Bland, J.M. and Altman, D.G. (1986) Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet*, **327**, 307–310
- Bland, J.M. and Altman, D.G. (1995) Multiple significance tests: the Bonferroni method. *British Medical Journal*, **310**, 170
- Bland, J.M. and Altman, D.G. (1999) Measuring agreement in method comparison studies. *Statistical Methods in Medical Research*, **8**, 135–160
- Bland, J.M. and Altman, D.G. (2007) Agreement between methods of measurement with multiple observations per individual. *Journal of Biopharmaceutical Statistics*, **17**, 571–582
- Blanton, J.D., Krebs, J.W., Hanlon, C.A. and Rupprecht, C.E. (2006) Rabies surveillance in the United States during 2005. *Journal of the American Veterinary Medical Association*, **229**, 1897–1911
- Blayney, D., Dyck, J. and Harvey, D. (2006) Economic effects of animal diseases linked to trade dependency. *Amber Waves*, April 2006. Economic Research Service, United States Department of Agriculture. <http://www.ers.usda.gov/amber-waves/2006-april/economic-effects-of-animal-diseases-linked-to-trade-dependency.aspx#.VroFLlLgosI> (accessed 9 March 2016)
- Blomme, E.A.G. and Spear, B.B. (2010) Theranostics in veterinary medicine: where are we heading? *The Veterinary Journal*, **185**, 237–238
- Blood, D.C. (1976) The principles of herd health programmes. In: *Proceedings No. 28. Refresher Course for Veterinarians on Mastitis*, pp. 47–64. University of Sydney Post-graduate Committee on Veterinary Science in association with the Australian Veterinary Association and the Veterinary Clinic Centre, University of Melbourne, Melbourne
- Blood, D.C. and Henderson, J.A. (1960) *Veterinary Medicine*, Baillière, Tindall and Cox, London
- Blood, D.C. and Studdert, V.P. (2002) *Saunders Comprehensive Veterinary Dictionary*, 2nd edn. W.B. Saunders, London
- Blood, D.C., Morris, R.S., Williamson, N.B., Cannon, C. M. and Cannon, R.M. (1978) A health program for commercial dairy herds. 1. Objectives and methods. *Australian Veterinary Journal*, **54**, 207–215
- Blot, W.J. and Day, N.E. (1979) Synergism and interaction: are they equivalent? *American Journal of Epidemiology*, **110**, 99–100
- Blowey, R. and Edmondson, P. (2000) The environment and mastitis. In *Practice*, **22**, 382–394
- Blowey, R. and Edmondson, P. (2010) *Mastitis Control in Dairy Herds*, 2nd edn. CABI, Wallingford
- Boardman, A.E., Greenberg D.H., Vining A.R. and Weimer D.L. (2011) *Cost-Benefit Analysis: Concepts and Practice*, 4th edn. Pearson Education, Upper Saddle River
- Boccaletti, S., Latora, V., Moreno, Y., Chavez, M. and Hwang, D.-U. (2006) Complex networks: structure and dynamics. *Physics Reports*, **424**, 175–308
- Boden, L.A. and McKendrick, I.J. (2017) Model-based policymaking: a framework to promote ethical “good practice” in mathematical modelling for public health policymaking. *Frontiers in Public Health*, **5**, 68; doi 10.3389/fpubh.2017.00068
- Boden, L.A., Parkin, T.D.H., Yates, J., Mellor, D. and Kao, R.R. (2012) Summary of current knowledge of the size and spatial distribution of the horse population with Great Britain. *BMC Veterinary Research*, **8**, 43; doi 10.1186/1746-6148-8-43

- Boden, L.A., Parkin, T.D.H., Yates, J., Mellor, D. and Kao, R.R. (2013) An online survey of horse-owners in Great Britain. *BMC Veterinary Research*, **9**, 188; doi 10.1186/1746-6148-9-188
- Bodin, J. (1580) *De la Demonomanie des Sorciers*. Jacques du Puys, Paris. (Quoted by Summers, 1994)
- Bodson, L. (1994) Ancient views on pests and parasites of livestock. *Argos*, **10**, 303–310
- Boehler, K.E. (2013) The evolution of animal identification in beef cattle. *Nebraska Law Review*, **91**, 568–599
- Boerlin, P., Eugster, S., Gaschen, F., Straub, R. and Schawalder, P. (2001) Transmission of opportunistic pathogens in a veterinary teaching hospital. *Veterinary Microbiology*, **82**, 347–359
- Boersema, J.S.C., Noordhuizen, J.P.T.M., Vieira, A., Lievaart, J.J. and Baumgartner, W. (2008) Imbedding HACCP principles in dairy herd health and production management: case report on calf rearing. *Irish Veterinary Journal*, **61**, 594–602
- Boghurst, W. (1665) *Loimographia: An Account of the Great Plague of London in the Year 1665. Now First Printed from the British Museum Sloane MS. 349, for the Epidemiological Society of London*. Ed. Payne, J.F. (1894). Shaw and Sons, London
- Bolajoko, M.B., Moses, G.D., Gambari-Bolajoko, K.O., Ifende, V.I., Emenna, P. *et al.* (2011) Participatory rural appraisal of livestock diseases among the Fulani community of the Barkin Ladi Local Government Area, Plateau State, Nigeria. *Journal of Veterinary Medicine and Animal Health*, **3**, 11–13
- Bolboacă, S. and Jäntschi, L. (2005) Binomial distribution sample confidence interval estimation for positive and negative likelihood ratio medical key parameters. *American Medical Informatics Association Annual Symposium Proceedings*, **2005**, 66–70
- Boldin, B. and Kisdi, É. (2012) On the evolutionary dynamics of pathogens with direct and environmental transmission. *Evolution*, **66**, 2514–2527
- Boliar, S., Stanislawek, W. and Chambers, T.M. (2006) Inability of kaolin treatment to remove nonspecific inhibitors from equine serum for the hemagglutination inhibition test against H7N7 influenza virus. *Journal of Veterinary Diagnostic Investigation*, **18**, 264–267
- Bollett, A.J. (1973) Pierre Louis: the numerical method and the foundation of quantitative medicine. *American Journal of Medical Science*, **266**, 92–101
- Bolton, J.F. (1968) A case of foot-and-mouth disease in sheep. *State Veterinary Journal*, **23**, 61–64
- Bonde, M. and Sørensen, J.T. (2004) Herd health management in organic pig production using a quality assurance system based on Hazard Analysis and Critical Control Points. *NJAS – Wageningen Journal of Life Sciences*, **52**, 133–143
- Bonde, M., Toft, N., Thomsen, P.T. and Sørensen, J.T. (2010) Evaluation of sensitivity and specificity of routine meat inspection of Danish slaughter pigs using latent class analysis. *Preventive Veterinary Medicine*, **94**, 165–169
- Boneau, C. (1960) The effects of violations of assumptions underlying the *t* test. *Psychological Bulletin*, **57**, 49–64
- Boni, M.F., Gog, J.R., Andreasen, V. and Christiansen, F.B. (2004) Influenza drift and epidemic size: the race between generating and escaping immunity. *Theoretical Population Biology*, **65**, 179–191
- Bonnett, B.N. and Egenvall, A. (2010) Age patterns of disease and death in insured Swedish dogs, cats and horses. *Journal of Comparative Pathology*, **142**, S33–S38
- Bonnett, B.N., Egenvall, A., Olson, P. and Hedhammar, Å. (1997) Mortality in insured Swedish dogs: rates and causes of death in various breeds. *Veterinary Record*, **141**, 40–44
- Bookchin, M. (1995) *The Philosophy of Social Ecology: Essays on Dialectical Naturalism*, 2nd edn. Black Rose Books, Montréal
- Booth, A.J., Staggdale, L. and Gregor, J.A. (1984) Salmon poisoning disease in dogs in southern Vancouver Island. *Canadian Veterinary Journal*, **25**, 2–6
- Booth, J.M. and Warren, M.E. (1984) The use of a computerised herd fertility monitor. In: *Society for Veterinary Epidemiology and Preventive Medicine. Proceedings, Edinburgh, 10–11 July 1984*. Ed. Thrusfield, M.V., pp. 166–173
- Booth, M. (1998) The application of attributable risk analysis in helminth epidemiology. *Parasitology Today*, **14**, 497–500
- Bording, A. (1990) The clinical trial in veterinary practice. In: *Proceedings of the International Pig Veterinary Society, 11th Congress, Lausanne, 1–5 July 1990*, p. 413. Swiss Association of Swine Medicine, Berne
- Borenstein, M., Hedges, L.V., Higgins, J.P.T. and Rothstein, H.R. (2009) *Introduction to Meta-Analysis*. John Wiley and Sons, Chichester
- Borenstein, M., Hedges, L.V., Higgins, J.P.T. and Rothstein, H.R. (2010) A basic introduction to fixed effect and random effects models for meta-analysis. *Research Synthesis Methods*, **1**, 97–111
- Borg, K. and Hugoson, G. (1980) Wildlife as an indicator of environmental disturbances. In: *Veterinary Epidemiology and Economics. Proceedings of the Second International Symposium, Canberra, 7–11 May, 1979*. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 250–253. Australian Government Publishing Service, Canberra
- Borissavlievitch, M. (1958) *The Golden Number and the Scientific Aesthetics of Architecture*. Alec Tiranti, London

- Bos, K.I., Schuenemann, V.S., Golding, G.B., Burbano, H. A., Waglechner N, *et al.* (2011) A draft genome of *Yersinia pestis* from victims of the Black Death. *Nature*, **478**, 506–510
- Bosch, F.X., Lorincz, A., Muñoz, N., Meijer, C.J.L.M. and Shah, K.V. (2002) The causal relation between human papillomavirus and cervical cancer. *Journal of Clinical Pathology*, **55**, 244–265
- Bossuyt, P.M.M. and Smidt, N. (2009) Standards for reporting on diagnostic accuracy studies. In: *The Evidence Base of Clinical Diagnosis: Theory and Methods of Diagnostic Research*, 2nd edn. Eds Knottnerus, J.A. and Buntinx, F., pp. 167–179. Blackwell Publishing, Oxford
- Bossuyt, P.M., Lijmer, J.G. and Mol, B.W. (2000) Randomised comparisons of medical tests: sometimes invalid, not always efficient. *The Lancet*, **356**, 1844–1847
- Bossuyt, P.M., Reitsma, J.B., Bruns, D.E., Gatsonis, C.A., Glasziou, P.P. *et al.* (2003) Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. *British Medical Journal*, **326**, 41–44
- Bostock, D.E. and Curtis, R. (1984) Comparison of canine oropharyngeal malignancy in various geographical locations. *Veterinary Record*, **114**, 341–342
- Bouma, A., de Jong, M.C.M. and Kimman, T.G. (1995) Transmission of pseudorabies virus within populations is independent of the size of the population. *Preventive Veterinary Medicine*, **23**, 163–172
- Bouma, A., Elbers, A.R.W., Dekker, A., de Koeijer, A., Bartels, C. *et al.* (2003) The foot-and-mouth disease epidemic in The Netherlands in 2001. *Preventive Veterinary Medicine* **57**, 155–166
- Bourke, C.A. (2007) A review of kikuyu grass (*Pennisetum clandestinum*) poisoning in cattle. *Australian Veterinary Journal*, **85**, 261–267
- Bourn, D. and Blench, R. (1999) *Can Livestock and Wildlife Co-exist? An Interdisciplinary Approach*. Overseas Development Institute, London
- Bourne, F.J. (1993) Biotechnology and farm animal medicine. In: *The Advancement of Veterinary Science. The Bicentenary Symposium Series. Volume 1: Veterinary Medicine Beyond 2000*. Ed. Michell, A.R., pp. 41–57. CAB International, Wallingford
- Bowling, M.B., Pendell, D.L., Morris, D.L., Yoon, Y., Katoh, K. *et al.* (2008) Identification and traceability of cattle in selected countries outside of North America. *The Professional Animal Scientist*, **24**, 287–294
- Boycott, J.A. (1971) *Natural History of Infectious Disease*. Studies in Biology No. 26. Edward Arnold, London
- Brace, S., Taylor, D. and O'Connor, A.M. (2010) The quality of reporting and publication status of vaccines trials presented at veterinary conferences from 1988 to 2003. *Vaccine*, **28**, 5306–5314
- Bradbury, J.A. (1989a) The policy implications of differing concepts of risk. *Science, Technology, & Human Values*, **14**, 380–399
- Bradbury, T. (1989b) *Meteorology and Flight*. A&C Black, London
- Bradhurst, R.A., Roche, S.E., Garner, M.G., Sajeev, A.S.M. and Kwan, P. (2013) Modelling the spread of livestock disease on a national scale: the case for a hybrid approach. In: *MODSIM2013, 20th International Congress on Modelling and Simulation, Adelaide, Australia, 1–6 December 2013*. Eds Piantadosi, J., Anderssen, R.S. and Boland, J., pp. 345–351. Modelling and Simulation Society of Australia and New Zealand
- Bradley, D.J. (2004) An exploration of chronotones: a concept for understanding the health processes of changing ecosystems. *EcoHealth*, **1**, 165–171
- Brand, A., Noordhuizen, J.P.T.M. and Schukken, Y.H. (1996) *Herd Health and Production Management in Dairy Practice*. Wageningen Pers, Wageningen
- Brandeis, L.J. (1971) Business: a profession. In: *Reprints of Economic Classics*, pp. 1–12. A.M. Kelley, New York
- Brandstätter, E. (1999) Confidence intervals as an alternative to significance testing. *Methods of Psychological Research Online*, **4**, (2), 34–46
- Branscum, A.J., Gardner, I.A. and Johnson, W.O. (2005) Estimation of diagnostic-test sensitivity and specificity through Bayesian modeling. *Preventive Veterinary Medicine*, **68**, 145–163
- Brauer, F. (2008) Epidemic models with heterogeneous mixing and treatment. *Bulletin of Mathematical Biology*, **70**, 1869–1885
- Brauer, F. (2012) Heterogeneous mixing in epidemic models. *Canadian Applied Mathematics Quarterly*, **20**, (1), 1–13
- Braukmann, M., Barrow, P.A., Berndt, A. and Methner, U. (2016) Combination of competitive exclusion and immunization with a live *Salmonella* vaccine in newly hatched chickens: immunological and microbiological effects. *Research in Veterinary Science*, **107**, 34–41
- Braverman, Y. and Chechik, F. (1993) Air streams and the introduction of animal diseases borne on *Culicoides* (Diptera, Ceratopogonidae) into Israel. *Israeli Journal of Veterinary Medicine*, **48**, 1–9
- Bravo de Rueda, C., de Jong, M.C.M., Eblé, P.L. and Dekker, A. (2015) Quantification of transmission of foot-and-mouth disease virus caused by an environment contaminated with secretions and excretions from infected calves. *Veterinary Research*, **46**, 43; doi 10.1186/s13567-015-0156-5
- Bregman, D.J. and Langmuir, A.D. (1990) Farr's law applied to AIDS projections. *Journal of the American Medical Association*, **263**, 1522–1525

- Brennan, M.L., Kemp, R. and Christley, R.M. (2008) Direct and indirect contacts between cattle farms in north-west England. *Preventive Veterinary Medicine*, **84**, 242–260
- Brenner, H. (1996) Correcting for exposure misclassification using an alloyed gold standard. *Epidemiology*, **7**, 406–410
- Brenner, H. and Gefeller, O. (1997) Variation in sensitivity, specificity, likelihood ratios and predictive values with disease prevalence. *Statistics in Medicine*, **16**, 981–991
- Bresalier, M. and Worboys, M. (2014) 'Saving the lives of our dogs': the development of canine distemper vaccine in interwar Britain. *British Journal of the History of Science*, **47**, 305–334
- Bresalier, M., Cassidy, A. and Woods, A. (2015) One health in history. In: *One Health: The Theory and Practice of Integrated Health Approaches*. Eds Zinsstag, J., Schelling, E., Whittaker, M., Tanner, M. and Waltner-Toews, D., pp. 1–15. CABI, Wallingford
- Breslow, N.E. (2003) Are statistical contributions to medicine undervalued? *Biometrics*, **59**, 1–8
- Breslow, N.E. and Day, N.E. (1980) Statistical Methods in Cancer Research, Volume 1: The Analysis of Case-Control Studies. IARC Scientific Publications No. 32. International Agency on Cancer Research, Lyon
- Breslow, N.E. and Day, N.E. (1987) *Statistical Methods in Cancer Research, Volume 2: The Design and Analysis of Cohort Studies*. IARC Scientific Publications No. 82. International Agency on Cancer Research, Lyon
- Bretzke, J.T. (2011) Casuistry. In: *Cambridge Dictionary of Christian Theology*. Eds McFarland, I.A., Fergusson, D.A.S., Kilby, K. and Torrance, I.R., pp. 85–86. Cambridge University Press, Cambridge.
- Briggs, J.H.Y. (1982) *A History of Longton: I. The Birth of a Community*. University of Keele, Keele
- Brink, H. (1996) *Fundamentals of Research Methodology for Health Care Professionals*. Juta and Co., Kenwyn
- Britanica (1992) *The New Encyclopædia Britannica*, 15th edn, Macropædia, Vol. 25, pp. 630–636. Encyclopædia Britannica, Chicago
- Broadbent, D.W. (1979) Field collection of animal disease data in developing countries. *World Animal Review*, **29**, 38–42
- Broberg, E., Nicoll, A. and Amato-Gauci, A. (2011) Seroprevalence to influenza A(H1N1) 2009 virus – where are we? *Clinical and Vaccine Immunology*, **18**, 1205–1212
- Brocchi, E., Bergmann, I.E., Dekker, A., Paton, D.J., Sammin, D.J. *et al.* (2006) Comparative evaluation of six ELISAs for the detection of antibodies to the non-structural proteins of foot-and-mouth disease virus. *Vaccine*, **24**, 6966–6979
- Brock, W.H. (2002) Crookes, carbolic and cattle plague. *Endeavour*, **26**, 87–91
- Brodbeck, D., Middleton, S., O'Neill, D., Summers, J. and Church, D. (2011) Companion animal practice based disease surveillance in the UK. *Épidémiologie et Santé Animale*, **59/60**, 38–40
- Brodey, R.S. (1970) Canine and feline neoplasia. *Advances in Veterinary Science and Comparative Medicine*, **14**, 309–354
- Brodey, R.S. (1979) The use of naturally occurring cancer in domestic animals for research into human cancer: general considerations and a review of canine skeletal osteosarcoma. *Yale Journal of Biology and Medicine*, **52**, 345–361
- Brodey, R.S. and Abt, D.A. (1976) Results of surgical treatment in 65 dogs with osteosarcoma. *Journal of the American Veterinary Medical Association*, **168**, 1032–1035
- Brodey, R.S., McDonough, S.F., Frye, F.L. and Hardy, W. D. (1970) Epidemiology of feline leukemia (lymphosarcoma). In: *Proceedings of the IVth International Symposium on Comparative Leukemia Research*, Cherry Hill, New Jersey, 1969. Ed. Dutcher, R.M. *Bibliotheca Haematologica*, **36**, 333–342
- Brønden, L.B., Eriksen, T. and Kristensen, A.T. (2009) Oral malignant melanomas and other head and neck neoplasms in Danish dogs – data from the Danish Veterinary Cancer Registry. *Acta Veterinaria Scandinavica*, **51**, 54; doi 10.1186/1751-0147-51-54
- Brønden, L.B., Flagstad, A. and Kristensen, A.T. (2007) Veterinary cancer registries in companion animal cancer: a review. *Veterinary and Comparative Oncology*, **5**, 133–144
- Brønden, L.B., Nielsen, S.S., Toft, N. and Kristensen, A.T. (2010) Data from the Danish Veterinary Cancer Registry on the occurrence and distribution of neoplasms in dogs in Denmark. *Veterinary Record*, **166**, 586–590
- Bronson, R.T. (1981) Age at death of necropsied intact and neutered cats. *American Journal of Veterinary Research*, **42**, 1606–1608
- Bronson, R.T. (1982) Variation in age at death of dogs of different sexes and breeds. *American Journal of Veterinary Research*, **43**, 2057–2059
- Bronsvort, B.M. deC., Toft, N., Bergmann, I.E., Sørensen, K.-J., Anderson, J. *et al.* (2006) Evaluation of three 3ABC ELISAs for foot-and-mouth disease non-structural antibodies using latent class analysis. *BMC Veterinary Research*, **2**, 30; doi 10.1186/1746-6148-2-30
- Brooks, R. (1990) Survey of the dog population of Zimbabwe and its level of rabies vaccination. *Veterinary Record*, **127**, 592–596
- Brooksby, J.B. (1972) Epizootiology of foot-and-mouth disease in developing countries. *World Animal Review*, **1**, 10–13

- Brooks-Pollock, E., Roberts, G.O. and Keeling, M.J. (2014) A dynamic model of bovine tuberculosis spread and control in Great Britain. *Nature*, **511**, 228–231
- Broom, D.M. and Fraser, A.F. (2015) *Domestic Animal Behaviour and Welfare*, 5th edn. CABI, Wallingford
- Brossard, M. (1998) The use of vaccines and genetically resistant animals in tick control. *Revue Scientifique et Technique, Office International des Epizooties*, **17**, 188–199
- Brothwell, D. and Sandison, A.T. (Eds) (1967) *Diseases in Antiquity*. Charles C. Thomas, Springfield
- Brown, C. and Bolin, C.A. (2000) *Emerging Diseases of Animals*. ASM Press, Washington DC
- Brown, D.C., Bell, M. and Rhodes, L. (2013) Power of treatment success definitions when the canine brief pain inventory is used to evaluate carprofen treatment for the control of pain and inflammation in dogs with osteoarthritis. *American Journal of Veterinary Research*, **74**, 1467–1473
- Brown, D.J. (2002) FMD in a parturient sheep flock (Letter). *Veterinary Record*, **151**, 127
- Brown, E.B.E., Adkin, A., Fooks, A.R., Stephenson, B., Medlock, J.M. *et al.* (2012a) Assessing the risks of West Nile virus-infected mosquitoes from transatlantic aircraft: implications for disease emergence in the United Kingdom. *Vector-Borne and Zoonotic Diseases*, **12**, 310–320
- Brown, J.D. (2011) Likert items and scales of measurement? *SHIKEN: JALT Testing & Evaluation SIG Newsletter*, **15** (1), 10–14
- Brown, J.L. (1964) The evolution of diversity in avian territorial systems. *Wilson Bulletin*, **76**, 160–169
- Brown, J.R. and Thornton, J.L. (1957) Percivall Pott (1714–1788) and chimney sweepers' cancer of the scrotum. *British Journal of Industrial Medicine*, **14**, 68–70
- Brown, L., Medlock, J. and Jakob-Hoff, V. (2014) Impact of drought on vector-borne diseases – how does one manage the risk? *Public Health*, **128**, 29–37
- Brown, L.D., Cai, T.T. and DasGupta, A. (2001) Interval estimation for a binomial proportion. *Statistical Science*, **16**, 101–123
- Brown, L.D., Cai, T.T. and DasGupta, A. (2002) Confidence intervals for a binomial proportion and asymptotic expansions. *The Annals of Statistics*, **30**, 160–201
- Brown, M. (Ed.) (2000) HACCP in the Meat Industry. Woodhead Publishing, Abington
- Brown, S.P., Cornforth, D.M. and Mideo, N. (2012b) Evolution of virulence in opportunistic pathogens: generalism, plasticity, and control. *Trends in Microbiology*, **20**, 336–342
- Brown, T. (2006) Did the US Army distribute smallpox blankets to Indians? Fabrication and falsification in Ward Churchill's genocide rhetoric. *Plagiary: Cross-Disciplinary Studies in Plagiarism, Fabrication and Falsification*, **1**, 100–129
- Brownlee, J. (1915) Historical note on Farr's theory of the epidemic. *British Medical Journal*, *ii*, 250–252
- Brownstein, J.S., Freifield, C.C. and Madoff, L.C. (2009) Digital disease detection – harnessing the Web for public health surveillance. *The New England Journal of Medicine*, **360**, 2153–2157
- Brownstein, J.S., Holford, T.R. and Fish, D. (2005) Effect of climate change on Lyme disease risk in North America. *EcoHealth*, **2**, 38–46
- Brozek, J., Oxman, A. and Schünemann, H. (2008) GRADEpro (Computer program). Version 3.2 for Windows. <http://www.grade-pro.org> (accessed 20 June 2016)
- Brun, A., Bárcena, J., Blanco, E., Borrego, B., Dory, D. *et al.* (2011) Current strategies for subunit and genetic viral veterinary vaccine development. *Virus Research*, **157**, 1–12
- Brunsdon, R.V. (1980) Principles of helminth control. *Veterinary Parasitology*, **6**, 185–215
- Bryans, J.T. (1964) Viral respiratory disease of horses. *Proceedings of the American Veterinary Medical Association*, **101**, 112–121
- BSI (1979) *Precision of Test Methods. I. Guide for the Determination of Repeatability and Reproducibility for a Standard Test Method for Inter-Laboratory Tests*. BS 5497, Part 1. British Standards Institution, London
- Buchanan, J.W. (1977) Chronic valvular disease (endocardiosis) in dogs. *Advances in Veterinary Science*, **21**, 75–106
- Bucher, H.C., Weinbacher, M. and Gyr, K. (1994) Influence of method of reporting a study on decision of physicians to prescribe drugs to lower cholesterol concentration. *British Medical Journal*, **309**, 761–764
- Buck, C. (1975) Popper's philosophy for epidemiologists. *International Journal of Epidemiology*, **4**, 159–168 (comment: 169–172)
- Buck, W.B. (1979) Animals as monitors of environmental quality. *Veterinary and Human Toxicology*, **21**, 277–284
- Buckingham, L. (2011) *Molecular Diagnostics: Fundamentals, Methods and Clinical Applications*, 2nd edn. F.A. Davis, Philadelphia
- Buckingham, S., McCalman, H., and Powell, H. (2013) *Grazing Systems*. Farming Connect/ADAS, Herts
- Buckland, A.W. (1882) Surgery and superstition in neolithic times. *Journal of the Anthropological Institute of Great Britain and Ireland*, **11**, 7–21
- Buckland, S.T., Anderson, D.R., Burnham, K.P. and Laake, J.L. (1993) *Distance Sampling*. Chapman and Hall, London

- Buckland, S.T., Anderson, D.R., Burnham, K.P., Laake, J. L., Borchers, D.L. *et al.* (2001) *Introduction to Distance Sampling: Estimating Abundance of Biological Populations*. Oxford University Press, Oxford
- Buddle, B.M., Skinner, M.A., Wedlock, D.N., Collins, D. M. and de Lisle, G.W. (2002) New generation vaccines and delivery systems for control of bovine tuberculosis in cattle and wildlife. *Veterinary Immunology and Immunopathology*, **87**, 177–185
- Buderer, N.M.F. (1996) Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. *Academic Emergency Medicine*, **3**, 895–900
- Bufalari, A., Adami, C., Angeli, G. and Short, C.E. (2007) Pain assessment in animals. *Veterinary Research Communications*, **31**, Suppl. 1, 55–58
- Buik, B. (2004) Flying high: air travel and lung disease. *Biologist*, **51**, 181–184
- Buisch, W.W., Hall, A.E., and McDaniel, H.A. (1984) 1983–1984 lethal avian influenza outbreak. *Proceedings of the United States Animal Health Association*, **88**, 430–446
- Bulfield, G., Siller, W.G., Wight, P.A. and Moore, K.J. (1984) X chromosome-linked muscular dystrophy (*mdx*) in the mouse. *Proceedings of the National Academy of Sciences of the United States of America*, **81**, 1189–1192
- Bullock, W.L. (1992) The origin of species and the origins of disease: a tale of two theories. *Perspectives on Science and the Christian Faith*, **44**, 36–44
- Bulmer, M.G. (1994) *Theoretical Evolutionary Ecology*. Sinauer Associates, Sunderland
- Bulpitt, C.J. (1996) *Randomized Controlled Clinical Trials*, 2nd edn. Kluwer Academic Publishers, Dordrecht
- Bumstead, N. (1998) Genetic resistance to avian viruses. *Revue Scientifique et Technique, Office International des Epizooties*, **17**, 249–255
- Buntinx, F., Aertgeerts, B. and Macaskill, P. (2009) Guidelines for conducting systematic reviews of studies evaluating the accuracy of diagnostic tests. In: *The Evidence Base of Clinical Diagnosis: Theory and Methods of Diagnostic Research*, 2nd edn. Eds Knottnerus, J.A. and Buntinx, F., pp. 180–212. Blackwell Publishing, Oxford
- Burch, P.R.J. (1966) Age and sex distributions for some idiopathic non-malignant conditions in man. Some possible implications for growth-control and natural and radiation-induced aging. In: *Radiation and Ageing*. Proceedings of a Colloquium held in Semmering, Austria, June 23–24, 1966. Eds Lindop, P.J. and Sacher, G.A., pp. 117–155. Taylor and Francis, London
- Burchell, A. (1983) Economic evaluation of health schemes. In: *Society for Veterinary Epidemiology and Preventive Medicine*, Proceedings, Southampton, 12–13 April 1983. Ed. Thrusfield, M.V., pp. 148–155
- Burg, J.G. (2001) Seasonal activity and spatial distribution of host-seeking adults of the tick *Dermacentor variabilis*. *Medical and Veterinary Entomology*, **15**, 413–421
- Burgdorfer, W. (1960) Colorado tick fever: II The behaviour of Colorado tick fever virus in rodents. *Journal of Infectious Diseases*, **107**, 384–388
- Burger, H.J. (1976) Trichostrongyle infestation in autumn on pastures grazed exclusively by cows or by calves. *Veterinary Parasitology*, **1**, 359–366
- Burgess, V.J., Kelly, D., Robertson, A.W. and Ladley, J.J. (2005) Positive effects of forest edges on plant reproduction: literature review and case study of bee visitation to flowers of *Peraxilla tetrapetala* (Loranthaceae). *New Zealand Journal of Ecology*, **30**, 179–190
- Burgin, L.E., Gloster, J., Sanders, C., Mellor, P.S., Gubbins, S. *et al.* (2013) Investigating incursions of bluetongue virus using a model of long-distance *Culicoides* biting midge dispersal. *Transboundary and Emerging Diseases*, **60**, 263–272
- Burke, J. (1985) What the doctor ordered. In: *The Day the Universe Changed*. pp. 195–236. British Broadcasting Corporation, London
- Burke, W., Tarini, B., Press, N.A. and Evans, J.P. (2011) Genetic screening. *Epidemiologic Reviews*, **33**, 148–164
- Burleigh, A., McMahon, S. and Kiely, S. (2015) Owned dog and cat populations in remote Indigenous communities in the Northern Territory: a retrospective study. *Australian Veterinary Journal*, **93**, 145–150
- Burnet, M. and White, D.O. (1972). *Natural History of Infectious Disease*, 4th edn. Cambridge University Press, London
- Burnham, K.P. and Anderson, D.R. (2004) Multimodal inference: understanding AIC and BIC in model selection. *Sociological Methods in Research*, **33**, 261–304
- Burns, P.B., Rohrich, R.D. and Chung, K.C. (2011) The levels of evidence and their role in evidence-based medicine. *Plastic & Reconstructive Surgery*, **128**, 305–310
- Burrell, G. and Seibert, F. (1914) Experiments with small animals and carbon monoxide. *Journal of Industrial and Engineering Chemistry*, **6**, 241–244
- Burridge, M.J. and McCarthy, S.M. (1979) The Florida veterinary clinical data retrieval system. In: *Proceedings of an International Symposium on Animal Health and Disease Data Banks*, 4–6 December 1978, Washington DC, pp. 155–161. Miscellaneous Publication No. 1381. United States Department of Agriculture, Washington DC
- Burridge, M.J., Simmons, L.-A., Peter, T.F. and Mahan, S. M. (2002) Increasing risks of introduction of heartwater onto the American Mainland associated with animal movements. In: *The Domestic Animal/*

- Wildlife Interface: Issues for Disease Control, Conservation, Sustainable Food Production, and Emerging Diseases. Annals of the New York Academy of Sciences*, Vol. 969. Eds Gibbs, E.P.J. and Bokma, B.H., pp. 269–274. The New York Academy of Sciences, New York
- Burrows, R. (1977) Herpes virus infections of animals – a brief review. *Journal of Antimicrobial Chemotherapy*, **3**, Suppl. A, 9–14
- Burt, W. (1943) Territoriality and home range concepts as applied to mammals. *Journal of Mammalogy*, **24**, 346–352
- Bush, E.G. and Gardner, A. (1995) Animal health surveillance in the United States via the National Animal Health Monitoring System (NAHMS). *Épidémiologie et Santé Animale*, **27**, 113–126
- Butler, J.R.A. and Bingham, J. (2000) Demography and dog-human relationships of the dog population in Zimbabwean communal lands. *Veterinary Record*, **147**, 442–446
- Butler, M.P., Graveling, R.A., Pilkington, A. and Boyle, A. L. (1999) *Non-Auditory Effects of Noise at Work: A Critical Review of the Literature Post 1988*. HSE Books, Sudbury
- BVA (2015) *Canine Health Schemes*. British Veterinary Association, London. <http://www.bva.co.uk/Canine-Health-Schemes/> (accessed 10 August 2015)
- Byaruhanga, C., Oosthuizen, M.C., Collins, N.E. and Knobel, D. (2015) Using participatory epidemiology to investigate management options and relative importance of tick-borne diseases among transhumant zebu cattle in Karamoja Region, Uganda. *Preventive Veterinary Medicine*, **122**, 287–297
- Byrt, T., Bishop, J. and Carlin, J.B. (1993) Bias, prevalence and kappa. *Journal of Clinical Epidemiology*, **46**, 423–429
- Cable, J. and Harris, P. (2003) Fish parasites: the fish farmer's foe. *Biologist*, **50**, 178–182
- Cabrera, V.E. (2014) Economics of fertility in high-yielding dairy cows on confined TMR systems. *Animal*, **8**, s1, 211–221
- Cain, C.E. (1999) Results from zooarcheological analysis at Axum, Ethiopia. *Archaeozoologia*, **10**, 27–46
- Calabrese, E.J. (1986) Animal extrapolation and the challenge of human heterogeneity. *Journal of Pharmaceutical Sciences*, **75**, 1041–1046
- Calba, C., Antione-Moussiaux, N., Charrier, F., Hendriks, P., Saegerman, C. *et al.* (2015a) Applying participatory approaches in the evaluation of surveillance systems: a pilot study on swine fevers in Corsica. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Ghent, 25–27 March 2015*. Eds Thulke, H.-H. and Verheyen, K., pp. 177–188
- Calba, C., Goutard, F.L., Hoinville, L., Hendriks, P., Lindberg, A. *et al.* (2015b) Surveillance systems evaluation: a systematic review of the existing approaches. *BMC Public Health*, **15**, 448; doi 10.1186/s12889-015-1791-5
- Calderón-Garcidueñas, L., Engel, R., Mora-Tiscareño, A., Styner, M., Gómez-Garza, G. *et al.* (2011) Exposure to severe urban air pollution influences cognitive outcomes, brain volume and systemic inflammation in clinically healthy children. *Brain and Cognition*, **77**, 345–355
- Calderón-Garcidueñas, L., Mora-Tiscareño, A., Ontiveros, E., Gómez-Garza, G., Barragán-Mejí, G. *et al.* (2008) Air pollution, cognitive defects and brain abnormalities: a pilot study with children and dogs. *Brain and Cognition*, **68**, 117–127
- Caldow, G.L. (1984) The potential for veterinary involvement in the reproductive management of beef suckler herds. MSc Thesis, University of Edinburgh
- Caldow, G., Gunn, G., Humphry, R., Crawshaw, M. and Rushbridge, S. (2001) Biosecurity and screening for disease. *Irish Veterinary Journal*, **54**, 461–470
- Caley, P. and Ramsay, D. (2001) Estimating disease transmission in wildlife, with emphasis on leptospirosis and bovine tuberculosis in possums, and effects on fertility control. *Journal of Applied Ecology*, **38**, 1362–1370
- Calisher, C.H., Childs, J.E., Field, I.E., Holmes, K.V. and Schountz, T. (2006) Bats: important reservoir hosts of emerging viruses. *Clinical Microbiology Reviews*, **19**, 531–545
- Callaos, N. and Callaos, B. (2002) Toward a systemic notation of information: practical consequences. *Informing Science*, **5**, 1–11
- Callens, M., De Clercq, K., Gruia, M. and Danes, M. (1998) Detection of foot and mouth disease by reverse transcription polymerase chain reaction and virus contact sheep without clinical signs of foot-and-mouth disease. *Veterinary Quarterly*, **20**, Suppl. 2, S37–S40
- Calnek, B.W. (Ed.) (1991) *Diseases of Poultry*, 9th edn. Iowa State University Press, Ames
- Calvete, C., Estrada, R., Villafuerte, R., Osácar, J.J. and Lucientes, J. (2002) Epidemiology of viral haemorrhagic disease and myxomatosis in a free-living population of wild rabbits. *Veterinary Record*, **150**, 776–782
- Cameron, A., Njeumi, F., Chibeu, D. and Martin, T. (2014) *Risk-Based Disease Surveillance*. FAO Animal Production and Health Manual No. 17. Food and Agriculture Organization of the United Nations, Rome
- Cameron, A.R. and Baldock, F.C. (1998a) A new probability formula for surveys to substantiate freedom from disease. *Preventive Veterinary Medicine*, **34**, 1–17

- Cameron, A.R. and Baldock, F.C. (1998b) Two-stage sampling in surveys to substantiate freedom from disease. *Preventive Veterinary Medicine*, **34**, 19–30
- Cameron, D. and Jones, I.G. (1983) John Snow, the Broad Street pump and modern epidemiology. *International Journal of Epidemiology*, **12**, 393–396
- Cameron, J. and Wade-Gery, W. (1995) Addressing uncertainty. In: *Environmental Policy in Search of New Instruments*. Ed. Dente, B., pp. 95–142. Kluwer Academic Publishers, Dordrecht
- Campbell, C.L. (1989) Geographical information system applications in animal health. In: Proceedings of the 93rd Annual Meeting of the United States Animal Health Association. Las Vegas, Nevada, 28 October–3 November 1989, pp. 47–62
- Campbell, M.J., Julious, S.A. and Altman, D.G. (1995) Estimating sample sizes for binary, ordered categorical and continuous outcomes in two group comparisons. *British Medical Journal*, **311**, 1145–1148
- Campbell, W.C. (Ed.) (1983) *Trichinella and Trichinosis*. Plenum Press, New York
- Cané, B.G., Rodríguez Toledo, J., Faczuk, A., Leanes, L.F., Manetti, J.C. *et al.* (1995) Quantitative risk analysis applied to innocuity and potency tests on the oil-adjuvanted vaccine against foot and mouth disease in Argentina. *Revue Scientifique et Technique, Office International des Epizooties*, **14**, 1109–1119
- Canner, P.L., Krol, W.F. and Forman, S.A., (1983) External quality control programs. *Controlled Clinical Trials*, **4**, 441–466
- Cannon, R. (1993) ANADIS: monitoring and eradication of brucellosis and tuberculosis in Australia. *Agricultural Systems and Information Technology*, **5** (1), 11–13
- Cannon, R.M. (2001) Sense and sensitivity – designing surveys based on an imperfect test. *Preventive Veterinary Medicine*, **49**, 141–163
- Cannon, R.M. and Roe, R.T. (1982) *Livestock Disease Surveys: A Field Manual for Veterinarians*. Australian Government Publishing Service, Canberra
- Cannon, R.M., Morris, R.S., Williamson, N.B., Cannon, C. M. and Blood, D.C. (1978) A health program for commercial dairy herds. 2. Data processing. *Australian Veterinary Journal*, **54**, 216–230
- Cannon, W.B. (1914) The emergency function of the adrenal medulla in pain and major emotions. *American Journal of Physiology*, **33**, 356–372
- Caplan, G. and Grunebaum, H. (1967) Perspectives on primary prevention: a review. *Archives of General Psychiatry*, **17**, 331–346
- Caporale, V., Giovannini, A., Di Francesco, C. and Calistri, P. (2001) Importance of traceability of animals and animal products in epidemiology. *Revue Scientifique et Technique, Office International des Epizooties*, **20**, 372–378
- Capron, A. and Dessaint, J.-P. (1992) Survival strategies of parasites and their immunocompetent hosts. *Advances in Neuroimmunology*, **2**, 181–198
- Capua, I. and Marangon, S. (2005) Currently available tools and strategies for emergency vaccination in case of avian influenza. In: *Avian Influenza: Prevention and Control*. Eds Schrijver, R.S. and Koch, G., pp. 59–74. Springer, Dordrecht
- Card, N.A. (2011) *Applied Meta-Analysis for Social Science Research*. Guilford Press, New York
- Cargo, M. and Mercer, S.L. (2008) The value and challenges of participatory research: strengthening its practice. *Annual Review of Public Health*, **29**, 325–350
- Carifio, J. and Perla, R.J. (2007) Ten common misunderstandings, misconceptions, persistent myths and urban legends about Likert scales and Likert response formats and their antidotes. *Journal of Social Sciences*, **3**, 106–116
- Carles, A.B. (1986) The levels of, and constraints to, productivity of goats and sheep at Ngurunit in Marsabit District. In: *Smallstock and Cattle Productivity, Nutrition and Disease in Northern Kenya*. Integrated Project in Arid Lands, Technical Report No. E-8. Ed. Field, C.R., pp. 33–118. UNESCO, Nairobi
- Carles, A.B. (1992) The non-medicinal prevention of livestock disease in African rangeland ecosystems. *Preventive Veterinary Medicine*, **12**, 165–173
- Carley, S., Dosman, S., Jones, S.R. and Harrison, M. (2005) Simple nomograms to calculate sample size in diagnostic studies. *Emergency Medicine Journal*, **22**, 180–181 (erratum: <http://emj.bmj.com/content/22/3/180/suppl/DC1?ck=nck>) (accessed 17 June 2015)
- Carlson, M.D.A. and Morrison, R.S. (2009) Study design, precision, and validity in observational studies. *Journal of Palliative Medicine*, **12**, 77–82
- Carmichael, L.E. and Binn, L.N. (1981) New enteric viruses in the dog. *Advances in Veterinary Science and Comparative Medicine*, **25**, 1–37
- Carmichael, L.E., Strandberg, J.D. and Barnes, F.D. (1965) Identification of a cytopathogenic agent infectious for puppies as a canine herpesvirus. *Proceedings of the Society of Experimental Biology and Medicine*, **120**, 644–650
- Carné, S., Gipson, T.A., Rovai, M., Merkel, R.C. and Caja, G. (2009) Extended field test on the use of visual ear tags and electronic boluses for the identification of different goat breeds in the United States. *Journal of Animal Science*, **87**, 2419–2427
- Carpenter, F.L., Paton, D.C. and Hixon, M.A. (1983) Weight gain and adjustment of feeding territory size in migrant hummingbirds. *Proceedings of the National*

- Academy of Sciences of the United States of America*, **80**, 7269–7263
- Carpenter, J.R., Schwarzer, G., Rücker, G. and Küntler, R. (2009) Empirical evaluation showed that the Copas selection model provided a useful summary in 80% of meta-analyses. *Journal of Clinical Epidemiology*, **62**, 624–631
- Carpenter, T.E. (1988) Stochastic epidemiologic modeling using a microcomputer spreadsheet package. *Preventive Veterinary Medicine*, **5**, 159–168
- Carpenter, T.E. (2001) Methods to investigate spatial and temporal clustering in veterinary epidemiology. *Preventive Veterinary Medicine*, **48**, 303–320
- Carpenter, T.E. (2002) Evaluation and extension of the cusum technique with an application to *Salmonella* surveillance. *Journal of Laboratory Diagnostic Investigation*, **14**, 211–218
- Carpenter, T.E., Berry, S.L. and Glenn, J.S. (1987) Economics of *Brucella ovis* control in sheep: computerized decision-tree analysis. *Journal of the American Veterinary Medical Association*, **190**, 983–987
- Carr, E.C.J. and Worth, A. (2001) The use of the telephone interview for research. *Nursing Times Research*, **6**, 511–524
- Carrique-Mas, J.J., Medley, G.F. and Green, L.E. (2008) Risks for bovine tuberculosis in British cattle farms restocked after the foot and mouth disease epidemic of 2001. *Preventive Veterinary Medicine*, **84**, 85–93
- Carruthers, H. (2009) Disease surveillance in small animal practice. *In Practice*, **31**, 356–358
- Carruthers, I. and Chambers, R. (1981) Rapid appraisal for rural development. *Agricultural Administration*, **8**, 407–422
- Carson, R.L. (1963) *Silent Spring*. Hamish Hamilton, London
- Carter, C.A. and Hule, J. (2004) Market effects of searching for mad cows. *ARE Update*, **8** (1), 5–7. Giannini Foundation of Agricultural Economics, University of California
- Carter, H.B. (1939) Longevity and fertility in S. African merino ewes. *Pastoral Review*, **49**, 266–267
- Carter, K.C. (Translator and Ed.) (1983) *Ignaz Semmelweis: The Etiology, Concept, and Prophylaxis of Childbed Fever*. University of Wisconsin Press, Madison
- Cartwright, S.F. and Huck, R.A. (1967) Viruses isolated in association with herd infertility, abortions and stillbirths in pigs. *Veterinary Research*, **81**, 196–197
- Casal, J., Moreso, J.M., Planas-Cuchí, E. and Casal, J. (1997) Simulated airborne spread of Aujeszky's disease and foot-and-mouth disease. *Veterinary Record*, **140**, 672–676
- Case, J.T. (1994) SNOMED (Review). *Preventive Veterinary Medicine*, **21**, 269–273
- Casella, G. and Berger, R.L. (1987) Reconciling Bayesian and frequentist evidence in the one-sided testing problem. *Journal of the American Statistical Association*, **82**, 106–111 (comment: 112–137)
- Cason, R. and Geering, W.A. (1980) An economic evaluation of possible Australian policies to meet the threat of an exotic pest – the screw-worm fly, *Chrysomya bezziana*. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 7–11 May 1979. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 463–470. Australian Government Publishing Service, Canberra
- Casswell, S.A. (1966) A simplified calculation of maximum vertical velocities in mountain lee waves. *Meteorological Magazine*, **95**, 68–80
- Castelhano-Carlos, M.J. and Baumans, V. (2009) The impact of light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats. *Laboratory Animals*, **43**, 311–327
- Caswell, J.A. and Hooker, N.H. (1996) HACCP as an international trade standard. *American Journal of Agricultural Economics*, **78**, 775–779
- Catanzaro, T.E. and Hall, T.H. (2002) *Veterinary Medicine and Practice 25 Years in the Future and the Economic Steps to Get There*. Iowa State Press, Ames
- Catley, A. (1999) *Methods on the Move: A Review of Veterinary Uses of Participatory Approaches and Methods Focussing on Experiences in Dryland Africa*. International Institute for Environment and Development, London
- Catley, A. (2000) The use of participatory appraisal by veterinarians in Africa. *Revue Scientifique et Technique, Office International des Epizooties*, **19**, 702–714
- Catley, A. (2004) Validation of participatory appraisal for use in animal-health information systems in Africa. PhD Thesis, University of Edinburgh
- Catley, A. (2005) *Participatory Epidemiology: A Guide for Trainers*. African Union/Interafrican Bureau for Animal Resources, Nairobi
- Catley, A. (2009) From marginal to normative: institutionalizing participatory epidemiology. In: *Farmer First Revisited: Innovation for Agricultural Research and Development*. Eds Scoones, I. and Thompson, J., pp. 247–254. Practical Action Publishing, Rugby
- Catley, A. and Leyland, T. (2001) Community participation and the delivery of veterinary services in Africa. *Preventive Veterinary Medicine*, **49**, 95–113
- Catley, A. and Mariner, J. (Eds) (2001) *Participatory Epidemiology: Lessons Learned and Future Directions*. Proceedings of an international workshop held in

- Addis Ababa, Ethiopia, 15th to 17th November 2001. Organization of African Unity/Interafrican Bureau of Animal Resources, Nairobi
- Catley, A. and Mariner, J. (2002) *Where There is No Data: Participatory Approaches to Veterinary Epidemiology in Pastoral Areas of the Horn of Africa*. Issue Paper No. 10. International Institute for Environment and Development, London
- Catley, A., Alders, R.G. and Wood, J.L.N. (2012) Participatory epidemiology: approaches, methods, experiences, *The Veterinary Journal*, **191**, 151–160
- Catley, A., Blakeway, S. and Leyland, T. (Eds) (2002a) *Community-based Animal Health Care: a Practical Guide to Improving Primary Veterinary Services*. ITDG Publishing, London
- Catley, A., Burns, J., Abebe, D. and Suji, O. (2013) *Participatory Impact Assessment: A Design Guide*. Feinstein International Center, Tufts University, Somerville
- Catley, A., Chibunda, R.T., Ranga, E., Makunu, S., Magayane, F.T. *et al.* (2004) Participatory diagnosis of heat-intolerance syndrome in cattle in Tanzania and association with foot-and-mouth disease. *Preventive Veterinary Medicine*, **65**, 17–30
- Catley, A., Irungu, P., Simiyu, K., Dadye, J., Mwakio, W. *et al.* (2002b) Participatory investigations of bovine trypanosomiasis in Tana River District, Kenya. *Medical and Veterinary Entomology*, **16**, 55–66
- Caughley, G. and Sinclair, A.R.E. (1994) *Wildlife Ecology and Management*. Blackwell Scientific, Oxford
- CDC (2010) *Emergency Preparedness and Response: Bioterrorism Agents/Diseases*. Centers for Disease Control, Atlanta. <http://emergency.cdc.gov/agent/agentlist.asp> (accessed 30 July 2015)
- CEC (2000) *Communication from the Commission on the Precautionary Principle*. Commission of the European Communities, Brussels
- CEC (2008) *Council Conclusions on Antimicrobial Resistance (AM)*, Luxembourg, 10 June 2008. Council of the European Union, Brussels. http://www.eu2008.si/en/News_and_Documents/Council_Conclusions/June/0609_EPSCO-AMR.pdf (accessed 28 July 2015)
- Cecchini, G., Bekele, T. and Kasali, O.B. (1992) The effect of repeated freezing and thawing of serum on the activity of antibodies. *Veterinary Research Communications*, **16**, 425–428
- CEPS (2002) *The Precautionary Principle in the European Union & Its Impact on International Trade Relations*. Centre for European Policy Studies, Brussels
- Cernicchiaro, N., Renter, D.G., White, B.J., Babcock, A.H. and Fox, J.T. (2012) Associations between weather conditions during the first 45 days after feedlot arrival and daily respiratory disease risks in autumn-placed feeder cattle in the United States. *Journal of Animal Science*, **90**, 1328–1337
- Çetinkaya, B., Erdogan, H.M. and Morgan, K.L. (1998) Prevalence, incidence and geographical distribution of Johne's disease in cattle in England and the Welsh borders. *Veterinary Record*, **143**, 265–269
- Cha, E., Hertl, J.A., Bar, D. and Gröhn, Y.T. (2010) The cost of different types of lameness in dairy cows calculated by dynamic programming. *Preventive Veterinary Medicine*, **97**, 1–8
- Chabner, B.A., Boral, A.L. and Multani, P. (1998) Translational research: walking the bridge between idea and cure. *Cancer Research*, **58**, 4211–4216
- Chain, P. (1980) Public participation and communications in Latin American disease control programs. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 4–11 May 1979. Eds Geering, W.A., Roe, R. T. and Chapman, L.A., pp. 335–340. Australian Government Publishing Service, Canberra
- Chalmers, A.W. (1968) African horse sickness. *Equine Veterinary Journal*, **1**, 83–86
- Chamberlain, A.T. and Wassell, T.R. (1995) The size and cost of the estimation error when using analysis of a sample of a dairy herd to assess the whole herd performance. *Preventive Veterinary Medicine*, **23**, 65–71
- Chambers, J.M., Cleveland, W.S., Kleiner, B. and Tukey, P.A. (1983) *Graphical Methods for Data Analysis*. Wadsworth International Group, Belmont
- Chambers, R. (1983) *Rural Development: Putting the Last First*. Longman Scientific and Technical, New York
- Chambers, R. (1992) *Rural Appraisal: Rapid, Relaxed and Participatory*. Institute of Development Studies IDS Discussion Paper 311. Institute of Development Studies, Brighton
- Chambers, R. (1994) Participatory Rural Appraisal (PRA): analysis of experience. *World Development*, **22**, 1253–1268
- Chambers, R. (1997) *Whose Reality Counts? Putting the Last First*. Intermediate Technology Publications, London
- Chambers, R. (2010) A revolution whose time has come? The win-win of quantitative participatory approaches and methods. *IDS Bulletin*, **41** (6), 45–55
- Chan, A.-W., Tetzlaff, J.M., Gøtzsche, P.C., Altman, D.G., Mann, H. *et al.* (2013) SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *British Medical Journal*, **346**, e7586; doi 10.1136/bmj.e7586 DOI:10.1136%2Fbmj.e7586#pmc_ext
- Chang, H., Ma, Y., Lin, T., Cong, G., Du, J. *et al.* (2013) Foot-and-mouth disease virus carrier status in Bos grunniens yaks. *Virology Journal*, **10**, 81; doi 10.1186/1743-422X-10-81

- Chang, P.H., Kuo, S.T., Lai, S.H., Yang, H.S., Ting, Y.Y. *et al.* (2005a) Herpes-like virus infection causing mortality of cultured abalone *Haliotis diversicolor supertexta* in Taiwan. *Diseases of Aquatic Organisms*, **65**, 23–27
- Chang, S.C., Chang, C.C., Chang, T.J. and Wong, M.L. (2005b) Prognostic factors associated with survival two years after surgery in dogs with malignant mammary tumors: 79 cases (1998–2002). *Journal of the American Veterinary Medical Association*, **227**, 1625–1629
- Chang, Y. and Moore, P.S. (2012) Merkel cell carcinoma: a virus-induced human cancer. *Annual Review of Pathology: Mechanisms of Disease*, **7**, 123–144
- Chapman, C.A. and Peres, C.A. (2001) Primate conservation in the new millennium: the role of scientists. *Evolutionary Anthropology*, **10**, 16–33
- Chapman, C.A., Gillespie, T.R. and Goldberg, T.L. (2005) Primates and the ecology of their infectious diseases: how will anthropogenic change affect host-parasite interactions? *Evolutionary Anthropology*, **14**, 134–144
- Chapman, P.A. (2000) Sources of *Escherichia coli* O157 and experiences over the past 15 years in Sheffield, UK. *Journal of Applied Microbiology Symposium Supplement*, **88**, 51S–60S
- Chapman, R.N. (1928) The quantitative analysis of environmental factors. *Ecology*, **9**, 111–122
- Chapman, R.N. (1931) *Animal Ecology with Special Reference to Insects*. McGraw-Hill, New York
- Charette, R.N. (1989) *Software Engineering Risk Analysis and Management*. McGraw-Hill, New York
- Charles, D.R. and Payne, C.G. (1966) The influence of graded levels of atmospheric ammonia on chickens. II Effects on the performance of laying hens. *British Poultry Science*, **7**, 189–198
- Charlier, J., Bennema, S.C., Caron, Y., Counotte, M., Ducheyne, E. *et al.* (2011) Towards assessing fine-scale indicators for the spatial transmission risk of *Fasciola hepatica* in cattle. *Geospatial Health*, **5**, 239–245
- Charrel, R.N., Zaki, A.M., Attoui, H., Fakhee, M., Billoir, F. *et al.* (2001) Complete coding sequence of the Alkhurma virus, a tick-borne flavivirus causing severe hemorrhagic fever in humans in Saudi Arabia. *Biochemical and Biophysical Research Communications*, **287**, 455–461
- Charron, D.F. (Ed.) (2012) *Ecohealth Research in Practice: Innovative Applications of an Ecosystem Approach to Health*. International Development Research Centre, Ottawa/Springer, New York
- Chatikobo, P., Choga, T., Ncube, C. and Mutambara, J. (2013) Participatory diagnosis and prioritization of constraints to cattle production in some smallholder farming areas of Zimbabwe. *Preventive Veterinary Medicine*, **109**, 327–333
- Chauvin, C., Le Bouquin-Leneveu, S., Hardy, A., Haguet, D., Orand, J.P. *et al.* (2005) An original system for the continuous monitoring of antimicrobial use in poultry production in France. *Journal of Veterinary Pharmacology and Therapeutics*, **28**, 525–523
- Chavance, M., Dellatolas, G. and Lellouch, J. (1992) Correlated nondifferential misclassifications of disease and exposure: application to a cross-sectional study of the relation between handedness and immune disorders. *International Journal of Epidemiology*, **21**, 537–546
- Chaves, L.F., Cohen, J.M., Pascual, M. and Wilson, M. (2008) Social exclusion modifies climate and deforestation impacts on a vector-borne disease. *PLoS Neglected Tropical Diseases*, **2**, 2, e176; doi 10.1371/journal.pntd.0000176
- Cheeseman, C.L., Jones, G.W., Gallagher, J. and Mallinson, P.J. (1981) The population structure, density and prevalence of tuberculosis (*Mycobacterium bovis*) in badgers (*Meles meles*) from four areas in south-west England. *Journal of Applied Ecology*, **18**, 795–804
- Cheeseman, C.L., Wilesmith, J.W. and Stuart, F.A. (1989) Tuberculosis: the disease and its epidemiology in the badger, a review. *Epidemiology and Infection*, **103**, 113–125
- Cheetham, B.F. and Katz, M.E. (1995) A role for bacteriophages in the evolution and transfer of bacterial virulence determinants. *Molecular Microbiology*, **18**, 201–208
- Chen, P. and Popovich, P. (2002) *Correlation: Parametric and Nonparametric Measures*. Sage Publications, Thousand Oaks
- Chen, R. and Holmes, E.C. (2006) Avian influenza virus exhibits rapid evolutionary dynamics. *Molecular Biology and Evolution*, **23**, 2336–2341
- Chen, Z., Peto, R., Collins, R., MacMahon, S., Lu, J. *et al.* (1991) Serum cholesterol concentration and coronary heart disease in population with low cholesterol concentrations. *British Medical Journal*, **303**, 276–282
- Chenoweth, P.J. and Sanderson, M.W. (2001) Health and production management in beef cattle breeding herds. In: *Herd Health: Food Animal Production Medicine*, 3rd edn. Ed. Radostits, O.M., pp. 509–580. Saunders, Philadelphia
- Chernick, M.R. (2008) *Bootstrap Methods: A Guide for Practitioners and Researchers*, 2nd edn. John Wiley and Sons, Hoboken
- Chesney, C.J. (2001) Systematic review of evidence for the prevalence of food sensitivity in dogs. *Veterinary Record*, **148**, 445–448
- Chevalier, V., Dupressoir, A., Tran, A., Diop, O.M., Gottland, C. *et al.* (2010) Environmental risk factors of

- West Nile infection in the Senegal River basin. *Epidemiology and Infection*, **138**, 1601–1609
- Cheville, N.F. (1975) The gray collie syndrome (cyclic neutropenia). *Journal of the American Animal Hospital Association*, **11**, 350–352
- Chiang, C.C. (1984) *The Life Table and its Applications*. Robert E. Kreiger, Malabar
- Chibeu, D.M. (2011) Animal Resources Information System (ARIS) of the AU-IBAR. In: *Challenges of Animal Health Information Systems and Surveillance for Animal Diseases and Zoonoses*. Proceedings of the International Workshop organized by FAO, 23–26 November 2010, Rome, Italy. Animal Production and Health Proceedings No. 14, pp. 31–32. Food and Agriculture Organization of the United Nations, Rome
- Childs, J.E., Robinson, L.E., Sadek, R., Madden, A., Miranda, M.E. *et al.* (1998) Density estimates of rural dog populations and an assessment of marking methods during a rabies vaccination campaign in the Philippines. *Preventive Veterinary Medicine*, **33**, 207–218
- Choi, E.C.K. and Pak, A.W.P. (2005) Bias, overview. In: *Encyclopedia of Biostatistics*, 2nd edn, Vol. 1. Eds Armitage, P. and Colton, T., pp. 331–338. John Wiley and Sons, Chichester
- Choi, H. and Varian, H. (2012) Predicting the present with Google Trends. *Economic Record*, **88**, Suppl. s1, 2–9
- Choi, J., Peters, M. and Mueller, R.O. (2010) Correlational analysis of ordinal data: from Pearson's *r* to Bayesian polychoric correlation. *Asia Pacific Education Review*, **11**, 459–466
- Chomel, B.B. (1998) New emerging zoonoses: a challenge and an opportunity for the veterinary profession. *Clinical Immunology, Microbiology and Infectious Diseases*, **21**, 1–14
- Chowell, G., Nishiura, H. and Bettencourt, L.M. (2007) Comparative estimation of the reproduction number for pandemic influenza from daily case notification data. *Journal of the Royal Society Interface*, **4**, 155–166
- Christensen, J., Ellegaard, B., Petersen, B.K., Willeberg, P. and Mousing, J. (1994) Pig health and production surveillance in Denmark: sampling design, data recording and measures of disease frequency. *Preventive Veterinary Medicine*, **20**, 47–61
- Christensen, L.S., Mortensen, S., Bøtner, A., Strandbygaard, S.B., Rønsholt, L. *et al.* (1993) Further evidence of long distance airborne transmission of Aujeszky's disease (pseudorabies) virus. *Veterinary Record*, **132**, 317–321
- Christensen, L.S., Mousing, J., Mortensen, S., Soerensen, K.J., Strandbygaard, S.B. *et al.* (1990) Evidence of long distance transmission of Aujeszky's disease (pseudorabies) virus. *Veterinary Record*, **127**, 471–474
- Christenson, J. and Robinson, J. (1980) *Community Development in America*. Iowa State University Press, Iowa
- Christiansen, K.H. (1980) Laboratory management and disease surveillance information system. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 7–11 May, 1979. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 59–64. Australian Government Publishing Service, Canberra
- Christiansen, K.H. and Carpenter, T.E. (1983) Linear programming as a planning tool in the New Zealand brucellosis eradication scheme. In: Proceedings of the Third International Symposium on Veterinary Epidemiology and Economics. Arlington, Virginia, 6–10 September 1982, pp. 369–376. Veterinary Medical Publishing Company, Edwardsville
- Christley, R.M. and French, N.P. (2003) Small-world topology of UK racing: the potential for rapid spread of infectious agents. *Equine Veterinary Journal*, **35**, 586–589
- Christley, R.M. and Reid, S.W.J. (2003) No significant difference: use of statistical methods for testing equivalence in clinical veterinary literature. *Journal of the American Veterinary Medical Association*, **222**, 433–437
- Christley, R.M., Morgan, K.L., Parkin, T.D.H. and French N.P. (2003) Factors related to the risk of neonatal mortality, birth weight and serum immunoglobulin concentration in lambs. *Preventive Veterinary Medicine*, **57**, 209–226
- Christley, R.M., Mort, M., Wynne, B., Wastling, J.M., Heathwaite, A.L. *et al.* (2013) 'Wrong, but useful': negotiating uncertainty in infectious disease modelling. *PloS ONE*, **8**, e76277; doi 10.1371/journal.pone.0076277. (Correction: Christley, R.M., Mort, M., Wynne, B., Wastling, J.M., Heathwaite, A.L. *et al.* (2014) Correction: 'Wrong, but useful': negotiating uncertainty in infectious disease modelling. *PLoS ONE*, **9**; doi 10.1371/annotation/240b4b75-2b98-4c22-bf3b-f700369f594b)
- Christopher, M.M. (2007) Improving the quality of reporting of studies of diagnostic accuracy: let's STARD now. *Veterinary Clinical Pathology*, **36**, 6
- Chung, S.I., Livingston, C.W., Jones, C.W. and Collisson, E.W. (1991) Cache Valley virus infection in Texas sheep flocks. *Journal of the American Veterinary Medical Association*, **199**, 337–340
- Chyou, P.-H. (2007) Patterns of bias due to differential misclassification by case-control status in a case-control study. *European Journal of Epidemiology*, **22**, 7–17
- Cicchetti, D.V. and Allison, T. (1971) A new procedure for assessing reliability of scoring EEG sleep

- recordings. *American Journal of EEG Technology*, **11**, 101–109
- Cimino, J.J. (1998) Desiderata for controlled medical vocabularies in the twenty-first century. *Methods of Information in Medicine*, **37**, 394–403
- Cintrón-Arias, A., Castillo-Chávez, C., Bettencourt, L.M. A., Lloyd, A.L. and Banks, H.T. (2009) The estimation of the effective reproductive number from disease outbreak data. *Mathematical Biosciences and Engineering*, **6**, 261–282
- Clapham, C. and Nicholson, J. (2009) *The Concise Oxford Dictionary of Mathematics*, 4th edn. Oxford University Press, Oxford
- Clark, B., Stewart G.B., Panzone L.A., Kyriakis I. and Frewer L.J. (2016) A systematic review of public attitudes, perceptions and behaviours towards production diseases associated with farm animal welfare. *Journal of Agricultural and Environmental Ethics*, **29**, 455–478
- Clark, J.D., Rager, D.R. and Calpin, J.P. (1997) Animal well-being. I. General considerations. *Laboratory Animal Science*, **47**, 564–570
- Clark, T.J., Khan, K.S. and Gupta, J.K. (2001) Provision of a pen along with questionnaire does not increase the response rate to a postal survey: a randomised controlled trial. *Journal of Epidemiology & Community Health*, **55**, 595–596
- Clarke, A. (1987) Air hygiene and equine respiratory disease. *In Practice*, **9**, 196–204
- Clarke, K.C., Osleeb, J.P., Sherry, J.M., Meert, J.P. and Larsson, R.W. (1991) The use of remote sensing and geographic information systems in UNICEF's dracunculiasis (Guinea worm) eradication effort. *Preventive Veterinary Medicine*, **11**, 229–235
- Clarkson, M.J. and Winter, A.C. (1997) *A Handbook for the Sheep Clinician*, 5th edn. Liverpool University Press, Liverpool
- Clatterbaugh, K.C. (1999) *The Causation Debate in Modern Philosophy, 1637–1739*. Routledge, London
- Clavijo, A. and Kitching, P. (2003) Foot-and-mouth disease: epidemiology and current situation. *Biologist*, **50**, 208–212
- Clavijo, A., Wright, P. and Kitching, R.P. (2004) Developments in diagnostic techniques for differentiating infection from vaccination in foot-and-mouth disease. *The Veterinary Journal*, **167**, 9–22
- Clayton, D. and McKeigue, P.M. (2001) Epidemiological methods for studying genes and environmental factors in complex diseases. *The Lancet*, **358**, 1356–1360
- Cleaveland, S., Hess, G.R., Dobson, A.P., Laurenson, M. K., McCallum, H.I. et al. (2002) The role of pathogens in biological conservation. In: *The Ecology of Wildlife Diseases*. Eds Hudson, P.J., Rizzoli, A., Grenfell, B., Heesterbeek, H. and Dobson, A., pp. 139–150. Oxford University Press, Oxford
- Clemens, J., Sack, D., Rao, M., Chakraborty, J., Kay, B. et al. (1993) The design and analysis of cholera vaccine trials: recent lessons from Bangladesh. *International Journal of Epidemiology*, **22**, 724–730
- Clements, A.C.A., Pfeiffer, D.U., Otte, M.J., Morteo, K. and Chen, L. (2002) A global livestock production and health atlas (GLiPHA) for interactive presentation, integration and analysis of livestock data. *Preventive Veterinary Medicine*, **56**, 19–32
- Clements, F.E. (1905) *Research Methods in Ecology*. The University Publishing Company, Lincoln
- Clements, F.E. (1916) *Plant Succession: An Analysis of the Development of Vegetation*. Carnegie Institution of Washington, Washington DC
- Cliff, A.D. and Haggett, P. (1988) *Atlas of Disease Distributions: Analytic Approaches to Epidemiological Data*. Basil Blackwell, Oxford
- Cliff, A.D. and Ord, J.K. (1981) *Spatial Processes. Models and Applications*. Pion, London
- Clifton-Hadley, R. (1993) The use of a geographical information system (GIS) in the control and epidemiology of bovine tuberculosis in south-west England. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Exeter, 31 March–2nd April 1993. Ed. Thrusfield, M.V., pp. 166–179
- Clopper, C.J. and Pearson, E.S. (1934) The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika*, **26**, 404–413
- Clough, H. E., Clancy, D. and French, N.P. (2006) Verocytotoxigenic *Escherichia coli* O157 in pasteurized milk containers at the point of retail: a qualitative approach to exposure assessment. *Risk Analysis*, **26**, 1291–1309
- Coaley, K. (2010) *An Introduction to Psychological Assessment and Psychometrics*. Sage Publications, London
- Cobb, A.D. (2012) Inductivism in practice: experiment in John Herschel's philosophy of science. *HOPOS*, **2**, 21–53
- Cobb, S.P. (2011) The spread of pathogens through trade in poultry hatching eggs: overview and recent developments. *Revue Scientifique et Technique, Office International des Epizooties*, **30**, 165–175
- Cobb, S.P., Pharo, H., Stone, M., Groenendaal, H. and Zagmutt, F.J. (2015) Quantitative risk assessment of the likelihood of introducing porcine reproductive and respiratory disease syndrome virus into New Zealand through the importation of pig meat. *Revue Scientifique et Technique, Office International des Epizooties*, **34**, 961–975
- Coburn, H.L., Snary, E.L. and Wooldridge, M. (2003) *Hazards and Risks from Wild Game: A Qualitative Risk*

- Assessment. Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency, Weybridge
- Coburn, H.L., Snary, E.L., Kelly, L.A. and Wooldridge, M. (2005) Qualitative risk assessment of the hazards and risks from wild game. *Veterinary Record*, **157**, 321–322
- Cockburn, A. (1963) *The Evolution and Eradication of Infectious Diseases*. Johns Hopkins Press, Baltimore
- Cockcroft, P.D. (2002) Clinical sign profile likelihood ratios for bovine spongiform encephalopathy suspects. *Research in Veterinary Science*, **68**, 285–290
- Cockcroft, P.D. and Holmes, M.A. (2003) *Handbook of Evidence-Based Veterinary Medicine*. Blackwell Publishing, Oxford
- Cockcroft, P.D. and Whiteley, P. (1999) Hypocalcaemia in 23 ataxic/recumbent ewes: clinical signs and likelihood ratios. *Veterinary Record*, **144**, 529–532
- Codd, G.A., Edwards, C., Beattie, K.A., Barr, W.M. and Gunn, G.J. (1992) Fatal attraction to cyanobacteria? (Letter). *Nature*, **359**, 110–111
- Codex Alimentarius Commission (1999) *Principles and Guidelines for the Conduct of Microbial Risk Assessment*. CAC/GL-30. Food and Agriculture Organization of the United Nations, Rome
- Coetzee, P., Stokstad, M., Venter, E.H., Myrmel, M. and Van Vuuren, M. (2012) Bluetongue: a historical and epidemiological perspective with the emphasis on South Africa. *Virology Journal*, **9**, 198; doi 10.1186/1743-422X-9-198
- Cohen, D., Booth, S. and Sussman, O. (1959) An epidemiological study of canine lymphoma and its public health significance. *American Journal of Veterinary Research*, **20**, 1026–1031
- Cohen, D.J. and Cohen, J.D. (2006) The sectioned density plot. *The American Statistician*, **60**, 167–174
- Cohen, J. (1994) The earth is round ($p < .05$). *American Psychologist*, **49**, 997–1003
- Cohen, J.M. and Uphof, N.T. (1980) Participation's place in rural development: seeking clarity through specificity. *World Development*, **8**, 213–235
- Cohen, J.T., Duggar, K., Gray, G.M., Kreindel, S., Abdelrahman, H. et al. (2001) *Evaluation of the Potential for Bovine Spongiform Encephalopathy in the United States*. <http://www.aphis.usda.gov/lpa/issues/bse/bse-riskassmt.html> (accessed 23 January 2006)
- Cohen, M.L. (2000) Changing patterns of infectious disease. *Nature*, **406**, 762–767
- Cohen, M.R. and Nagel, E. (1934) *An Introduction to Logic and Scientific Method*. Routledge and Keegan Paul, London
- Cohn, S.K. (2008) Epidemiology of the Black Death and successive waves of plague. *Medical History*, **52**, Suppl. S27, 74–100
- Coker-Vann, M., Brown, P. and Gajdusek, D.C. (1984) Serodiagnosis of human cysticercosis using a chromatofocused antigen preparation of *Taenia solium* cysticerci in an enzyme-linked immunosorbent assay (ELISA). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **78**, 492–496
- Cole, P. and MacMahon, B. (1971) Attributable risk percent in case-control studies. *British Journal of Preventive and Social Medicine*, **25**, 242–244
- Colinvaux, P.A. (1973) *Introduction to Ecology*. John Wiley and Sons, New York
- Collett, D. (2003) *Modelling Binary Data*, 2nd edn. Chapman and Hall, Boca Raton and London
- Collett, S.R. (2013) Principles of disease prevention, diagnosis, and control. In: *Diseases of Poultry*, 13th edn. Eds Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. et al., pp. 3–60. John Wiley and Sons, Ames
- Collins, D.S. and Huey, R.J. (Eds) (2015) *Gracey's Meat Hygiene*, 11th edn. John Wiley and Sons, Chichester
- Collins, M. and Algers, B. (1986) Effects of stable dust on farm animals – a review. *Veterinary Research Communications*, **10**, 415–428
- Colvin, A.F., Walkden-Brown, S.W., Knox, M.R. and Scott, J.M. (2008) Intensive rotational grazing assists control of gastrointestinal nematodosis of sheep in a cool temperate environment with summer-dominant rainfall. *Veterinary Parasitology*, **153**, 108–120
- Comben, N. (1969) The early English printed literature on the diseases of poultry and other birds. *The Veterinarian*, **6**, 17–25
- Comer, P.J. and Huntley, P.J. (2003) TSE risk assessments: a decision support tool. *Statistical Methods in Medical Research*, **12**, 279–291
- Comer, P.J. and Huntley, P.J. (2004) Exposure of the human population to BSE infectivity over the course of the BSE epidemic in Great Britain and the impact of changes to the Over Thirty Month Rule. *Journal of Risk Research*, **7**, 523–543
- Comfort, A. (1955) The maximum ages reached by domestic cats. *Journal of Mammalogy*, **37**, 118–119
- Comfort, A. (1956) Longevity and mortality of Irish wolfhounds. *Proceedings of the Zoological Society of London*, **127**, 27–34
- Comfort, A. (1957) Survival curves of mammals in captivity. *Proceedings of the Zoological Society of London*, **128**, 349–364
- Comfort, A. (1958) The longevity and mortality of thoroughbred mares. *Journal of Gerontology*, **13**, 342–350
- Comfort, A. (1960) Longevity and mortality in dogs of four breeds. *Journal of Gerontology*, **15**, 126–129
- Comin, A., Haesler, B., Hoineville, L., Peyre, M., Dörea, F. et al. (2016) RISKSUR tools: taking animal health surveillance into the future through interdisciplinary integration of scientific evidence. In: Society for

- Veterinary Epidemiology and Preventive Medicine, Proceedings, Elsinore, 16–18 March 2016. Eds Lindberg, A. and Thulke, H.-H., pp. 243–254
- Conan, A., Goutard, F.L., Sorn, S. and Vong, S. (2012) Biosecurity measures for backyard poultry in developing countries: a systematic review. *BMC Veterinary Research*, **8**, 240; doi 10.1186/1746-6148-8-240
- Condy, J.B., Hedger, R.S., Hamblin, C. and Barnett, I.T.R. (1985) The duration of the foot-and-mouth disease virus carrier state in African buffalo: (i) in the individual animal and (ii) in a free-living herd. *Comparative Immunology, Microbiology and Infectious Diseases*, **8**, 259–265
- Condy, J.B., Herniman, K.A.J. and Hedger, R.S. (1969) Foot-and-mouth disease in wildlife in Rhodesia and other African territories. *Journal of Comparative Pathology*, **79**, 27–31
- Conenello, G.M., Zamarin, D., Perrone, L.A., Tumpey, T. and Palese, P. (2007) A single mutation in the PB1-F2 of H5N1 (HK/97) and 1918 influenza A viruses contributes to increased virulence. *PLoS Pathogens*, **3**, 10, e141; doi 10.1371/journal.ppat.0030141
- Conlan, A.J.K., Brooks Pollock, E., McKinley, T.J., Mitchell, A.P., Jones, G.J. *et al.* (2015) Potential benefits of cattle vaccination as a supplementary control for bovine tuberculosis. *PLoS Computational Biology*, **11**, 2, e1004038; doi 10.1371/journal.pcbi.1004038
- Conover, W.J. (1999) *Practical Nonparametric Statistics*, 3rd edn. John Wiley, New York
- Conrad, P.A., Miller, M.A., Kreuder, C., James, E.R., Mazet, J. *et al.* (2005) Transmission of *Toxoplasma*: clues from the study of sea otters as sentinels of *Toxoplasma gondii* flow in the marine environment. *International Journal of Parasitology*, **35**, 1155–1168
- Conroy, C. (2005) *Participatory Livestock Research: A Guide*. ITDG Publishing, Rugby
- Convery, I., Mort, M., Baxter, J. and Bailey, K. (2008) *Animal Disease and Human Trauma*. Palgrave Macmillan, Basingstoke
- Conzemius, M.G. and Evans, R.B. (2012) Caregiver placebo effect for dogs with lameness from osteoarthritis. *Journal of the American Veterinary Medical Association*, **241**, 1314–1319
- Cook, J. (2010) Measuring and monitoring reproductive performance in dairy herds. *In Practice*, **32**, 432–436
- Cook, R., Flesness, N., Munson, L. and Ullrey, D. (1993) Working group report: monitoring, investigation, and surveillance of disease in captive wildlife. *Journal of Zoo and Wildlife Medicine*, **24**, 374–388
- Cook, R.D. (1977) Detection of influential observations in linear regression. *Technometrics*, **19**, 15–18
- Cook, R.J. and Sackett, D.L. (1995) The number needed to treat: a clinically useful measure of treatment effect. *British Medical Journal*, **310**, 452–454
- Cooney, R. and Dickson, B. (Eds) (2005) *Biodiversity & the Precautionary Principle*. Earthscan, London
- Cooper, H.M., Hedges, L.V. and Valentine, J.C. (2009) *The Handbook of Research Synthesis and Meta-analysis*. Russell Sage Foundation, New York
- Cooper, J.C. (1990) *The Aquarian Dictionary of Festivals*. The Aquarian Press, Wellingborough
- Copeland, K.T., Checkoway, H., McMichael, A.J. and Holbrook, R.H. (1977) Bias due to misclassification in the estimation of relative risk. *American Journal of Epidemiology*, **105**, 488–495
- Copeland, N.G., Jenkins, N.A., Gilbert, D.J., Eppig, J.T., Maltais, L.J. *et al.* (1993) A genetic linkage map of the mouse: current applications and future prospects. *Science*, **262**, 57–66
- Coppieters, K.T., Boettler, T. and von Herrath, M. (2012) Virus infections in Type 1 diabetes. *Cold Spring Harbor Perspectives in Medicine*, **2**, a007732; doi 10.1101/cshperspect.a007682
- Cordes, D.O., Limer, K.L. and McKentee, K. (1981) Data management for the International Registry of Reproductive Pathology using SNOMED coding and computerization. *Veterinary Pathology*, **18**, 342–350
- Coria, M.F. and McClurkin, A.W. (1978) Specific immune tolerance in an apparently healthy bull persistently infected with bovine viral diarrhoea. *Journal of the American Veterinary Medical Association*, **172**, 449–451
- Cork, A. (1996) Olfactory basis of host location by mosquitoes and other haematophagous Diptera. In: *Olfaction in Mosquito-Host Interactions*. Eds Bock, G. R. and Cardew, G., pp. 71–88. John Wiley, Chichester
- Cork, L.C., Hadlow, W.J., Crawford, T.B., Gorham, J.R. and Piper, R.C. (1974) Infectious leukoencephalomyelitis of young goats. *Journal of Infectious Diseases*, **129**, 134–141
- Cork, S.C., Hall, D.C. and Liljebjelke, K.A. (Eds) (2016) *One Health Case Studies: Addressing Complex Problems in a Changing World*. 5M Publishing, Sheffield
- Cornfield, J. (1951) A method of estimating comparative rates from clinical data: applications to cancer of the lung, breast and cervix. *Journal of the National Cancer Institute*, **11**, 1269–1275
- Cornfield, J. (1956) A statistical problem arising from retrospective studies. In: *Proceedings of the Third Berkeley Symposium on Mathematical Statistics and Probability*. Ed. Newman, J., 135–148. University of California Press, Berkeley
- Corrigan, P.J. and Seneviratna, P. (1989) Pesticide residues in Australian meat. *Veterinary Record*, **125**, 181–182
- Corso, B. (1997) Likelihood of introducing selected exotic diseases to domestic swine in the continental United

- States of America through uncooked swill. *Revue Scientifique et Technique, Office International des Epizooties*, **16**, 199–206
- Cosgrove, A.S. (1962) An apparently new disease of chickens – avian nephrosis. *Avian Diseases*, **6**, 385–389
- Costanza, M.C. (1995) Matching. *Preventive Medicine*, **24**, 425–433
- Costanza, R. and Mageau, M. (1999) What is a healthy ecosystem? *Aquatic Epidemiology*, **33**, 105–115
- Costard, S., Jones, B.A., Martinez-Lopez, B., Mur, L., de la Torre, A. *et al.* (2013a) Introduction of African swine fever into the European Union through illegal importation of pork and pork products. *PLoS ONE*, **8**, e61104–e61104
- Costard, S., Mur, L., Lubroth, J., Sanchez-Vizcaino, J.M. and Pfeiffer, D.U. (2013b) Epidemiology of African swine fever virus. *Virus Research*, **173**, 191–197
- Coste, J., Fermanian, J. and Venot, A. (1995) Methodological and statistical problems in the construction of composite measurement scales: a survey of six medical and epidemiological journals. *Statistics in Medicine*, **14**, 331–345
- Cotchin, E. (1956) *Neoplasms of Domesticated Animals*. Commonwealth Agricultural Bureaux, Farnham Royal
- Côté, R.A., Rothwell, D.J., Palotay, J.L., Beckett, R.S. and Brochu, L. (Eds) (1993) *SNOMED International. The Systematized Nomenclature of Human and Veterinary Medicine*. 4 vols. College of American Pathologists, Northfield/American Veterinary Medical Association, Schaumburg
- Cottam, E.M., Haydon, D.T., Paton, D.J., Gloster, J., Wilesmith, J.W. *et al.* (2006) Molecular epidemiology of the foot-and-mouth disease virus outbreak in the United Kingdom in 2001. *Journal of Virology*, **80**, 11274–11282
- Cottam, E.M., Wadsworth, J., Shaw, A.E., Rowlands, R.J., Goatley, L. *et al.* (2008) Transmission pathways of foot-and-mouth disease virus in the United Kingdom in 2007. *PLoS Pathogens*, **4**, e1000050; doi 10.1371/journal.ppat.1000050
- Couëtil, L. (2009) Multimodal management of the horse with airway disease. In: *Current Therapy in Equine Medicine*, 6th edn. Eds Robinson, N.E. and Sprayberry, K.A., pp. 279–283. Saunders Elsevier, St Louis
- Couper, M.P., Baker, R.P., Bethlehem, J., Clark, C.Z.F., Martin, J. *et al.* (Eds) (1998) *Computer Assisted Survey Information Collection*. John Wiley, New York
- Covello, V.T., Peters, R.G., Wojtecki, J.G. and Hyde, R.C. (2001) Risk communication, the West Nile virus epidemic, and bioterrorism: responding to the communication challenges posed by the intentional or unintentional release of a pathogen in an urban setting. *Journal of Urban Health*, **78**, 382–391
- Cowling, B.J. and Nishiura, H. (2012) Virus interference and estimates of influenza vaccine effectiveness from test-negative studies (Letter). *Epidemiology*, **23**, 930–931
- Cowling, D., Gardner, I.A. and Johnson, W.O. (1999) Comparison of methods for estimation of individual-level prevalence based on pooled samples. *Preventive Veterinary Medicine*, **39**, 211–225
- Cox, C.S. (1987) *The Aerobiological Pathway of Microorganisms*. John Wiley and Sons, Chichester
- Cox, D.R. and Wermuth, N. (1996) *Multivariate Dependencies: Models, Analysis and Interpretation*. Chapman and Hall, London
- Cox, L.A. (2008) What's wrong with risk matrices? *Risk Analysis*, **28**, 497–512
- Cox, N. and Ryan, T. (1998) Introduction of anthrax via green hides: risk analysis revisited. *Surveillance*, **25** (3), 6–8
- Cox, N.A., Richardson, L.J., Bailey, J.S., Cosby, D.E., Cason, J.A. *et al.* (2005) Bacterial contamination of poultry as a risk to human health. In: *Food Safety Control in the Poultry Industry*. Ed. Mead, G., pp. 21–43. Woodhead Publishing, Abington
- Cox-Witton, K., Reiss, A., Woods, R., Grillo, V., Baker, R.T. *et al.* (2014) Emerging infectious diseases in free-ranging wildlife – Australian zoo based wildlife hospitals contribute to national surveillance. *PLoS ONE*, **9**, 5, e95127; doi 10.1371/journal.pone.0095127
- Craft, M.E., Volz, E., Packer, C. and Meyers, L.A. (2011) Disease transmission in territorial populations: the small-world network of Serengeti lions. *Journal of the Royal Society Interface*, **8**, 776–786
- Cramer, D.W., Welch, W.R., Scully, R.E. and Wojciechowski, C.A. (1982) Ovarian cancer and talc. A case-control study. *Cancer*, **50**, 372–376
- Crandell, R.A. and Maurer, F.D. (1958) Isolation of a feline virus associated with intranuclear inclusion bodies. *Proceedings of the Society of Experimental Biology and Medicine*, **97**, 487–490
- Crawley, M.J. (2007) *The R Book*. John Wiley and Sons, Chichester
- Cregier, S.E. (1990) Shocking docking: mutilation before education? *Journal of Equine Veterinary Science*, **10**, 252–255
- Creighton, C. (1965) *A History of Epidemics in Britain*, 2nd edn. Frank Cass and Co., London
- Creswell, J.W. (2003) *Research Design: Qualitative, Quantitative, and Mixed Methods Approaches*, 2nd edn. Sage Publications, Thousand Oaks
- Cringoli, G., Rinaldi, L., Veneziano, V. and Musella, V. (2005) Disease mapping and risk assessment in veterinary parasitology: some case studies. *Parassitologia*, **47**, 9–25

- Cringoli, G., Taddei, R., Rinaldi, L., Veneziano, V., Musella, V. *et al.* (2004) Use of remote sensing and geographical information systems to identify environmental features that influence the distribution of paramphistomosis in sheep from the southern Italian Apennines. *Veterinary Parasitology*, **122**, 15–26
- Crisp, A., Boschetti, C., Perry, M., Tunnacliffe, A. and Micklem, G. (2015) Expression of multiple horizontally acquired genes is a hallmark of both vertebrate and invertebrate genomes. *Genome Biology*, **16**, 50; doi 10.1186/s13059-015-0607-3
- Croll, N.A., Anderson, R.M., Gyorkos, T.W. and Ghadirian, E. (1982) The population biology and control of *Ascaris lumbricoides* in a rural community in Iran. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **76**, 187–197
- Cromie, R.L., Ash, N.J., Brown, M.J. and Stanford, J.L. (2000) Avian immune responses to *Mycobacterium avium*: the waterfowl example. *Developmental and Comparative Immunology*, **24**, 169–185
- Cross, D. (2003) Ethics and science: time for a critical review of water fluoridation. *Biologist*, **50**, 199
- Crossman, P.J. (1978) Gastric torsion in sows. *The Pig Veterinary Society Proceedings*, **2**, 47–49
- Crowther, J.R., Unger, H. and Viljoen, G.J. (2006) Aspects of kit validation for tests used for the diagnosis and surveillance of livestock diseases: producer and end-user responsibilities. *Revue Scientifique et Technique, Office International des Epizooties*, **25**, 913–935
- Csiza, C.K., Scott, F.W., Lahunta, A. de and Gillespie, J.H. (1971) Feline viruses. XIV: Transplacental infections in spontaneous panleukopenia of cats. *Cornell Veterinarian*, **61**, 423–439
- Cullor, J.S. (1995) Implementing the HACCP program on your clients' dairies. *Veterinary Medicine*, **90**, 290–295
- Cummings, S.R. and Richard, R.J. (1988) Optimum cutoff points for biochemical validation of smoking status. *American Journal of Public Health*, **78**, 575–575
- Cunha, M.G., Silva, E.S., Sepúlveda, N., Costa, S.P.T., Saboia, T.C. *et al.* (2014) Serologically defined variations in malaria endemicity in Pará State, Brazil. *PLoS ONE*, **9**, 11, e113357; doi 10.1371/journal.pone.0113357
- Cunningham, E.P. and Meghen, C.M. (2001) Biological identification systems: genetic markers. *Revue Scientifique et Technique, Office International des Epizooties*, **20**, 491–499
- Cunningham, M.P., McIntyre, W.I.M. and Ives, J.C.J. (1957) The incidence of canine leptospirosis in Glasgow. *Veterinary Record*, **69**, 903–906
- Curth, L.H. (2003) Lessons from the past: preventive medicine in early modern England. *Medical Humanities*, **29**, 16–20
- Curtis, C.R. and Farrar, J.A. (Eds) (1990) The National Animal Health Monitoring System in the United States. *Preventive Veterinary Medicine*, **8**, 87–225
- Curtis, R. and Barnett, K.C. (1989) A survey of cataracts in golden and labrador retrievers. *Journal of Small Animal Practice*, **30**, 277–286
- Cushing, H.B. (1928) Experimental epidemiology. *Canadian Medical Association Journal*, **18**, 429–431
- Cuthbert, R.J., Taggart, M.A., Prakash, V., Chakraborty, S. S., Soumya, S. *et al.* (2014) Avian scavengers and the threat from veterinary pharmaceuticals. *Philosophical Transactions of the Royal Society, Series B*, **369**, 20130574; doi 10.1098/rstb.2013.0574
- Cuthbertson, J.C. (1983) Sheep disease surveillance based on condemnations at three Scottish abattoirs. *Veterinary Record*, **112**, 219–221
- CVMP (1993) *Demonstration of Efficacy of Ectoparasiticides*. CVMP Working Party on the Efficacy of Veterinary Medicines, Notes for Guidance. Document No. III/3682/92-EN. Commission of the European Communities, Brussels
- Czaja, R. (2005) Sampling with probability proportional to size. In: *Encyclopedia of Biostatistics*, 2nd edn, Vol. 7. Eds Armitage, P. and Colton, T., pp. 4764–4770. John Wiley and Sons, Chichester
- D'Aoust, J.-Y. (2001) *Salmonella*. In: *Guide to Foodborne Pathogens*. Eds Labbé, R.G. and Garcia, S., pp. 163–191. John Wiley, New York
- D'Ieteren, G.D.M., Authié, E., Wissocq, N. and Murray, M. (1998) Trypanotolerance; an option for sustainable livestock production in areas at risk from trypanosomosis. *Revue Scientifique et Technique, Office International des Epizooties*, **17**, 154–175
- D'Oliveira, M.C., Souza, M.I.L., Corrêa Filho, R.A.C., Moraes, M. da G., Ítavo, C.C.B.F. *et al.* (2014) Effects of road transportation or droving on the weight and metabolism of young bulls. *Tropical Animal Health and Production*, **46**, 1447–1453
- D'Souza, R.M., Lysenko, M., Marino, S. and Kirschner, D. (2009) Data-parallel algorithms for agent-based model simulation of tuberculosis on graphics processing units. In: *Proceedings of the 2009 Agent-Directed Simulation Symposium*, March 22–27, 2009, Article No. 21. The Society for Modeling & Simulation International, San Diego
- da Cruz, J.C.M., Singh, D.K., Lamara, A. and Chebloune, Y. (2013) Small ruminant lentiviruses (SRLVs) break species barrier to acquire new host range. *Viruses*, **5**, 1867–1884
- Dabalen, A. and Nguyen, N. (undated) *The Short-Run Impact of Import Bans on Poverty in Nigeria (2008–2012)*. World Bank's Africa Region Poverty Reduction Unit (AFTPM)

- Daft, B., Nordhausen, R.W., Latimer, K.S. and Niagro, F. D. (1996) Interstitial pneumonia and lymphadenopathy associated with circoviral infection in a six-week-old pig. *Proceedings of the American Association of Veterinary Laboratory Diagnosticians*, **39**, 32
- Dahlstrand, S., Ringertz, O. and Zetterberg, B. (1971) Airborne tularemia in Sweden. *Scandinavian Journal of Infectious Diseases*, **3**, 7–17
- Daily Mail (2004) Chinese to cull 10,000 cats as SARS returns. *Daily Mail, Foreign Service*, 6 January 2004, 6
- Dalal, S.R., Fowlkes, E.B. and Hoadley, B. (1989) Risk analysis of the space shuttle: pre-Challenger prediction of failure. *Journal of the American Statistical Association*, **84**, 945–957
- Dalla Villa, P., Messori, S., Possenti, L., Barnard, S., Cianella, M. *et al.* (2013) Pet population management and public health: a web service based tool for the improvement of traceability. *Preventive Veterinary Medicine*, **109**, 349–353
- Dalton, J.P. and Mulcahy, G. (2001) Parasite vaccines – a reality? *Veterinary Parasitology*, **98**, 149–167
- Daly, L.E. (1998) Confidence limits made easy: interval estimation using substitution. *American Journal of Epidemiology*, **147**, 783–790
- Dampier, W.C.D. (1948) *A History of Science and its Relations with Philosophy and Religion*, 4th edn. Cambridge University Press, Cambridge
- Daniel, M. and Kolár, K. (1990) Using satellite data to forecast the occurrence of the common tick *Ixodes ricinus* (L.). *Journal of Hygiene, Epidemiology, Microbiology and Immunology*, **34**, 243–252
- Dannat, L. (1997) *Neospora caninum* antibody levels in an endemically infected dairy herd. *Cattle Practice*, **5**, 335–337
- Danon, L., Ford, A.P., House, T., Jewell, C.P., Keeling, M.J. *et al.* (2011) Networks and the epidemiology of infectious disease. *Interdisciplinary Perspectives on Infectious Disease*, **2011**, Article ID 284909; doi 10.1155/2011/284909
- Darakjy, S., Brady, J.E., DiMaggio, C.J. and Guogua, L. (2014) Applying Farr's Law to project the drug overdose mortality epidemic in the United States. *Injury Epidemiology*, **1**, 31; doi 10.1186/s40621-014-0031-2
- Darbyshire, J.H. (1968) Bovine adenoviruses. *Journal of the American Veterinary Medical Association*, **152**, 786–792
- DARD (2014) *Northern Ireland Food Animal Information System*. Department of Agriculture and Rural Development, Belfast. <http://www.dardni.gov.uk/index/animal-health-and-welfare/disease-control-prevention/identification-registration-and-movement/nifais.htm> (accessed 27 October, 2014)
- Darke, P.G.G. (1982) The value of clinical case records in small animal cases. In: *Epidemiology in Animal Health*. Proceedings of a Symposium held at the British Veterinary Association's Centenary Congress, Reading, 22–25 September, 1982, pp. 103–106. Society for Veterinary Epidemiology and Preventive Medicine
- Darke, P.G.G., Thrusfield, M.V. and Aitken, C.G.G. (1985) Association between tail injuries and docking in dogs. *Veterinary Record*, **116**, 409
- Das, K.M. and Tashjian, R.J. (1965) Chronic mitral valve disease in the dog. *Veterinary Medicine/Small Animal Clinician*, **60**, 1209–1216
- Dasgupta, A., Lawson, K.A. and Wilson, J.P. (2010) Evaluating equivalence and noninferiority trials. *American Journal of Health-System Pharmacy*, **67**, 1337–1343
- Daubney, R., Hudson, J.R. and Garnham, P.C. (1931) Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep, cattle and man from East Africa. *Journal of Pathology and Bacteriology*, **34**, 545–579
- David, F.N. and Barton, D.E. (1966) Two space-time interaction tests for epidemicity. *British Journal of Preventive and Social Medicine*, **20**, 44–48
- Davidson, A.P. and Pappagianis, D. (1996) Canine coccidioidomycosis: 1970 to 1993. In: *Coccidioidomycosis*. Proceedings of the 5th International Conference on Coccidioidomycosis, Stanford University, 24–27 August 1994. Eds Einstein, H.E. and Catanzaro, A.P., pp. 155–162. National Foundation for Infectious Diseases, Bethesda
- Davidson, M.K., Lindsey, J.R. and Davis, J.K. (1987) Requirements and selection of an animal model. *Israel Journal of Medical Sciences*, **23**, 551–555
- David-West, K.B. (1980) Planning and implementation of contagious bovine pleuropneumonia control programs in Nigeria. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 7–14 May 1979. Eds Geering, W.A., Roe, R. T. and Chapman, L.A., pp. 575–580. Australian Government Publishing Service, Canberra
- Davies, E.B. and Watkinson, J.H. (1966) Uptake of native and applied selenium by pasture species. I. Uptake of Se by browntop, ryegrass, cocksfoot, and white clover from Atiamuri sand. *New Zealand Journal of Agricultural Research*, **9**, 317–327
- Davies, F.G. (1975) Observations on the epidemiology of Rift Valley fever in Kenya. *Journal of Hygiene, Cambridge*, **75**, 219–230
- Davies, F.G., Shaw, T. and Ochieng, P. (1975) Observations on the epidemiology of ephemeral fever in Kenya. *Journal of Hygiene, Cambridge*, **75**, 231–235
- Davies, G. (1980) Animal disease surveillance. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra,

- 7–11 May, 1979. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 3–10. Australian Government Publishing Service, Canberra
- Davies, G. (1993) Do we need a European surveillance system? In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Exeter, 31st March–2nd April, 1993. Ed. Thrusfield, M.V., pp. 153–163
- Davies, G. (2002) Foot and mouth disease. *Research in Veterinary Science*, **73**, 195–199
- Davies, J.T. (1973) *The Scientific Approach*. Academic Press, London and New York
- Davies, O.L. and Goldsmith, P.L. (1972) *Statistical Methods in Research and Production*, 4th edn. Longman Group, New York and London
- Davis, S., Trapman, P., Leirs, H., Begon, M. and Heesterbeek, J.A.P. (2008) The abundance threshold for plague as a critical percolation phenomenon. *Nature*, **454**, 634–637
- Davison, A. and Hinckley, D. (1997) *Bootstrap Methods and their Applications*. Cambridge University Press, Cambridge
- Davison, H.C., Greiner, M. and Trees, A.J. (1999) Quantitative analysis of *Neospora caninum* serological data obtained from dairy cattle. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Bristol, 24–26 March 1999. Eds Goodall, E.A. and Thrusfield, M.V., pp. 172–181
- Dawson, A., Thevsagayam, S., Smith, S.C., Sherington, J. and Peters, A.R. (2002) The efficacy and duration of *Stellamune One*® a single dose *Mycoplasma hyopneumonia* vaccine for young pigs. In: Proceedings of the 17th Congress of the International Pig Veterinary Society Congress, June 2–5, 2002, Ames, Iowa, Vol. 2, p. 339
- Dawson, P.S. (1973) Epidemiological aspects of Newcastle disease. *Bulletin Office International des Epizooties*, **79**, 27–34
- Day, J.E.L. and Webster, S.D. (1998) Tail-biting and tail-docking in pigs (Letter). *Veterinary Record*, **142**, 468
- Day, M.J., Breitschwerdt, E., Cleaveland, S., Karkare, U., Khanna, C. *et al.* (2012) Surveillance of zoonotic infectious diseases transmitted by small companion animals. *Emerging Infectious Diseases*, **18**, 12; doi 10.3201/eid1812.120664
2Feid1812.120664#pmc_ext
- De Alwis, M.C.L. (1999) *Haemorrhagic Septicaemia*. ACIAR Monograph No. 57. Australian Centre for International Agricultural Research, Canberra
- De Balogh, K.K.I.M., Wandeler, A.I. and Meslin, F.-X. (1993) A dog ecology study in an urban and semi-rural area of Zambia. *Onderstepoort Journal of Veterinary Research*, **60**, 437–443
- De Boer, C.J. (1967) Studies to determine neutralizing antibody in sera from animals recovered from African swine fever and laboratory animals inoculated with African virus adjuvants. *Archiv für die Gesamte Virusforschung*, **20**, 164–179
- de Candolle, A.P.A. (1874) Constitution dans le regne vegetal de groupes physiologiques applicables a la geographie ancienne et moderne. *Archives des Sciences Physiques et Naturelles*, **50**, 5–42
- de Castro, F. and Bolker, B. (2005) Mechanisms of disease-induced extinction. *Ecology Letters*, **8**, 117–126
- de Hann, C. and Umali, D.L. (1992) Public and private sector roles in the supply of veterinary services. In: *Public and Private Roles in Agricultural Development*. Proceedings of the 12th Agricultural Sector Symposium. Eds Anderson, J.R. and de Hann, C., pp. 125–137. World Bank, Washington DC
- de Jong, M.C.M., Diekmann, O. and Heesterbeek, J.A.P. (1995) How does transmission of infection depend on population size? In: *Epidemic Models: Their Structure and Relation to Data*. Ed. Mollison, D., pp. 84–94. Cambridge University Press, Cambridge
- De Kruif, A. (1980) Efficiency of a fertility control programme in dairy herds. In: Proceedings of the Ninth International Congress on Animal Reproduction and Artificial Insemination, Madrid, 16–20 June 1980, Vol. II, pp. 381–388
- de la Rocque, S., Michel, J.F., Bouyer, J., De Wispelaere, G. and Cuisance, D. (2005) Geographical information systems in parasitology: a review of potential applications using the example of animal trypanosomosis in West Africa. *Parassitologia*, **47**, 97–104
- de la Rocque, S., Rioux, J.A. and Slingenbergh, J. (2008) Climate change: effects on animal disease systems and implications for disease control. *Revue Scientifique et Technique, Office International des Epizooties*, **27**, 339–354
- de la Rua, R., Watkins, G.H. and Watson, P.J. (2001) Idiopathic mouth ulcers in sheep (Letter). *Veterinary Record*, **149**, 30–31
- de La Rúa-Domènech, R., Mohammed, H.O., Atwill, E.R., Cummings, J.F., Divers, T.J. *et al.* (1995) Epidemiological evidence for clustering of equine motor neuron disease in the United States. *American Journal of Veterinary Research*, **56**, 1433–1439
- De Las Heras Guillamón, M., and Clau, L.B. (2010) The sheep as a large animal experimental model in respiratory diseases research. *Archivos de Bronconeumologia*, **46**, 499–501
- De Leo, G., Dobson, A. and Goodman, A. (2002) Wildlife perspectives on the evolution of virulence. In: *Adaptive Dynamics of Infectious Diseases: In Pursuit of Virulence Management*. Eds Dieckmann, U., Metz, A.J., Sabelis,

- M.W. and Sigmund, K., pp. 26–38. Cambridge University Press, Cambridge
- de Lisle, G.W., Wilson, T., Collins, D.M. and Buddle, B.M. (2001) *Mycobacterium bovis* in free-living and captive wildlife, including farmed deer. *Revue Scientifique et Technique, Office International des Epizooties*, **20**, 86–111
- de Martel, C., Ferlay, J., Franceschi, S., Vignat, J., Bray, F. *et al.* (2012) Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *The Lancet Oncology*, **13**, 607–615
- De Risio, L. and Platt, S. (2014) *Canine and Feline Epilepsy: Diagnosis and Management*. CABI, Wallingford
- De Smet, J.-J. (1856) *Breve chronicon clerici anonymi. Recueil des Chroniques de Flandres*, III, 1–36
- De Vos, C.J., Hoek, C.A. and Nodelijk, G. (2011) Risk of introducing African horse sickness into The Netherlands by importation of equines. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Leipzig, 23–25 March 2011. Eds Fourichon, C. and Pfeiffer, D.U., pp. 217–231
- Deardon, R., Brooks, S., Grenfell, B., Keeling, M., Tildesley, M. *et al.* (2010) Inference for individual-level models of infectious diseases in large populations. *Statistica Sinica*, **20**, 239–261
- DeBach, P. and Rosen, D. (1991) *Biological Control by Natural Enemies*, 2nd edn. Cambridge University Press, Cambridge
- Dee, S.A., Molitor, T.W. and Rossow, K.D. (2000) Epidemiological and diagnostic observations following the elimination of porcine reproductive and respiratory syndrome virus from a breeding herd of pigs by the test and removal protocol. *Veterinary Record*, **146**, 211–213
- Dee, S., Otake, S., Omiveira, S. and Deen, J. (2009) Evidence of long distance airborne transport of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae*. *Veterinary Research*, **40**, 39; doi 10.1051/vetres/2009022
- Deeks, J.J. (2002) Issues in the selection of a summary statistic for meta-analysis of clinical trials with binary outcomes. *Statistics in Medicine*, **21**, 1575–1600
- Deeks, J.J. and Altman, D.G. (2004) Diagnostic tests 4: likelihood ratios. *British Medical Journal*, **329**, 168–169
- Deeks, J.J., Altman, D.G. and Bradburn, M.J. (2001) Statistical methods for examining heterogeneity and combining results from several studies in meta-analysis. In: *Systematic Reviews in Health Care: Meta-Analysis in Context*, 2nd edn. Eds Egger, M., Smith, G.D. and Altman, D.G., pp. 285–312. BMJ Publishing Group, London
- Deeks, J.J., Higgins, J.P.T. and Altman, D.G. (2011) Analysing data and undertaking meta-analyses. In: *Cochrane Handbook for Systematic Reviews of Interventions*, Version 5.1.0 (updated March 2011). Eds Higgins, J. and Green, S. The Cochran Collaboration. <http://handbook.cochrane.org>
- Deem, S.L., Perry, B.D., Katende, J.M., McDermott, J.J., Mahan, S.M. *et al.* (1993) Variation in prevalence rates of tick-borne diseases in zebu cattle by agro-ecological zone: implications for East Coast fever immunization. *Preventive Veterinary Medicine*, **16**, 171–187
- Deen, J., Dee, S., Morrison, R.B. and Radostits, O.M. (2001) Health and production management in swine herds. In: *Herd Health: Food Animal Production Medicine*, 3rd edn. Ed. Radostits, O.M., pp. 635–764
- Deen, J., Martin, S.W. and Wilson, M.R. (1995) An evaluation of personal interviews as a method of estimating production indices on Ontario swine farms. *Preventive Veterinary Medicine*, **22**, 263–271
- DEFRA (2001) *Condition Scoring of Dairy Cows*. Department for Environment, Food and Rural Affairs, London
- DEFRA (2002a) *Foot and Mouth Disease in the United Kingdom 2001. Report to the OIE. Part I: Great Britain*. Department for Environment Food and Rural Affairs, London
- DEFRA (2002b) *Origin of the UK Foot and Mouth Disease Epidemic in 2001*. Department for Environment Food and Rural Affairs, London
- DEFRA (2002c) *Partnership, Priorities and Professionalism. A Proposed Strategy for Enhancing Veterinary Surveillance in the UK*. Department for Environment, Food and Rural Affairs, London
- DEFRA (2002d) *Technical Review – Bluetongue*, Version 1.5. Department for the Environment, Food and Rural Affairs, London
- DEFRA (2003) *Qualitative Hazard Assessment – Foot and Mouth Disease Outbreak in South Africa (Recognised Buffer Zone)*. International Animal Health Division, Department for Environment, Food and Rural Affairs, London. http://www.defra.gov.uk/animalh/diseases/monitoring/pdf/fmd_rsa.pdf (accessed 2 July 2006)
- DEFRA (2004) *Preparing for a New GB Strategy on Bovine Tuberculosis*. Consultation document. Department for Environment, Food and Rural Affairs, London
- DEFRA (2005) *Qualitative Hazard Assessment – Highly Pathogenic Avian Influenza (H5N1) in Russia*. International Animal Health Division, Department for Environment, Food and Rural Affairs, London. <http://www.defra.gov.uk/animalh/diseases/monitoring/pdf/hpai-russia090805.pdf> (accessed 2 July 2006)

- DEFRA (2006) *National Scrapie Plan for Great Britain*. NSP1 (Revised July 2006). Department for Environment Food and Rural Affairs, London
- DEFRA (2010) *Vaccination as a Control Tool for Exotic Animal Disease: Key Considerations*. Department for Environment, Food and Rural Affairs, London
- DEFRA (2011a) *A Review of the Implementation of the Veterinary Surveillance Strategy (VSS)*. Department for Environment, Food and Rural Affairs, London
- DEFRA (2011b) *Qualitative Analysis of the Risk of Introduction of Equine Infectious Anaemia (EIA) into Great Britain from an EIA Endemic Area Through Temporary Movement of UK Origin Horses*, v. 1.0, 20 June 2011. (Roberts, R. and Paterson, A.) Veterinary Science Team, Department for Environment, Food and Rural Affairs, London
- DEFRA (2012a) *Zoos Expert Committee Handbook*, PB13815. Department for Environment Food and Rural Affairs, London
- DEFRA (2012b) *Agriculture in the United Kingdom 2011*. Department for Environment, Food and Rural Affairs, London
- DEFRA (2012c) *Farming Statistics: Final Crop Areas, Yields, Livestock Populations and Agricultural Workforce at 1 June 2012, United Kingdom*. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/183200/defra-stats-foodfarm-landuslivestock-farmingstats-june-statsrelease-june12finaluk-121220.pdf (accessed 17 September 2013)
- DEFRA (2012d) *What is the Risk of Introducing Echinococcus multilocularis to the UK Wildlife Population by Importing European Beavers? Qualitative Risk Assessment. June 2012*. Department for Environment, Food and Rural Affairs, London. <http://webarchive.nationalarchives.gov.uk/20140507133914/http://www.defra.gov.uk/animal-diseases/files/qra-non-native-species-echinococcus-120627.pdf> (accessed 6 October 2015)
- DEFRA (2013) *Poultry Health Scheme (PHS) Handbook*, September 2013. Department for Environment, Food and Rural Affairs, London
- Degner, R.L., Rodan, L.W., Mathis, W.K. and Gibbs, E.P.J. (1983) The recreational and commercial importance of feral swine in Florida: relevance to the possible introduction of African swine fever into the USA. *Preventive Veterinary Medicine*, **1**, 371–381
- Degregorio, O.J., Cerverizzo, I.J. and Eyherabide, R.E. (1995) Animal population in the Municipal District of General San Martín, Province of Buenos Aires, Argentina. 1 Demographic aspects. *Revista de Medicina Veterinaria*, **76**, 255–258 (in Spanish; English abstract)
- DeGroote, J.P., Larson, S.R., Zhang, Y. and Sugumaran, R. (2012) Application of geospatial technologies for understanding and predicting vector populations and vector-borne disease incidence. *Geography Compass*, **6**, 645–659
- Dela Cruz, F.N., Giannitti, F., Li, L., Woods, L.W., Del Valle, L. *et al.* (2013) Novel polyomavirus associated with brain tumors in free-ranging raccoons, western United States. *Emerging Infectious Diseases*, **19**, 77–84
- Delahay, J., Smith, G.C., Barlow, A.M., Walker, N., Harris, A. *et al.* (2007) Bovine tuberculosis infection in wild animals in the south-west region of England: a survey of prevalence and a semi-quantitative assessment of the relative risks to cattle. *The Veterinary Journal*, **173**, 287–301
- Delgado, C., Rosegrant, M., Steinfeld, H., Ehui, S. and Courbois, C. (1999) *Livestock to 2020: The Next Food Revolution*. Food, Agriculture, and Environment Discussion Paper No. 28. International Food Policy Research Unit/Food and Agriculture Organization of the United Nations/International Livestock Research Institute/International Food Policy Research Unit, Washington DC
- Delgado-Rodríguez, M. and Llorca, J. (2004) Bias. *Journal of Epidemiology & Community Health*, **58**, 635–641
- DeLong, E.R., DeLong, E.M. and Clarke-Pearson, D.L. (1988) Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*, **44**, 837–845
- Delves, P.J., Martin, J., Burton, D.R. and Roitt, I.M. (2013) *Roitt's Essential Immunology*, 12th edn. Wiley-Blackwell, Chichester
- Demets, D.L. and Ware, J.H. (1980) Group sequential methods in clinical trials with a one-sided hypothesis. *Biometrika*, **67**, 651–660
- Dennis, R. (2012) Interpretation and use of BVA/KC hip scores in dogs. *In Practice*, **34**, 178–194
- Denny, G.O., Wilesmith, J.W., Clements, R.A. and Hueston, W.D. (1992) Bovine spongiform encephalopathy in Northern Ireland: epidemiological observations 1988–1990. *Veterinary Record*, **130**, 113–116
- Dent, J.B. and Blackie, M.J. (1979) *Systems Simulation in Agriculture*. Applied Science Publishers, London
- Deresinski, S.C. (1980) History of coccidioidomycosis: 'dust to dust'. In: *Coccidioidomycosis*. Ed. Stevens, D.A., pp. 1–20. Plenum, New York
- Derks, M., van Werven, T., Hogeveen, H. and Kremer, W. D.J. (2013a) Veterinary herd health management programs on dairy farms in the Netherlands: use, execution, and relations to farmer characteristics. *Journal of Dairy Science*, **96**, 1623–1637
- Derks, M., van Werven, T., Hogeveen, H. and Kremer, W. D.J. (2014) Associations between farmer participation in veterinary herd health management programs and

- farm performance. *Journal of Dairy Science*, **97**, 1336–1347
- Derks, M., van Woudenberg, B., Boender, M., Kremer, W. and van Werven, T. (2013b) Veterinarian awareness of farmer goals and attitudes to herd health management in The Netherlands. *The Veterinary Journal*, **198**, 224–228
- Dermer, O.C. and Ham, G.E. (1969) *Ethyleneimine and Other Aziridines*. Academic Press, New York
- DerSimonian, R. and Laird, N. (1986) Meta-analysis in clinical trials. *Controlled Clinical Trials*, **7**, 177–188
- Descour, L. (1922) *Pasteur and His Work*. Wedd, A.F. and Wedd, B.H. (translators). T. Fisher Unwin, London
- Despommier, D., Ellis, B.R. and Wilcox, B.A. (2007) The role of ecotones in emerging infectious diseases. *EcoHealth*, **3**, 281–289
- Detilleux, J.C. and Serteyn, D. (2005) Cost-effectiveness analysis in veterinary medicine: illustration with packed cell value in the prognosis of horse surgical colic in Belgium. *International Journal of Applied Research in Veterinary Medicine*, **3**, 309–318
- Detsky, A.S., Baker, J.P., O'Rourke, K. and Goel, V. (1987) Perioperative parenteral nutrition: a meta-analysis. *Annals of Internal Medicine*, **107**, 195–203
- Devereaux, P.J., Manns, B.J., Ghali, W.A., Quan, H., Lacchetti, C. *et al.* (2001) Physician interpretations and textbook definitions of blinding terminology in randomized controlled trials. *Journal of the American Medical Association*, **285**, 2000–2003
- Dhama, K., Verma, A.K., Tiwari, R., Chakraborty, S., Vora, K. *et al.* (2013) A perspective on applications of Geographical Information System (GIS): an advanced tracking tool for disease surveillance and monitoring in veterinary epidemiology. *Advances in Animal and Veterinary Sciences*, **1**, 14–24
- DHHPs (2014) *Dairy Herd Health and Productivity Service*. Royal (Dick) School of Veterinary Studies, Edinburgh. www.ed.ac.uk/vet/dhpps (accessed 27 October 2014)
- Dhillon, R.S. and Kelly, J.D. (2015) Community trust and the Ebola endgame. *The New England Journal of Medicine*, **373**, 787–789
- Dhingra M.S., Dissanayake, R., Negi, A.B., Oberoi, M., Castellan, D. *et al.* (2014) Spatio-temporal epidemiology of highly pathogenic avian influenza (subtype H5N1) in poultry in eastern India. *Spatial and Spatio-temporal Epidemiology*, **11**, 45–57
- Di Nardo, A., Rossi, D., Saleh, S.M.L., Lejlifa, M., Hamdi, S.J. *et al.* (2014) Evidence of Rift Valley fever seroprevalence in the Sahrawi semi-nomadic pastoralist system, Western Sahara. *BMC Veterinary Research*, **10**, 92; doi 10.1186/1746-6148-10-92
- Di Salvo, A.F. and Johnson, W.M. (1979) Histoplasmosis in South Carolina: support for the microfocus concept. *American Journal of Epidemiology*, **109**, 408–492
- Diamond, J. (1997) *Guns, Germs and Steel: the Fates of Human Societies*. Jonathan Cape, London
- Dias, R.A., Garcia, R. de C., de Silva, D.F., Amaku, M., Neto, J.S.F. *et al.* (2004) Estimate of the owned canine and feline populations in urban area in Brazil. *Revista de Saúde Pública*, **38**, 565–570 (in Portuguese; English abstract)
- Diaz, N.M., Mendez, G.S., Grijalva, C.J., Walden, H.S., Cruz, M. *et al.* (2016) Dog overpopulation and burden of exposure to canine distemper virus and other pathogens on Santa Cruz Island, Galapagos. *Preventive Veterinary Medicine*, **123**, 128–137
- Dicker, R.C., Coronado, F., Koo, D. and Parrish, R.G. (2006) *Principles of Epidemiology in Public Health Practice*, 3rd edn. US Department of Health and Human Services, Atlanta. <http://stacks.cdc.gov/view/cdc/6914/> (accessed 8 September 2015)
- Dickersin, K. and Berlin, J.A. (1992) Meta-analysis: state-of-the-science. *Epidemiologic Reviews*, **14**, 154–176
- Dickinson, A.G. and Barlow, R.M. (1967) The demonstration of the transmissibility of Border disease in sheep. *Veterinary Record*, **81**, 114
- Didion, S.P., Sigmund, C.D. and Faraci, F.M. (2000) Impaired endothelial function in transgenic mice expressing both human renin and human angiotensin. *Stroke*, **31**, 760–765
- Diekmann, O., Heesterbeek, J.A.P. and Metz, J.A.J. (1990) On the definition and the computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. *Journal of Mathematical Biology*, **28**, 365–382
- Diesch, S.L. (1979) Disease recording at the farm level. In: *Proceedings of an International Symposium on Animal Health and Disease Data Banks*, 4–6 December 1978, Washington DC, pp. 97–100. United States Department of Agriculture, Miscellaneous Publications No. 1381
- Diesch, S.L. and Ellinghausen, H.C. (1975) Leptospiroses. In: *Diseases Transmitted from Animals to Man*, 6th edn. Eds Hubbert, W.T., McCulloch, W.F. and Schnurrenberger, P.R., pp. 436–462. Charles C. Thomas, Springfield
- Dietz, K. (1993) The estimation of the basic reproduction number for infectious diseases. *Statistical Methods in Medical Research*, **2**, 23–41
- Dietz, K. and Heesterbeek, J.A.P. (2002) Daniel Bernoulli's epidemiological model revisited. *Mathematical Biosciences*, **180**, 1–21
- Diggle, P.J. (2013) *Statistical Analysis of Spatial and Spatio-Temporal Point Patterns*, 3rd edn. CRC Press, Boca Raton

- Diggle, P.J. and Chetwynd, A.G. (2011) *Statistics and Scientific Method*. Oxford University Press, Oxford
- Dijkhuizen, A.A. Morris, R.S. and Morrow, M. (1986) Economic optimization of culling strategies in swine breeding herds, using the PorkCHOP computer program. *Preventive Veterinary Medicine*, **4**, 431–435
- Dijkhuizen, A.A., Renkema, J.A. and Stelwagen, J. (1987) An economic simulation model to support management decisions in dairy herds. *European Review of Agricultural Economics*, **14**, 395–412
- Dillon, J.L. and Anderson, J.R. (1990) *The Analysis of Response in Crop and Livestock Production*, 3rd edn. Pergamon Press, Oxford
- Dimock, W.W. and Edwards, P.R. (1932) Infections of foetuses and foals. *Kentucky Agriculture Experimental Station Bulletin*, **333**, 291–339
- Dion, E., VenSchalkwyk, L. and Lambin, E.F. (2011) The landscape epidemiology of foot-and-mouth disease in South Africa: a spatially explicit multi-agent simulation. *Ecological Modelling*, **222**, 2059–2072
- Dobben, W.H. van (1952) The food of the cormorants in the Netherlands. *Ardea*, **40**, 1–63
- Dobson, A.P. (1988) Restoring island ecosystems: the potential of parasites to control introduced mammals. *Conservation Biology*, **2**, 31–39
- Dobson, A.P. (2014) Yellowstone wolves and the forces that structure natural systems. *PLoS Biology*, **12**, 12; doi 10.1371/journal.pbio.1002025
- Dobson, A.P., Hudson, P.J. and Lyles, A.M. (1992) Macroparasites: worms and others. In: *Natural Enemies: The Population Biology of Predators, Parasites and Diseases*. Ed. Crawley, M.J., pp. 329–348. Blackwell Scientific Publications, Oxford
- Dobson, J.M. (2013) Breed-predispositions to cancer in pedigree dogs. *ISRN Veterinary Science*, **2013**; doi 10.1155/2013/941275
- Dobson, J.M., Samuel, S., Milstein, H., Rogers, K. and Wood, J.L.N. (2002) Canine neoplasia in the UK: estimates of incidence rates from a population of insured dogs. *Journal of Small Animal Practice*, **43**, 240–246
- Dobson, R.J., Sangster, N.C., Besier, R.B. and Woodgate, R.G. (2009) Geometric means provide a biased efficacy result when conducting a faecal egg count reduction test (FECRT). *Veterinary Parasitology*, **161**, 162–167
- Dodd, F.H. and Neave, F.K. (1970) *Mastitis Control*. Biennial Review, Paper No. 3559. National Institute for Research in Dairying, Reading
- Dodd, S. (1923) Cancer of the ear of sheep. *Journal of Comparative Pathology*, **36**, 231–242
- Dodds, W.J. (2001) Vaccine protocols for dogs predisposed to vaccine reactions. *Journal of the American Animal Hospital Association*, **37**, 1–4
- Dodson, S.I., Allen, T.F.H., Carpenter, S.R., Ives, A.R., Jeanne, R.L. et al. (1998) *Ecology*. Oxford University Press, New York
- Doel, T.R. (2003) FMD vaccines. *Virus Research*, **91**, 81–99
- Doherr, M.G., Baumgarten, L. and Heim, D. (2000) The need for an active (targeted) surveillance system for BSE and scrapie in addition to mandatory reporting of clinical suspect cases. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Edinburgh, 29–31 March 2000. Eds Thrusfield, M.V. and Goodall, E.A., pp. 198–203
- Doherr, M.G., Carpenter, T.E., Wilson, W.D. and Gardner, I.A. (1997) Effect of different sampling techniques on odds ratio estimates using hospital-based cases and controls. *Preventive Veterinary Medicine*, **32**, 77–93
- Doherr, M.G., Carpenter, T., Wilson, W.D. and Gardner, I.A. (1999) Evaluation of temporal and spatial clustering of horses with *Corynebacterium pseudotuberculosis* infection. *American Journal of Veterinary Research*, **60**, 284–291
- Dohms, J.E. and Metz, A. (1991) Stress-mechanisms of immunosuppression. *Veterinary Immunology and Immunopathology*, **30**, 89–109
- Dohoo, I.R. (1988) Animal Productivity and Health Information Network. *Canadian Veterinary Journal*, **29**, 281–287
- Dohoo, I.R. (1992) Dairy APHIN – an information service for the dairy industry in Prince Edward Island, Canada. *Preventive Veterinary Medicine*, **12**, 259–268
- Dohoo, I.R. (1993) Monitoring livestock health and production: service – epidemiology's last frontier? *Preventive Veterinary Medicine*, **18**, 43–52
- Dohoo, I.R., DesCôteaux, L., Leslie, K., Fredeen, A., Shewfelt, W. et al. (2003) A meta-analysis review of the effects of recombinant bovine somatotropin 2. Effects on animal health, reproductive performance, and culling. *Canadian Journal of Veterinary Research*, **67**, 252–264
- Dohoo, I., Martin, W. and Stryhn, H (2009) *Veterinary Epidemiologic Research*, 2nd edn. VER, Charlottetown
- Dohoo, I.R., Morris, R.S., Martin, S.W., Perry, B.D., Bernado, T. et al. (1994) Epidemiology (Letter). *Nature*, **368**, 284
- Doll, R. (1959) Smoking and cancer. Report to the sub-committee for the study of the risks of cancer from air pollution and the consumption of tobacco. *Acta Uniaris Internationalis Contra Cancrum*, **15**, 1283–1296
- Doll, R. (1977) Strategy for detection of cancer hazards to man. *Nature*, **265**, 589–596
- Doll, R. (1992) Sir Austin Bradford Hill and the progress of medical science. *British Medical Journal*, **305**, 1521–1528

- Doll, R. and Hill, A.B. (1964a) Mortality in relation to smoking. Ten years' observations of British doctors. *British Medical Journal*, **i**, 1399–1410
- Doll, R. and Hill, A.B. (1964b) Mortality in relation to smoking. Ten years' observations of British doctors. *British Medical Journal*, **i**, 1460–1467
- Doll, R., Peto, R., Boreham, J. and Sutherland, I. (2004) Mortality in relation to smoking: 50 years' observations on male British doctors. *British Medical Journal*, **328**, 1519–1528
- Domingo, E., Escarmís, C., Menéndez-Arias, L. and Holland, J.J. (1999) Viral quasispecies and fitness variations. In: *Origin and Evolution of Viruses*. Eds Domingo, E., Webster, R. and Holland, J., pp. 141–161. Academic Press, San Diego
- Dominguez, M., Münstermann, S.M., de Guindos, I. and Timoney, P. (2016) Equine disease events resulting from international horse movements: Systematic review and lessons learned. *Equine Veterinary Journal*, **48**, 641–653
- Donaldson, A.I. (1987) Foot and mouth disease: the principal features. *Irish Veterinary Journal*, **41**, 325–327
- Donaldson, A.I. (1993) Epidemiology of foot-and-mouth disease: the current situation and new perspectives. In: *Diagnosis and Epidemiology of Foot-and-Mouth Disease in Southeast Asia*, Proceedings of an international workshop held at Lampang, Thailand, September 6–9, 1993. Eds Copland, J.W., Gleeson, L.J. and Chamnanpood, C., pp. 9–15
- Donaldson, A.I. (1997) Risks of spreading foot and mouth disease through milk and dairy products. *Revue Scientifique et Technique, Office International des Epizooties*, **16**, 117–124
- Donaldson, A.I. (2000) The role of sheep in the epidemiology of foot-and-mouth disease and proposals for control and eradication in animal populations with a high density of sheep. In: Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Borovets, Bulgaria, 5th–8th September 2000. AGA: EUFMD/RG/00, pp. 107–116. Food and Agriculture Organization of the United Nations, Rome
- Donaldson, A.I. and Alexandersen, S. (2001) Relative resistance of pigs to infection by natural aerosols of FMD virus. *Veterinary Record*, **148**, 600–602
- Donaldson, A.I. and Alexandersen, S. (2002) Predicting the spread of foot and mouth disease by airborne virus. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 569–575
- Donaldson, A.I. and Doel, T.R. (1992) Foot-and-mouth disease: the risk for Great Britain after 1992. *Veterinary Record*, **131**, 114–120
- Donaldson, A.I. and Kitching, R.P. (1989) Transmission of foot-and-mouth disease by vaccinated cattle following natural challenge. *Research in Veterinary Science*, **46**, 9–14
- Donaldson, A.I., Alexandersen, S., Sørensen, J.H. and Mikkelsen, T. (2001) Relative risks of the uncontrollable (airborne) spread of FMD by different species. *Veterinary Record*, **148**, 602–604
- Donaldson, A.I., Gloster, J., Harvey, L.D.J. and Deans, D. H. (1982) Use of prediction models to forecast and analyse airborne spread during the foot-and-mouth disease outbreaks in Brittany, Jersey and the Isle of Wight in 1981. *Veterinary Record*, **110**, 53–57
- Done, J.T. (1985) Eradication, elimination or control? *Veterinary Record*, **117**, 253
- Donham, K.J. and Thorne, P.S. (1994) Agents in organic dust: criteria for a causal relationship. *American Journal of Industrial Medicine*, **25**, 33–39
- Donham, K.J., Berg, J.W. and Sawin, R.S. (1980) Epidemiologic relationships of the bovine population and human leukemia in Iowa. *American Journal of Epidemiology*, **112**, 80–92
- Donnellan, M.B. and Lucas, R.E. (2013) Secondary data analysis. In: *The Oxford Handbook of Quantitative Methods. Volume 2: Statistical Analysis*. Ed. Little, T. D., pp. 665–677. Oxford University Press, New York
- Donnelly, C.A., Woodroffe, R., Cox, D.R., Bourne, J., Gettinby, G. *et al.* (2003) Impact of localized badger culling on tuberculosis incidence in British cattle. *Nature*, **426**, 834–837
- Donner, A. (1993) The comparison of proportions in the presence of litter effects. *Preventive Veterinary Medicine*, **18**, 17–26
- Donoghue, A.M. (2001) The design of hazard risk assessment matrices for ranking occupational health risks and their application in mining and minerals processing. *Occupational Medicine*, **51**, 118–123
- Donoghue, D.J. (2005) Modelling risks from antibiotics and other residues in poultry and eggs. In: *Food Safety Control in the Poultry Industry*. Ed. Mead, G., pp. 83–100. Woodhead Publishing, Abington
- Dórea, F.C., Berghaus, R., Hofacre, C. and Cole, D.J. (2010) Survey of biosecurity protocols and practices adopted by growers on commercial poultry farms in Georgia, USA. *Avian Diseases*, **54**, 1007–1015
- Dórea, F.C., Lindberg, A., McEwen, B.J., Revie, C.W. and Sanchez, J. (2014) Syndromic surveillance using laboratory test requests: a practical guide informed by experience with two systems. *Preventive Veterinary Medicine*, **116**, 313–324
- Dórea, F.C., McEwen, B.J., McNab, W.B., Revie, C.W. and Sanchez, J. (2013) Syndromic surveillance using veterinary laboratory data: data pre-processing and algorithm performance evaluation. *Journal of the Royal*

- Society Interface*, **10**, 20130114; doi 10.1098/rsif.2013.0114
- Dórea, F.C., Sanchez, J. and Revie, C.W. (2011) Veterinary syndromic surveillance: current initiatives and potential for development. *Preventive Veterinary Medicine*, **101**, 1–17
- Dorjee, S., Poljak, Z., Revie, C.W., Bridgland, J., McNab, B. *et al.* (2012) A review of simulation modelling approaches used for the spread of zoonotic influenza viruses in animal and human populations. *Zoonoses and Public Health*, **60**, 383–411
- Dorjee, S., Revie, C.W., Poljak, Z., McNab, B., McLure, J. T. *et al.* (2016) One-Health simulation modelling: a case study of influenza spread between human and swine populations using the NAADSM. *Transboundary and Emerging Diseases*, **63**, 36–55
- Dorn, C.R. (1966) An animal tumour registry as a source of morbidity information. In: Proceedings of the United States Livestock Sanitation Association, pp. 443–454
- Dorn, C.R. and Priester, W.A. (1987) Epidemiology. In: *Veterinary Cancer Medicine*, 2nd edn. Eds Theilen, G. H. and Madewell, B.R., pp. 27–52. Lea and Febiger, Philadelphia
- Dorn, C.R., Taylor, D.O.N. and Schneider, R. (1971) Sunlight exposure and risk of developing cutaneous and oral squamous cell carcinomas in white cats. *Journal of the National Cancer Institute*, **46**, 1073–1081
- Dorn, C.R., Taylor, D.O.N., Schneider, R., Hibbard, H.H. and Klauber, M.R. (1968) Survey of animal neoplasms in Alameda and Contra Costa Counties, California. II. Cancer morbidity in dogs and cats from Alameda County. *Journal of the National Cancer Institute*, **40**, 307–318
- Dorn, C.R., Terbrusch, F.G. and Hibbard, H.H. (1967) Zoographic and Demographic Analysis of Dog and Cat Ownership in Alameda County, California, 1965. State of California Department of Public Health, Berkeley
- Dosemeci, M., Wacholder, S. and Lubin, J.H. (1990) Does nondifferential misclassification of exposure always bias a true effect toward the null value? *American Journal of Epidemiology*, **132**, 746–748
- Douglas, A. (1984) Disease prevention in pig herds. *In Practice*, **6**, 108–115
- Dowdle, W.R. (1998) The principles of disease elimination and eradication. *Bulletin of the World Health Organization*, **76**, Suppl. 2, 22–25
- Downes, M., Canty, M.J. and More, S.J. (2009) Demography of the pet dog and cat population on the island of Ireland and human factors influencing pet ownership. *Preventive Veterinary Medicine*, **92**, 140–149
- Doyle, K.A. (1995) Models used in Australia in risk assessments for veterinary biologicals. *Revue Scientifique et Technique, Office International des Epizooties*, **14**, 1021–1042
- Doyle, L.P. and Hutchings, L.M. (1946) A transmissible gastroenteritis of pigs. *Journal of the American Veterinary Medical Association*, **108**, 257–259
- Doyle, T.M. (1927) A hitherto unrecorded disease of fowls due to a filter-passing virus. *Journal of Comparative Pathology and Therapeutics*, **40**, 144–169
- Dragon, D.C. and Rennie, R.P. (1995) The ecology of anthrax spores: tough but not invincible. *Canadian Veterinary Journal*, **36**, 295–301
- Draper, S.J. and Heeney, J.L. (2010) Viruses as vaccine vectors for infectious diseases and cancer. *Nature Reviews Microbiology*, **8**, 62–73
- Drazen, J.M., Kanapathipillai, R., Campion, E.W., Rubin, E.J., Hammer, S.M. *et al.* (2014) Ebola and quarantine. *The New England Journal of Medicine*, **371**, 2029–2030
- Drewe, J.A., Eames, K.T.D., Madden, J.R. and Pearce, G.P. (2010) Application of epidemiological tools to the investigation of a wildlife disease. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Nantes, 24–26 March 2010. Eds Alban, L. and Kelly, L.A., pp. 53–66
- Drewe, J.A., Häslar, B., Rushton, J. and Stärk, K.D.C. (2014) Assessing the expenditure distribution of animal health surveillance: the case of Great Britain. *Veterinary Record*, **174**, 16; doi 10.1136/vr.101846
- Drewe, J.A., Hoinville, L.J., Cook, A.J.C., Floyd, T., Gunn, G. *et al.* (2015) SERVAL: a new framework for the evaluation of animal health surveillance. *Transboundary and Emerging Diseases*, **62**, 33–45
- Drewe, J.A., Hoinville, L.J., Cook, A.J.C., Floyd, T. and Stärk, K.D.C. (2012) Evaluation of animal and public health surveillance systems: a systematic review. *Epidemiology and Infection*, **140**, 575–590
- Drummond, J.C. and Wilbraham, A. (1957) *The Englishman's Food: A History of Five Centuries of English Diet*. Jonathan Cape, London
- Drummond, J.G., Curties, S.E., Meyer, R.C., Simon, J. and Norton, H.W. (1981) Effects of atmospheric ammonia on young pigs experimentally infected with *Bordetella bronchiseptica*. *American Journal of Veterinary Research*, **42**, 963–968
- Dubben, H.-H. and Beck-Bornholdt, H.-P. (2005) Systematic review of publication bias in studies on publication bias. *British Medical Journal*, **331**, 433–434
- Dubé, C., Ribble, C., Kelton, D. and McNab, B. (2009) A review of network analysis terminology and its application to foot-and-mouth disease modelling and

- policy development. *Transboundary and Emerging Diseases*, **56**, 73–85
- Dubos, R. (1965) *Man Adapting*. Yale University Press, New Haven
- Dudden, R.F. and Protzko, S.L. (2011) The systematic review team: contributions of the health sciences librarian. *Medical Reference Services Quarterly*, **30**, 301–315
- Duffy, C.E. (1944) Interference between St. Louis encephalitis virus and equine encephalomyelitis (Western type) in the chick embryo. *Science*, **99**, 517–518
- Dufour, B. and Audigé, L. (1997) A proposed classification of veterinary epidemicsurveillance networks. *Revue Scientifique et Technique, Office International des Epizooties*, **16**, 745–758
- Dufour, B. and Hendrikx, P. (2009) *Epidemiological Surveillance in Animal Health*, 2nd edn. FAO, Rome
- Dufour, B. and La Vieille, S. (2000) Epidemiological surveillance of infectious diseases in France. *Veterinary Research*, **31**, 169–185
- Dukes, T.W. (2000) The other branch of medicine: an historiography of veterinary medicine from a Canadian perspective. *Canadian Bulletin of Medical History*, **17**, 229–243
- Dunbar, I. (1979) *Dog Behaviour: Why Dogs Do What They Do*. T.F.H. Publications, Neptune
- Duncan, A. (2000) Cattle Health Certification Standards (UK) (CHeCS). *Cattle Practice*, **8**, 105–110
- Duncan, I.J.H. (1996) Animal welfare defined in terms of feelings. *Acta Agriculturae Scandinavica, Section A, Animal Science*, Suppl. **27**, 29–35
- Dunne, H.W., Gobble, J.L., Hokanson, J.F., Kradel, D.C. and Bubash, G.R. (1965) Porcine reproductive failure associated with a newly identified 'SMEDI' groups of picorna viruses. *American Journal of Veterinary Research*, **26**, 1284–1297
- Dupont, N. and Stege, H. (2013) Vetstat – monitoring usage of antimicrobials in animals. In: *Challenges and Benefits of Health Data Recording in the Context of Food Chain Quality, Management and Breeding*. ICAR Technical Series No. 17. Eds Egger-Danner, C., Hansen, O.K., Stock, K., Pryce, J.E., Cole, J. *et al.*, pp. 21–37. ICAR, Rome
- Duppong, B.L. and Ettinger, S.J. (1983) The medical record. In: *Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat*, 2nd edn, Vol. 2. Ed. Ettinger, S.J., pp. 3–29. W.B. Saunders Company, Philadelphia
- Dupuy, C., Bronner, A., Watson, E., Wuyckhuise-Sjouke, L., Reist, M. *et al.* (2013) Inventory of veterinary syndromic surveillance initiatives in Europe (Triple-S project): current situation and perspectives. *Preventive Veterinary Medicine*, **111**, 220–229
- Durr, P.A. and Froggatt, A.E.A. (2002) How best to geo-reference farms? A case study from Cornwall, England. *Preventive Veterinary Medicine*, **56**, 51–62
- Durr, P.A., Argyraki, A., Ramsey, M.H. and Clifton-Hadley, R.S. (2000) Agro-ecological databases for spatial correlation studies: methodological issues. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Edinburgh*, 29–31 March 2000. Eds Thrusfield, M.V. and Goodall, E.A., pp. 225–235
- Dürr, S. and Ward, M.P. (2014) Roaming behaviour and home range estimation of domestic dogs in Aboriginal and Torres Strait Islander communities in northern Australia using four different methods. *Preventive Veterinary Medicine*, **117**, 340–357
- Dutton, W.H. and Blank, G., with Groselj, D. (2013) *Cultures of the Internet: The Internet in Britain*. Oxford Internet Survey 2013. Oxford Internet Institute, University of Oxford, Oxford
- Dwan, K., Altman, D.G., Arnaiz, J.A., Bloom, J., Chan, A.-W. *et al.* (2008) Systematic review of the empirical evidence of study publication and outcome reporting bias. *PloS ONE*, **3**, e3081; doi 10.1371/journal.pone.0003081
- Dwan, K., Gamble, C., Kolamunnage-Dona, R., Mohammed, S., Powell, C. *et al.* (2010) Assessing the potential for outcome reporting bias in a review: a tutorial. *Trials*, **11**, 52; doi 10.1186/1745-6215-11-52
- Dwinger, R., Luckins, A.G., Murray, M., Rae, P. and Moloo, S.H.K. (1986) Interference between different serodemes of *Trypanosoma congolense* in the establishment of superinfections in goats following transmission by tsetse. *Parasite Immunology*, **8**, 293–305
- Dye, C. (1999) PETS: Risk of Rabies from North America. Department for Environment, Food and Rural Affairs, London. <http://www.defra.gov.uk/animalh/quarantine/pets/safety/annex.htm> (accessed 18 April 2006)
- Dye, C., Scheele, S., Dolin, P., Pathania, V. and Ravigliione, M. (1999) Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country: WHO Global Surveillance and Monitoring Project. *Journal of the American Medical Association*, **282**, 677–686
- Dyer, J. (1990) *Ancient Britain*. Batsford, London
- Dykhuizen, A.A. (1993) Modelling animal health economics. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Exeter*, 31 March–2 April 1993. Ed. Thrusfield, M.V., pp. ix–xx
- Dzienciowski, R. (1976) Estimating ungulate numbers in a forest by track counts. *Acta Theriologica*, **21**, 217–222

- EAEMP (2000) *Points to Consider on Switching Between Superiority and Non-Inferiority*. CPMP/EWP/482/99. The European Agency for the Evaluation of Medicinal Products, London. (Reproduced in *British Journal of Clinical Pharmacology* (2001), **52**, 223–228)
- Eaglesome, M.D. and Garcia, M.M. (1997) Disease risks to animal health from artificial insemination with bovine semen. *Revue Scientifique et Technique, Office International des Epizooties*, **16**, 215–225
- Eames, I., Tang, J.W. and Wilson, P. (2009) Airborne transmission of disease in hospital. *Journal of the Royal Society Interface*, **6**, S697–S702
- Earn, D.J.D. (2008) A light introduction to modelling recurrent epidemics. In: *Mathematical Epidemiology*. Eds Brauer, F., van den Driessche, P. and Jianhong, W., pp. 3–17. Springer-Verlag, Berlin and Heidelberg
- Ebel, E.D., Hornbaker, R.H. and Nelson, C.H. (1992) Welfare effects of the national pseudorabies eradication program. *American Journal of Agricultural Economics*, **74**, 638–645
- Ebert, D. and Bull, J.J. (2008) The evolution and expression of virulence. In: *Evolution in Health and Disease*, 2nd edn. Eds Stearns, S.C. and Koella, J.C., pp. 153–167. Oxford University Press, Oxford
- Ebert, D. and Mangin, K.L. (1995) The evolution of virulence – when familiarity breeds death. *Biologist*, **42**, 154–156
- ECDC (2006) *The Public Health Risks from Highly Pathogenic Avian Influenza Viruses Emerging in Europe with Specific Reference to Type A/H5N1. Interim ECDC Risk Assessment – October 19th 2005* (updated January 5th 2006). European Centre for Disease Prevention and Control, Stockholm
- Eddy, R.G. (1992) Private health schemes. In: *Livestock Health and Welfare*. Ed. Moss, R., pp. 183–291. Longman Scientific and Technical, Harlow
- Edelsten, R.M. (1995) *Livestock Diseases in the Northern Regions of Somalia. A Report of the British Veterinary Team, 1969–1972*, 1994 Condensed Version. VetAid, Midlothian
- Edney, A.T.B. and Smith, P.M. (1986) Study of obesity in dogs visiting veterinary practices in the United Kingdom. *Veterinary Record*, **118**, 391–396
- Edney, A.T.B., Gaskell, C.J. and Sharp, N.J.H. (Eds) (1987) Feline dysautonomia – an emerging disease. *Journal of Small Animal Practice*, **28**, 333–416
- Edwards, D. (2002) On-farm hazard analysis critical control points (HACCP) for improving the safety of lamb and beef. In: *Food Safety Assurance and Veterinary Public Health. Volume 1. Food Safety Assurance in the Pre-harvest Phase*. Eds Smulders, F.J.M. and Collins, J.D., pp. 299–301. Wageningen Academic, Wageningen
- Edwards, D.S., Henley, W.E., Harding, E.F., Dobson, J.M. and Wood, J.L.N. (2003) Breed incidence of lymphoma in the UK. *Veterinary and Comparative Oncology*, **1**, 200–206
- Edwards, J.R. (1990) Surveys and questionnaires: design, conduct and analysis. In: *Epidemiological Skills in Animal Health. Refresher Course for Veterinarians*. Proceedings 143, Sydney, 1–5 October 1990. Ed. Kennedy, D., pp. 151–158. Post Graduate Committee in Veterinary Science, University of Sydney, Sydney
- Edwards, J.T. (1928) Rinderpest: active immunization by means of the serum simultaneous method; goat virus. *Agricultural Journal of India*, **23**, 185–189
- Edwards, P. (Ed.) (1967) *The Encyclopedia of Philosophy*, Vol. 8. The Macmillan Company and The Free Press, New York
- Edwards, S., Fukusho, A., Lefèvre, P.-C., Lipowski, A., Pejsak, Z. et al. (2000) Classical swine fever: the global situation. *Veterinary Microbiology*, **73**, 103–119
- Efron, B. and Tibshirani, R.J. (1993) *An Introduction to the Bootstrap*. Chapman and Hall, New York
- EFSA (2005) Scientific Report: *Animal Health and Welfare Aspects of Avian Influenza*. Annex to EFSA (2005). *EFSA Journal*, **266**, 1–1. http://www.efsa.eu.int/science/ahaw/ahaw_opinions/1145_en.html (accessed 1 March 2006)
- EFSA (2010) Application of systematic review methodology to food and feed safety assessments to support decision making. *EFSA Journal*, **8** (6), 1637; doi 10.2903/j.efsa.2010.1637
- EFSA (2015) African swine fever: EFSA Panel on Animal Health and Welfare (AHAW). *EFSA Journal*, **13** (7), 4163; doi 10.2903/j.efsa.2015.4163. <http://www.efsa.europa.eu/en/efsajournal/pub/4163> (accessed 11 December 2015)
- Egeberg, R.O. (1954) Coccidioidomycosis: its clinical and climatological aspects with remarks on treatment. *American Journal of Medical Science*, **227**, 268–271
- Egenvall, A., Bonnett, B.N., Olson, P. and Hedhammar, A. (1998) Validation of computerized Swedish dog and cat insurance data against veterinary practice records. *Preventive Veterinary Medicine*, **36**, 51–65
- Egenvall, A., Bonnett, B.N., Shoukri, M., Olson, P., Hedhammar, Å. et al. (2000) Age pattern and mortality in eight breeds of insured dogs in Sweden. *Preventive Veterinary Medicine*, **46**, 1–14
- Egenvall, A., Hedhammar, Å., Bonnett, B.N. and Olson, P. (1999) Survey of the Swedish dog population: gender, breed, location and enrolment in animal insurance. *Acta Veterinaria Scandinavica*, **40**, 231–240
- Egenvall, A., Penell, J.C., Bonnett, B.N., Olson, P. and Pringle, J. (2005) Morbidity of Swedish horses insured for veterinary care between 1997 and 2000: variations

- with age, sex, breed and location. *Veterinary Record*, **157**, 436–443
- Egerton, F.N. (1973) Changing concepts of the balance of nature. *Quarterly Review of Biology*, **48**, 322–350
- Egerton, F.N. (2009) A history of ecological sciences, Part 32: Humboldt, nature's geographer. *Bulletin of the Ecological Society of America*, **90**, 253–282
- Egger, M., Zellweger-Zahner, T., Schneider, M., Junker, C., Lengeler, C. *et al.* (1997) Language bias in randomized controlled trials published in English and German. *The Lancet*, **350**, 326–329
- Eisinger, D. and Thulke, H.-H. (2005) Saving budget in rabies control – revising a classic epidemiological threshold. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Nairn, 30 March–1 April 2004. Eds Reid, S.J.W., Menzies, F.D. and Russell, A.M., pp. 101–110
- Eijigou, A. and McHugh, R. (1977) On the factorization of the crude relative risk. *American Journal of Epidemiology*, **106**, 188–191
- Eksebo, I. (1992) Monitoring systems using clinical, subclinical and behavioural records for improving health and welfare. In: *Livestock Health and Welfare*. Ed. Moss, R., pp. 20–50. Longman Scientific and Technical, Harlow
- Eksebo, I., Oltenacu, P.A., Vilson, B. and Nilsson, J. (1994) A disease monitoring system for dairy herds. *Veterinary Record*, **134**, 270–273
- Ekong, P.S., Ducheyne, E., Carpenter, T.E., Owolodun, O. A., Lombin, L.H. *et al.* (2012) Spatio-temporal epidemiology of highly pathogenic avian influenza (H5N1) outbreaks in Nigeria, 2006–2008. *Preventive Veterinary Medicine*, **103**, 170–177
- Elandt-Johnson, R.C. (1977) Various estimators of conditional probabilities of death in follow-up studies. *Journal of Chronic Diseases*, **30**, 247–256
- Elbers, A.R.W. and Schukken, Y.H. (1995) Critical features of veterinary field trials. *Veterinary Record*, **136**, 187–192
- Elbers, A.R.W., Backx, A., Meroc, E., Gerbier, G., Staubach, C. *et al.* (2008) Field observations during the bluetongue serotype 8 epidemic in 2006 – I. Detection of first outbreaks and clinical signs in sheep and cattle in Belgium, France, and the Netherlands. *Preventive Veterinary Medicine*, **87**, 21–30
- Elbers, A.R.W., Moser, H., Ekker, H.M., Crauwels, P.A.A., Stegeman, J.A. *et al.* (2001) Tracing systems used during the epidemic of classical swine fever in the Netherlands, 1997–1998. *Revue Scientifique et Technique, Office International des Epizooties*, **20**, 614–629
- Elder, J.K. (1976) A functional computer recording system for a veterinary diagnostic laboratory. *Australian Veterinary Journal*, **52**, 24–35
- Elelu, N., Lawal, A., Bolu, S.A., Jaji, Z. and Eisler, M.C. (2016) Participatory epidemiology of cattle diseases among the Fulani pastoralists in Bacita Market, Edu Local Government Area, Kwara State, North-central Nigeria. *EC Veterinary Science*, **2**, 133–144
- Ellenberg, S.S., Fleming, T.R. and DeMets, D.L. (2002) *Data Monitoring Committee in Clinical Trials*. John Wiley, New York
- Ellis, A.K. and McCommon, G.W. (2018) Preventive health programs. In: *McCurnin's Clinical Textbook for Veterinary Technicians*, 9th edn. Eds Basset, J.M., Beal, A.D. and Samples, O.M., pp. 241–273. Elsevier, St Louis
- Ellis, P.D. (2010) *The Essential Guide to Effect Sizes*. Cambridge University Press, Cambridge
- Ellis, P.R. (1980) International aspects of animal disease surveillance. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 7–11 May, 1979. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 11–15. Australian Government Publishing Service, Canberra
- Ellis, P.R., James, A.D. and Shaw, A.P. (1977) *Studies on the Epidemiology and Economics of Swine Fever Eradication in the EEC EUR 5738e*. Commission of the European Communities, Luxembourg
- Elson, C.O., Sartor, R.B., Tennyson, G.S. and Riddell, R.H. (1995) Experimental models of inflammatory bowel disease. *Gastroenterology*, **109**, 1344–1367
- Elton, C.S. (1927) *Animal Ecology*. Macmillan, New York
- Elwell, L.P. and Shipley, P.L. (1980) Plasmid-mediated factors associated with virulence of bacteria to animals. *Annual Review of Microbiology*, **34**, 465–496
- Elwood, J.M. (2007) *Critical Appraisal of Epidemiological Studies and Clinical Trials*, 3rd edn. Oxford University Press, Oxford
- EMA (2011) *Principles for Ensuring Confidentiality of Data Supplied to the European Surveillance of Veterinary Antimicrobial Agent Consumption (ESVAC) Project*. EMA/327935/2010. European Medicines Agency, London. http://www.ema.europa.eu/docs/en_GB/document_library/Other/2011/03/WC500104214.pdf (accessed 16 July 2015)
- EMA (2012) *Guideline on Statistical Principles for Clinical Trials for Veterinary Medicinal Products (Pharmaceuticals)*. EMA/CVMP/EWP/81976/2010. European Medicines Agency, London
- EMEA (2014) *Guideline on Specific Efficacy Requirements for Ectoparasiticides in Cattle*. EMEA/CVMP/625/03/Final. European Medicines Agency, London
- Emori, T.G. and Gaynes, R.P. (1993) An overview of nosocomial infections, including the role of the microbiology laboratory. *Clinical Microbiology Reviews*, **6**, 428–442
- EMPRES (2003) Clarifying disease spread in the Eurasian ruminant street. *EMPRES Transboundary Animal Disease Bulletin*, **23**, 19–23

- EMPRES (2009) Peste des petits ruminants (PPR): an increasing threat to small ruminant production in Africa and Asia. *EMPRES Transboundary Animal Diseases Bulletin*, **33**, 1–8
- Engel, G.L. (1977) The need for a new medical model: a challenge for biomedicine. *Science*, **196**, 129–136
- Engel, M., Wierup, M. and Novén B. (1999) Patterns of seroconversion to Aujeszky's disease virus in unvaccinated chronically infected Swedish weaner pig-producing herds. *Journal of Veterinary Medicine, B*, **46**, 525–533
- Engelken, T.J., Trejo, C. and Voss, K. (2007) Reproductive health programs for beef herds: analysis of record for assessment of reproductive performance. In: *Current Therapy in Large Animal Theriogenology*, 2nd edn. Eds Youngquist, R.S. and Threlfall, W.R., pp. 490–496. Saunders-Elsevier, St Louis
- Engelkirk, P.G. and Duben-Engelkirk, J. (2008) *Laboratory Diagnosis of Infectious Diseases*. Lippincott Williams and Wilkins, Baltimore
- England, J.J. (2002) Biosecurity: safeguarding your veterinarian:client relationship. *Veterinary Clinics of North America: Food Animal Practice*, **18**, 373–378
- Enøe, C., Christensen, G., Andersen, S. and Willeberg, P. (2003) The need for built-in validation of surveillance data so that changes in diagnostic performance of post-mortem meat inspection can be detected. *Preventive Veterinary Medicine*, **57**, 117–125
- Enserink, M. (2007) Tropical disease follows mosquitoes to Europe. *Science*, **317**, 1485
- Enticott, G. (2008) The ecological paradox: social and natural consequences of the geographies of animal health promotion. *Transactions of the Institute of British Geographers*, **33**, 433–446
- Enticott, G., Maye, D., Carmody, P., Naylor, R., Ward, K. *et al.* (2015) Farming on the edge: farmer attitudes to bovine tuberculosis in newly endemic areas. *Veterinary Record*, **177**, 439; doi 10.1136/vr.103187
- EPA (1998) *United States Environmental Protection Agency (EPA) Guidance for Quality Assurance Project Plans*. EPA QA/G-5. EPA Office of Research and Development, Washington DC
- Erb, H. (1984) Economics in veterinary farm practice. *In Practice*, **6**, 33–37
- Erickson, B.H. and Nosanchuk, T.A. (1979) *Understanding Data*. Open University Press, Milton Keynes
- Ernst, S.N. and Fabrega, F. (1989) A time series analysis of the rabies control programme in Chile. *Epidemiology and Infection*, **103**, 651–657
- Erskine, R.J. (2001) Mastitis control in dairy herds In: *Herd Health: Food Animal Production Medicine*, 3rd edn. Ed. Radostits, O.M., pp. 397–433. W.B. Saunders, Philadelphia
- Ertl, H.C.J. (2016) Viral vectors as vaccine carriers. *Current Opinion in Virology*, **21**, 1–8
- Espevedt, M., Lind, A.-K., Wolff, C., Rintakoski, S., Virtala, A.-M. *et al.* (2013) Nordic farmers' threshold for contacting a veterinarian and consequences for disease recording: mild clinical mastitis as an example. *Preventive Veterinary Medicine*, **108**, 114–124
- Esslemont, R.J. (1993a) Measuring fertility in dairy herds. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Exeter, 31 March–2 April 1993*. Ed. Thrusfield, M.V., pp. 96–109
- Esslemont, R.J. (1993b) *The Development of Decision Support Systems in Agriculture: DAISY – The Dairy Information System*. Farm Management Unit Publication No. 30. Department of Agriculture, University of Reading, Reading
- Esslemont, R.J. and Kossaibati, M.A. (2002) *The Costs of Poor Fertility and Disease in UK Dairy Herds*. DAISY Research Report No. 5. Intervet UK, Walton Manor
- Esslemont, R.J., Wassell, B.R., Wassell, T.R., Grimbleby, L., Lamb, J.M. *et al.* (1991) The application of planned animal health and production to dairy farms: DAISY – the Dairy Information System. *The Bovine Practitioner*, **26**, 38–41
- Estes, R.D. (1976) The significance of breeding synchrony in the wildebeest. *East African Wildlife Journal*, **14**, 135–152
- Etherington, W.G., Kinsel, M.L. and Marsh, W.E. (1995) Options in dairy data management. *Canadian Veterinary Journal*, **36**, 28–33
- Eugster, A.K., Bende, R.A. and Hones, C.P. (1978) Parvovirus infection in dogs. *Journal of the American Veterinary Medical Association*, **173**, 1340–1341
- EUROPA (2003) *Regulation (EC) No. 998/2003 of the European Parliament and of the Council of 26 May 2003 on the Animal Health Requirements Applicable to Non-Commercial Movement of Pet Animals and Amending Council Directive 92/65/EEC*. <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=URISERV%3Af83009> (accessed 22 March 2016)
- EUROPA (2008) Commission Regulation (EC) No. 504/2008 of 6 June 2008 implementing Council Directives 90/426/EEC and 90/427/EEC as regards methods for the identification of equidae (text with EEA relevance). *Official Journal of the European Union*, **7.6.2008**, L149/3–L149/32. Commission of the European Communities, Brussels. <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32008R0504> (accessed 22 August 2016)
- EUROPA (2015a) *ANIMO*. http://ec.europa.eu/food/animal/diseases/animo/index_en.htm (accessed 22 July 2015)

- EUROPA (2015b) *TRACES*. http://ec.europa.eu/food/animal/diseases/traces/index_en.htm (accessed 22 July 2015)
- European Commission (2002) *Proposal for a Council Directive on Community Measures for the Control of Foot-and-Mouth Disease and Amending Directive 92/46/EEC*. COM(2002) 736 final, 2002/0299 (CNS). Commission of the European Communities, Brussels
- European Commission (2006) *Working Document on Bluetongue Monitoring and Surveillance in the EU*. SANCO/10581/2006 Rev 4. European Commission Health & Consumer Protection Directorate-General, Brussels. http://ec.europa.eu/food/animal/diseases/controlmeasures/bluetongue_wd_en.pdf (accessed 19 August 2015)
- Evans, A.S. (1976) Causation and disease. The Henle-Koch postulates revisited. *Yale Journal of Biology and Medicine*, **49**, 175–195
- Evans, A.S. (1984) Surveillance and seroepidemiology. In: *Viral Infections of Humans: Epidemiology and Control*. Ed. Evans, A. S., pp. 43–64. Plenum Publishing Company, New York
- Evans, A.S. (1987) Subclinical epidemiology. *American Journal of Epidemiology*, **125**, 545–555
- Evans, A.S. (1993) *Causation and Disease: A Chronological Journey*. Plenum Publishing Corporation, New York
- Evans, A.S. and Mueller, N.E. (1992) The past is prologue: use of serum banks in cancer research. *Cancer Research*, **52**, Suppl., 5557 s–5560 s
- Evans, M., Hastings, N. and Peacock, B. (2000) *Statistical Distributions*, 3rd edn. John Wiley, New York
- Evans-Pritchard, E.E. (1956) *Nuer Religion*. Oxford University Press, London
- Everitt, B.S. (1989) *Statistical Methods for Medical Investigations*. Oxford University Press, New York/Edward Arnold, London
- Everitt, B.S. (1995) *The Cambridge Dictionary of Statistics in the Medical Sciences*. Cambridge University Press, Cambridge
- Everitt, B.S. (2005) *An R and S-PLUS Companion to Multivariate Analysis*. Springer-Verlag, London
- Everitt, B. (2006) *Medical Statistics from A to Z*, 2nd edn. Cambridge University Press, Cambridge
- Everitt, B.S. and Dunn, G. (2001) *Applied Multivariate Data Analysis*, 2nd edn. Arnold, London/Oxford University Press, New York
- Everitt, B.S. and Skrondal, A. (2010) *The Cambridge Dictionary of Statistics*, 4th edn. Cambridge University Press, Cambridge
- Evermann, J.F., Bryan, G.M. and McKiernan, A.J. (1981) Isolation of a calicivirus from a case of canine glossitis. *Canine Practice*, **8**, 36–39
- Evermann, J.F., Laurenson, M.K., McKeirnan, A.J. and Caro, T.M. (1993) Infectious disease surveillance in captive and free-living cheetahs: an integral part of the species survival plan. *Zoo Biology*, **12**, 125–133
- Evers, E.G. and Nauta, M.J. (2001) Estimation of animal-level prevalence from pooled samples in animal production. *Preventive Veterinary Medicine*, **49**, 175–190
- Ewald, P.W. (1995) The evolution of virulence: a unifying link between parasitology and ecology. *Journal of Parasitology*, **81**, 465–485
- Ewald, P.W. (2000) *Plague Time: How Stealth Infections Cause Cancers, Heart Disease, and Other Deadly Ailments*. The Free Press, New York
- Ewald, P.W. and Swain Ewald, H.A. (2014) Joint infectious causation of human cancers. *Advances in Parasitology*, **84**, 1–26
- Ewbank, D. and Wray, J.D. (1980) Population and public health. In: *Maxcy-Rosenau: Public Health and Preventive Medicine*, 11th edn. Ed. Last, J.M., p. 1504–1548. Appleton-Century-Crofts, New York
- Ewbank, R. (1986) Animal welfare – is an epidemiological approach possible? In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings*, Edinburgh, 2–4 April 1986. Ed. Thrusfield, M.V., pp. 92–96
- Ewbank, R. and Cullinan, A. (1984) The effect of simulated sonic booms on horses. In: *Proceedings of the International Congress on Applied Ethology in Farm Animals*, Kiel. Eds Unschem, J., van Putten, G. and Zeeb, K., pp. 325–328. Kuratorium für Technik und Bauwesen in der Landwirtschaft, Darmstadt
- Eylenbosch, W.J. and Noah, N.D. (Eds) (1988) *Surveillance in Health and Disease*. Oxford University Press, Oxford
- Eyler, J.M. (2001) The changing assessments of John Snow's and William Farr's cholera studies. *Sozial- und Präventivmedizin/Social and Preventive Medicine*, **46**, 225–232
- Eysenbach, G. (2009) Infodemiology and infoveillance: framework for an emerging set of public health informatics methods to analyse search, communication and publication behavior on the Internet. *Journal of Medical Internet Research*, **11** (1), e11; doi 10.2196/jmir.1157
- Fabia, J., Boivin, J.-F., Bernard, P.-M., Ducic, S. and Thuriaux, M. (1988) English-French and French-English dictionary of terms used in epidemiology. *International Journal of Epidemiology*, **17** (1), Suppl., 1–49
- Fabricant, C.G. and Gillespie, J.H. (1974) Identification and characterization of a second feline herpesvirus. *Infection and Immunity*, **9**, 460–466
- Fabrigar, L.R., Krosnick, J.A. and MacDougall, B.L. (2005) Attitude measurement: techniques for measuring the unobservable. In: *Persuasion: Psychological Insights and Perspectives*, 2nd edn. Eds Brock, T.C. and Green, M.C., pp. 17–40. Sage Publications, Thousand Oaks

- Faes, C., Aerts, M., Litière, S., Méroc, E., Van der Stede, Y. *et al.* (2011) Estimating herd prevalence on the basis of aggregate testing of animals. *Journal of the Royal Statistical Society, Series A*, **174**, 155–174
- Fagan, T.J. (1975) Nomogram for Bayes's Theorem (Letter). *The New England Journal of Medicine*, **293**, 257
- Fagerstone, K.A. and Biggins, D.E. (1986) Comparison of capture-recapture and visual count indices of prairie dog densities in black-footed ferret habitat. *Great Basin Naturalist Memoirs*, **8**, 94–98
- Fahey, J.E. and Crawley, J.F. (1954) Studies on chronic respiratory disease of chickens. II. Isolation of a virus. *Canadian Journal of Comparative Medicine*, **18**, 13–21
- FAHRMX (1984) FAHRMX dairy herd health management computer network. In: Proceedings of the Second Symposium on Computer Applications in Veterinary Medicine, College of Veterinary Medicine, Mississippi State University, 23–25 May 1984, pp. 45–50
- Falconer, D.S. and Mackay, T.F.C. (1996) *Introduction to Quantitative Genetics*, 4th edn. Longman, Harlow
- Fall, T., Hamlin, H.H., Hedhammar, A., Kämpe, O. and Egenvall, A. (2007) Diabetes mellitus in a population of 180,000 insured dogs: incidence, survival, and breed distribution. *Journal of Veterinary Internal Medicine*, **21**, 1209–1216
- Fallon, R.J. (2001) The development and use of electronic ruminal boluses as a vehicle for bovine identification. *Revue Scientifique et Technique, Office International des Epizooties*, **20**, 480–490
- Falzon, L.C., O'Neill, T.J., Menzies, P.I., Peregrine, A.S., Jones-Bitton, A. *et al.* (2014) A systematic review and meta-analysis of factors associated with anthelmintic resistance in sheep. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Dublin, 26–28 March 2014. Eds Mintiens, K. and Parkin, T.D.H., pp. 35–50
- Fan, N. and Lai, L. (2013) Genetically modified pig models for human diseases. *Journal of Genetics and Genomics*, **40**, 67–73
- Fang, D.Y. and Wang, Y.K. (1981) Studies on the aetiology and specific control of goose parvovirus infection. *Scientia Agricultura Sinica*, **4**, 1–8 (in Chinese)
- Fanshawe, T.R., Diggle, P.J., Rushton, S., Sanderson, R., Lurz, P.W.W. *et al.* (2008) Modelling spatio-temporal variation in exposure to particulate matter: a two-stage approach. *Environmetrics*, **19**, 549–566
- FAO (1999) *Agro-ecological Zones, Farming Systems and Land Pressure in Africa and Asia*. Consultancy Report by ERGO Ltd to the Animal Health Division of the Food and Agriculture Organization of the United Nations, Rome
- FAO (2014) *GAEZ Global Agro-Ecological Zones*. Food and Agriculture Organization of the United Nations, Rome. <http://www.fao.org/nr/gaez/en/> (accessed 10 March 2014)
- FAO (2015) *Building Veterinary Laboratory Diagnostic Capacity in Africa: The VETLAB Network*. Food and Agriculture Organization of the United Nations, Rome. <http://www.fao.org/emergencies/resources/documents/resources-detail/en/c/295768/> (accessed 18 March 2016)
- FAO/OIE (2011) *Joint FAO/OIE Committee on Global Rinderpest Eradication Final Report, May 2011*. Food and Agriculture Organization of the United Nations, Rome/World Organisation for Animal Health (OIE), Paris
- FAO/WHO (1967) *Report of a Joint Food and Agriculture Organization/World Health Organization Expert Committee on Zoonoses*. Technical Report Series No. 3. World Health Organization, Geneva
- FAO/WHO (2002) *Risk Assessments of Salmonella in Eggs and Broiler Chickens*. Microbiological Risk Assessment Series 2. Food and Agriculture Organization of the United Nations, Rome/World Health Organization, Geneva
- FAO/WHO/OIE (1997) *Animal Health Yearbook 1995*. Food and Agriculture Organization of the United Nations, World Health Organization, Office International des Epizooties. Food and Agricultural Organization of the United Nations, Rome
- Fara, P. (2009) *Science: A Four Thousand Year History*. Oxford University Press, Oxford
- Farina A. (2000a) *Landscape Ecology in Action*. Kluwer Academic Publishers, Dordrecht
- Farina, A. (2000b) *Principles and Methods in Landscape Ecology*. Kluwer Academic Publishers, Dordrecht
- Farley, J.U. and Lehmann, D. (1986) *Meta-Analysis in Marketing Generalization of Response Models*. Lexington Books, Lexington
- Farmer, P. (1999) *Infections and Inequalities: The Modern Plagues*. University of California Press, Berkeley
- Farmer, P.E., Nizeye, B., Stulac, S. and Keshavjee, S. (2006) Structural violence and clinical medicine. *PLoS Med*, **3**, 10, e449; doi 10.1371/journal.pmed.0030449
- Farr, W. (1840) Appendix to the Second Annual Report of the Registrar-General of Births, Deaths, and Marriages in England, pp. 3–22. William Clowes and Sons, London. In: *House of Commons Parliamentary Papers, Vol. XVII. Reports from Commissioners, 2, Births; Deaths; Marriages; Poor Laws, Session 16 January–11th August, 1840*, pp. 39–58
- Farr, W. (1852) *Report on the Mortality of Cholera in England*. W. Clowes, London
- Farver, T.B. (2008) Concepts of normality in clinical chemistry. In: *Clinical Biochemistry of Domestic*

- Animals*, 6th edn. Eds Kaneko, J.J., Harvey, J.W. and Bruss, M.L., pp. 1–25. Academic Press, Burlington, San Diego and London
- Fascetti, A.J., Morris, J.G. and Rogers, Q.R. (2010) Nutrition of healthy dogs and cats in various adult stages. In: *Textbook of Veterinary Internal Medicine*, 7th edn. Eds Ettinger, S.J. and Feldman, E.C., pp. 661–666. Saunders-Elsevier, St Louis
- Fastier, L.B. (1957) A new feline virus isolated in tissue culture. *American Journal of Veterinary Research*, **18**, 382–389
- Fauci, A.S. and Morens, D.M. (2012) The perpetual challenge of infectious diseases. *The New England Journal of Medicine*, **366**, 454–461
- Fawcett, J. and Head, R. (2001a) *Foot and Mouth Disease: Business Impact Tracking Survey Scotland (June 2001), Second Wave*. Scottish Executive Central Research Unit, Edinburgh
- Fawcett, J. and Head, R. (2001b) *Foot and Mouth Disease: Business Impact Tracking Survey Scotland (September 2001), Third Wave*. Scottish Executive Central Research Unit, Edinburgh
- Fearon, R. (2004) Vets warned against microchip anomalies. *Veterinary Times*, **34** (42), 1–2
- Feeney, C. (2004) PETS travel scheme. *Irish Veterinary Journal*, **57**, 406–407
- Feinstein, A.R. and Cicchetti, D.V. (1990) High agreement but low kappa, I: the problems of two paradoxes. *Journal of Clinical Epidemiology*, **43**, 543–549
- Fekadu, M. and Baer, G.M. (1980) Recovery from clinical rabies of 2 dogs inoculated with a rabies virus strain from Ethiopia. *American Journal of Veterinary Research*, **41**, 1632–1634
- Felder, S. and Mayrhofer, T. (2011) *Medical Decision Making: A Health Economic Primer*. Springer-Verlag, Berlin and Heidelberg
- Felton, M.R. and Ellis, P.R. (1978) *Studies in the Control of Rinderpest in Nigeria*. Study No. 23. University of Reading, Reading
- Fennell, C. (1975) Some demographic characteristics of the domestic cat population in Great Britain with particular reference to feeding habits and the incidence of the feline urological syndrome. *Journal of Small Animal Practice*, **16**, 775–783
- Fenner, F. and Ratcliffe, F.N. (1965) *Myxomatosis*. Cambridge University Press, Cambridge
- Fenner, F., Henderson, D.A., Arita, I., Jezek, Z. and Ladnyi, I.D. (1988) *Smallpox and its Eradication*. World Health Organization, Geneva
- Fenton, A. (2013) Dances with worms: the ecological and evolutionary impacts of deworming on coinfecting pathogens. *Parasitology*, **140**, 1119–1132
- Ferber, D. (2002) Livestock feed ban preserves drugs' power. *Science*, **295**, 27–28
- Ferguson, N.M., Donnelly, C.A. and Anderson, R. M. (2001) The foot-and-mouth epidemic in Great Britain: patterns of spread and impact of interventions. *Science*, **292**, 1155–1160
- Ferguson, N.M., Galvani, A.P. and Bush, R.M. (2003) Ecological and immunological determinants of influenza evolution. *Nature*, **422**, 428–433
- Fernández-Guerrero, I.M., Torralbo, M. and Fernández-Cano, A. (2014) A forerunner of qualitative health research: Risueño's report against the use of statistics. *Qualitative Health Research*, **24**, 124–135
- Fernandez-Mehler, P., Gloor, P., Sager, E., Lewis, F.I. and Glaus, T.M. (2013) Veterinarians' role for pet owners facing pet loss. *Veterinary Record*, **172**, 555–562
- Ferrè, N., Mulatti, P., Mazzucato, M., Lorenzetto, M., Trolese, M. et al. (2011) GeoCREV: veterinary geographical information system and the development of a practical sub-national spatial data infrastructure. *Geospatial Health*, **5**, 275–283
- Ferreira-Paim, K., Andrade-Silva, L., Mora, D.J., Pedrosa, A.L. et al. (2010) Genotyping of *Cryptococcus neoformans* isolated from captive birds in Uberaba, Minas Gerais, Brazil. *Mycoses*, **54**, e294–e300; doi 10.1111/j.1439-0507.2010.01901.x
- Ferris, D.H. (1967) Epizootiology. *Advances in Veterinary Science*, **11**, 261–320
- Ferris, D.H. (1971) Epizootiologic features of transmissible swine gastroenteritis. *Journal of the American Veterinary Medical Association*, **159**, 184–194
- Fetrow, J., Madison, J.B. and Galligan, D. (1985) Economic decisions in veterinary practice: a method for field use. *Journal of the American Veterinary Medical Association*, **186**, 792–797
- Fetrow, J., Stewart, S. and Eicker, S. (1997) Reproductive health programs for dairy herds: analysis of records for assessment of reproductive performance. In: *Current Therapy in Large Animal Theriogenology*, Ed. Youngquist, R.S., pp. 441–451. W.B. Saunders, Philadelphia
- Fetrow, J., Stewart, S., Eicker, S. and Rapnicki, P. (2007) Reproductive health programs for dairy herds: analysis of record for assessment of reproductive performance. In: *Current Therapy in Large Animal Theriogenology*, 2nd edn. Eds Youngquist, R.S. and Threlfall, W.R., pp. 473–489. Saunders-Elsevier, St Louis
- Fèvre, E.M., Coleman, P.G., Odiit, M., Magona, J.W., Welburn, S.C. et al. (2001) The origins of a new *Trypanosoma brucei rhodesiense* sleeping sickness outbreak in eastern Uganda. *The Lancet*, **358**, 625–628
- Fey, P.D., Safraneck, T.J., Rupp, M.E., Dunne, E.F., Ribot, E. et al. (2000) Ceftriaxone-resistant Salmonella infection acquired by a child from cattle. *The New England Journal of Medicine*, **342**, 1242–1249

- Feyerabend, P. (1975) *Against Method: Outline of Anarchistic Theory of Knowledge*. Verso, London
- Feynman, R.P. (1988) *What Do You Care What Other People Think? Further Adventures of a Curious Character*. W.W. Norton, New York and London
- Fidgett, A.L., Pullen, P.K. and Brunger, D. (2008) *Zoo Research Guidelines*. British and Irish Association of Zoos and Aquariums, London
- Fidler, I.J., Brodey, R.S., Howson, A.E. and Cohen, D. (1966) Relationship of estrus irregularity, pseudopregnancy, and pregnancy to canine pyometra. *Journal of the American Veterinary Medical Association*, **149**, 1043–1046
- Field, C.R. and Simkin, S.P. (1985) Camel production in Kenya. In: *Significance and Prospects of Camel Pastoralism*. IDS Occasional Paper No.45. Ed. Migot-Adholla, S.E., pp. 144–168. Institute for Development Studies, University of Nairobi, Nairobi
- Fielding, R.D. (2004) Ethnoveterinary medicine in the tropics. In: *Bovine Medicine: Diseases and Husbandry of Cattle*, 2nd edn. Eds Andrews, A.H., Blowey, R.W., Boyd, H. and Eddy, R.G., pp. 83–87. Blackwell Science, Oxford
- Findlay, G.M. and MacCallum, F.O. (1937) An interference phenomenon in relation to yellow fever and other viruses. *Journal of Pathology and Bacteriology*, **44**, 405–424
- Fine, P.E.M. (2003) The interval between successive cases of an infectious disease. *American Journal of Epidemiology*, **158**, 1039–1047
- Fine, P., Eames, K. and Heymann, D.L. (2011) 'Herd immunity': a rough guide. *Clinical Infectious Diseases*, **52**, 911–916
- Finlay, B.B. and Falkow, S. (1997) Common themes of microbial pathogenicity revisited. *Microbiology and Molecular Biology Reviews*, **61**, 136–169
- Finney, D.J. (1971) *Probit Analysis*, 3rd edn. Cambridge University Press, Cambridge
- Finney, D.J. (1978) *Statistical Method in Biological Assay*, 3rd edn. Charles Griffin, London and High Wycombe
- Firth, A.M. and Haldane, S.L. (1999) Development of a scale to evaluate postoperative pain in dogs. *Journal of the American Veterinary Medical Association*, **214**, 651–659
- Fisch, M.H. (Ed.) (1982) *Writings of Charles S. Peirce, A Chronological Edition, Volume 1, 1857–1866*. Indiana University Press, Bloomington
- Fischhoff, B., Slovic, P. and Lichtenstein, S. (1978) How safe is enough? A psychometric study of attitudes towards technological risks and benefits. *Policy Sciences*, **9**, 127–152
- Fisher, R.B. (1991) *Edward Jenner: 1749–1823*. André Deutsch, London
- Flasse, S., Walker, C., Biggs, H., Stephenson, P. and Hutchinson, P. (1998) Using remote sensing to predict outbreaks of *Oestrus ovis* in Namibia. *Preventive Veterinary Medicine*, **33**, 31–38
- Flegal, K.M., Keyl, P.M. and Nieto, F.J. (1991) Differential misclassification arising from nondifferential errors in exposure measurement. *American Journal of Epidemiology*, **134**, 1233–1244
- Fleiss, J.L. (1986) Analysis of data from multiclinic trials. *Controlled Clinical Trials*, **7**, 267–275
- Fleiss, J.L., Levin, B. and Paik, M.C. (2003) *Statistical Methods for Rates and Proportions*, 3rd edn. John Wiley, Hoboken
- Flesness, N.R. (2003) International Species Information System, (ISIS): over 25 years of compiling global animal data to facilitate collection and population management. *International Zoo Yearbook*, **38**, 53–61
- Fletcher, J. (2012) What is heterogeneity and is it important? *British Medical Journal*, **334**, 94–96
- Fletcher, R. (1896) *The Witches' Pharmacopoeia*. The Freidenwald Co., Baltimore. Reprinted 2003 by The Kessinger Publishing Co., Kila
- Fletcher, R.H., Fletcher, S.W. and Fletcher, G.S. (2014) *Clinical Epidemiology: the Essentials*, 5th edn. Lippincott Williams and Wilkins, Baltimore
- Fletcher, R.H., Fletcher, S.W. and Wagener, E.H. (1988) *Clinical Epidemiology: the Essentials*, 2nd edn. Williams and Wilkins, Baltimore
- Flint, J.C., and Moss, L.C. (1953) Infectious anaemia in cats. *Journal of the American Veterinary Medical Association*, **122**, 45–48
- Flower, S.S. (1931) Contributions to our knowledge of the duration of life in vertebrates. V. Mammals. *Proceedings of the Zoological Society of London*, **1931**, 145–234
- Fluss, R., Faraggi, D. and Reiser, B. (2005) Estimation of Youden index and its associated cutoff point. *Biometrical Journal*, **47**, 458–472
- Fock, K.M., Graham, D.Y. and Malfertheiner, P. (2012) *Helicobacter pylori* research: historical insights and future directions. *Nature Reviews Gastroenterology and Hepatology*, **10**, 495–500
- Foggia, C.M. (1983) Mokola virus infection in cats and a dog in Zimbabwe. *Veterinary Record*, **113**, 115
- Foley, C.W., Lasley, J.F. and Osweiler, G.D. (1979) *Abnormalities of Companion Animals: Analysis of Heritability*. Iowa State University Press, Ames
- Foley, J.E. (2012) Prevention and management of infection in multiple-cat environments. In: *Infectious Diseases of the Dog and Cat*, 4th edn. Ed. Greene, C.E., pp. 1130–1136. Elsevier-Saunders, St Louis
- Folk, L.C., Hahn, A.W., Patrick, T.B., Allen, G.K., Smith, A.B. et al. (2002) Salvaging legacy data: mapping an obsolete medical nomenclature to a modern one. *Biomedical Sciences Instrumentation*, **38**, 405–410

- Fooks, A.R., McElhinney, L.M., Brookes, S.M., Johnson, N., Keene, V. *et al.* (2002) Rabies antibody testing and the UK Pet Travel Scheme. *Veterinary Record*, **150**, 428–430
- Forbes, N.A., Simpson, G.N., Higgins, R.J. and Gough, R. E. (1997) Adenovirus infection in Mauritius kestrels (*Falca punctatus*). *Journal of Avian Medicine and Surgery*, **11**, 31–33
- Forbes, R.N., Sanson, R.L. and Morris, R.S. (1994) Application of subjective methods to the determination of the likelihood and consequences of the entry of foot-and-mouth disease into New Zealand. *New Zealand Veterinary Journal*, **42**, 81–88
- Ford, J. (1971) *The Role of the Trypanosomiases in African Ecology: A Study of the Tsetse Fly Problem*. Clarendon Press, Oxford
- Forman, A.J. and Garland, A.J.M. (2002) Foot and mouth disease: the future of vaccine banks. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 601–612
- Forman, R.T.T. and Godron, M. (1981) Patches and structural components for a landscape ecology. *BioScience*, **31**, 733–740
- Forman, R. and Godron, M. (1986) *Landscape Ecology*. John Wiley and Sons, New York
- Forman, S., Le Gall, F., Belton, D., Evans, B., François, J.L. *et al.* (2009) Moving towards the global control of foot and mouth disease: an opportunity for donors. *Revue Scientifique et Technique, Office International des Epizooties*, **28**, 883–896
- Forrow, L., Taylor, W.C. and Arnold, R. (1992) Absolutely relative: how research results are summarised can affect treatment decisions. *American Journal of Medicine*, **92**, 121–124
- Forster, F. (1966) Use of a demographic base map for the presentation of aerial data in epidemiology. *British Journal of Preventive and Social Medicine*, **20**, 156–171
- Fosgate, G.T. (2009) Practical sample size calculations for surveillance and diagnostic investigations. *Journal of Veterinary Diagnostic Investigation*, **21**, 3–14
- Fouchier, R.A.M., Osterhaus, A.D.M.E. and Brown, I.H. (2003) Animal influenza virus surveillance. *Vaccine*, **21**, 1754–1757
- Fourichon, C.S.H., Seegers, H. and Malher, X. (2000) Effect of disease on reproduction in the dairy cow: a meta analysis. *Theriogenology*, **53**, 1729–1759
- Fowlkes, E.B. (1987) *A Folio of Distributions: A Collection of Theoretical Quantile-Quantile Plots*. Marcel Dekker, New York
- Fox, J.P. (1958) Prophylaxis against rabies in humans. *Annals of the New York Academy of Sciences*, **70**, 480–494
- Fox, M.T. and Sykes, T.J. (1985) Establishment of the tropical dog tick, *Rhipicephalus sanguineus*, in a house in London. *Veterinary Record*, **116**, 661–662
- France, J. and Kebreab, E. (Eds) (2008) *Mathematical Modelling in Animal Nutrition*. CABI, Wallingford
- Francis, J. (1947) *Bovine Tuberculosis: Including a Contrast with Human Tuberculosis*. Staple Press, London
- Frankena, K., Stassen, E.N., Noordhuizen, J.P. and Goelema, J.O. (1991) Lameness in dairy cattle during housing: general descriptive results and risk analysis for dermatitis digitalis. In: Proceedings of the Sixth International Symposium on Veterinary Epidemiology and Economics, Ottawa, 12th–16th August 1991. Ed. Martin, S.W., pp. 276–279
- Franti, C.E. and Kraus, J.F. (1974) Aspects of pet ownership in Yolo County, California. *Journal of the American Veterinary Medical Association*, **164**, 166–171
- Franti, C.E., Kraus, J.F. and Borhani, N.O. (1974) Pet ownership in a suburban-rural area of California, 1970. *Public Health Reports*, **89**, 473–484
- Franti, C.E., Kraus, J.F., Borhani, N.O., Johnson, S.L. and Tucker, S.D. (1980) Pet ownership in rural northern California (El Dorado County). *Journal of the American Veterinary Medical Association*, **176**, 143–149
- Frape, D. (2010) *Equine Nutrition and Feeding*, 4th edn. Wiley-Blackwell, Chichester
- Fraser, A.C. (1969) Advisory and preventive medicine in equine practice. *Veterinary Record*, **85**, 249–250
- Fraser, A.F. (1976) *Topics in Theriogenology. A Supplementary Handbook on Special Practical and Clinical Features of Veterinary Reproduction*. Western College of Veterinary Medicine, Saskatoon
- Frazer, J.G. (1890) *The Golden Bough: A Study in Comparative Religion*, Vol. 2. Macmillan, London
- Freer, M. and Dove, H. (Eds) (2002) *Sheep Nutrition*. CABI Publishing, Wallingford
- French, N.P., Berriatua, E., Kaya, G. and Morgan K.L. (1998) Case control study of diarrhoea and faecal soiling in two- to six-month-old lambs. *Veterinary Record*, **143**, 408–412
- French, N.P., Berriatua, E., Wall, R., Smith, K. and Morgan, K.L. (1999) Sheep scab outbreaks in Great Britain between 1973 and 1992: spatial and temporal patterns. *Veterinary Parasitology*, **83**, 187–200
- French, N.P., Wall, R. and Morgan K.L. (1994) Lamb tail docking: a controlled field study of the effects of tail amputation on health and productivity. *Veterinary Record*, **124**, 463–467
- Frenkel, J.K. (1969) Choice of animal models for the study of disease processes in man. *Federation Proceedings*, **28**, 160–161
- Freshman, J.L., Reif, J.S., Allen, T.A., and Jones, R.L. (1989) Risk factors associated with urinary tract infection in female dogs. *Preventive Veterinary Medicine*, **7**, 59–67

- Frey, J.H. (1989) *Survey Research by Telephone*, 2nd edn. Sage, Newbury Park
- Friedman, L.M., Furberg, C.D., DeMets, D.L., Reboussin, D.M. and Granger, C.B. (2015) *Fundamentals of Clinical Trials*, 5th edn. Springer Cham, Heidelberg
- Friedrichs, K.R., Harr, K.E., Freeman, K.P., Szladovits, B., Walton, R.M. *et al.* (2012) ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. *Veterinary Clinical Pathology*, **41**, 441–453
- Fries, C.L. and Remedios, A.M. (1995) The pathogenesis and diagnosis of canine hip dysplasia: a review. *Canadian Veterinary Journal*, **36**, 494–502
- Fritschen, R.D. (1979) Housing and its effect on feet and leg problems. *The Pig Veterinary Society Proceedings*, **5**, 95–98
- Frössling, J., Bonnett, B., Lindberg, A. and Björkman, C. (2003) Validation of *Neospora caninum* iscom ELISA without a gold standard. *Preventive Veterinary Medicine*, **57**, 141–153
- Frost, W.H. (1976) Some conceptions of epidemics in general. *American Journal of Epidemiology*, **103**, 141–151
- Frumkin, H. and Berlin, J. (1988) Asbestos exposure and gastrointestinal malignancy: review and meta-analysis. *American Journal of Industrial Medicine*, **14**, 79–95
- Fry, R. and Green, R. (1999) Biological and migrational characteristics of microchips (Letter). *Veterinary Record*, **145**, 564
- Frye, G.H. (1995) Bovine tuberculosis eradication: the program in the United States. In: *Mycobacterium bovis Infection in Animals and Humans*. Eds Thoen, C.O. and Steele, J.H., pp. 119–129. Iowa State University Press, Ames
- Fryxell, J.M., Greever, J. and Sinclair, A.R.E. (1988) Why are migratory ungulates so abundant? *American Naturalist*, **131**, 781–798
- Fuentes, M.V., Valero, M.A., Bargues, M.D., Esteban, J.G., Angles, R. *et al.* (1999) Analysis of climatic data and forecast indices for human fascioliasis at very high altitude. *Annals of Tropical Medicine & Parasitology*, **93**, 835–850
- Fuller, S. (2003) *Kuhn vs Popper*. Icon Books, Cambridge
- Fulton, R.W., Purdy, C.W., Confer, A.W., Saliki, J.T., Loan, R.W. *et al.* (2000) Bovine viral diarrhoea infections in feeder calves with respiratory disease: Interactions with *Pasteurella* spp. parainfluenza-3 virus, and bovine respiratory syncytial virus. *Canadian Journal of Veterinary Research*, **64**, 151–159
- Furtado, A.R.R. and Constantino-Casas, F. (2013) Histopathology inflammation scoring misclassification in 34 dogs with inflammatory nasal disease. *Veterinary Record*, **173**, 71; doi 10.1136/vr.101120
- Gabel, M.J., Foster, N.L., Heidebrink, J.L., Higdon, R., Aizenstein, H.J. *et al.* (2010) Validation consensus panel diagnosis in dementia. *Archives of Neurology*, **67**, 1506–1512
- Gaddum, J.H. (1933) *Reports of Biological Standards. III. Methods of Biological Assay Depending on a Quantal Response*. Special Report Series No. 183. Medical Research Council, London
- Gage, K.L. and Montenieri, J.A. (1994) The role of predators in the ecology, epidemiology, and surveillance of plague in the United States. In: *16th Vertebrate Pest Conference*. Eds Halverson, W.S. and Crabb, A.H., pp. 200–206. University of California, Davis
- Gagnon, F. (1950) Contribution to the study of the etiology and prevention of cancer of the cervix of the uterus. *American Journal of Obstetrics and Gynecology*, **60**, 516–522
- Gagnon, R., Charlin, B., Coletti, M. and Sauve, E. and Van, D.V. (2005) Assessment in the context of uncertainty: how many members are needed on the panel of a reference script concordance test? *Medical Education*, **39**, 284–291
- Gail, M.H. (2005) Case-control study. In: *Encyclopedia of Biostatistics*, 2nd edn, Vol. 1. Eds Armitage, P. and Colton, T., pp. 503–514. John Wiley and Sons, Chichester
- Gale, P. and Stanfield, G. (2001) Towards a quantitative risk assessment for BSE in sewage sludge. *Journal of Applied Microbiology*, **91**, 563–569
- Gale, P., Brouwer, A., Ramnial, V., Kelly, L., Kosmider, R. *et al.* (2010) Assessing the impact of climate change on vector-borne viruses in the EU through the elicitation of expert opinion. *Epidemiology and Infection*, **138**, 214–225
- Gale, P., Stephenson, B., Brouwer, A., Martinez, M., de la Torre, A. *et al.* (2012) Impact of climate change on risk of incursion of Crimean-Congo haemorrhagic fever virus in livestock in Europe through migratory birds. *Journal of Applied Microbiology*, **112**, 246–257
- Gale, P., Young, C., Stanfield, G. and Oakes, D. (1998) Development of a risk assessment for BSE in the aquatic environment. *Journal of Applied Microbiology*, **84**, 467–477
- Galen, R.S. (1982) Application of the predictive value model in the analysis of test effectiveness. *Clinical and Laboratory Medicine*, **2**, 685–699
- Galen, R.S. and Gambino, S.R. (1975) *Beyond Normality – The Predictive Value and Efficiency of Medical Diagnoses*. John Wiley, New York
- Gallagher, E., Kelly, L., Pfeiffer, D.U. and Wooldridge, M. (2003) A quantitative risk assessment for badger to cattle transmission of *Mycobacterium bovis*. In: Society for Veterinary Epidemiology and Preventive Medicine,

- Proceedings, Warwick, 31 March–2 April 2003. Eds Reid, S.W.J. and Menzies, F.D, pp. 33–44
- Galuzo, I.G. (1975) Landscape epidemiology (epizootiology). *Advances in Veterinary Science and Comparative Medicine*, **19**, 73–96
- Galvani, A. (2003) Epidemiology meets evolutionary ecology. *Trends in Ecology and Evolution*, **18**, 132–139
- Gani, R. and Leach, S. (2001) Transmission potential of smallpox in contemporary populations. *Nature*, **414**, 748–751
- Ganter, M. (2008) Veterinary consultancy and health schemes in sheep: experiences and reflections from a local German outlook. *Small Ruminant Research*, **76**, 55–67
- Gantner, G.E. (1977) History of coding. *Pathologist*, **31**, 390–395
- Ganusov, V.V., Bergstrom, C.T. and Antia, R. (2002) Within-host population dynamics and the evolution of microparasites in a heterogeneous host population. *Evolution*, **56**, 213–223
- Garcia, C., Thébault, A., Dégremont, L., Arzul, I., Miossec, L. *et al.* (2011) Ostreid herpesvirus 1 detection and relationship with *Crassostrea gigas* spat mortality in France between 1998 and 2006. *Veterinary Research*, **42**, 73; doi 10.1186/1297-9716-42-73
- García-Lastra, R., Leginagoikoa, I., Plazaola, J.M., Ocabo, B., Aduriz, G. *et al.* (2012) Bluetongue virus serotype 1 outbreak in the Basque Country (northern Spain) 2007–2008. Data support a primary vector windborne transport. *PLoS ONE* **7** (3), e34421; doi 10.1371/journal.pone.0034421
- Gardiner, A. (2014) The ‘dangerous’ women of animal welfare: how British veterinary medicine went to the dogs. *Social History of Medicine*, **27**, 466–487
- Gardiner, W.P. and Gettinby, G. (1983) A weather-based prediction model for the life-cycle of the sheep tick *Ixodes ricinus* (L). *Veterinary Parasitology*, **13**, 77–84
- Gardner, I.A. (2010) Quality standards are needed for reporting test accuracy studies for animal diseases. *Preventive Veterinary Medicine*, **97**, 136–143
- Gardner, I.A. and Greiner, M. (2006) Receiver-operating characteristic curves and likelihood ratios: improvements over traditional methods for the evaluation and application of veterinary clinical pathology tests. *Veterinary Clinical Pathology*, **35**, 8–17
- Gardner, I.A., Stryhn, H., Lind, P. and Collins, M.T. (2000) Conditional dependence between tests affects the diagnosis and surveillance of animal diseases. *Preventive Veterinary Medicine*, **45**, 107–122
- Gargadennec, L. and Lalanne, A. (1942) La peste des petits ruminants. *Bulletin des Services Zootechniques et des Epizooties de l'Afrique Occidentale Française*, **5**, 16–21
- Garland, A.J.M. and Donaldson, A.I. (1990) Foot and mouth disease. *Surveillance*, **17** (4), 6–8
- Garner, G. (2011) Surveillance for animal diseases and animal health information management in Australia. In: *Challenges of Animal Health Information Systems and Surveillance for Animal Diseases and Zoonoses*. Proceedings of the International Workshop organized by FAO, 23–26 November 2010, Rome, Italy. Animal Production and Health Proceedings No. 14, pp. 19–21. Food and Agriculture Organization of the United Nations, Rome
- Garner, J.P., Falcone, C., Wakenell, P., Martin, M. and Mench, J.A. (2002) Reliability and validity of a modified gait scoring system and its use in assessing tibial dyschondroplasia in broilers. *British Poultry Science*, **43**, 355–363
- Garner, M.G. and Nunn, M.J. (1995) The Australian national animal health information system. *Épidémiologie et Santé Animale*, **27**, 143–160
- Garraway, L.A. and Lander, E.S. (2013) Lessons from the cancer genome. *Cell*, **153**, 17–37
- Gaskell, C.J. (1983) Dilated pupil (Key-Gaskell) syndrome in cats. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Southampton, 12–13 April 1983. Ed. Thrusfield, M.V., pp. 99–100
- Gause, G.F. (1934) *The Struggle for Existence*. Williams and Wilkins, Baltimore
- GBWDSP (2015) *The GB Wildlife Disease Surveillance Partnership*. http://webarchive.nationalarchives.gov.uk/20130822084033/http://www.defra.gov.uk/vla/reports/docs/rep_survrep_gbwsdp.pdf (accessed 25 August 2015)
- Geering, W.A. and Amanfu, W. (2002) *Preparation of Contagious Bovine Pleuropneumonia Contingency Plans*. FAO Animal Health Manual No. 14. Food and Agriculture Organization of the United Nations, Rome
- Geering, W.A., Penrith, M.-L. and Nyakahuma (2001) *Manual on Procedures for Disease Eradication and Stamping Out*. FAO Animal Health Manual No. 12. Food and Agriculture Organization of the United Nations, Rome
- Gelfand, M., Weinberg, R.W. and Castle, W.M. (1967) Relation between carcinoma of the bladder and infestation with *Schistosoma haematobium*. *The Lancet*, **289**, 1249–1251
- Genders, T.S.S., Spronk, S., Stijnen, T., Steyerberg, E.W., Lesaffre, E. *et al.* (2012) Methods of calculating sensitivity and specificity of clustered data: a tutorial. *Radiology*, **265**, 910–916
- Genovese, K.J., Anderson, R.C., Harvey, R.B. and Nisbet, D.J. (2000) Competitive exclusion treatment reduces the mortality and fecal shedding associated with

- enterotoxigenic *Escherichia coli* infection in nursery-raised neonatal piglets. *Canadian Journal of Veterinary Research*, **64**, 204–207
- George, L. (1995) *The Encyclopedia of Heresies and Heretics*. Robson Books, London
- George, L.W. (1993) Clinical infectious bovine keratoconjunctivitis. In: *Infectious Disease in Food Animal Practice*, pp.159–168. Veterinary Learning Systems, Trenton
- Germonpré, M., Sablin, M.V., Stevens, R.E., Hedges, R.E. M., Hofreiter, M. *et al.* (2009) Fossil dogs and wolves from Paleolithic sites in Belgium, the Ukraine and Russia: osteometry, ancient DNA and stable isotopes. *Journal of Archeological Science*, **36**, 473–490
- Gershman, K.A., Sacks, J.J. and Wright, J.C. (1994) Which dogs bite? A case-control study of risk factors. *Pediatrics*, **93**, 913–917
- Gettinby, G. and Byrom, W. (1989) ECFXPRT: a computer model for the study of East Coast fever. In: Eighth Biennial Conference and Bushfire Dynamics Workshop Proceedings, 25–27 September 1989, Australian National University, Canberra. Simulation Society of Australia Inc. affiliated with the International Association for Mathematics and Computers in Simulation, pp. 123–127
- Gettinby, G. and McClean, S. (1979) A matrix formulation of the life cycle of liver fluke. *Proceedings of the Royal Irish Academy*, **79B**, 155–167
- Gettinby, G., Bairden, K., Armour, J. and Benitez-Usher, C. (1979) A prediction model for bovine ostertagiasis. *Veterinary Record*, **105**, 57–59
- Ghirotti, M. (1992) Rapid appraisal techniques: a tool for planning and managing animal health and production programmes. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Edinburgh, 1–3 April 1992. Ed. Thrusfield, M.V., pp. 190–206
- Giavarina, D. (2015) Understanding Bland Altman analysis. *Biochemica Medica*, **25**, 141–151
- Gibbens, J.C. and Wilesmith, J.W. (2002) Temporal and geographical distribution of cases of foot-and-mouth disease during the early weeks of the 2001 epidemic in Great Britain. *Veterinary Record*, **151**, 407–412
- Gibbens, J.C., Pascoe, S.J.S., Evans, S.J., Davies, R.H. and Sayers, A.R. (2001a) A trial of biosecurity as a means to control *Campylobacter* infection of broiler chickens. *Preventive Veterinary Medicine*, **48**, 85–99
- Gibbens, J.C., Sharpe, C.E., Wilesmith, J.W., Mansley, L. M., Michalopoulou, E. *et al.* (2001b) Descriptive epidemiology of the 2001 FMD epidemic in Great Britain: the first five months. *Veterinary Record*, **149**, 729–743
- Gibson, M. and Warren, K.S. (1970) Capture of *Schistomsoma mansoni* miracidia and cercariae by carnivorous aquatic vascular plants of the genus *Utricularia*. *Bulletin of the World Health Organization*, **42**, 833–835
- Gibson, T.E. (1978) The 'Mt' system for forecasting the prevalence of fascioliasis. In: *Weather and Parasitic Animal Disease*. World Meteorological Organization Technical Note No. 159. Ed. Gibson, T.E., pp. 3–5. World Meteorological Organization, Geneva
- Gibson, W. (2001) Sex and evolution in trypanosomes. *International Journal for Parasitology*, **31**, 643–647
- Giddens, A. (1987) *Social Theory and Modern Society*. Blackwell Publications, Oxford
- Gilbert, G.N. and Mulkay, M. (1984) *Opening Pandora's Box*. Cambridge University Press, Cambridge
- Gill, G.J. (1993) *OK, The Data's Lousy, But it's All We've Got (Being a Critique of Conventional Methods)*. Gatekeeper Series No. 38. International Institute for Environment and Development, London
- Gill, J.L. (1987) Biased statistical analysis when the animal is not the experimental unit (Letter). *Journal of the American Veterinary Medical Association*, **190**, 5–6
- Gillespie, I.A., Mook, P., Adak, G.K., O'Brien, S.J., McCarthy, N.D. (2012) The 'case-chaos study' as an adjunct or alternative to conventional case-control study methodology. *American Journal of Epidemiology*, **176**, 497–505
- Gillespie, I.A., O'Brien, S.J., Frost, J.A., Adak, G.K., Horby, P. *et al.* (2002) A case-case comparison of *Campylobacter coli* and *Campylobacter jejuni* infection: a tool for generating hypotheses. *Emerging Infectious Diseases*, **8**, 937–942
- Gillette, E.L. (1979) Radiotherapy. In: *Veterinary Cancer Medicine*. Eds Theilen, G.H. and Madwell, B.R., pp. 85–94. Lea and Febiger, Philadelphia
- Gillette, E.L. (1982) Spontaneous animal neoplasms as models for therapeutic agents. In: *Design of Models for Testing Cancer Therapeutic Agents*. Eds Figler, I.J. and White, R.J., pp. 185–192. Van Nostrand Reinhold, New York
- Gillingwater, K., Mamabolo, M.V. and Majiwa, P.A.O. (2010) Prevalence of mixed *Trypanosoma congolense* infections in livestock and tsetse in KwaZulu-Natal, South Africa. *Journal of the South African Veterinary Association*, **81**, 219–223
- Gilmour, J.S. (1989) *Grass Sickness in Horses*. Animal Diseases Research Association News Sheet No. 17. Moredun Research Institute, Edinburgh
- Gilovich, T., Griffin, D. and Kahneman, D. (Eds) (2002) *Heuristics and Biases: The Psychology of Intuitive Judgement*. Cambridge University Press, Cambridge
- Girmay, G., Arega, B., Tesfaye, D., Berkvens, D. Muleta, G. *et al.* (2016) Community-based tsetse fly control significantly reduces fly density and trypanosomosis prevalence in Metekel Zone, Northwest, Ethiopia. *Tropical Animal Health and Production*, **48**, 633–642

- Gittinger, J.P. (1972) *Economic Analysis of Agricultural Projects*. The Johns Hopkins University Press, Baltimore and London
- Glas, A.S., Lijmer, J.G., Prins, M.H., Bonsel, G.J. and Bossuyt, P.M.M. (2003) The diagnostic odds ratio: a single indicator of test performance. *Journal of Clinical Epidemiology*, **56**, 1129–1135
- Glass, G.V. (1976) Primary, secondary, and meta-analysis of research. *Educational Research*, **5**, 3–8
- Glass, G.V., McGaw, B. and Smith, M.L. (1981) *Meta-analysis in Social Research*. Sage Publications, Beverly Hills
- Glickman, L.T., Domanski, L.M., Maguire, T.G., Dubielzig, R.R. and Churg, A. (1983) Mesothelioma in pet dogs associated with exposure of their owners to asbestos. *Environmental Research*, **32**, 305–313
- Glickman, L.T., Domanski, L.M., Patronek, G.J. and Visintainer, F. (1985) Breed-related risk factors for canine parvovirus enteritis. *Journal of the American Veterinary Medical Association*, **187**, 589–594
- Glickman, L.T., Moore, G.E., Glickman, N.W., Caldanaro, R.J., Aucoin, D. *et al.* (2006) Purdue University-Banfield National Companion Animal Surveillance Program for emerging and zoonotic diseases. *Vector-Borne and Zoonotic Diseases*, **6**, 14–23
- Glickman, L.T., Raghavan, M., Knapp, D.W., Bonney, P.L. and Dawson, M.H. (2004) Herbicide exposure and the risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. *Journal of the American Veterinary Medical Association*, **224**, 1290–1297
- Glickman, L.T., Schofer, F.S., McKee, L.J., Reif, J.S. and Goldschmidt, M.H. (1989) Epidemiologic study of insecticide exposures, obesity, and risk of bladder cancer in household dogs. *Journal of Toxicology and Environmental Health*, **28**, 407–414
- GLiPHA (2014) *Global Livestock Production and Health Atlas*. Animal Health Division, Food and Agriculture Organization of the United Nations, Rome. <http://kids.fao.org/glipha/> (accessed 31 October 2014)
- Glosser, J.W. (1988) Back to the future: the Animal Health Monitoring System – a political necessity being addressed in the United States. *Acta Veterinaria Scandinavica*, Suppl. 84, 42–48
- Gloster, J., Blackall, R.M., Sellers, R.F. and Donaldson, A.I. (1981) Forecasting the airborne spread of foot-and-mouth disease. *Veterinary Record*, **108**, 370–374
- Gloster, J., Donaldson, A.I. and Hough, M.N. (1984) Analysis of a series of outbreaks of Aujeszky's disease in Yorkshire in 1981–82: the possibility of airborne disease spread. *Veterinary Record*, **114**, 234–239
- Gloster, J., Freshwater, A., Sellers, R.F. and Alexandersen, S. (2005) Re-assessing the likelihood of airborne spread of foot-and-mouth disease at the start of the 1967–68 UK foot-and-mouth disease epidemic. *Epidemiology and Infection*, **133**, 767–783
- Gloster, J., Hewson, H., Mackay, D., Garland, T., Donaldson, A. *et al.* (2001) Spread of foot-and-mouth disease from the burning of animal carcasses on open pyres. *Veterinary Record*, **148**, 585–586
- Gloster, J., Jones, A., Redington, A., Burgin, L., Sørensen, J.H. *et al.* (2010) Airborne spread of foot-and-mouth disease – model intercomparison. *Veterinary Journal*, **183**, 278–286
- Gloster, J., Sellars, R.F. and Donaldson, A.I. (1982) Long distance transport of foot and mouth disease virus over the sea. *Veterinary Record*, **110**, 47–52
- Glymour, M.M. and Greenland, S. (2008) Causal diagrams. In: *Modern Epidemiology*, 3rd edn. Eds Rothman, K.J., Greenland, S. and Lash, L.T., pp. 183–209. Lippincott Williams and Wilkins, Philadelphia
- Gobar, G.M., Case, J.T. and Kass, P.H. (1998) Program for surveillance of causes of death of dogs, using the Internet to survey small animal veterinarians. *Journal of the American Veterinary Medical Association*, **213**, 251–256
- Gochenour, P.H. (2006) Distributed communities and nodal subjects. *New Media & Society*, **18**, 33–51
- Goddard, A.D., Donaldson, N.M., Horton, D.L., Kosmider, R., Kelly, L.A. *et al.* (2012) A quantitative release assessment for the noncommercial movement of companion animals: risk of rabies reintroduction to the United Kingdom. *Risk Analysis*, **32**, 1769–1783
- Godfroid J., Nielsen, K. and Saegerman, C. (2010) Diagnosis of brucellosis in livestock and wildlife. *Croatian Medical Journal*, **51**, 296–305
- Godsall, S.A., Clegg, S.R., Stavisky, J.H., Radford, A.D. and Pinchbeck, G.L. (2010) Epidemiology of canine parvovirus and coronavirus in dogs presented with severe diarrhoea to PDSA PetAid hospitals. *Veterinary Record*, **167**, 196–201
- Gogolin-Ewens, K.J., Meeusen, E.N.T., Scott, P.C., Adams, T.E. and Brandon, M.R. (1990) Genetic selection for disease resistance and traits of economic importance in animal production. *Revue Scientifique et Technique, Office International des Epizooties*, **9**, 865–896
- Goines, L. and Hagler, L. (2007) Noise pollution: a modern plague. *Southern Medical Journal*, **100**, 287–294
- Golan, E., Krissoff, B., Kuchler, F., Calvin, L., Nelson, K. *et al.* (2004) *Traceability in the US Food Supply: Economic Theory and Industry Studies*. Agricultural Economic Report No. 830. United States Department of Agriculture, Washington DC
- Gold, R.Z. (1963) Tests auxiliary to χ^2 tests in a Markov chain. *Annals of Mathematical Statistics*, **34**, 56–74

- Goldberger, J., Waring, C.H. and Tanner, W.F. (1923) Pellagra prevention by diet among institutional inmates. *Public Health Reports*, **38**, 2361–2368
- Goldschmidt, M.H. and Shofer, F.S. (1994) *Skin Tumours of the Dog and Cat*. Butterworth-Heinemann, Oxford
- Goldspink, G.E. (1993) The impact of recombinant DNA techniques on veterinary diagnosis and genetic screening. In: *The Advancement of Veterinary Science. The Bicentenary Symposium Series. Volume 1: Veterinary Medicine Beyond 2000*. Ed. Michell, A.R., pp. 59–71. CAB International, Wallingford
- Goldspink, G. and Gerlach, G.-F. (1990) Prospective use of recombinant DNA methods in animal disease control. In: *Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine*, Belfast, 4th–6th April 1990. Ed. Thrusfield, M.V., pp. 1–14
- Goldstein, H., Browne, W. and Rasbash, J. (2002) Partitioning variation in multilevel models. *Understanding Statistics*, **1**, 223–231
- Gonzales-Barron, U., Butler, F., McDonnell, K. and Ward, S. (2009) The end of the identity crisis? Advances in biometric markers for animal identification. *Irish Veterinary Journal*, **62**, 204–208
- Gooch, P. (2004) Van Gujjar: the persistent forest pastoralists. *Nomadic Peoples*, **8**, 125–135
- Good, P.I. (2005) *Permutation, Parametric and Bootstrap Tests of Hypotheses*, 3rd edn. Springer Science + Business Media, New York
- Goodall, E.A., McCaughey, W.J., McMurray, C.H. and Rice, D. (1984) SIRO – a computer system for dairy herd recording. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings*, Edinburgh, 10–11 July 1984. Ed. Thrusfield, M.V., pp. 174–183
- Goodall, E.A., Menzies, F.D., McConachy, D.A. and McIlroy, S.G. (1997) A disease management information system for profitable broiler production. In: *Society for Veterinary Epidemiology and Preventive Medicine*, Chester, 9–11 April 1997. Eds Goodall, E.A. and Thrusfield, M.V., pp. 268–278
- Goodger, W.J. and Ruppner, R. (1982) Why the dairy industry does not make greater use of veterinarians. *Journal of the American Veterinary Medical Association*, **181**, 706–710
- Goodman, R.A. and Buehler, J.W. (2008) Field epidemiology defined. In: *Field Epidemiology*, 3rd edn. Ed. Gregg, M.B., pp. 3–15. Oxford University Press, New York
- Goodman, R.A., Munson, J.W., Dammers, K., Lazzarini, Z. and Barkley, J.P. (2003) Forensic epidemiology: law at the intersection of public health and criminal investigations. *Journal of Law, Medicine & Ethics*, **31**, 684–700
- Goodman, S.N. (1993) *p* values, hypothesis tests, and likelihood: implications for epidemiology of a neglected historical debate. *American Journal of Epidemiology*, **137**, 485–496
- Goodman, S.N. (1999a) Towards evidence-based medical statistics. 1: The *P* value fallacy. *Annals of Internal Medicine*, **130**, 995–1004
- Goodman, S.N. (1999b) Towards evidence-based medical statistics. 2: The Bayes factor. *Annals of Internal Medicine*, **130**, 1005–1013
- Goodman, S.N. and Berlin, J.A. (1994) The use of predicted confidence intervals when planning experiments and the misuse of power when interpreting results. *Annals of Internal Medicine*, **121**, 200–206
- Goodwin, R.W.F. (1985) Apparent re-infection of enzootic-pneumonia-free pig herds: search for possible causes. *Veterinary Record*, **116**, 690–694
- Gopal, T. (1977) Carcinogenesis. In: *Current Veterinary Therapy VI. Small Animal Practice*. Ed. Kirk, R.W., pp. 164–166. W.B. Saunders Company, Philadelphia
- Gordis, L. (2014) *Epidemiology*, 5th edn. Elsevier Life Sciences, London
- Gordon, E.R., Curns, A.T., Krebs, J.W., Rupprecht, C.E., Real, L.A. *et al.* (2004) Temporal dynamics of rabies in a wildlife host and the risk of cross-species transmission. *Epidemiology and Infection*, **132**, 515–524
- Gordon, I. and Khanna, C. (2010) Modeling opportunities in comparative oncology for drug development. *ILAR Journal*, **51**, 214–220
- Gordon, J.C. and Angrick, E.J. (1985) Stray dogs as sentinels for canine parvovirus. *Preventive Veterinary Medicine*, **3**, 311–316
- Gordon Smith, C.E. (1976) *Epidemiology and Infections*. Meadowfield Press, Shildon
- Gordon Smith, C.E. (1982) Major factors in the spread of infections. In: *Animal Disease in Relation to Animal Conservation*. Symposium of the Zoological Society of London No. 50. Eds Edwards, M.A. and McDonald, U., pp. 207–235. Academic Press, London and New York
- Gosling, S.D., Vazire, S., Srivastava, S. and John, O.P. (2004) Should we trust Web-based studies? *American Psychologist*, **59**, 95–104
- Gosz, J.R. (1992) Gradient analysis of ecological change in time and space: implications for forest management. *Ecological Applications*, **2**, 248–261
- Gosz, J.R. (1993) Ecotone hierarchies. *Ecological Applications*, **3**, 369–376
- Gottfreid, R.S. (1983) *The Black Death: Natural and Human Disaster in Medieval Europe*. The Free Press, New York
- Gottstein, B. (1984) An immunoassay for the detection of circulating antigens in human echinococcosis.

- American Journal of Tropical Medicine and Hygiene*, **33**, 1185–1191
- Gough, A. and Thomas, A. (2010) *Breed Predispositions to Disease in Dogs and Cats*, 2nd edn. Wiley-Blackwell, Chichester
- Gould, L.C., Gardner, G.T., DeLuca, G.R., Tiemann, A. R., Doob, L.W. *et al.* (1988) *Perceptions of Technological Risks and Benefits*. Russell Sage Foundation, New York
- Gourley, S.A., Liu, R. and Wu, J. (2007) Eradicating vector-borne diseases via age-structured culling. *Journal of Mathematical Biology*, **54**, 309–335
- Graat, E.A.M. and Frankena, K. (2001) Introduction to theoretical epidemiology. In: *Application of Quantitative Methods in Veterinary Epidemiology*, 2nd edn. Noordhuizen, J.P.T.M., Frankena, K., Thrusfield, M.V. and Graat, E.A.M., pp. 239–259. Wageningen Pers, Wageningen
- Grace, D. (2014) The business case for One Health. *Onderstepoort Journal of Veterinary Research*, **81** (2), Article 725; doi org/10.4102/ojvr.v81i2.725
- Grace, D., Randolph, T., Affognon, H., Dramane, D., Diall, O. *et al.* (2009) Characterisation and validation of farmers' knowledge and practice of cattle trypanosomiasis management in the cotton zone of West Africa. *Acta Tropica*, **111**, 137–143
- Grace, D., Randolph, T., Olawoye, J., Dipelou, M. and Kang'ethe, E. (2008) Participatory risk assessment: a new approach for safer food in vulnerable African communities. *Development in Practice*, **18**, 611–618
- Gracey, J.F. (1960) *Survey of Livestock Diseases in Northern Ireland*. Her Majesty's Stationery Office, Belfast
- Gracey, J.F., Collins, D.S. and Huey, R.J. (1999) *Meat Hygiene*, 10th edn. W.B. Saunders, London
- Grachev, M.A., Kumarev, V.P., Mamaev, L.V., Zorin, V.L., Baranova, L.V. *et al.* (1989) Distemper virus in Baikal seals. *Nature*, **338**, 209
- Græsbøll, K., Andresen, L.O., Halasa, T. and Toft, N. (2016) How few pooled tests are needed to detect a single positive sample? In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Elsinore, 16–18 March 2016. Eds Lindberg, A. and Thulke, H.-H., pp. 11–21
- Graham, J.P., Leibler, J.H., Price, L.B., Otte, J.M., Pfeiffer, D.U. *et al.* (2008) The animal-human interface and infectious disease in industrial food animal production: rethinking biosecurity and biocontainment. *Public Health Reports*, **123**, 282–299
- Graham, J.W. (2012) *Missing Data: Analysis and Design*. Springer, New York
- Grais, R.F., Ellis, J.H. and Glass, G.E. (2003) Forecasting the geographical spread of smallpox cases by air travel. *Epidemiology and Infection*, **131**, 849–857
- Grant, M.J. and Booth, A. (2009) A typology of reviews: an analysis of 14 review types and associated methodologies. *Health Information and Libraries Journal*, **26**, 91–108
- Grant, R.J., Scheidt, A.B. and Ruff, L.R. (1994) Aerosol transmission of a viable virus affecting swine: explanation of an epizootic of pseudorabies. *International Journal of Biometeorology*, **38**, 33–39
- Grant, W. (2009) Intractable policy failure: the case of bovine TB and badgers. *British Journal of Politics & International Relations*, **11**, 557–573
- Grauballe, P.C., Baagoe, H.J., Fekadu, M., Westergaard, J. M. and Zoffman, H. (1987) Bat rabies in Denmark. *The Lancet*, **329**, 379–380
- Graunt, J. (1662) *Natural and Political Observations Mentioned in a Following Index, and Made Upon the Bills of Mortality*. Thomas Roycroft, London. Reprinted by The Johns Hopkins Press, Baltimore, 1939. Reproduced in facsimile, with an introduction by P. Laslett, in *The Earliest Classics*. Gregg International Publishers, Farnborough, 1973
- Grave, K., Greko, C., Kvaale, M.K., Torren-Edo, J., Mackay, D. *et al.* (2012) Sales of veterinary antibacterial agents in nine European countries during 2005–09: trends and patterns. *Journal of Antimicrobial Chemotherapy*, **67**, 3001–3008
- Green, L. (2015) Lameness control in flock health planning. *Cattle Practice*, **23**, 107–111
- Green, L.E. and George, T.R.N. (2008) Assessment of current knowledge of footrot in sheep with particular reference to *Dichelobacter nodosus* and implications for elimination or control strategies for sheep in Great Britain. *The Veterinary Journal*, **175**, 173–180
- Green, L.E. and Medley, G.E. (2002) Mathematical modelling of the foot-and-mouth disease epidemic of 2001: strengths and weaknesses. *Research in Veterinary Science*, **73**, 201–205
- Green, M. and Bradley, A. (2013) The changing face of mastitis control. *Veterinary Record*, **173**, 517–521
- Green, R.G. and Stulberg, C.S. (1946) Distemperoid virus interference in canine distemper. *Science*, **103**, 497–498
- Green, S.B. and Byar, D.P. (1984) Using observational data from registries to compare treatments: the fallacy of omnimetrics. *Statistics in Medicine*, **3**, 361–370
- Greene, C.E. and Hall, G.G. (2012) Recommendations for core and noncore vaccinations of dogs and cats. In: *Infectious Diseases of the Dog and Cat*, 4th edn. Ed. Greene, C.E., Web appendices 1 and 2. Elsevier, Saunders, St Louis
- Greene, C.E., Weese, J.S. and Calpin, J.P. (2012) Environmental factors in infectious disease. In: *Infectious Diseases of the Dog and Cat*, 4th edn. Ed.

- Greene, C.F., pp. 1078–1100. Elsevier Saunders, St Louis
- Greenland, S. (1987) Quantitative methods in the review of epidemiologic literature. *Epidemiologic Reviews*, **9**, 1–30
- Greenland, S. and Finkle, W.D. (1995) A critical look at methods for handling missing covariates in epidemiologic regression analyses. *American Journal of Epidemiology*, **142**, 1255–1264
- Greenland, S. and Kleinbaum, D.G. (1983) Correcting for misclassification in two-way tables and matched-pair studies. *International Journal of Epidemiology*, **12**, 93–97
- Greenland, S. and Lash, T.L. (2008) Bias analysis. In: *Modern Epidemiology*, 3rd edn. Eds Rothman, K.J., Greenland, S. and Lash, L.T., pp. 345–380. Lippincott Williams and Wilkins, Philadelphia
- Greenland, S., Gago-Dominguez, M. and Castela, J.E. (2004) The value of risk-factor ('black-box') epidemiology. *Epidemiology*, **15**, 529–535
- Greenland, S., Morgenstern, H. and Thomas, D.C. (1981) Considerations in determining matching criteria and stratum sizes for case-control studies. *International Journal of Epidemiology*, **10**, 389–392
- Greenwood, Major (1943) Medical statistics from Graunt to Farr. VI. Some English medical statisticians in the Eighteenth Century. *Biometrika*, **33**, 1–24
- Greenwood, P. (2004) Animal identification and the veterinary practitioner. *Canadian Veterinary Journal*, **45**, 332–333
- Greger, M. (2012) Transgenesis in animal agriculture and zoonotic disease resistance. In: *Animal Science Reviews 2011*. Ed. Hemming, D., pp. 123–128. CABI, Wallingford
- Gregory, Fr. CSWG (2002) War in Heaven. *New Directions*, **5** (80), 6–8
- Greiner, M. and Gardner, I.A. (2000) Epidemiologic issues in the validation of veterinary diagnostic tests. *Preventive Veterinary Medicine*, **45**, 3–22
- Greiner, M., Böhning, D. and Dahms, S. (1997) Meta-analytic review of ELISA tests for the diagnosis of human and porcine trichinellosis: which factors are involved in diagnostic accuracy? In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Chester, 9–11 April 1997. Eds Goodall, E. A. and Thrusfield, M.V., pp. 12–21
- Greiner, M., Pfeiffer, D. and Smith, R.D. (2000) Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. *Preventive Veterinary Medicine*, **45**, 23–41
- Greiser-Wilke, I., Grummer, B. and Moennig, V. (2003) Bovine viral diarrhoea eradication and control programmes in Europe. *Biologicals*, **31**, 113–118
- Grelotti, D.J. and Kaptchuk, T.J. (2011) Placebo by proxy. *British Medical Journal*, **343**, d4345; doi 10.1136/bmj.d4345
- Grenfell, B.T. and Dobson, A.P. (Eds) (1995) *Ecology of Infectious Diseases in Natural Populations*. Cambridge University Press, Cambridge
- Grenfell, B.T., Pybus, O.G., Gog, J.R., Wood, J.L.N., Daly, J.M. et al. (2004) Unifying the epidemiological and evolutionary dynamics of pathogens. *Science*, **303**, 327–332
- Grenfell, B.T., Wilson, K., Finkenstädt, B.F., Coulson, T.N., Murray, S. et al. (1998) Noise and determinism in synchronized sheep dynamics. *Nature*, **394**, 674–677
- Griffiths, A.J.F., Wessler, S.R., Lewontin, R.C., Gelbart, W. M., Suzuki, D.T. et al. (2005) *An Introduction to Genetic Analysis*, 8th edn. W.H. Freeman and Company, New York
- Griffiths, A.O. and Brenner, A. (1977) Survey of cat and dog ownership in Champagne County, Illinois, 1976. *Journal of the American Veterinary Medical Association*, **170**, 1333–1340
- Griffiths, D. (2008) *Introduction to Elementary Particles*, 2nd edn. Wiley-VCH, Weinheim
- Griffiths, D.A. (1974) A catalytic model of infection for measles. *Journal of the Royal Statistical Society, Series C*, **23**, 330–339
- Griffiths, I.B. and Done, S.H. (1991) Citrinin as a possible cause of the pruritis, pyrexia, haemorrhagic syndrome in cattle. *Veterinary Record*, **129**, 113–117
- Grilli, L. and Rampichini, C. (2012) Multilevel models for ordinal data. In: *Modern Analysis for Customer Surveys: With Applications using R*. Eds Kenett, R. and Salini, S., pp. 391–411. John Wiley and Sons, Chichester
- Grindlay, D.J., Brennan, M.L. and Dean, R.S. (2012) Searching the veterinary literature: a comparison of the coverage of veterinary journals by nine bibliographic databases. *Journal of Veterinary Medical Education*, **39**, 404–412
- Grindle, R.J. (1980). Appropriate methodology in economic analysis of disease control projects. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 7–11 May 1979. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 506–510. Australian Government Publishing Service, Canberra
- Grindle, R.J. (1986) The use and abuse of economic methods as applied to veterinary problems. In: Proceedings of the Fourth International Symposium on Veterinary Epidemiology and Economics, Singapore, 18–22 November 1985, pp. 60–70. Singapore Veterinary Association, Singapore
- Griner, L.A. (1980) Storage and retrieval of necropsy records at San Diego zoo. In: *The Comparative Pathology of Zoo Animals*. Eds Montali, R.J. and Migaki, G., pp. 663–667. Smithsonian Institution Press, Washington DC

- Griner, L.A. and Hutton, N.E. (1968) Pathology data retrieval system adopted at a zoological garden. *Journal of the American Veterinary Medical Association*, **153**, 885–892
- Grisel, J.E. (2000) Quantitative trait locus analysis. *Alcohol Health & Research*, **24**, 169–174
- Grogan, L.F., Berger, L., Rose, K., Grillo, V., Cashins, S.D. *et al.* (2014) Surveillance for emerging biodiversity diseases of wildlife. *PLOS Pathogens*, **10**, 5, e1004015; doi 10.1371/journal.ppat.1004015
- Grønvold, J., Henriksen, S.A., Nansen, P., Wolstrup, J. and Thylin, J. (1989) Attempts to control infection with *Ostertagia ostertagi* (Trichostrongylidae) in grazing calves by adding mycelium of the nematode-trapping fungus *Arthrobotrys oligospora* (Hyphomycetales) to cow pats. *Journal of Helminthology*, **63**, 115–126
- Grønvold, J., Wolstrup, J., Nansen, P., Henriksen, S.A., Larsen, M. *et al.* (1993) Biological control of nematode parasites in cattle with nematode-trapping fungi: a survey of Danish studies. *Veterinary Parasitology*, **48**, 311–325
- Gross, L. (1955) Mouse leukaemia: an egg-borne virus disease (with a note on mouse salivary gland carcinoma). *Acta Haematologica*, **13**, 13–29
- Groves, R. (1990) Theories and methods of telephone surveys. *Annual Review of Sociology*, **16**, 221–240
- Groves, R.M. (2004) *Survey Errors and Survey Costs*. John Wiley and Sons, Hoboken
- Groves, R.M., Biemer, P.P., Lyberg, L.E., Massey, J.T., Nicholls, W.L. *et al.* (Eds) (1988) *Telephone Survey Methodology*. John Wiley, New York
- Grubb, B.R. and Boucher, R.C. (1999) Pathophysiology of gene-targeted mouse models for cystic fibrosis. *Physiological Reviews*, **79**, Suppl., S193–S214
- Gruen, M.E., Griffith, E.H., Thomson, A.E., Simpson, W. and Lascelles, B.D.X. (2015) Criterion validation testing of clinical metrology instruments for measuring degenerative joint disease associated mobility impairment in cats. *PLoS ONE*, **10**; doi 10.1371/journal.pone.0131839
- Grufferman, S. and Kimm, S.Y.S. (1984) Clinical epidemiology defined. *The New England Journal of Medicine*, **311**, 541–542
- Grunkemeier, G.L., Wu, Y.X. and Furnary, P. (2003) Cumulative sum techniques for assessing surgical results. *Annals of Thoracic Surgery*, **76**, 663–667
- Grunsell, C.S., Penny, R.H.C., Wragg, S.R. and Allcock, J. (1969) The practicability and economics of veterinary preventive medicine. *Veterinary Record*, **84**, 26–41
- Grüntzig, K., Graf, R., Hässig, M., Welle, M., Meier, D. *et al.* (2015) The Swiss Canine Cancer Registry: a retrospective study on the occurrence of tumours in dogs in Switzerland from 1955 to 2008. *Journal of Comparative Pathology*, **152**, 161–171
- Gsell, A.S., Knobel, D.L., Cleaveland, S., Kazwala, R.R., Vounatsou, P. *et al.*, (2012) Domestic dog demographic structure and dynamics relevant to rabies control planning in urban areas in Africa: the case of Iringa, Tanzania. *BMC Veterinary Research*, **8**, 236; doi 10.1186/1746-6148-8-236
- Guernier, V., Milinovich, G.J., Santos, M.A.B., Haworth, M., Coleman, G. *et al.* (2016) Use of big data in the surveillance of veterinary diseases: early detection of tick paralysis in companion animals. *Parasites & Vectors*, **9**, 303; doi 10.1186/s13071-016-1590-6
- Guerra, M., Walker, E.C., Paskewitz, S.M. Cortinas, M.R., Stancil, A. *et al.* (2002) Predicting the risk of Lyme disease: habitat suitability for *Ixodes scapularis* in the North Central United States *Emerging Infectious Diseases*, **8**, 289–297
- Guerra, R. and Golstein, D.R. (2009) *Meta-Analysis and Combining Information in Genetics and Genomics*. CRC Press, Boca Raton
- Guerrero, J., Newcomb, K.M. and Fukase, T. (1992) Prevalence of *Dirofilaria immitis* in dogs and cats in the USA and Japan. In: Proceedings of the 17th World Small Animal Veterinary Association Congress, Rome, 24–27 September 1992, Vol. 1. Ed. Dolfino, A., pp. 211–217
- Guest, G., Bunce, A. and Johnson, L. (2003) How many interviews are enough? An experiment with data saturation and variability. *Field Methods*, **18**, 59–82
- Guis, H., Camanide, C., Calvete, C., Morse, A.P., Tran, A. *et al.* (2012) Modelling the effects of past and future climate on the risk of bluetongue emergence in Europe. *Journal of the Royal Society Interface*, **9**, 339–350
- Gummow, B. (1996) Experimentally induced chronic copper toxicity in cattle. *Onderstepoort Journal of Veterinary Research*, **63**, 277–288
- Gummow, B., Botha, C.J., Basson, A.T. and Bastianello, S. S. (1991) Copper toxicity in ruminants: air pollution as a possible cause. *Onderstepoort Journal of Veterinary Research*, **58**, 33–39
- Gunn, G.J. and Stott, A.W. (1996) Combining economic arguments and active surveillance to promote better management of endemic cattle disease. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Glasgow, 27–29 March 1996. Eds Thrusfield, M.V. and Goodall, E.A., pp. 1–7
- Gunn, G.J., Heffernan, C., Hall, M., McLeod, A. and Hovi, M. (2008) Measuring and comparing constraints to improved biosecurity amongst GB farmers, veterinarians and the auxiliary industries. *Preventive Veterinary Medicine*, **84**, 310–323
- Gunnarsson, S., Keeling, L.J. and Svedberg, J. (1998) Effects of rearing conditions on behavioural problems in laying hens: an epidemiological approach. In: Proceedings of the 32nd Congress of the International

- Society for Applied Ethology, 21–25 July 1998, Clermont-Ferrand, France. Eds Veissier, I. and Boissy, A., p. 57. Institut National de la Recherche Agronomique, France
- Gupta, A.K. and Nadaraja, S. (Eds) (2004) *Handbook of Beta Distribution and its Application*. Marcel Dekker, New York
- Gupta, S. (2001) Avoiding ambiguity. *Nature*, **412**, 589
- Gurarie, D. and Seto, E.Y.W. (2009) Connectivity sustains disease transmission in environments with low potential for endemicity: modelling schistosomiasis with hydrologic and social connectivities. *Journal of the Royal Society Interface*, **6**, 495–508
- Gustavsson, I. (1969) Cytogenetics, distribution and phenotypic effects of a translocation in Swedish cattle. *Hereditas*, **63**, 68–169
- Guthrie, R.S. and Pidduck, H. (1990) Heritability of elbow osteochondrosis within a closed population of dogs. *Journal of Small Animal Practice*, **31**, 93–96
- Guyatt, G., Oxman, A.D., Akl, E.A., Kunz, R., Vist, G. *et al.* (2011a) GRADE guidelines: Introduction – GRADE evidence profiles and summary of findings tables. *Journal of Clinical Epidemiology*, **64**, 383–394
- Guyatt, G.H., Oxman, A.D., Kunz, R., Atkins, D., Brozek, J. *et al.* (2011b) GRADE guidelines: 2. Framing the question and deciding on important outcomes. *Journal of Clinical Epidemiology*, **64**, 395–400
- Guyatt, G., Oxman, A.D., Kunz, R., Brozek, J., Alonso-Coello, P. *et al.* (2011c) GRADE guidelines 6. Rating the quality of evidence – imprecision. *Journal of Clinical Epidemiology*, **64**, 1283–1293
- Guyatt, G.H., Oxman, A.D., Kunz, R., Woodcock, J., Brozek, J. *et al.*, (2011d) GRADE guidelines: 7. Rating the quality of evidence – inconsistency. *Journal of Clinical Epidemiology*, **64**, 1294–1302
- Guyatt, G.H., Oxman, A.D., Kunz, R., Woodcock, J., Brozek, J. *et al.* (2011e) GRADE guidelines: 8. Rating the quality of evidence – indirectness. *Journal of Clinical Epidemiology*, **64**, 1303–1310
- Guyatt, G.H., Oxman, A.D., Montori, V., Vist, G., Kunz, R. *et al.*, (2011f) GRADE guidelines: 5. Rating the quality of evidence – publication bias. *Journal of Clinical Epidemiology*, **64**, 1277–1282
- Guyatt, G.H., Oxman, A.D., Sultan, S., Glasziou, P., Akl, E. A. *et al.* (2011g) GRADE guidelines: 9. Rating up the quality of evidence. *Journal of Clinical Epidemiology*, **64**, 1311–1316
- Guyatt, G.H., Oxman, A.D., Vist, G., Kunz, R., Brozek, J. *et al.* (2011h) GRADE guidelines: 4. Rating the quality of evidence – study limitations (risk of bias). *Journal of Clinical Epidemiology*, **64**, 407–415
- Guyatt, G.H., Oxman, A.D., Vist, G.E., Kunz, R., Falck-Ytter, Y. *et al.* (2008) GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *British Medical Journal*, **336**, 924–926
- H.M. Treasury (2003) *The Green Book: Appraisal and Evaluation in Central Government. Treasury Guidance*. The Stationery Office, London
- Habermas, J. (1972) *Knowledge and Human Interests*. Heinemann Educational, London
- Habtemariam, T., Howitt, R., Ruppanner, R. and Riemann, H.P. (1984) Application of a linear programming model to the control of African trypanosomiasis. *Preventive Veterinary Medicine*, **3**, 1–14
- Hacking, I. (1975) *The Emergence of Probability: A Philosophical Study of Early Ideas about Probability, Induction and Statistical Inference*. Cambridge University Press, Cambridge
- Hackstadt, T. (1998) The diverse habitats of obligate intracellular parasites. *Current Opinions in Microbiology*, **1**, 82–87
- Hadgu, A. (1996) The discrepancy in discrepant analysis. *The Lancet*, **348**, 592–593
- Hadgu, A., Dendukuri, N. and Hilden, J. (2005) Evaluation of nucleic acid amplification tests in the absence of a perfect gold-standard test: a review of the statistical and epidemiologic issues. *Epidemiology*, **16**, 604–612
- Hadorn, D.C. and Stärk, K.D.C. (2008) Evaluation and optimization of surveillance systems for rare and emerging infectious diseases. *Veterinary Research*, **39**, 57; doi 10.1051/vetres:2008033
- Haelterman, E.O. (1963) Epidemiological studies of transmissible gastroenteritis of swine. Proceedings of the 66th Annual Meeting of the United States Livestock Sanitary Association, October 29–November 2, 1962, Washington DC, pp. 305–315
- Hagerman, A.D., McCarl, B.A., Carpenter, T.E., Ward, M. P. and O'Brien, J. (2012) Emergency vaccination to control foot-and-mouth disease as a US policy option. *Applied Economic Perspectives and Policy*, **34**, 119–146
- Hahn, K.A., Bravo, L., Adams, W.H. and Frazier, D.L. (1994) Naturally-occurring tumors in dogs as comparative models for cancer therapy and research. *In Vivo*, **8**, 133–144
- Haig, D.A. (1977) Rabies in animals. In: *Rabies: The Facts*. Ed. Kaplan, C., pp. 53–69. Oxford University Press, Oxford
- Haig, D.A., Clarke, M.C., and Pereira, M.S. (1964) Isolation of an adenovirus from a pig. *Journal of Comparative Pathology*, **74**, 81–84
- Hair, J.F., Black, W.C., Babin, B.J. and Anderson, R.E. (2014) *Multivariate Data Analysis*. Pearson Education, Harlow
- Hajian-Tilaki, K. (2014) Sample size estimation in diagnostic test studies of biomedical informatics. *Journal of Biomedical Informatics*, **48**, 193–204

- Haladej, S. and Hurcik, V. (1988) Computerised management system of the Veterinary Service in the Slovak Socialist Republic. *Revue Scientifique et Technique, Office International des Epizooties*, **7**, 517–541
- Hall, D.C., Putra, A.A.G., Willyanto, I. and Basuno, E. (2016) One Health approaches to rabies control in Bali, Indonesia. In: *One Health Case Studies: Addressing Complex Problems in a Changing World*. Eds Cork, S. C., Hall, D.C. and Liljebjelke, K.A., pp. 13–25. 5M Publishing, Sheffield
- Hall, J. and Wapenaar, W. (2012) Opinions and practices of veterinarians and dairy farmers towards herd health management in the UK. *Veterinary Record*, **170**, 441; doi 10.1136/vr.100318
- Hall, J.P.J., Wang, H. and Barry, J.D. (2013) Mosaic VSGs and the scale of *Trypanosoma brucei* antigenic variation. *PLoS Pathogens*, **9** (7), e1003502; doi 10.1371/journal.ppat.1003502
- Hall, S.A. (1978) Farm animal disease data banks. *Advances in Veterinary Science and Comparative Medicine*, **22**, 265–286
- Hall, S.A., Dawson, P.S. and Davies, G. (1980) VIDA II: a computerised diagnostic recording system for veterinary investigation centres in Great Britain. *Veterinary Record*, **106**, 260–264.
- Hall, S.J.G. (2011) Number of females in cattle, sheep, pig, goat and horse breeds predicted from a single year's registration data. *Animal*, **5**, 980–985
- Hall, S.R., Lafferty, K.D., Brown, J.H., Cáceres, C.E., Chase, J.M. *et al.* (2008) Is infectious disease just another type of predator-prey interaction? In: *Infectious Disease Ecology*. Eds Ostfield, R.S., Keesing, F. and Eviner, V., pp. 223–241. Princeton University Press, Princeton
- Halliday, J., Meredith, A.L., Knobel, D.L., Shaw, D.J., Bronsvoort, B.M. de C. *et al.* (2007) A framework for evaluating animals as sentinels for infectious disease surveillance. *Journal of the Royal Society Interface*, **4**, 973–984
- Halliday, S. (2000) William Farr: a campaigning statistician. *Journal of Medical Biography*, **8**, 220–227
- Halliwell, R.E.W. (1978) Autoimmune disease in the dog. *Advances in Veterinary Science*, **22**, 221–263
- Halloran, M.E. (1998) Concepts of infectious disease epidemiology. In: *Modern Epidemiology*, 2nd edn. Rothman, K.J. and Greenland, S., pp. 529–554. Lippincott-Raven, Philadelphia
- Halloran, M.E., Longini, I.M. and Struchiner, C.J. (2010) *Design and Analysis of Vaccine Studies*. Springer Science + Business Media, New York
- Hallstrom, A.P. and Trobaugh, G.B. (1985) Specificity, sensitivity, and prevalence in the design of randomized trials: a univariate analysis. *Controlled Clinical Trials*, **6**, 128–135
- Hamilton, H.L. and Dillard, J.P. (2006) Natural transformation of *Neisseria gonorrhoeae*: from DNA donation to homologous recombination. *Molecular Microbiology*, **59**, 376–385
- Hamilton, W. (1852) *Discussions on Philosophy and Literature, Education and University Reform*. Longman, Brown, Green and Longmans, London
- Hammer, C.J., Ostby, S.M., Reetz, C.T. and Wagner, S.A. (2014) Preventive health programs. In: *McCurnin's Clinical Textbook for Veterinary Technicians*, 8th edn. Eds Bassert, J.M. and Thomas, J.A., pp. 258–290. Elsevier Saunders, St Louis
- Hammond, R.F. and Lynch, K. (1992) The use and application of geographical information system technology in the Tuberculosis Investigation Unit. Tuberculosis Investigation Unit, University College Dublin, selected papers, pp. 28–30
- Hampson, K., Dushoff, J., Cleaveland, S., Haydon, D.T., Kaare, M. *et al.* (2009) Transmission dynamics and prospects for the elimination of canine rabies. *PLoS Biology*, **7**, 3, e1000053; doi 10.1371/journal.pbio.1000053
- Hanahan, D. and Weinberg R.A. (2000) The hallmarks of cancer. *Cell*, **100**, 57–70
- Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: the next generation. *Cell*, **144**, 646–674
- Hancock, R.D. and Prado, J.A.P. (1993) Foot-and-mouth disease in a flock of sheep in southern Brazil. *Veterinary Record*, **132**, 278–279
- Hand, M.S., Thatcher, C.D., Remillard, R.L., Roudebush, P. and Novotny, B.J. (Eds) (2010) *Small Animal Clinical Nutrition*, 5th edn. Mark Morris Institute, Topeka
- Hanks, J.D. (2009) InterHerd and Herd Companion: using information to focus veterinary input. *Cattle Practice*, **17**, 50–56
- Hanks, J. and Kossaihati, M. (2013) *Key Performance Indicators for the UK National Dairy Herd. A Study of Herd Performance in 500 Holstein/Friesian herds for the Year Ending 31st August 2013*. Veterinary Epidemiology and Economics Research Unit, Reading
- Hanley, J.A. and Hajian-Tilaki, K.O. (1997) Sampling variability of nonparametric estimates of the areas under receiver operating characteristic curves: an update. *Academic Radiology*, **4**, 49–58
- Hanley, J.A. and McNeil, B.J. (1982) The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology*, **143**, 29–36
- Hanley, J.A., and McNeil, B.J. (1983) A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology*, **148**, 839–843

- Hanna, S.R., Briggs, G.A. and Hosker, R.P. (1982) *Handbook on Atmospheric Diffusion*. Technical Information Center, US Department of Energy, Document DE82002045 (DOE/TIC-11223). National Technical Information Service, US Department of Commerce, Springfield
- Hanrahan, C.E. and Becker, G.S. (2004) *Mad Cow Disease and the US Beef Trade. CRS Report for Congress, 4 August 2004*. Congressional Research Service, The Library of Congress, Washington, DC
- Hanski, I. and Woiwod, I.P. (1993) Spatial synchrony in the dynamics of moth and aphid populations. *Journal of Animal Ecology*, **62**, 656–668
- Harada, M. (1995) Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. *Critical Reviews in Toxicology*, **25**, 1–24
- Hardie, E.M. (2000) Recognition of pain behaviour in animals. In: *Animal Pain: A Practice-Oriented Approach to an Effective Pain Control in Animals*. Ed. Heelebrekers, I.J., pp. 51–69. Van der Wees, Utrecht
- Hardstaff, J.L., Häslar, B. and Rushton, J.R. (2015) Livestock trade networks for guiding animal health surveillance. *BMC Veterinary Research*, **11**, 82; doi 10.1186/s12817-015-0354-4
- Hare, K.M. and Biggs, H.C. (1996) Design and evaluation of a veterinary information system for Namibia. *Preventive Veterinary Medicine*, **26**, 239–251
- Harel, O. and Zhou, X.-H. (2006) Multiple imputation for correcting verification bias. *Statistics in Medicine*, **25**, 3769–3786
- Haresnape, J.M. and Wilkinson, P.J. (1989) A study of African swine fever virus infected ticks (*Ornithodoros moubata*) collected from villages in the ASF enzootic area of Malawi following an outbreak of the disease in domestic pigs. *Epidemiology and Infection*, **102**, 507–522
- Harley, S., More, S., Boyle, L., O'Connell, N. and Hanlon, A. (2012) Good animal welfare makes economic sense: potential of pig abattoir meat inspection as a welfare surveillance tool. *Irish Veterinary Journal*, **65**, 11; doi 10.1186/2046-0481-65-11
- Harper, G., and Henson, S. (1998) *Consumer Concerns about Animal Welfare and the Impact on Food Choice: Comparative Literature Review. EU FAIR – CT98 – 3678 November*. Centre for Food Economics Research, University of Reading, Reading
- Harrell, F.E., Lee, K.L. and Mark, D.B. (1996) Multivariable prognostic models: issues in developing models evaluating assumptions and adequacy, and measuring and reducing errors. *Statistics in Medicine*, **15**, 361–387
- Harremöes, P., Gee, D., MacGarvin, M., Stirling, A., Keys, J. *et al.* (2001) *Late Lessons from Early Warnings: the Precautionary Principle 1896–2000*. European Environmental Agency, Environmental Issue Report No. 22. Office for Official Publications of the European Communities, Luxembourg
- Harrison, D.J., Bissonette, J.A. and Sherburne, J.A. (1989) Spatial relationships between coyotes and red foxes in eastern Maine. *Journal of Wildlife Management*, **53**, 181–185
- Harrison, M. (2012) *Contagion: How Commerce Has Spread Disease*. Yale University Press, New Haven and London
- Hartemink, N., Vanwambeke, S.O., Heesterbeek, H., Rogers, D., Morley, D. *et al.* (2011) Integrated mapping of establishment risk for emerging vector-borne infections: a case study of canine leishmaniasis in southwest France. *PLoS ONE*, **6**, 8, e20817; doi 10.1371/journal.pone.0020817
- Hartley, D.M., Nelson, N.P., Arthur, R.R., Barboza, P., Collier, N. *et al.* (2013) An overview of Internet biosurveillance. *Clinical Microbiology and Infection*, **19**, 1006–1013
- Hartman, A., van Wuijkhuise, L., Frankena, K., Franken, P., Wever, P. *et al.* (1997) Within-herd BHV-1 prevalence prediction from an ELISA on bulk milk. *Veterinary Record*, **140**, 484–485
- Hartnack, S., Budke, C.M., Craig, P.S., Jiamin, Q., Boufana, B. *et al.* (2013) Latent-class methods to evaluate diagnostics tests for *Echinococcus* infections in dogs. *PLoS Neglected Tropical Diseases*, **7**, 2, e2068; doi 10.1371/journal.pntd.0002068
- Hartnett, E., Adkin, A., Seaman, M., Cooper, J., Watson, E. *et al.* (2007) A quantitative assessment of the risks from illegally imported meat contaminated with foot and mouth disease virus to Great Britain. *Risk Analysis*, **27**, 187–202
- Hartsough, G.R. and Burger, D. (1965) Encephalopathy of mink. I. Epizootiologic and clinical observations. *Journal of Infectious Diseases*, **115**, 387–392
- Hartsough, G.R. and Gorham, J.R. (1956) Aleutian disease in mink. *National Fur News*, **28**, 10–11
- Harvell, C.D., Kim, K., Burkholder, J.M., Colwell, R.R., Epstein, P.R. *et al.* (1999) Emerging marine diseases – climate links and anthropogenic factors. *Science*, **285**, 1505–1510
- Hashmi, H.A. and Connan, R.M. (1989) Biological control of ruminant trichostrongylids by *Arthrobotrys oligospora*, a predacious fungus. *Parasitology Today*, **5**, 28–30
- Häslar, B., Hiby, E., Gilbert, W., Obeyesekere, N., Bennani, H. *et al.* (2014) A One Health framework for the evaluation of rabies control programmes: a case study from Colombo City, Sri Lanka. *PLoS Neglected Tropical Diseases*, **8**, 10, e3270; doi 10.1371/journal.pntd.0003270

- Hassan, M.K., Afify, M.A. and Aly, M.M. (2004) Genetic resistance of Egyptian chickens to infectious bursal disease and Newcastle disease. *Tropical Animal Health and Production*, **36**, 1–9
- Håstein, T., Hellström, A., Jonsson, G., Olsen, N.J. and Pärnänen, E.R. (2001) Surveillance of fish diseases in the Nordic countries. *Acta Veterinaria Scandinavica*, Suppl. 94, 43–50
- Hastie, T. and Tibshirani, R. (1986) Generalized additive models. *Statistical Science*, **1**, 297–318
- Hathaway, S.C. (1981) Leptospirosis in New Zealand: an ecological view. *New Zealand Veterinary Journal*, **29**, 109–112
- Hauck, W.W. and Donner, A. (1977) Wald's test as applied to hypotheses in logit analysis. *Journal of the American Statistical Association*, **72**, 851–853
- Haughton, D., Legrand, P. and Woolford, S. (2009) Review of three latent class cluster analysis packages: Latent GOLD, poLCA, and MCLUST. *The American Statistician*, **63**, 81–91
- Havelaar, A.H., van Pelt, W., Ang, C.W., Wagenaar, J.A., van Putten, J.P.M. *et al.* (2009) Immunity to *Campylobacter*: its role in risk assessment and epidemiology. *Critical Reviews in Microbiology*, **35** (1) 1–22
- Hawkins, S.A.C., Simmons, H.A., Gough, K.C. and Maddison, B.C. (2015) Persistence of ovine scrapie infectivity in a farm environment following cleaning and decontamination. *Veterinary Record*, **176**, 99; doi 10.1136/vr.102743
- Hay, F.C. and Westwood, O.M.R. (2002) *Practical Immunology*, 4th edn. Blackwell Science, Oxford
- Hay, S.I., Randolph, S.E. and Rogers, D.J. (2000) Remote sensing and geographical information systems in epidemiology. *Advances in Parasitology*, **47**, 1–351
- Hayashidani, H., Omi, Y., Ogawa, M. and Fukutomi, K. (1988) Epidemiological studies on the expectation of life for dogs computed from animal cemetery records. *Japanese Journal of Veterinary Science*, **50**, 1003–1008
- Hayashidani, H., Omi, Y., Ogawa, M. and Fukutomi, Y. (1989) Epidemiological studies on the expectation of life for cats computed from animal cemetery records. *Japanese Journal of Veterinary Science*, **51**, 905–908
- Haydon, D.T., Chase-Topping, M., Shaw, D.J., Matthews, L., Friar, J.K. *et al.* (2003) The construction and analysis of epidemic trees with reference to the 2001 UK foot-and-mouth outbreak. *Proceedings of the Royal Society of London, Series B*, **270**, 121–127
- Haydon, D.T., Cleaveland, S., Taylor, L.H. and Laurenson, M.K. (2002) Identifying reservoirs of infection: a conceptual and practical challenge. *Emerging Infectious Diseases*, **8**, 1468–1473
- Haydon, D.T., Kao, R.R. and Kitching, R.P. (2004) The UK foot-and-mouth disease outbreak – the aftermath. *Nature Reviews Microbiology*, **2**, 675–681
- Haydon, D.T., Randall, D.A., Matthews, L., Knobel, D.L., Tallents, L.A. *et al.* (2006) Low-coverage vaccination strategies for the conservation of endangered species. *Nature*, **443**, 692–695
- Hayes, H.M. (1976) Canine bladder cancer: epidemiologic features. *American Journal of Epidemiology*, **104**, 673–677
- Hayes, H.M. and Wilson, G.P. (1986) Hospital incidence of hypospadias in dogs in North America. *Veterinary Record*, **18**, 605–606
- Hayes, H.M., Hoover, R. and Tarone, R.E. (1981) Bladder cancer in pet dogs: a sentinel for environmental cancer? *American Journal of Epidemiology*, **114**, 229–233
- Hayes, M.L., Bonaventura, J., Mitchell, T.P., Prospero, J. M., Shinn, E.A. *et al.*, (2001) How are climate and marine biological outbreaks functionally linked? *Hydrobiologia*, **460**, 213–220
- Hayes, R.J. and Bennett, S. (1999) Simple sample size calculation for cluster-randomized trials. *International Journal of Epidemiology*, **28**, 319–326
- Haygarth, J. (1800) *Of the Imagination, as a Cause and as a Cure of Disorders of the Body; Exemplified by Fictitious Tractors, and Epidemical Convulsions*. R. Cruttwell, Bath
- Haynes, R.B., Sackett, D.L., Guyatt, G.H. and Tugwell, P. (2006) *Clinical Epidemiology: How to Do Clinical Practice Research*, 3rd edn. Lippincott Williams and Wilkins, Philadelphia
- He, Y. (2010) Missing data analysis using multiple imputation: getting to the heart of the matter. *Circulation: Cardiovascular Quality and Outcomes*, **3**, 98–105
- Head, S.J., Kaul, S., Bogers, A.J.J.C. and Kappetein, A.P. (2012) Non-inferiority study design: lessons to be learned from cardiovascular trials. *European Heart Journal*, **33**, 1318–1324
- Heady, E.O. and Dillon, J.L. (1961) *Agricultural Production Functions*. Iowa State University Press, Ames
- Heath, S.E. (2006) Challenges and options for animal and public health services in the next two decades. *Revue Scientifique et Technique, Office International des Epizooties*, **25**, 403–419
- Heath, T.J. (1998) Longitudinal study of career plans and directions of veterinary students and recent graduates during the first five years after graduation. *Australian Veterinary Journal*, **76**, 181–186
- Hedges, L.V. (1981) Distribution theory of Glass's estimator of effect size and related estimators. *Journal of Educational Statistics*, **6**, 106–128

- Hedges, L.V. and Vevea, J.L. (1998) Fixed- and random-effects models in meta-analysis. *Psychological Methods*, **3**, 486–504
- Hedhammar, Å., Egenvalle, A., Olson, P., Sallander, M., Uddman, U. *et al.* (1999) Hund i Sverige. Diags in Sewden. *Svensk Veterinärtidning*, **51**, 355–362 (in Swedish)
- Heffernan, C. (2013) The climate change-infectious disease nexus: is it time for climate change syndemics? *Animal Health Research Reviews*, **14**, 151–154
- Heffernan, C., Azbel-Jackson, L., Brownlie, J. and Gunn, G. (2016) Farmer attitudes and livestock disease: exploring citizenship behaviour and peer monitoring across the BVD control schemes in the UK. *PLoS ONE*, **11**, 30; doi 10.1371/journal.pone.0152295
- Heffernan, C., Misturelli, F., Nielsen, L., Gunn, G.J. and Yu, J. (2009) Analysis of Pan-European attitudes to the eradication and control of bovine viral diarrhoea. *Veterinary Record*, **164**, 163–167
- Heffernan, C., Nielsen, L., Thomson, K. and Gunn, G. (2008) An exploration of the drivers of bio-security collective action among a sample of UK cattle and sheep farmers. *Preventive Veterinary Medicine*, **87**, 358–372
- Heffernan, C., Salman, M. and York, L. (2013) Livestock infectious disease and climate change: a review of selected literature. In: *Animal Science Reviews 2012*. Ed. Hemming, D., pp. 19–44. CABI, Wallingford
- Heino, M., Kaitala, V., Ranta, S. and Lindström, J. (1997) Synchronous dynamics and rates of extinction in spatially structured populations. *Proceedings of the Royal Society of London, Series B*, **264**, 481–486
- Helle, O. (1971) The effect on sheep parasites of grazing in alternate years by sheep and cattle. A comparison with set-stocking, and the use of anthelmintics with these grazing arrangements. *Acta Veterinaria Scandinavica*, Suppl. 33, 1–59
- Heller, J., Innocent, G., Denwood, M., Reid, S., Kelly, L. *et al.* (2011) Assessing the probability of acquisition of methicillin-resistant *Staphylococcus aureus* (MRSA) in a dog using a nested stochastic simulation model and logistic regression sensitivity analysis. *Preventive Veterinary Medicine*, **99**, 211–224
- Heller, J., Kelly, L., Reid, S. and Mellor, D. (2010) Qualitative risk assessment of the acquisition of methicillin-resistant *Staphylococcus aureus* in pet dogs. *Risk Analysis*, **30**, 458–472
- Hellmén, E., Bergström, R., Holmberg, L., Spånberg, I.-B., Hannson, K. *et al.* (1993) Prognostic factors in canine mammary tumors: a multivariate study of 202 consecutive cases. *Veterinary Pathology*, **30**, 20–27
- Hempel, C.G. (1965) *Aspects of Scientific Explanation*. The Free Press, New York
- Hempel, S. (2006) *The Medical Detective: John Snow and the Mystery of Cholera*. Granta Books, London
- Henao-Restrepo, A.M., Longini, I.M., Egger, M., Dean, N. E., Edmunds, W.J. *et al.* (2015) Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial. *The Lancet*, **386**, 857–866
- Henderson, D.A. (1998) Bioterrorism as a public health threat. *Emerging Infectious Diseases*, **4**, 488–492
- Henderson, R.J. (1969) The outbreak of foot and mouth disease in Worcestershire. An epidemiological study: with special reference to spread of the disease by wind-carriage of the virus. *Journal of Hygiene, Cambridge*, **67**, 21–33
- Henderson, W.M. (1982) Priorities for research. In: *The Control of Infectious Diseases in Farm Animals*. British Veterinary Association Trust Project on the Future of Animal Health Control, London, pp. 50–55
- Hendrickx, G., Napala, A., Slingenbergh, J.H.W., De Deken, R.J., Vercruysse, J. *et al.* (2000) The spatial patterns of trypanosomosis predicted with the aid of satellite imagery. *Parasitology*, **120**, 121–134
- Hendrikx, P., Gay, E., Chazel, M., Moutou, F., Danan, C. *et al.* (2011) OASIS: an assessment tool of epidemiological surveillance systems in animal health and food safety. *Epidemiology and Infection*, **139**, 1486–1496
- Heng, N.H. and Wilson, D.W. (1993) Risk assessment on the importation of milk and milk products (excluding cheese) from countries not free from foot and mouth disease. *Revue Scientifique et Technique, Office International des Epizooties*, **12**, 1135–1146
- Hennebelle, J.H., Sykes, J.E., Carpenter, T.E. and Foley, J. (2013) Spatial and temporal patterns of *Leptospira* infection in dogs from northern California: 67 cases (2001–2010). *Journal of the American Veterinary Medical Association*, **242**, 941–947
- Henneke, D.R., Potter, G.D., Kreider, J.L. and Yeates, B.F. (1983) Relationship between condition score, physical measurement, and body fat percentage in mares. *Equine Veterinary Journal*, **15**, 371–372
- Henry, C.J., Hahn, A.W., Tyler, J.W., Steinberg, H.S. and Caldwell, C.W. (2005) Comparative oncology solution to understanding hormonal impact in the epidemiology of lymphoma. *Veterinary and Comparative Oncology*, **3**, 33
- Henson, J.B., Gorham, J.R., McGuire, T.C. and Crawford, T.B. (1976) Pathology and pathogenesis of Aleutian disease. In: *Slow Virus Diseases of Animals and Man*. Ed. Kimberlin, R.H., pp. 175–205. North-Holland Publishing Company, Amsterdam
- Herbert, W.J. (1970) *Veterinary Immunology*. Blackwell Scientific Publications, Oxford

- Herd, R., Strong, L. and Wardhaugh, K. (Eds) (1993) Environmental impact of avermectin usage in livestock. *Veterinary Parasitology*, **48**, 1–340
- Herlihy, D. (1997) *The Black Death and the Transformation of the West*. Harvard University Press, Cambridge
- Herløv, L. and Vedel, T. (1992) An information system for pig producers and advisory service. In: Proceedings of EAAE Satellite Symposium on Pig Management Information Systems, 12 September 1992, pp. 17–23; 43rd Annual Meeting of the European Association of Animal Production, Madrid, 13–17 September 1992
- Hernán, M.A. (2008) Confounding. In: *Encyclopedia of Quantitative Risk Analysis and Risk Assessment*, Vol 1. Eds Melnick, E.L. and Everitt, B.S., pp. 353–362. John Wiley and Sons, Chichester
- Hernán, M.A. (2010) The hazards of hazard ratios. *Epidemiology*, **21**, 13–15
- Hernán, M.A., Hernández-Díaz, A., Werler, M.M. and Mitchell, A.A. (2002) Causal knowledge as a pre-requisite for confounding evaluation: an application to birth defects epidemiology. *American Journal of Epidemiology*, **155**, 176–184
- Hernandez-Diaz, S., Hernán, M.A., Meyer, K., Werler, M.M. and Mitchell, A.A. (2003) Case-crossover and case-time-control designs in birth defects epidemiology. *American Journal of Epidemiology*, **158**, 385–391
- Heske, L., Nødtvedt, A., Jäderlund, K.H., Berendt, M. and Egenvall, A. (2014) A cohort study of epilepsy among 665,000 insured dogs: incidence, mortality and survival after diagnosis. *The Veterinary Journal*, **202**, 471–476
- Heussner, J.C., Flowers, A.I., Williams, J.D. and Silvy, N.J. (1978) Estimating dog and cat populations in an urban area. *Animal Regulation Studies*, **1**, 203–212
- Hewitson, J.P. and Maizels, R.M. (2014) Vaccination against helminth parasite infections. *Expert Review of Vaccines*, **13**, 473–487
- Hibbing, M.E., Fuqua, C., Parsek, M.R. and Brook Peterson, S. (2010) Bacterial competition: surviving and thriving in the microbial jungle. *Nature Reviews Microbiology*, **8**, 15–25
- Hibi, J., Kurosawa, A., Watanabe, T., Kadowaki, H., Watari, M. *et al.* (2015) Post-traumatic stress disorder in participants of foot-and-mouth disease epidemic disease control in Miyazaki, Japan, in 2010. *Journal of Veterinary Medical Science*, **77**, 953–959
- Hidalgo, B. and Goodman, M. (2013) Multivariate or multivariable regression? *American Journal of Public Health*, **103**, 39–40
- Hide, G. (2003) The origins of sleeping sickness epidemics. *Biologist*, **50**, 268–272
- Hide, G., Tait, A., Maudlin, I. and Welburn, S.C. (1996) The origins, dynamics and generation of *Trypanosoma brucei rhodesiense* epidemics in East Africa. *Parasitology Today*, **12**, 50–55
- Hielm-Björkman, A.K., Kapatkin, A.S. and Rita, H.J.U. (2011) Reliability and validity of a visual analogue scale used by owners to measure chronic pain attributable to osteoarthritis in their dogs. *American Journal of Veterinary Research*, **72**, 601–607
- Hielm-Björkman, A.K., Kuusela, E., Liman, A., Markkola, A., Saarto, E. *et al.* (2003) Evaluation of methods for assessment of pain associated with chronic osteoarthritis in dogs. *Journal of the American Veterinary Medical Association*, **222**, 1552–1558
- Hielm-Björkman, A.K., Rita, H. and Tulamo, R.-M. (2009) Psychometric testing of the Helsinki chronic pain index by completion of a questionnaire in Finnish by owners of dogs with chronic signs of pain caused by osteoarthritis. *American Journal of Veterinary Research*, **70**, 727–734
- Higgins, J.P.T. and Green, S. (Eds) (2011) *Cochrane Handbook for Systematic Reviews of Interventions*, Version 5.1.0 (updated March 2011). The Cochrane Collaboration, www.cochrane-handbook.org
- Higgins, J.P.T. and Thompson, S.G. (2002) Quantifying heterogeneity in meta analysis. *Statistics in Medicine*, **21**, 1539–1558
- Higgins, J., Altman, D. and Sterne, J. (2011) Assessing risk of bias in included studies. In: *Cochrane Handbook for Systematic Reviews of Interventions*, Version 5.1.0 (updated March 2011). Eds Higgins, J., Green, S., chapter 8. The Cochrane Collaboration, www.cochrane-handbook.org
- Higgins, J.P., Thompson, S.G., Deeks, J.J. and Altman, D. G. (2003) Measuring inconsistency in meta-analyses. *British Medical Journal*, **327**, 557–560
- Higham, L.E., Ongeri W., Asena, K. and Thrusfield, M.V. (2016) Characterising and comparing drug-dispensing practices at animal health outlets in the Rift Valley, Kenya: an exploratory analysis (part II). *Tropical Animal Health and Production*, **48**, 1633–1643 (erratum: **48**, 1645–1646)
- Hill, A.B. (1965) The environment and disease: association or causation? *Proceedings of the Royal Society of Medicine*, **58**, 295–300
- Hill, B.H. (2002) National and international impacts of white spot disease of shrimp. *Bulletin of the European Association of Fish Pathologists*, **22**, 58–65
- Hill, H.A. and Kleinbaum, D.G. (2005) Bias in observational studies. In: *Encyclopedia of Biostatistics*, 2nd edn, Vol. 1. Eds Armitage, P., and Colton, T., pp. 323–330. John Wiley and Sons, Chichester
- Hill, I.D. (1987) 95% confidence limits for the median. *Journal of Statistics and Computer Simulation*, **28**, 80–81

- Hill, R.B. and Cambournac, F.C.D. (1941) Intermittent irrigation in rice cultivation and its effect on yield, water consumption and *Anopheles* production. *American Journal of Tropical Medicine and Hygiene*, **21**, 123–144
- Hills, M. and Armitage, P. (1979) The two-period cross-over clinical trial. *British Journal of Clinical Pharmacology*, **8**, 7–20
- Hillyer, M.H., Taylor, F.G.R., Proudman, C.J., Edwards, G. B., Smith, J.E. *et al.* (2002) Case control study to identify risk factors for simple colonic obstruction and distension colic in horses. *Equine Veterinary Journal*, **34**, 455–463
- Hindmarsh, F., Fraser, J. and Scott, K. (1989) Efficacy of a multivalent *Bacteroides nodosus* vaccine against foot rot in sheep in Britain. *Veterinary Record*, **125**, 128–130
- Hindson, J. (1982) Sheep health schemes. *In Practice*, **4**, 53–58
- Hinrichs, J., Otte, J. and Rushton, J. (2010) Technical, epidemiological and financial implications of large-scale national vaccination campaigns to control HPAI H5N1. *CABI Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, **5** (021), 1–20
- Hintze, J.L. and Nelson, R.D. (1998) Violin plots: a boxplot-density trace synergism. *The American Statistician*, **52**, 181–184
- Hird, D.W. and Christiansen, K.H. (1991) California national animal health monitoring system for meat turkey flocks. In: *Proceedings of the 6th International Symposium on Veterinary Epidemiology and Economics*, Ottawa, 12–16 August 1991. Ed. Martin, S. W., pp. 504–506
- Hirst, P.H. (1965) Liberal education and the nature of knowledge. In: *Philosophical Analysis and Education*. Ed. Archambault, R.D., pp. 113–138. Routledge and Kegan Paul, London
- Hitchcock, P., Chamberlain, A., Van Wagoner, M., Inglesby, T.V. and O'Toole, T. (2007) Challenges to global surveillance and response to infectious disease outbreaks of international importance. *Biosecurity and Bioterrorism*, **5**, 206–227
- HMSO (1954) *Mortality and Morbidity during the London fog of December 1952*. Reports on Public Health and Medical Subjects, No. 95. Her Majesty's Stationery Office, London
- HMSO (1958) *1951 Occupational Mortality*, Part II, Vol. 2. Registrar General's Decennial Supplement, 1958. Her Majesty's Stationery Office, London
- HMSO (1968) *A Century of Agricultural Statistics, Great Britain, 1866–1966*. Ministry of Agriculture, Fisheries and Food; Department of Agriculture and Fisheries for Scotland. Her Majesty's Stationery Office, London
- HMSO (1969) *Report of the Committee of Enquiry on Foot-and-Mouth Disease 1968*. 2 vols. Her Majesty's Stationery Office, London
- HMSO (1982) *Agricultural Statistics, United Kingdom 1980 and 1981*. Ministry of Agriculture, Fisheries and Food; Department of Agriculture and Fisheries for Scotland; Department of Agriculture for Northern Ireland; Welsh Agricultural Department. Her Majesty's Stationery Office, London
- HMSO (1991) *Agricultural Statistics, United Kingdom 1989*. Ministry of Agriculture, Fisheries and Food; Department of Agriculture and Fisheries for Scotland; Department of Agriculture for Northern Ireland; Welsh Agricultural Department. Her Majesty's Stationery Office, London
- HMSO (1998) *The Digest of Agricultural Statistics, United Kingdom 1997*. Ministry of Agriculture, Fisheries and Food; The Scottish Office Agriculture, Environment and Fisheries Department; Department of Agriculture for Northern Ireland; Welsh Office. The Stationery Office, London
- Hoberg, E.P., Kutz, S.J., Nagy, J., Jenkins, E., Elkin, B. *et al.* (2002) *Protostrongylus stilesi* (Nematoda: Protostrongylidae): ecological isolation and putative host-switching between Dall's sheep and muskoxen in a contact zone. *Comparative Parasitology*, **69**, 1–9
- Hoekman, B.M. and Kostecki, M.M. (2011) *The Political Economy of the World Trading System: The WTO and Beyond*, 3rd edn. Oxford University Press, Oxford
- Höfle, U., Blanco, J.M. and Kaleta, E.F. (2002) Seroprevalence of avian paramyxovirus 1, 2, and 3 in captive and free-living birds of prey in Spain (preliminary results). In: *The Domestic Animal/Wildlife Interface: Issues for Disease Control, Conservation, Sustainable Food Production, and Emerging Diseases. Annals of the New York Academy of Sciences*, Vol. 969. Eds Gibbs, E.P.J. and Bokma, B.H., pp. 213–216. The New York Academy of Sciences, New York
- Hoffmann, B., Beer, M., Reid, S.M., Mertens, P., Oura, C. A.L. *et al.* (2009) A review of RT-PCR technologies used in veterinary virology and disease control: sensitive and specific diagnosis of five livestock diseases notifiable to the World Organisation for Animal Health. *Veterinary Microbiology*, **139**, 1–23
- Hoffmann, B., Scheuch, M., Hoper, D., Jungblut, R., Holsteg, M. *et al.* (2012) Novel orthobunyavirus in cattle, Europe, 2011. *Emerging Infectious Diseases*, **18**, 469–472
- Hogenesch, H., Azcona-Olivera, J., Scott-Moncreiff, C., Snyder, P.W. and Glickman, L.T. (1999) Vaccine-induced autoimmunity in the dog. *Advances in Veterinary Medicine*, **41**, 733–744

- Hogeveen, H., Noordhuizen-Stassen, E.N., Nielen, M. and Brand, A. (1993) Use of knowledge representation methodologies to diagnose mastitis. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Exeter, 31 March–2 April 1993. Ed. Thrusfield, M.V., pp. 225–235
- Hoinville, L.J. (2013) *Animal Health Surveillance Terminology Final Report from Pre-ICAHS Workshop, July 2013 (version 1.2)*. http://www.fp7-risksur.eu/sites/fp7-risksur.eu/files/partner_logos/icahs-workshop-2011_surveillance_terminology_report_V1.2.pdf (accessed 23 July 2015)
- Hoinville, L.J., Alban, L., Drewe, J.A., Gibbens, J.C., Gustafson, L. *et al.* (2013) Proposed terms and concepts for describing and evaluating animal-health surveillance systems. *Preventive Veterinary Medicine*, **112**, 1–12
- Holden, S., Ashley, S. and Bazeley, P. (1996) *Improving the Delivery of Animal Health Services in Developing Countries: A Literature Review*. Livestock in Development, Crewkerne
- Holland, P.W. (1986) Statistics and causal inference. *Journal of the American Statistical Association*, **81**, 945–960
- Holland, W. (1984) A standard advisory system for lowland sheep flocks. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Edinburgh, 10–11 July 1984. Ed. Thrusfield, M.V., pp. 39–52
- Holling, C.S. (1966) The strategy of building models of complex ecological systems. In: *Systems Analysis and Ecology*. Ed. Watt, K.E.F., pp.195–214. Academic Press, New York
- Holling, C.S. (Ed.) (1978) *Adaptive Environmental Assessment and Management*. John Wiley, Chichester
- Holmes, G. (1996) *Renaissance*. Weidenfeld and Nicolson, London.
- Holmes, J.C. (1973) Site segregation by parasitic helminths: interspecific interaction, site segregation, and their importance to the development of helminth communities. *Canadian Journal of Zoology*, **51**, 333–347
- Holst, P.A., Kromhout, D. and Brand, R. (1988) For debate: pet birds as an independent risk factor for lung cancer. *British Medical Journal*, **297**, 1319–1321
- Holt, P.E. and Thrusfield, M.V. (1993) Association in bitches between breed, size, neutering and docking, and acquired urinary incontinence due to incompetence of the urethral sphincter mechanism. *Veterinary Record*, **133**, 177–180
- Holton, L., Reid, J., Scott, E.M., Pawson, P. and Nolan, A. (2001) Development of a behaviour-based scale to measure acute pain in dogs. *Veterinary Record*, **148**, 525–531
- Holton, L.L., Scott, E.M., Nolan, A., Reid, J., Welsh, E. *et al.* (1998) Comparison of three methods used for assessment of pain in dogs. *Journal of the American Veterinary Medical Association*, **212**, 61–66
- Honhold, N., Taylor, N.M., Mansley, L.M. and Paterson, A.D. (2004a) Relationship of speed of slaughter on infected premises and intensity of culling of other premises to the rate of spread of the foot-and-mouth disease in Great Britain, 2001. *Veterinary Record*, **155**, 287–204
- Honhold, N., Taylor, N. M., Wingfield, A., Einshoj, P., Middlemiss, C. *et al.* (2004b) Evaluation of the application of veterinary judgement in the pre-emptive cull of contiguous premises during the epidemic of foot-and-mouth disease in Cumbria in 2001. *Veterinary Record*, **155**, 349–355
- Hoogstraal, H. (1966) Ticks in relation to human diseases caused by viruses. *Annual Review of Entomology*, **11**, 261–308
- Hoogstraal, H. (1979) The epidemiology of tick-borne Crimean-Congo haemorrhagic fever in Asia, Europe and Africa. *Journal of Medical Entomology*, **15**, 307–417
- Hooper, L.V. and Gordon, J.I. (2001) Commensal host-bacterial relationships in the gut. *Science*, **292**, 1115–1118
- Hoover, R. and Cole, P. (1973) Temporal aspects of occupational bladder carcinogenesis. *The New England Journal of Medicine*, **288**, 1040–1043
- Hope-Cawdery, M.J., Gettinby, G. and Grainger, J.N. (1978) Mathematical models for predicting the prevalence of liver-fluke disease and its control from biological and meteorological data. In: *Weather and Parasitic Animal Disease*. Ed. Gibson, T.E., pp. 21–38. World Meteorological Organization Technical Note No. 159. World Meteorological Organization, Geneva
- Hopewell, S., McDonald, S., Clarke, M.J. and Egger, M. (2007) Grey literature in meta-analyses of randomized trials of health care interventions. *Cochrane Database of Systematic Reviews*, Issue 2. Article No. MR000010; doi 10.1002/14651858.MR000010.pub3
- Horrobin, D.F. (Ed.) (1990) *Omega-6 Essential Fatty Acids: Pathophysiology and Roles in Clinical Medicine*. Alan R. Liss, New York
- Horse and Hound (1992) *The Horse and Hound Equestrian Survey*, October 1992. IPC Magazines, London
- Horst, H.S., Huirne, R.B.M. and Dijkhuizen, A.A. (1997) Risks and economic consequences of introducing classical swine fever into the Netherlands by feeding swill to swine. *Revue Scientifique et Technique, Office International des Epizooties*, **16**, 207–214

- Horton, D.L., Lawson, B., Egbertade, A., Jeffries, C., Johnson, N. *et al.* (2013) Targeted surveillance for Usutu virus in British birds (2005–2011). *Veterinary Record*, **172**, 17; doi 10.1136/vr.101275
- Horton, N.J. and Keinman, K.P. (2007) Much ado about nothing: a comparison of missing data methods and software to fit incomplete data regression models. *The American Statistician*, **61**, 79–90
- Horwitz, R.I. (1987) Complexity and contradiction in clinical trial research. *American Journal of Medicine*, **82**, 498–510
- Hoskins, J.D., Eades, S.C. and Gill, M.S. (2006) Preventive health programs. In: *Clinical Textbook for Veterinary Technicians*, 6th edn. Eds McCurnin, D.M. and Bassert, J.M., pp. 332–381. Elsevier Saunders, St Louis
- Hosmer, D.W. and Lemeshow, S. (1980) A goodness-of-fit test for the multiple logistic regression model. *Communications in Statistics*, **A10**, 1043–1069
- Hosmer, D.W., Lemeshow, S. and Klar, J. (1988) Goodness-of-fit testing for multiple logistic regression analysis when the estimated probabilities are small. *Biometrical Journal*, **30**, 911–924
- Hosmer, D.W., Lemeshow, S. and Sturdivant, R.X. (2013) *Applied Logistic Regression*, 3rd edn. John Wiley and Sons, Hoboken
- Houben, E.H.P., Huirne, R.B.M., Dijkhuizen, A.A. and Kristensen, A.R. (1994) Optimal replacement of mastitic cows determined by a hierarchic Markov process. *Journal of Dairy Science*, **77**, 2975–2993
- Howe, H. and Meyling, A. (1991) Prevalence of bovine virus diarrhoea (BVD) in 19 Danish dairy herds and estimation of rate of infection in early pregnancy. *Preventive Veterinary Medicine*, **11**, 9–16
- Howe, H., Baker, J.C., Maes, R.K., Lloyd, J.W. and Enevoldsen, C. (1995) Comparison of the prevalence and incidence of infection with bovine virus diarrhoea virus (BVDV) in Denmark and Michigan and association with possible risk factors. *Acta Veterinaria Scandinavica*, **36**, 521–531
- Houston, R. (2001) A computerised database system for bovine traceability. *Revue Scientifique et Technique, Office International des Epizooties*, **20**, 652–661
- Howard, C.R. (2009) Arenaviruses. In: *Principles and Practice of Clinical Virology*, 6th edn. Eds Zuckerman, A.J., Banatvala, J.E., Schoub, B.D., Griffiths, P.D. and Mortimer, P., pp. 733–754. Wiley-Blackwell, Chichester
- Howe, K.S. (1985) An economist's view of animal disease. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Reading, 27–29 March 1985. Ed. Thrusfield, M.V., pp. 122–129
- Howe, K.S. (1989) An economist's view of veterinary epidemiology. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Exeter, 12–14 April 1989. Ed. Thrusfield, M.V., pp. 60–71
- Howe, K.S. (1992) Epidemiologists' views of economics – an economist's reply. In: Society for Veterinary Epidemiology and Preventive Medicine, Denary Proceedings, Edinburgh, 1–3 April 1992. Ed. Thrusfield, M.V., pp. 157–167
- Howe, K.S. and Christiansen, K. (2004) The state of animal health economics: a review. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Martigny, 24–26 March 2004. Eds Reid, S.W.J., Menzies, F.D. and Russell, A.M., pp. 153–165
- Hoye, B.J., Munster, V.J., Nishiura, H., Klaassen, M. and Fouchier, R.A.M. (2010) Surveillance of wild birds for avian influenza virus. *Emerging Infectious Diseases*, **16**, 1827–1834
- Hoyningen-Huene, P. (1993) *Reconstructing Scientific Revolutions: Thomas S. Kuhn's Philosophy of Science*. University of Chicago Press, Chicago
- Hsia, J. (2005) Sampling with and without replacement. In: *Encyclopedia of Biostatistics*, 2nd edn, Vol. 7. Eds Armitage, P. and Colton, T., pp. 4763–4764. John Wiley and Sons, Chichester
- HSVMA (2011) *Guide to Congenital and Heritable Disorders in Dogs*. The Humane Society Veterinary Medical Association, Davis. <http://www.hsvma.org/assets/pdfs/guide-to-congenital-and-heritable-disorders.pdf> (accessed 4 November 2015)
- Huang, X., Lin, J. and Demner-Fushman, D. (2006) Evaluation of PICO as a knowledge representation for clinical questions. *AMIA Annual Symposium Proceedings*, **2006**, 359–363
- Huang, Y. and Rohani, P. (2006) Age-structured effects and disease interference in childhood infections. *Proceedings of the Royal Society, Series B*, **273**, 1229–1237
- Hubbard, R. and Ryan, P.A. (2000) The historical growth of statistical significance testing in psychology – and its future prospects. *Educational and Psychological Measurement*, **60**, 661–681
- Hubbert, W.T. and Hermann, G.J. (1970) A winter epizootic of infectious bovine keratoconjunctivitis. *Journal of the American Veterinary Medical Association*, **157**, 452–454
- Huberty, C.J. and Pike, C.J. (1999) On some history regarding statistical testing. In: *Advances in Social Science Methodology*, Vol. 2. Ed. Thompson, B., pp. 1–23. JAI Press, Stamford
- Hudak, D.G. (1994) Adjusting triangular distributions for judgemental bias. *Risk Analysis*, **14**, 1025–1031
- Hudson, P.J. (1986) *Red Grouse: The Biology and Management of a Wild Gamebird*. The Game Conservancy Trust, Fordingbridge

- Hudson, P.J. and Cattadori, I.M. (1999) The Moran effect: a cause of population synchrony. *Trends in Ecology and Evolution*, **14**, 1–2
- Hudson, P.J., Rizzoli, A., Grenfell, B.T., Heesterbeek, H. and Dobson, A.P. (Eds) (2001) *The Ecology of Wildlife Diseases*. Oxford University Press, Oxford
- Huebner, R.J. (1957) The virologist's dilemma. *Annals of the New York Academy of Sciences*, **67**, 430–445
- Huertas, S.M., Gil, A.D., Piaggio, J.M. and van Eerdenburg, F.J.C.M. (2010) Transportation of beef cattle to slaughterhouses and how this relates to animal welfare and carcase bruising in an extensive production system. *Animal Welfare*, **19**, 281–285
- Hueston, W.D. (2001) Science, politics and animal health policy: epidemiology in action. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Noordwijkerhout, 28–30 March 2001. Eds Menzies, F.D. and Reid, S.W.J., pp. xi–xix
- Hueston, W.D. (2007) The risk communication challenges of mass animal destruction. *Veterinaria Italiana*, **43**, 303–315
- Hueston, W.D. (2013) BSE and variant CJD: Emerging science, public pressure and the vagaries of policy-making. *Preventive Veterinary Medicine*, **109**, 179–184
- Hueston, W.D. and Walker, K.D. (1993) Macroepidemiological contributions to quantitative risk assessment. *Revue Scientifique et Technique, Office International des Epizooties*, **12**, 1197–1201
- Hufkens, K., Scheunders, P. and Ceulemans, R. (2009) Ecotones in vegetation ecology: methodologies and definitions revisited. *Ecological Research*, **24**, 977–986
- Hughes, D.E., Pugh, G.W. and McDonald, T.J. (1965) Ultraviolet radiation and *Moraxella bovis* in the etiology of bovine infectious keratoconjunctivitis. *American Journal of Veterinary Research*, **26**, 1331–1338
- Hugh-Jones, M.E. (1972) Epidemiological studies on the 1967/68 foot and mouth disease epidemic: attack rates and cattle density. *Research in Veterinary Science*, **13**, 411–417
- Hugh-Jones, M.E. (1975) Some pragmatic aspects of animal disease monitoring. In: *Animal Disease Monitoring*. Eds Ingram, D.G., Mitchell, W.F. and Martin, S.W., pp. 220–236. Charles C. Thomas, Springfield
- Hugh-Jones, M. (1983) Conclusions for the symposium and lessons for the future. In: Proceedings of the Third International Symposium on Veterinary Epidemiology and Economics, Arlington, Virginia, 6–10 September 1982, pp. 650–654. Veterinary Medical Publishing Company, Edwardsville
- Hugh-Jones, M.E. (1986) Utilization of a working serum bank. In: Proceedings of the Fourth International Symposium on Veterinary Epidemiology and Economics, Singapore, 18–22 November 1985, pp. 179–182. Singapore Veterinary Association, Singapore
- Hugh-Jones, M. (1989) Applications of remote sensing to the identification of the habitats of parasites and disease vectors. *Parasitology Today*, **5**, 244–251
- Hugh-Jones, M.E. (1991a) Remote sensing and geographic information systems, epidemiology and Igor Piskun's rule. In: Proceedings of the 6th International Symposium on Veterinary Epidemiology and Economics, Ottawa, 12–16 August 1991. Ed. Martin, S.W., pp. 541–543
- Hugh-Jones, M. (1991b) Satellite imaging as a technique for obtaining disease-related data. *Revue Scientifique et Technique, Office International des Epizooties*, **10**, 197–204
- Hugh-Jones, M. (1991c) The remote recognition of tick habitats. *Journal of Agricultural Entomology*, **8**, 309–315
- Hugh-Jones, M.E. (2006) Distinguishing between natural and unnatural outbreaks of animal diseases. *Revue Scientifique et Technique, Office International des Epizooties*, **25**, 173–186
- Hugh-Jones, M.E. and Hubbert, W. (1988) Seroconversion rates to bovine leucosis virus, blue tongue virus, *Leptospira hardjo*, and *Anaplasma marginale* infections in a random series of Louisiana beef cattle. *Acta Veterinaria Scandinavica*, Suppl. 84, 107–109
- Hugh-Jones, M.E. and O'Neil, P. (1986) The epidemiological uses of remote sensing and satellites. In: Proceedings of the 4th International Conference on Veterinary Epidemiology and Economics, 18–22 November 1985, Singapore, pp. 113–118. Singapore Veterinary Association
- Hugh-Jones, M.E. and Wright, P.B. (1970) Studies on the 1967–68 foot-and-mouth disease epidemic. The relation of weather to spread of disease. *Journal of Hygiene, Cambridge*, **68**, 253–271
- Hugh-Jones, M., Barre, N., Nelson, G., Wehnes, K., Warner, J. et al. (1992) Landstat-TM identification of *Amblyomma variegatum* (Acari: Ixodidae) habitats in Guadeloupe. *Remote Sensing of Environment*, **40**, 43–55
- Hugh-Jones, M., Wilson, K. and Scheffler, S. (1996) GIS mapping of expected *Vibrio vulnificus* levels in southern Louisiana oysterbays. In: The Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Glasgow, 27–29 March 1996. Eds Thrusfield, M.V. and Goodall, E.A., pp. 169–177
- Hugoson, G. and Wallén, C. (Eds) (2000) Proceedings of the Symposium on Antibiotic Resistance with Emphasis on Animal-Human Transfer, Falkenberg, Sweden, 13–14 September 1999. *Acta Veterinaria Scandinavica*, Suppl. 93, 1–164

- Hugoson, G., Vennström, R. and Hentricksson, K. (1968) The occurrence of bovine leucosis following introduction of babesiosis vaccination. *Bibliotheca Haematologica*, **31**, 157–161
- Huhn, J.E. and Baumann, M.P.O. (1996) The situation of animal health care in Sub-Saharan Africa: a compilation of constraints and required improvements of animal health systems, research and education. In: Primary Animal Health Activities in Southern Africa, Proceedings of an International Seminar held in Mzuzu, Malawi, February 26 to March 8, 1996. Eds Zimmerman, W., Pfeiffer, D.U. and Zessin, K.-H., pp. 16–26. Deutsche Stiftung für internationale Entwicklung (DSE) and Zentralstelle für Ernährung Landwirtschaft (ZEL), Feldafing
- Hui, S.L. and Walter, S.D. (1980) Estimating the error rates of diagnostic tests. *Biometrics*, **36**, 167–171
- Huirne, R.B.M. and Dijkhuizen, A.A. (1994) CHESS: a decision support system to analyze individual sow-herd performance. In: Society for Veterinary Epidemiology and Preventive Medicine Proceedings, Belfast, 13–15 April 1994. Ed. Thrusfield, M.V., pp. 136–145
- Huirne, R.B.M., Dijkhuizen, A.A., Pijpers, A., Verheijden, J.H.M. and van Gulick, P. (1991) An economic expert system on the personal computer to support sow replacement strategies. *Preventive Veterinary Medicine*, **11**, 79–93
- Huirne, R.B.M., Dijkhuizen, A.A., Renkema, J.A. and Van Beek, P. (1992) Computerized analysis of individual sow-herd performance. *American Journal of Agricultural Economics*, **74**, 388–399
- Hulka, B.S., Wilcosky, T.C. and Griffith, J.D. (Eds) (1990) *Biological Markers in Epidemiology*. Oxford University Press, New York
- Humbert, F., Carramiñana, J.J., Lalande, F. and Salvat, G. (1997) Bacteriological monitoring of *Salmonella enteritidis* carrier birds after decontaminating using competitive exclusion and movement of birds. *Veterinary Record*, **141**, 297–299
- Hume, D. (1739–1740) *A Treatise of Human Nature: Being an Attempt to Introduce the Experimental Method of Reasoning into Moral Subjects*. 3 vols. Printed for John Noon (vols 1 and 2) and Thomas Longman (vol. 3), London
- Humphry, R.W., Cameron, A. and Gunn, G.J. (2004) A practical approach to calculate sample size for herd prevalence surveys. *Preventive Veterinary Medicine*, **65**, 173–188
- Huneau, A., Le Bouquin-Leneveu, S., Dia, M., Mateus, A., Stärk, K. *et al.* (2012) *External Scientific Report Submitted to EFSA on the Contribution of Meat Inspection to Animal Health Surveillance in Poultry*. Service Contract No. CT/EFSA/AHAW/2010/05. <http://www.efsa.europa.eu/en/search/doc/287e.pdf> (accessed 27 July 2015)
- Hunt, M. (1997) *How Science Takes Stock: The Story of Meta-analysis*. Russell Sage Foundation, New York
- Hunt, W.G., Burnham, W., Parish, C.N., Burnham, K.K., Mutch, B. *et al.* (2006) Bullet fragments in deer remains: implication for lead exposure in avian scavengers. *Wildlife Society Bulletin*, **34**, 167–170
- Hunter, L., Miller, J.K. and Poxton, I.R. (1999) The association of *Clostridium botulinum* type C with equine grass sickness: a toxicoinfection? *Equine Veterinary Journal*, **31**, 492–499
- Hurd, H.S. and Kaneene, J.B. (1993) The application of simulation models and systems analysis in epidemiology: a review. *Preventive Veterinary Medicine*, **15**, 81–99
- Hurley, K. and Baldwin, C.J. (2012) Prevention and management of infection in canine populations. In: *Infectious Diseases of the Dog and Cat*, 4th edn. Ed. Greene, C.E., pp. 1124–1130. Elsevier-Saunders, St Louis
- Hutchinson S.E. (1996) *Nuer Dilemmas: Coping with Money, War and the State*. University of California Press, Berkeley
- Hutton, J.L. (2000) Number needed to treat: properties and problems. *Journal of the Royal Statistical Society, Series A*, **163**, 403–419
- Hutton, N.E. (1969) *Veterinary medical information systems*. MSc Thesis, Iowa State University
- Hutton, N.E. and Halvorson, L.C. (1974) *A Nationwide System for Animal Health Surveillance*. National Academy of Sciences, Washington DC
- Hutton, N.E. and Seaton, V.A. (1966) Use of computers in veterinary public health activities. *Journal of the American Veterinary Medical Association*, **149**, 1681–1684
- Huxley, T.E. (1868) On the classification and distribution of the Alectoromorphae and Heteromorphae. *Proceedings of the Zoological Society of London*, 214–219
- Huynh, M., Vilmouth, S., Gonzalez, M.S., Calvo Carrasco, D., Di Girolamo, N. *et al.* (2014) Retrospective cohort study of gastrointestinal stasis in pet rabbits. *Veterinary Record*, **175**, 225; doi 10.1136/vr.102460
- Huzurbazar, S., Van Campen, H. and McLean, M.B. (2004) Sample size calculations for Bayesian prediction of bovine viral-diarrhoea-virus infection in beef herds. *Preventive Veterinary Medicine*, **62**, 217–232
- IAEA (1998) *Towards Livestock Disease Diagnosis and Control in the 21st Century*. Proceedings of an international symposium on the diagnosis and control of livestock diseases using nuclear and related techniques jointly organized by the International Atomic Energy Agency and the Food and Agriculture

- Organization of the United Nations, Vienna, 7–11 April 1997. International Atomic Energy Agency, Vienna
- Ianovich, T.D., Bliznichenko, A.G., Zaburina, L.V., Mstibovskii, S.A., Berkovich, A.I. *et al.* (1957) Leptospirosis of the *canicola* type in one of the districts of Rostov-on-the-Don. *Journal of Microbiology, Epidemiology and Immunology*, **28**, 259–264
- IASP (2012) *IASP Taxonomy*. International Association for the Study of Pain. <http://www.iasp-pain.org/Education/Content.aspx?ItemNumber=1698> (accessed 12 June 2014)
- Ibarra, M.L., Cisternas, P.L., Valencia, J.G. and Morales, M.M.A. (1997) Dog and cat population indexes and existences of other species in the County El Bosque, metropolitan region, Chile. *Avances en Ciencias Veterinarias*, **12**, 80–84 (in Spanish; English abstract)
- Ibrahim, A., Junaidu, A. and Garba, M.N. (2009) Multiple antibiotic residues in meat from slaughtered cattle in Nigeria. *The Internet Journal of Veterinary Medicine*, **8** (1). <http://ispub.com/IJVM/8/1/11167#> (accessed 29 December 2013)
- ICH (1997) *International Harmonised Tripartite Guideline: General Considerations for Clinical Trials: E8*. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, July 17, 1997. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E8/Step4/E8_Guideline.pdf (accessed 8 August 2017)
- Ifende, V.I., Derks, M., Hooijer, G.A. and Hogeveen, H. (2014) Financial aspects of veterinary herd health management programmes. *Veterinary Record*, **175**, 224; doi 10.1136/vr.102183
- IGD (2007) *Consumer Attitudes to Animal Welfare: A Report for Freedom Food, March 2007*. Institute for Grocery Distribution, Watford
- IHTSDO (2014) *SNOMED CT*. International Health Terminology Development Organisation, Copenhagen. <http://www.ihtsdo.org/snomed-ct/> (accessed 22 June 2014)
- Ingwersen, W. (2000) Electronic identification and retrieval systems. In: *Kirk's Current Veterinary Therapy XIII*. Ed. Bonagura J.D., pp. 93–95. W.B. Saunders, Philadelphia
- Inoue, M., Hasegawa, A., Hosoi, Y. and Sugiura, K. (2015a) Breed, gender and age pattern of diagnosis for veterinary care in insured dogs in Japan during fiscal year 2010. *Preventive Veterinary Medicine*, **119**, 54–60
- Inoue, M., Hasegawa, A., Hosoi, Y. and Sugiura, K. (2015b) A current life table and causes of death for insured dogs in Japan. *Preventive Veterinary Medicine*, **120**, 210–218
- Inoue, M., Hasegawa, A. and Sugiura, K. (2015c) Morbidity pattern by age, sex and breed in insured cats in Japan (2008–2013). *Journal of Feline Medicine and Surgery*, 1–10; doi 10.1177/1098612X15616433
- Int'Hout, J., Ioannidis, J.P.A. and Brom, G.F. (2014) The Hartung-Knapp-Sidik-Jonkman method for random effects meta-analysis is straightforward and considerably outperforms the standard DerSimonian-Laird method. *BMC Medical Research Methodology*, **14**, 25; doi 10.1186/1471-2288-14-25
- Ioannidis, J.P.A. (1998) Effect of the statistical significance of results on the time to completion and publication of randomized efficacy trials. *Journal of the American Medical Association*, **279**, 281–286
- Ioannidis, J.P.A., Schmid, C.H. and Lau, S. (2000) Meta-analysis in hematology and oncology. *Hematology/Oncology Clinics of North America*, **14**, 973–991
- Ioannidis, J.P.A. (2005) Contradicted and initially stronger effects in highly cited clinical research. *Journal of the American Medical Association*, **294**, 218–228
- Irwig, L.M., Bossuyt, P.M.M., Glasziou, P.M., Gatsonis, C. and Lijmer, J.G. (2009) Designing studies to ensure that estimates of test accuracy will travel. In: *The Evidence Base of Clinical Diagnosis: Theory and Methods of Diagnostic Research*, 2nd edn. Eds Knottnerus, J.A. and Buntinx, F., pp. 96–117. Blackwell Publishing, Oxford
- Irwin, D.A. (1996) *Against the Tide: An Intellectual History of Free Trade*. Princeton University Press, Princeton
- Isaacson, R.E., Moon, H.W. and Schneider, R.A. (1978) Distribution and virulence of *Escherichia coli* in the small intestines of calves with and without diarrhoea. *American Journal of Veterinary Research*, **39**, 1750–1755
- ISG (2007) *Bovine TB: The Scientific Evidence. Final Report of the Independent Scientific Group on Cattle TB*. Presented to the Secretary of State for Environment, Food and Rural Affairs. DEFRA, London
- ISIS (2015) *International Species Information System*. www.isis.org (accessed 12 July 2015)
- Ismaiel, M.O., Greenman, J., Morgan, K., Glover, M.G., Rees, A.S. *et al.* (1989) Periodontitis in sheep: a model for human periodontal disease. *Journal of Periodontology*, **60**, 279–284
- Iverson, T. and Perry, C. (2011) *The Precautionary Principle and Global Environmental Change*. Paper No. 15. Division of Environmental Policy Implementation, The United Nations Environment Programme, Nairobi
- Ivey, E. and Morrissey, J. (1999) Ferrets: examination and preventive medicine. *Veterinary Clinics of North America: Exotic Animal Practice*, **2**, 471–494
- Jachmann, H. (2002) Comparison of aerial counts with ground counts for large African herbivores. *Journal of Applied Ecology*, **39**, 841–852

- Jack, J.M.D. (1961) The Sudan. In: *A History of the Overseas Veterinary Services*, Part 1. Ed. West, G.P., pp. 123–143. British Veterinary Association, London
- Jackson, C.H. (2008) Displaying uncertainty with shading. *The American Statistician*, **62**, 340–347
- Jackson, G.H. and Baker, J.R. (1981) The occurrence of unthriftiness in piglets post-weaning. *The Pig Veterinary Society Proceedings*, **7**, 63–67
- Jackson, M. (2013) *The Age of Stress: Science and the Search for Stability*. Oxford University Press, Oxford
- Jackson, R.M., Roe, J.D., Wangchuk, R. and Hunter, D.O. (2006) Estimating snow leopard population abundance using photography and capture-recapture techniques. *Wildlife Society Bulletin*, **34**, 772–781
- Jacobs, A. (2009) The pathologies of big data. *Acm Queue*, **7** (6), 1–10
- Jacobson, E.R. (1994) Causes of mortality and diseases in tortoises: a review. *Journal of Zoo and Wildlife Medicine*, **25**, 2–17
- Jacobson, E.R., Brown, M.B., Schummercher, I.M., Collins, B.R., Harris, R.K. *et al.* (1995). Mycoplasmosis and the desert tortoise (*Gopherus agassizii*) in Las Vegas Valley, Nevada. *Chelonian Conservation and Biology*, **1**, 279–284
- Jacobson, R. (2000) Principles of validation of diagnostic assays for infectious diseases. In: *Manual of Standards for Diagnostic Tests and Vaccines (2000)*. Office International des Epizooties, Paris. http://www.oie.int/eng/normes/mmanual/A_00013.htm (accessed 7 October 2003)
- Jafari, S., Copes, R., Baharlou, S., Etminan, M. and Buxton, J. (2010) Tattooing and the risk of transmission of hepatitis C: a systematic review and meta-analysis. *International Journal of Infectious Diseases*, **14**, e928–e940
- Jakab, G.J. (1977) Pulmonary defense mechanisms and the interaction between viruses and bacteria in acute respiratory infections. *Bulletin Européen de Physiopathologie Respiratoire*, **13**, 119–135
- Jakob-Hoff, R.M., MacDiarmid, S.C., Lees, C., Miller, P.S., Travis, D. *et al.* (2014) *Manual of Procedures for Wildlife Disease Risk Analysis*. World Organisation for Animal Health (OIE [Office International des Epizooties]), Paris/International Union for Conservation of Nature, Gland
- Jalvingh, A.W. (1993) *Dynamic livestock modelling for on-farm decision support*. PhD Thesis, Wageningen Agricultural University
- James, A.D. (1977) Models of animal health problems. *Agricultural Systems*, **2**, 183–187
- James, A.D. (1998) Guide to epidemiological surveillance for rinderpest. *Revue Scientifique et Technique, Office International des Epizooties*, **17**, 796–809
- Jamieson, S. (2004) Likert scales: how to (ab)use them. *Medical Education*, **38**, 1212–1218
- Janzen, D.H. (1971) Seed predation by animals. *Annual Review of Ecology and systematics*, **2**, 465–492
- Järhult, J.D. (2015) One Health: a doctor's perspective. *Veterinary Record*, **176**, 351–353
- Jarrett, O. (1985) Update: feline leukaemia virus. *In Practice*, **7**, 125–125
- Jarrett, W.F.H. (1980) Bracken fern and papilloma virus in bovine alimentary cancer. *British Medical Bulletin*, **36**, 79–81
- Jarrett, W.F.H., Martin, W.B., Crichton, G.W., Dalton, R. G. and Stewart, M.F. (1964) Transmission experiments with leukaemia (lymphosarcoma). *Nature*, **202**, 566–567
- Jarvis, A. and Cockram, M.S. (1994) Effects of handling and transport on bruising of sheep sent directly from farms to slaughter. *Veterinary Record*, **135**, 523–527
- Jasper, D.E., Dellinger, J.D., Rollins, H. and Hakanson, H. D. (1979) Prevalence of mycoplasmal bovine mastitis in California. *American Journal of Veterinary Research*, **40**, 1043–1047
- Jebara, K.B. (2011) The OIE World Animal Health Information System. In: *Challenges of Animal Health Information Systems and Surveillance for Animal Diseases and Zoonoses*. Proceedings of the International Workshop organized by FAO, 23–26 November 2010, Rome, Italy. Animal Production and Health Proceedings No. 14, pp. 103–108. Food and Agriculture Organization of the United Nations, Rome
- Jebara, K.B., Cáceres, P., Berlingieri, F. and Weber-Vintzel, L. (2012) Ten years' work on the World Organisation for Animal Health (OIE) Worldwide Animal Disease Notification System. *Preventive Veterinary Medicine*, **107**, 149–159
- Jeffery, R.A., Lankester, M.W., McGrath, M.J. and Whitney, H.G. (2004) *Angiostrongylus vasorum* and *Crenosoma vulpis* in red foxes (*Vulpes vulpes*) in Newfoundland, Canada. *Canadian Journal of Zoology*, **82**, 66–74
- Jeglum, K.A. (1982) The use of spontaneous feline neoplasms for studies of therapeutic agents. In: *Design of Models for Testing Cancer Therapeutic Agents*. Eds Figler, I.J. and White, R.J., pp. 193–205. Van Nostrand Reinhold, New York
- Jelinski, M.D., Ribble, C.S., Campbell, J.R. and Janzen, E. D. (1996) Investigating the relationship between abomasal hairballs and perforating abomasal ulcers in unweaned beef calves. *Canadian Veterinary Journal*, **37**, 23–26
- Jellum, E., Andersen, A., Lund-Larsen, P., Theodorsen, L. and Ørjasæter, H. (1995) Experiences of the Janus serum bank in Norway. *Environmental Health Perspectives*, **103**, Suppl. 3, 85–88

- Jenick, M. (2013) *A Primer on Clinical Experience in Medicine*. CRC Press, Boca Raton
- Jennings, D.E. (1986) Judging inference adequacy in logistic regression. *Journal of the American Statistical Association*, **81**, 471–476
- Jessen, L.R., Damborg, P.P., Spohr, A., Schjøth, B. and Wiinberg, B. *et al.* (2012) *Antibiotic Use Guidelines for Companion Animal Practice*. The Danish Small Animal Veterinary Association (SvHKS), Copenhagen
- Jia, B. (2011) FAO EMPRESS-*i* Asia: regional animal disease information system for Asia. In: *Challenges of Animal Health Information Systems and Surveillance for Animal Diseases and Zoonoses*. Proceedings of the International Workshop organized by FAO, 23–26 November 2010, Rome, Italy. Animal Production and Health Proceedings No. 14, pp. 71–72. Food and Agriculture Organization of the United Nations, Rome
- Jin, H.J., Skripnitchenko, A. and Koo, W.W. (2004) *The Effects of the BSE Outbreak in the United States on the Beef and Cattle Industry*. Center for Agricultural Policy and Trade Studies, Department of Agribusiness and Applied Economics, North Dakota State University, Fargo
- Joffe, M. (2011) Causality and evidence discovery in epidemiology. In: *Explanation, Prediction, and Confirmation*. Eds Dieks, D., Wenceslao, J.G., Hartmann, S., Uebel, T. and Weber, M., pp. 153–166. Springer, Dordrecht
- Joffe, M., Gambhir, M., Chadeau-Hyam, M. and Vineis, P. (2012) Causal diagrams in systems epidemiology. *Emerging Themes in Epidemiology*, **9**, 1; doi 10.1186/1742-7622-9-1
- Johnson, B.M., Fry, M.M., Flatland, B. and Kirk, C.A. (2009) Comparison of a human portable blood glucose meter, veterinary portable blood glucose meter, and automated chemistry analyzer for measurement of blood glucose concentrations in dogs. *Journal of the American Veterinary Medical Association*, **235**, 1309–1313
- Johnson, D. (1997) The triangular distribution as a proxy for the beta distribution in risk analysis. *Journal of the Royal Statistical Society, Series D*, **46**, 387–398
- Johnson, R.E., Cameron, T.P. and Kinard, R. (1968a) Canine lymphoma as a potential model for experimental therapeutics. *Cancer Research*, **28**, 2562–2564
- Johnson, R.E., Cameron, T.P. and Kinard, R. (1968b) Total-body irradiation of canine lymphoma. *Radiation Research*, **36**, 629–632
- Johnston, A.M. (2000) HACCP and farm production. In: *HACCP in the Meat Industry*. Ed. Brown, M., pp. 38–79. Woodhead Publishing, Abington
- Johnston, P.I., Henry, N., De Boer, R. and Braidwood, J.C. (1992) Phenoxyethyl penicillin potassium as an in-feed medication for pigs with streptococcal meningitis. *Veterinary Record*, **130**, 138–139
- Johnstone, I.B. and Crane, S. (1981) Von Willebrand's disease in two families of Doberman pinchers. *Canadian Veterinary Journal*, **22**, 239–243
- Jolly, R.D. (2002) Mannosidosis in cattle and its control. *New Zealand Veterinary Journal*, **50**, Suppl., 90
- Jolly, R.D. and Townsley, R.J. (1980) Genetic screening programmes: an analysis of benefits and costs using the bovine mannosidosis scheme as a model. *New Zealand Veterinary Journal*, **28**, 3–6
- Jolly, R.D., Dodds, W.J., Ruth, G.R. and Trauner, D.B. (1981) Screening for genetic diseases: principles and practice. *Advances in Veterinary Science and Comparative Medicine*, **25**, 245–275
- Jolly, R.D., Tse, C.A. and Greenway, R.M. (1973) Plasma α -mannosidase activity as a means of detecting mannosidosis heterozygotes. *New Zealand Veterinary Journal*, **21**, 64–69
- Jones, B. and Kenward, M.G. (2015) *Design and Analysis of Cross-Over Trials*, 3rd edn. CRC Press, Boca Raton
- Jones, B.A., Grace, D., Kock, R., Alonso, S., Rushton, J. *et al.* (2013) Zoonosis emergence linked to agricultural intensification and environmental change. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 8399–8404
- Jones, D. (2006) History and physical examination. In: *Saunders Manual of Small Animal Practice*, 3rd edn. Eds Birchard, S.J. and Sherding, R.G., pp. 1–17. Saunders Elsevier, St Louis
- Jones, E.E. (1932) An encephalomyelitis in the chicken. *Science*, **76**, 331–332
- Jones, G.E. and Gilmour, J.S. (1983) Atypical pneumonia. In: *Diseases of Sheep*. Ed. Martin, W.B., pp. 17–23. Blackwell, Oxford
- Jones, J.B. and Fletcher, W.J. (2012) *Assessment of the Risks Associated with the Release of Abalone Sourced from Abalone Hatcheries for Enhancement of Marine Grow-out in the Open Ocean Areas of WA*. Fisheries Research Report No. 227. Department of Fisheries, Western Australia
- Jones, P.H., Dawson, S., Gaskell, R.M., Coyne, K.P., Tierney, Á. *et al.* (2014) Surveillance of diarrhoea in small animal practice through the Small Animal Veterinary Surveillance Network (SAVSNET). *The Veterinary Journal*, **201**, 412–418
- Jones, R. (1969) Firestick farming. *Australian Natural History*, **16**, 224–231
- Jones, R., Kelly, L., England, T., MacMillan, A. and Wooldridge, M. (2004a) A quantitative risk assessment for the importation of brucellosis-infected breeding cattle into Great Britain from selected countries. *Preventive Veterinary Medicine*, **63**, 51–61

- Jones, R.D., Kelly, L., Fooks, A.R. and Wooldridge, M. (2002) Quantitative Risk Assessment to Compare the Risk of Rabies entering Great Britain from North America via Quarantine and PETS. <http://www.defra.gov.uk/animalh/quarantine/pets/safety/vla2002.PDF> (accessed 19 April 2006)
- Jones, R.D., Kelly, L., Fooks, A.R. and Wooldridge, M. (2005) Quantitative risk assessment of rabies entering Great Britain from North America via cats and dogs. *Risk Analysis*, **25**, 533–542
- Jones, R., Kelly, L., French, N., England, T., Livesey, C. *et al.* (2004b) Quantitative estimates of the risk of new outbreaks of foot-and-mouth disease as a result of burning pyres. *Veterinary Record*, **154**, 161–165
- Jones, W.H.S. (Translator) (1923) *Hippocrates*, Vol. 1. The Loeb Classical Library. William Heinemann, London
- Jordan, A.M. (1986) *Trypanosomiasis Control and African Rural Development*. Longman Group Limited, Harlow and New York
- Jordan, D. and McEwen, S.A. (1998) Herd-level test performance based on uncertain estimates of individual test performance, individual true prevalence and herd true prevalence. *Preventive Veterinary Medicine*, **36**, 187–209
- Jordan, D., McEwen, S.A., Lammerding, A.M., McNab, W.B. and Wilson, J.B. (1999) A simulation model for studying the role of pre-slaughter factors on the exposure of beef carcasses to human microbial hazards. *Preventive Veterinary Medicine*, **41**, 37–54
- Jordan, K., Porcheret, M. and Croft, P. (2004) Quality of morbidity coding in general practice computerized medical records: a systematic review. *Family Practice*, **21**, 396–412
- Joseph, L., Gyorkos, T.W., Coupal, L. (1995) Bayesian estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard. *American Journal of Epidemiology*, **141**, 263–272
- Jost, C.C., Mariner, J.C., Roeder, P.L., Sawitri, E. and Macgregor-Skinner, G.L. (2007) Participatory epidemiology in disease surveillance and research. *Revue Scientifique et Technique, Office International des Epizooties*, **26**, 537–549
- Jourdain, E., Zeller, H.G., Sabatier, P., Murri, S., Kayser, Y. *et al.* (2008) Prevalence of West Nile virus neutralizing antibodies in wild birds from the Camargue area, southern France. *Journal of Wildlife Diseases*, **44**, 766–771
- Julian, A.F. (1981) Tuberculosis in the possum (*Trichosurus vulpecula*). In: Proceedings of the First Symposium on Marsupials in New Zealand. Zoology Publications from Victoria University of Wellington No. 74. Ed. Bell, B.D., pp. 163–174. Victoria University, Wellington
- Julious, S.A. and Campbell, M.J. (1998) Sample size calculations for paired or matched ordinal data. *Statistics in Medicine*, **17**, 1635–1642
- Junker, F., Komorowska, J. and van Tongeren, F. (2009) Impact of Animal Disease Outbreaks and Alternative Control Practices on Agricultural Markets and Trade: The Case of FMD. OECD Food, Agriculture and Fisheries Working Papers, No. 19. OECD Publishing; doi 10.1787/221275827814
- Kagan, I.G. (1951) Aspects of the life history of *Neoleucochloridium problematicum* (Magath, 1902) new com. and *Leucochloridium cynanocittae* McIntosh, 1932 (Trematoda: Brachylaemidae). *Transactions of the American Microscopical Society*, **70**, 281–318
- Kahn, H.A. and Sempos, C.T. (1989) *Statistical Methods in Epidemiology*. Oxford University Press, New York
- Kahn, L.H., Kaplan, B., Monath, T.P. and Steele, J.H. (2008) Teaching 'One Medicine, One Health'. *American Journal of Medicine*, **121**, 169–170
- Kahn, L.H., Kaplan, B. and Steele, J.H. (2007) Confronting zoonoses through closer collaboration between medicine and veterinary medicine (as 'one medicine'). *Veterinaria Italiana*, **43**, 5–19
- Kahneman, D., Slovic, P. and Tversky, A. (Eds) (1982) *Judgement Under Uncertainty: Heuristics and Biases*. Cambridge University Press, Cambridge
- Kaler, J. and Green, L.E. (2013) Sheep farmer opinions on the current and future role of veterinarians in flock management on sheep farms: a qualitative study. *Preventive Veterinary Medicine*, **112**, 370–377
- Kaleta, E.F., Alexander, D.J. and Russell, P.H. (1985) The first isolation of the avian PMV1 virus responsible for the current panzootic in pigeons. *Avian Pathology*, **14**, 553–557
- Kaminjolo, J.S., Nyaga, P.N. and Gicho, J.N. (1974) Isolation, cultivation and characterization of a pox virus from some horses in Kenya. *Zentralblatt für Veterinärmedizin, Reihe B*, **21**, 592–601
- Kampstra, P. (2008) Beanplot: a boxplot alternative for visual comparison of distributions. *Journal of Statistical Software, Code Snippets*, **28**, 1–9
- Kaneene, J.B., Miller, R. and Meyer, R.M. (2006) Abattoir surveillance: the US experience. *Veterinary Microbiology*, **112**, 273–282
- Kaneko, J.J., Harvey, J.W. and Bruss, M.L. (Eds) (2008) *Clinical Biochemistry of Domestic Animals*, 6th edn. Academic Press, Burlington, San Diego and London
- Kao, R.R. (2002) The role of mathematical modelling in the control of the 2001 FMD epidemic in the UK. *Trends in Microbiology*, **10**, 279–286
- Kao, R.R., Danon, L., Green, D.M. and Kiss, I.Z. (2006) Demographic structure and pathogen dynamics on the network of livestock movements in Great Britain.

- Proceedings of the Royal Society, Series B*, **273**, 1999–2007
- Kao, R.R., Green, D.M., Johnson, J. and Kiss, I.Z. (2007) Disease dynamics over very different time-scales: foot-and-mouth disease and scrapie on the network of livestock movements in the UK. *Journal of the Royal Society Interface*, **4**, 907–916
- Kapetsky, J.M., Wijkstrom, U.N., Macpherson, N.J., Vincke, M.M.J., Ataman, E. *et al.* (1991) Where are the best opportunities for fish farming in Ghana? The Ghana aquaculture geographical information system as a decision-making tool. In: *Geographical Information Systems and Remote Sensing in Inland Fisheries*. FAO Fisheries Technical Paper No. 318, pp. 228–233. Food and Agriculture Organization of the United Nations, Rome
- Kaplan, E.L. and Meier, P. (1958) Non-parametric estimation from incomplete observations. *Journal of the American Statistical Association*, **53**, 457–481
- Kaplan, M.M. (1982) Influenza in nature. In: *Animal Diseases in Relation to Animal Conservation*. Symposium of the Zoological Society of London No. 50. Eds Edwards, M.A. and McDonald, U., pp. 121–135. Academic Press, London and New York
- Kapoor, A., Dubovi, E.J., Henriquez-Rivera, J.A. and Lipkin, W.I. (2012) Complete genome sequence of the first canine circovirus. *Journal of Virology*, **86**, 7018
- Kappas, M., Groß, U. and Kelleher, D. (Eds) (2012) *Global Health: A Challenge for Interdisciplinary Research*. Universitätsverlag Göttingen, Göttingen
- Kappeler, A. (1992) Biological and ecological approaches to the management of reservoir species. In: *Wildlife Rabies Control*. Eds Bögel, K., Meslin, F.X. and Kaplan, M., pp. 184–186. Wells Medical, Tunbridge Wells
- Kaptchuck, T.J. (2003) Effect of interpretative bias on research evidence. *British Medical Journal*, **326**, 1453–1455
- Karasszon, D. (1988) *A Concise History of Veterinary Medicine*. Akadémiai Kiadó, Budapest
- Kärber, G. (1931) Beitrag zur kollektiven behandlung pharmakologischer reihenversuche. *Archiv für experimentelle Pathologie und Pharmakologie*, **162**, 480–487
- Kark, S. and Rensburg, B.J. van (2006) Ecotones: marginal or central areas of transition? *Israel Journal of Ecology & Evolution*, **52**, 29–53
- Karki, S., Lupiani, B., Budke, C.M., Karki, N.P.S., Rushton, J. *et al.* (2015) Cost-benefit analysis of avian influenza control in Nepal. *Revue Scientifique et Technique, Office International des Epizooties*, **34**, 813–827
- Karlsen, A.E. and Dyrberg, T. (1998) Molecular mimicry between non-self, modified self and self in autoimmunity. *Seminars in Immunology*, **10**, 25–34
- Karstad, L. (1962) Epizootiology in disease investigations. *Canadian Veterinary Journal*, **3**, 145–149
- Kasl, S.V. and Jones, B.A. (2002) Social epidemiology: towards a better understanding of the field. *International Journal of Epidemiology*, **31**, 1094–1097
- Kasper, R.G. (1980) Perceptions of risk and their effects on decision making. In: *Societal Risk Assessment: How Safe is Enough?*. Eds Schwing, R.C. and Albers, A., pp. 71–80 (discussion: pp. 80–84). Plenum Press, New York
- Kasperson, R.E., Jhaveri, N. and Kasperson, J.X. (2001) Stigma and the social amplification of risk: toward a framework of analysis. In: *Risk, Media, and Stigma: Understanding Public Challenges to Modern Science and Technology*. Eds Flynn, J., Slovik, P. and Kunreuther, H., pp. 9–27. Earthscan, London
- Kass, P.H. and Greenland, S. (1991) Conflicting definitions of confounding and their ramifications for veterinary epidemiologic research: collapsibility vs comparability. *Journal of the American Veterinary Medical Association*, **199**, 1569–1573
- Katz, D., Baptista, J., Azen, S.P. and Pike, M.C. (1978) Obtaining confidence intervals for the risk ratio in cohort studies. *Biometrics*, **34**, 469–474
- Katz, J.M., Hancock, K. and Xu, X. (2011) Serologic assays for influenza surveillance, diagnosis and vaccine evaluation. *Expert Review of Anti-infective Therapy*, **9**, 669–683
- Kaufman, J.A. (2008) China's health care system and avian influenza preparedness. *Journal of Infectious Diseases*, **197**, Suppl. 1, S7–S13
- Kausrud, K.L., Viljugrein, H., Figessi, A., Begon, M., Davis, S. *et al.* (2007) Climatically driven synchrony of gerbil populations allows large-scale plague outbreaks. *Proceedings of the Royal Society of London, Series B*, **274**, 1963–1969
- Kaye, K.S., Harris, A.D., Samore, M. and Cameli, Y. (2005) The case-case-control study design: addressing the limitation of risk factor studies for antimicrobial resistance. *Infection Control and Hospital Epidemiology*, **26**, 346–351
- Kearsley-Fleet, L., O'Neill, D.G., Volk, H.A., Church, D. B. and Brodbelt, D.C. (2013) Prevalence and risk factors for canine epilepsy of unknown origin in the UK. *Veterinary Record*, **172**, 338; doi 10.1136/vr.101133
- Kedmi, M., Herziger, Y., Galon, N., Cohen, R.M., Perel, M. *et al.* (2010) The association of winds with the spread of EHDV in dairy cattle in Israel during an outbreak in 2006. *Preventive Veterinary Medicine*, **96**, 152–160
- Keeling, M.J. (2005) Models of foot-and-mouth disease. *Proceedings of the Royal Society, Series B*, **272**, 1195–1202
- Keeling, M.J. and Eames, K.T.D. (2005) Networks and epidemic models, *Journal of the Royal Society Interface*, **2**, 295–307

- Keeling, M.J. and Grenfell, B.T. (1997) Disease extinction and community size: modeling the persistence of measles. *Science*, **275**, 65–67
- Keeling, M.J. and Rohani, P. (2008) *Modeling Infectious Diseases in Humans and Animals*. Princeton University Press, Princeton and Oxford
- Keeping, D. and Pelletier, R. (2014) Animal density and track counts: understanding the nature of observations based on animal movements. *PLoS ONE*, **9** (5), e96598; doi 10.1371/journal.pone.0096598
- Keet, D.F., Hunter, P., Bengis, R.G., Bastos, A. and Thomson, G.R. (1996) The foot-and-mouth disease epizootic in the Kruger National Park. *Journal of the South African Veterinary Association*, **67**, 83–87
- Keffaber, K.K. (1989) Reproductive failure of unknown aetiology. *American Association of Swine Practitioners Newsletter*, **1** (2), 1, 4–9
- Keighley, E. (1968) Carcinoma of the cervix among prostitutes in a women's prison. *British Journal of Venereal Diseases*, **44**, 254–255
- Kellar, J.A. (1983) Canada's bovine serum bank – a practical approach. In: Proceedings of the Third International Symposium on Veterinary Epidemiology and Economics, Arlington, Virginia, 6–10 September 1982, pp. 138–143. Veterinary Medical Publishing Company, Edwardsville
- Keller, G.H. and Manak, M.M. (Eds) (1989) *DNA Probes*. Macmillan, London
- Kelley, K. and Preacher, K.J. (2012) On effect size. *Psychological Methods*, **17**, 137–152
- Kelly, J.M. and Whitaker, D.A. (2001) A multidisciplinary approach to dairy herd health and productivity management. In: *Fertility in the High-Producing Dairy Cow*. British Society of Animal Science Occasional Publication No. 26, Vol. 1. Ed. Diskin, M.G., pp. 209–222
- Kelly, L. (2005) Microbial risk assessment in poultry production and processing. In: *Food Safety Control in the Poultry Industry*. Ed. Mead, G., pp. 255–272. Woodhead Publishing, Abington
- Kelly, L.A., Hartnett, E., Gettinby, G., Fazil, A., Snary, E. *et al.* (2003) Microbial safety of poultry meat: risk assessment as a way forward. *World's Poultry Science Journal*, **59**, 495–508
- Kelly, L., Murchie, L., Xia, B., Whyte, P. and Madden, R. (2009) Probabilistic model for contamination of egg dishes with *Salmonella* spp. made from shell eggs produced on the island of Ireland. *International Journal of Food Microbiology*, **135**, 187–192
- Kelly, L., Smith, D.L., Snary, E.L., Johnson, J.A., Harris, A. D. *et al.* (2004) Animal growth promoters: to ban or not to ban? A risk assessment approach. *International Journal of Antimicrobial Agents*, **24**, 7–14
- Kelton, D.F., Martin, S.W. and Hansen, D.S. (1992) Development and implementation of the Ontario dairy monitoring and analysis program. In: Proceedings of the 17th World Buiatrics Congress and the 25th American Association of Bovine Practitioners Conference, St Paul, Minnesota, 31 August– 4 September 1992, Vol. 1. Ed. Williams, I.E., pp. 285–289
- Kempf, A.M. and Remington, P.L. (2007) New challenges for telephone survey research in the twenty-first century. *Annual Review of Public Health*, **28**, 113–126
- Kendall, D.G. (1957) La propagation d'une épidémie ou d'un bruit dans une population limitée. *Publications of the Institute of Statistics, University of Paris*, **6**, 307–331
- Kendall, M.G. and Buckland, W.R. (1982) *A Dictionary of Statistical Terms*, 4th edn. Longman Group, London and New York
- Kendler, K.S. and Gardner, C.O. (2010) Interpretation of interactions: guide for the perplexed. *British Journal of Psychiatry*, **197**, 170–171
- Kennedy, R.C., Lane, K.E., Arifin, S.M.N., Fuentes, A., Hollocher, H. *et al.* (2009) A GIS aware agent-based model of pathogen transmission. *International Journal of Intelligent Control and Systems*, **14**, 51–61
- Kennel Club (2015) *DNA Screening Schemes and Results*. The Kennel Club, London. <http://www.thekennelclub.org.uk/health/breeding-for-health/dna-screening-schemes-and-results/> (accessed 5 November 2015)
- Kent, J.E., Meikle, L., Molony V. and McKendrick, I.J. (2001) Qualitative versus quantitative assessment of an acute pain in lambs. *Proceedings of the Sheep Veterinary Society*, **25**, 65–66
- Kent, J.E., Thrusfield, M.V., Molony, V., Hosie, B.D. and Sheppard, B.W. (2004) Randomised, controlled field trial of two new techniques for the castration and docking of lambs less than two days of age. *Veterinary Record*, **154**, 193–200
- Kenyon, F., Sargison, N.D., Skuce, P.J. and Jackson, F. (2009) Sheep helminth parasitic disease in southeastern Scotland arising as a possible consequence of climate change. *Veterinary Parasitology*, **163**, 293–297
- Kermack, W.O. and McKendrick, A.G. (1927) A contribution to the mathematical theory of epidemics. *Proceedings of the Royal Society of London, Series A*, **115**, 700–721
- Kernaghan, G. and Harper, K.A. (2001) Community structure and ectomycorrhizal fungi across an alpine/subalpine ecotone. *Ecography*, **24**, 181–188
- Kernot, K. (2016) Designer drugs a possibility in 10 years, says prof. *Veterinary Times*, **46** (36), 1
- Kerr, N.L. (1998) HARKing: Hypothesizing After the Results are Known. *Personality and Social Psychology Review*, **2**, 196–217
- Keszei, A.P., Novak, M. and Streiner, D.L. (2010) Introduction to health measurement scales. *Journal of Psychosomatic Research*, **68**, 319–323

- Khaitov, M.R., Laza-Stanca, V., Edwards, M.R., Walton, R.P., Rohde, G. *et al.* (2009) Respiratory virus induction of alpha-, beta- and lambda-interferons in bronchial epithelial cells and peripheral blood mononuclear cells. *Allergy*, **64**, 375–386
- Khan, K.S., Ball, E., Fox, C.E. and Meads, C. (2012) Systematic reviews to evaluate causation: an overview of methods and application. *Evidence Based Medicine*, **17**, 137–141
- Khoury M.J. and Flanders W.D. (1996) Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls. *American Journal of Epidemiology*, **144**, 207–213
- Khoury, M.J., Beaty, T.H. and Cohen, B.H. (1993) *Fundamentals of Genetic Epidemiology*. Oxford University Press, New York
- Kick, A.R., Tompkins, M.B. and Almond, G.W. (2012) Stress and immunity in the pig. In: *Animal Science Reviews 2011*. Ed. Hemming, D., pp. 51–67. CABI, Wallingford
- Kida, K. (2003) Relationships of metabolic profiles to milk production and feeding in dairy cows. *Journal of Veterinary Medical Science*, **65**, 671–677
- Kihu, S.M., Njagi, L.M., Njogu, G.N., Kamande, J.N. and Gitao, C.G. (2012) Peste des petits ruminants in Kenya; pastoralist knowledge of the disease in goats in Samburu and Baringo Counties. *Research Opinions in Veterinary & Animal Sciences*, **2**, 544–553
- Kim, L.M., Morley, P.S., Traub-Dargatz, J.L., Salman, M. D. and Gentry-Weeks, C. (2001) Factors associated with *Salmonella* shedding among equine colic patients at a veterinary teaching hospital. *Journal of the American Veterinary Medical Association*, **218**, 740–748
- Kimura, K.C., Zanini, D. de A., Nishiya, A.T. and Dagli, M.L.Z. (2013) Domestic animals as sentinels for environmental carcinogenic agents. *BMC Proceedings*, **7**, Suppl 2, K13; doi 10.1186/1753-6561-7-S2-K13
- King, G. (1696) *Natural and Political Observations and Conclusions upon the State and Condition of England 1696*. Published in 1804 by G.E. Barnett, London. Reproduced in facsimile, with an introduction by P. Laslett, in *The Earliest Classics*. Gregg International Publishers, Farnborough, 1973
- King, L.J. (1985) Unique characteristics of the National Animal Disease Surveillance System in the United States. *Journal of the American Veterinary Medical Association*, **186**, 35–39
- Kingwill, R.G., Neave, F.K., Dodd, F.H., Griffin, T.K. and Westgarth, D.R. (1970) The effect of a mastitis control system on levels of subclinical and clinical mastitis in two years. *Veterinary Record*, **87**, 94–100
- Kirk, G.S., Raven, J.E. and Schofield, M. (1983) *The Presocratic Philosophers*, 2nd edn. Cambridge University Press, Cambridge
- Kirkham, J.J., Dwan, K.M., Altman, D.G., Gamble, C., Dodd, S. *et al.* (2010) The impact of outcome reporting bias in randomized controlled trials on a cohort of systematic reviews. *British Medical Journal*, **340**, c365; doi 10.1136/bmj.c365
- Kirkland, T.N. and Fierer, J. (1996) Coccidioidomycosis: a reemerging infectious disease. *Emerging Infectious Diseases*, **2**, 192–199
- Kirkness, E.F., Bafna, V., Halpern, A.L., Levy, S., Remington, K. *et al.* (2003) The dog genome: survey sequencing and comparative analysis. *Science*, **301**, 1898–1903
- Kirkwood, J.K. (2003) Welfare, husbandry and veterinary care of wild animals in captivity; changes in attitudes, progress in knowledge and techniques. In: *International Zoo Yearbook 38*. Eds Olney, P.J.S. and Fiskin, F.A., pp. 124–130. The Zoological Society of London, London
- Kirkwood, T.B.L. (1985) Comparative and evolutionary aspects of longevity. In: *Handbook of the Biology of Aging*, 2nd edn. Eds Finch, C.E. and Schneider, E.L., pp. 27–44. Van Nostrand Reinhold Company, New York
- Kiss, I.Z., Green, D.M. and Kao, R.R. (2006) The network of sheep movements within Great Britain: network properties and their implications for infectious disease spread. *Journal of the Royal Society Interface*, **3**, 669–677
- Kiss, I.Z., Green, D.M. and Kao, R.R. (2008) The effect of network mixing patterns on epidemic dynamics and the efficacy of disease contact patterns. *Journal of the Royal Society Interface*, **5**, 791–799
- Kissling, R.E. (1958) Host relationships of the arthropod-borne encephalitides. *Annals of the New York Academy of Sciences*, **70**, 320–327
- Kissling, R.E., Chamberlain, R.W., Eidson, E., Sikes, R.K. and Bucca, M.A. (1954) Studies on the North American arthropod-borne encephalitides. II. Eastern equine encephalitis in horses. *American Journal of Hygiene*, **60**, 237–250
- Kitala, P.M., McDermott, J.J., Kyule, M.N. and Cathuma, J.M. (1993) Features of dog ecology relevant to rabies spread in Machokos District, Kenya. *Onderstepoort Journal of Veterinary Research*, **60**, 445–449
- Kitala, P.M., McDermott, J.J., Kyule, M.N. and Gathuma, J.M. (2000) Community-based active surveillance for rabies in Machakos District, Kenya. *Preventive Veterinary Medicine*, **44**, 73–85
- Kitcher, P. and Salmon, W.C. (Eds) (1989) *Scientific Explanation. Minnesota Studies in the Philosophy of Science, Volume XIII*. University of Minnesota Press, Minneapolis

- Kitchin, P.A., Almond, N., Szotyori, Z., Fromhole, C.E., McAlpine, L. *et al.* (1990) The use of the polymerase chain reaction for the detection of Simian immunodeficiency virus in experimentally infected macaques. *Journal of Virological Methods*, **28**, 85–100
- Kitching, R.P. (1998) A recent history of foot-and-mouth disease. *Journal of Comparative Pathology*, **118**, 89–108
- Kitching, R.P. (2004) Predictive models and FMD: the emperor's new clothes? *The Veterinary Journal*, **167**, 127–128
- Kitching, R.P. and Hughes, G.J. (2002) Clinical variation in foot and mouth disease: sheep and goats. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 505–512
- Kitching, R.P., Hutber, A.M. and Thrusfield, M.V. (2005) A review of foot-and-mouth disease with special consideration for the clinical and epidemiological factors relevant to predictive modelling of the disease. *The Veterinary Journal*, **169**, 197–209
- Kitching, R.P., Thrusfield, M.V. and Taylor, N.M. (2006) Use and abuse of mathematical models: an illustration from the 2001 foot and mouth disease epidemic in the United Kingdom. *Revue Scientifique et Technique, Office International des Epizooties*, **25**, 294–311
- Kitron, U., Bouseman, J.K. and Jones, C.J. (1991) Use of the ARC/INFO GIS to study the distribution of Lyme disease ticks in an Illinois county. *Preventive Veterinary Medicine*, **11**, 243–248
- Kivara, F.M., Kwiatek, O., Kapaga, A.M., Swai, E.S., Libeau, G. *et al.* (2013) The incursion, persistence and spread of peste des petits ruminants in Tanzania: epidemiological patterns and predictions. *Onderstepoort Journal of Veterinary Research*, **80**, Article 593; doi 10.4102/ojvr.v80i1.593
- Klaucke, D.N., Buehler, J.W., Thacker, S.B., Parrish, R.G., Trowbridge, F.L. *et al.* (1988) Guidelines for evaluating surveillance systems. *Morbidity and Mortality Weekly Report*, **37** (S-5), 1–18. <http://www.cdc.gov/mmwr/preview/mmwrhtml/00001769.htm> (accessed 2 August 2015)
- Klauder, J.V. (1958) Interrelations of human and veterinary medicine. *The New England Journal of Medicine*, **258**, 170–177
- Kleen, J.L., Atkinson, O. and Noordhuizen, J.P.T.M. (2011) Communication in production animal medicine: modelling a complex interaction with the example of dairy herd medicine. *Irish Veterinary Journal*, **64**, 8; doi 10.1186/2046-0481-64-8
- Klein, M. (1962) The relationship of two bovine adenoviruses to human adenoviruses. *Annals of the New York Academy of Sciences*, **101**, 493–497
- Kleinbaum, D.G. and Klein, M. (2012) *Survival Analysis: A Self-learning Text*, 3rd edn. Springer, New York
- Kleinbaum, D.G., Kupper, L.L. and Morgenstern, H. (1982) *Epidemiologic Research. Principles and Quantitative Measures*. Lifetime Learning Publications, Belmont
- Kleinbaum, D.G., Kupper, L.L., Nizam, A. and Rosenberg, E. (1998) *Applied Regression Analysis and Other Multivariable Methods*, 5th edn. Cengage Learning, Boston
- Kleinbaum, D.G., Sullivan, K.M. and Barker, N.D. (2013) *ActivEpi Companion Textbook*, 2nd edn. Springer-Verlag, New York
- Kleinman, K.P. and Abrams, A.M. (2006) Assessing surveillance using sensitivity, specificity and timeliness. *Statistical Methods in Medical Research*, **15**, 445–464
- Kleypas, J., Danabasoglu, G. and Lough, J. (2008) Potential role of the ocean thermostat in determining regional differences in coral reef bleaching events. *Geophysical Research Letters*, **35**, L03613; doi 10.1029/2007GL032257
- Klimczak, J.C. (1994) SNOMED (Review). *Preventive Veterinary Medicine*, **21**, 266–269
- Kloeze, H., Mukhi, S., Kitching, P., Lees, V.W. and Alexandersen, S. (2010) Effective animal health disease surveillance using a network-enabled approach. *Transboundary and Emerging Diseases*, **57**, 414–419
- Knight, H.D. (1972) Bacterial and spirochetal diseases: other bacterial infections. In: *Equine Medicine and Surgery – A Textbook and Reference Work*, 2nd edn. Eds Catcott, E.J. and Smithcors, J.F., pp. 85–113. American Veterinary Publications, Wheaton
- Knol, M.J. and VanderWeele, T.J. (2012) Recommendations for presenting analyses of effect modification and interaction. *International Journal of Epidemiology*, **41** (2), 514–520
- Knottnerus, A.J., Buntinx, F. and van Weel, C. (2009) General introduction: evaluation of diagnostic procedures. In: *The Evidence Base of Clinical Diagnosis: Theory and Methods of Diagnostic Research*, 2nd edn. Eds Knottnerus, J.A. and Buntinx, F., pp. 1–19. Blackwell Publishing, Oxford
- Knowles, D.P. and Gorham, J.R. (1990) Diagnosis of viral and bacterial diseases *Revue Scientifique et Technique, Office International des Epizooties*, **9**, 733–757
- Knox, G.A. (1964) The detection of space-time interactions. *Applied Statistics*, **13**, 25–29
- Knox, K.M.G., Reid, S.W.J., Irwin, T. and Gettinby, G. (1996) Interrogation of a hospital database: from data to decision support. In: *Society for Veterinary Epidemiology and Preventive Medicine Proceedings*, Glasgow, 27–29 March 1996. Eds Thrusfield, M.V. and Goodall, E.A., pp. 94–101
- Knox, S.S. (2010) From 'omics' to complex disease: a systems biology approach to gene-environment

- interactions in cancer. *Cancer Cell International*, **10**, 11; doi 10.1186/1475-2867-10-11
- Koch, A. (1891) *Encyclopädie der gesamten Thierheilkunde und Thierzucht*, Vol. 8. Verlag Moritz Perles, Vienna and Leipzig
- Koch, R. (1892) *Ueber bakteriologische Forschung*. Hirschwald, Berlin. Translation: Rivers, T.M. (1937) Viruses and Koch's postulates. *Journal of Bacteriology*, **33**, 1–12
- Koch, T. (2005) *Cartographies of Disease: Maps, Mapping, and Medicine*. Esri Press, Redlands
- Kochin, B.F., Bull, J.J. and Antia, R. (2010) Parasite evolution and life history theory. *PLoS Biol*, **8**, 10, e1000524; doi 10.1371/journal.pbio.1000524
- Komarov, A. and Kalmar, E. (1960) A hitherto undescribed disease – turkey meningo-encephalitis. *Veterinary Record*, **72**, 257–261
- Konig, C.D.W. (1985) Bedrijfsdiergeneeskundige aspecten van de schapenhouderij (Planned animal health and production service on sheep farms. An inventory and evaluation). PhD Thesis, University of Utrecht
- Konner, M. (1993) *The Trouble with Medicine*. BBC Books, London
- Koo, D. and Thacker, S.B. (2010) In Snow's footsteps: commentary on shoe-leather and applied epidemiology. *American Journal of Epidemiology*, **172**, 737–739
- Koontz, S.R., Hoag, D.L., Thilmany, D.D., Green, J.W. and Grannis, J.L. (Eds) (2006) *The Economics of Livestock Disease Insurance: Concepts, Issues and International Case Studies*. CABI Publishing, Wallingford
- Koops, H. and Hartmann, F. (1989) *Anguillicola*-infestations in Germany and in German eel imports. *Journal of Applied Ichthyology*, **5**, 41–45
- Kopelman, L.M. (2002) If HIV/AIDS is punishment, who is bad? *Journal of Medicine and Philosophy*, **27**, 231–242
- Kopinski, J.S. and McKenzie, R.A. (2007) Oesophagogastric ulceration in pigs: a visual morphological scoring guide. *Australian Veterinary Journal*, **85**, 356–361
- Körmeny, B. (1988) Diagnostic value of mammalian, avian and johnin PPD tuberculin in cattle herds infected with *Mycobacterium paratuberculosis*. *Acta Veterinaria Hungarica*, **36**, 177–183.
- Kosinski, A.S., Chen, Y. and Lules, R.H. (2009) Sample size calculations for evaluating a diagnostic test when the gold standard is missing at random. *Statistics in Medicine*, **29**, 1572–1579
- Kosmider, R.D., Kelly, L., Laurenson, K., Coleman, P., Fooks, A.R. *et al.* (2006) Risk assessment informs policy decisions regarding pet importation. *Veterinary Record*, **158**, 694–695
- Kossaibati, M.A. and Esslemont, R.J. (1997) The cost of production diseases in dairy herds in England. *Veterinary Journal*, **154**, 41–51
- Kossaibati, M.A., Hovi, M. and Esslemont, R.J. (1998) Incidence of clinical mastitis in dairy herds in England. *Veterinary Record*, **143**, 649–653
- Kottner, J., Audigé, L., Brorson, S., Donner, A., Gajewski, B.J. *et al.* (2011) Guidelines for reporting reliability and agreement studies (GRRAS) were proposed. *Journal of Clinical Epidemiology*, **64**, 96–106
- Kovacs, A.B. and Beer, G.Y. (1979) The mechanical properties and qualities of floors for pigs in relation to limb disorders. *The Pig Veterinary Society Proceedings*, **5**, 99–104
- Krabbe, H. (1864) *Observations for Icelanders on Hydatids and Precautions Against Them*. J. Schultz, Copenhagen. Translation: Maxwell, I.R. (1973) *Australian Veterinary Journal*, **49**, 395–401
- Kraemer, H.C. (1992) *Evaluating Medical Tests*. Sage Publications, Newbury Park
- Kraus, C., Pavard S. and Promislow, D.E.L. (2013) The size-life span trade-off decomposed: why large dogs die young. *The American Naturalist*, **181**, 492–505
- Krebs, C.J. and Boutin, S. (1995) Impact of food and predation on the snowshoe hare cycle. *Science*, **269**, 112–115
- Krebs, C.J., Boonstra, R., Boutin, S. and Sinclair, A.R.E. (2001) What drives the 10-year cycle of snowshoe hares? *Bioscience*, **51**, 25–35
- Krebs, J.R. (1997) *Bovine Tuberculosis in Cattle and Badgers*. Ministry of Agriculture, Fisheries and Food, London
- Kreso, A. and Dick, J.E. (2014) Evolution of the cancer stem cell model. *Cell Stem Cell*, **14**, 275–291
- Kricher, J. (2009) *The Balance of Nature: Ecology's Enduring Myth*. Princeton University Press, Princeton
- Kricka, L.J. (Ed.) (1992) *Nonisotopic DNA Probe Techniques*. Academic Press, San Diego
- Krieger, N. (1994) Epidemiology and the web of causation: has anyone seen the spider? *Social Science & Medicine*, **39**, 887–903
- Krishnamoorthy, K. and Peng, J. (2007) Some properties of the exact and score methods for binomial proportion and sample size calculation. *Communications in Statistics – Simulation and Computation*, **36**, 1171–1186
- Kristensen, P. (1992) Bias from nondifferential but dependent misclassification of exposure and outcome. *Epidemiology*, **3**, 210–215
- Kristjanson, P.M., Swallow, B.M., Rowlands, G.J., Kruska, R.L. and de Leeuw, P.N. (1999) Measuring the costs of African animal trypanosomosis, the potential benefits of control and returns to research. *Agricultural Systems*, **59**, 79–98

- Krone, L.M., Brown, C.M. and Lindenmayer, J.M. (2014) Survey of electronic veterinary medical record adoption and use by independent small animal veterinary medical practices in Massachusetts. *Journal of the American Veterinary Medical Association*, **245**, 324–332
- Kroneman, A., Vellenga, L., van der Wilt, F.J. and Vermeer, H.M. (1993) Review of health problems in group-housed sows, with special emphasis on lameness. *Veterinary Quarterly*, **15**, 26–29
- Krouwer, J.S. (2008) Why Bland-Altman plots should use X , not $(Y + X)/2$ when X is a reference method. *Statistics in Medicine*, **27**, 778–780
- Kryter, K.D. (1985) *The Effects of Noise on Man*, 2nd edn. Academic Press, Orlando
- Kryzanowski, W.J. and Hand, D.J. (2009) *ROC Curves for Continuous Data*. Chapman and Hall/CRC Press, Boca Raton
- Krzyzanowska, M.K., Pintilie, M., Brezden-Masley, C., Dent, R. and Tannock, I.F. (2004) Quality of abstracts describing randomized trials in the proceedings of American Society of Clinical Oncology meetings: guidelines for improved reporting. *Journal of Clinical Oncology*, **22**, 1993–1999
- Kubes, V. and Rios, F.A. (1939) The causative agent of infectious equine encephalomyelitis in Venezuela. *Science*, **90**, 20–21
- Kuerpick, B., Conraths, F.J., Staubach, C., Froehlich, A., Schnieder, T. *et al.* (2013) Seroprevalence and GIS-supported risk factor analysis of *Fasciola hepatica* infections in dairy herds in Germany. *Parasitology*, **140**, 1051–1060
- Kues, W.A. and Niemann, H. (2011) Advances in farm animal transgenesis. *Preventive Veterinary Medicine*, **102**, 146–156
- Kuhn, K., Campbell-Lendrum, D., Haines, A. and Cox, J. (2005) *Using Climate to Predict Infectious Disease Epidemics*. World Health Organization, Geneva
- Kuhn, T.S. (1970) *The Structure of Scientific Revolutions*. International Encyclopaedia of Unified Science, 2nd edn, Vol. 2, No. 2. University of Chicago Press, Chicago and London
- Kühne, M. and Lhafi, S.K. (2005) Ist erregerefreiheit in tierbeständen für die lebensmittelsicherheit notwendig? [Do we need pathogen-free livestock for food safety?] *Deutsche tierärztliche Wochenschrift*, **112**, 300–303 (in German)
- Kuiken, T., Holmes, E.C., McCauley, J., Rimmelzwaan, G.F., Williams, C.S. *et al.* (2006) Host species barriers to influenza virus infections. *Science*, **312**, 394–397
- Kuiken, T., Ryser-Degiorgis, M.-P., Gavier-Widén, D. and Gortázar, C. (2011) Establishing a European network for wildlife health surveillance. *Revue Scientifique et Technique, Office International des Epizooties*, **30**, 755–761
- Kulathinal, S., Karvanen, J., Saarela, O. and Kuulasmaa, K. (2007) Case-cohort study in practice – experiences from the MORGAM project. *Epidemiologic Perspectives & Innovations*, **4**, 15; doi 10.1186/1742-5573-4-15
- Kumar, K. (2006) *Conducting Mini Surveys in Developing Countries*. Bureau for Policy and Program Coordination, USAID Program Design and Evaluation Methodology Report, PN-ADG-566. USAID, Silver Spring
- Kumar, S. (2002) *Methods for Community Participation: A Complete Guide for Practitioners*. Vistaar Publications, New Delhi/ITDG Publishing, Rugby
- Kumar, S., Mirajkar P.P., Singh Y.P. and Singh, R. (2011) Analysis of willingness to pay for veterinary services of the livestock owners of Sangli District of Maharashtra. *Agricultural Economics Research Review*, **24**, 149–153
- Kummar, S. and Doroshov, J.H. (2011) Phase 0 trials: expediting the development of chemoprevention agents. *Cancer Prevention Research*, **4**, 288–292
- Kummar, S., Rubinstein, L., Kinders, R., Parchment, R.E., Gutierrez, M.E. *et al.* (2008) Phase 0 clinical trials: conceptions and misconceptions. *The Cancer Journal*, **14**, 133–137
- Kupper, L.L. and Hogan, M.D. (1978) Interaction in epidemiologic studies. *American Journal of Epidemiology*, **108**, 447–453
- Kurshid, A. and Ageel, M.I. (2010) Binomial and Poisson confidence intervals and its variants: a bibliography. *Pakistan Journal of Statistics and Operation Research*, **6**, 76–100
- Kutlina-Dimitrova, Z. (2015) The economic impact of the Russian import ban: a CGE analysis. TRADE, Chief Economist Note. European Commission. Issue No. 3, December 2013. <http://trade.ec.europa.eu/doclib/html/154025.htm> (accessed 22 August 2016)
- Kvale, S. and Brinkmann, S. (2009) *InterViews: Learning the Craft of Qualitative Research Interviewing*, 2nd edn. Sage Publications, Thousand Oaks
- Kwaghe, A.V., Ndahi, M.D., Usman, J.G., Sambo, E., Waziri, I. *et al.* (2011) Highly pathogenic avian influenza participatory disease surveillance in Plateau State, Nigeria. *Archives Des Sciences*, **65**, 132–142
- Kwon, J.M. and Goate, A.M. (2000) The candidate gene approach. *Alcohol Research & Health*, **24**, 164–168
- Kyriacou, D.N., Adamski, A. and Khardori, N. (2006) Anthrax: from antiquity and obscurity to a front-runner in bioterrorism. *Infectious Disease Clinics of North America*, **20**, 227–251
- L'Estrange Ewen, C. (1933) *Witchcraft and Demonism. A Concise Account derived from Sworn*

- Depositions and Confessions obtained in the Courts of England and Wales*. Heath Cranton, London
- La Ragione, R. and Mead, G. (2013) Competitive exclusion. In: *Salmonella in Domestic Animals*, 2nd edn. Eds Barrow, P.A. and Methner, U., pp. 426–454. CABI International, Wallingford
- Ladds, P.W. and Entwistle, K.W. (1977) Observations on squamous cell carcinomas of sheep in Queensland, Australia. *British Journal of Cancer*, **35**, 110–114
- Lafferty, K.D. (2009) The ecology of climate change and infectious diseases. *Ecology*, **90**, 888–900
- Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., De Leo, G. *et al.* (2008) Parasites in food webs: the ultimate missing links. *Ecology Letters*, **11**, 533–546
- LaFond, E., Breur, G.J. and Austin, C.C. (2002) Breed susceptibility for developmental orthopedic diseases in dogs. *Journal of the American Animal Hospital Association*, **38**, 467–477
- Laing, S.E., Buckland, S.T., Burn, R.W., Lambie, D. and Amphlett, A. (2003) Dung and nest surveys: estimating decay rates. *Journal of Applied Ecology*, **40**, 1102–1111
- Lam, T.G.J.M., van den Borne, B.H.P., Jansen, J., Huijps, K., van Weeren, J.C.L. *et al.* (2013) Improving bovine udder health: a national mastitis control program in the Netherlands. *Journal of Dairy Science*, **96**, 1301–1311
- Lambin, E.F., Tran, A., Vanwambeke, S.O., Linard, C. and Soti, V. (2010) Pathogenic landscapes: interactions between land, people, disease vectors, and their animal hosts. *International Journal of Health Geographics*, **9**, 54; doi 10.1186/1476-072X-9-54
- Lancaster, H.O. (1994) *Quantitative Methods in Biological and Medical Sciences: A Historical Essay*. Springer-Verlag, New York
- Landa, L. (2012) Pain in domestic animals and how to assess it: a review. *Veterinarni Medicina*, **57**, 185–192
- Lane, D.M., Rendleman, M. and Ott, S.L. (1997) Using partial budgets to analyse selected management practices associated with reducing preweaning mortality. *Swine Health and Production*, **5**, 95–102
- Lane-Clayton, J.E. (1926) A further report on cancer of the breast. *Reports on Public Health and Medical Subjects*, 32. His Majesty's Stationery Office, London
- Lang, Z. and Reiczig, J. (2014) Confidence limits for prevalence adjusted for estimated sensitivity and specificity. *Preventive Veterinary Medicine*, **113**, 13–22
- Langbein, J. (1996) The red deer of Exmore and the Quantocks. *Deer*, **9**, 492–498
- Langholz, B. (2005) Case-control study, nested. In: *Encyclopedia of Biostatistics*, 2nd edn, Vol. 1. Eds Armitage, P. and Colton, T., pp. 646–655. John Wiley and Sons, Chichester
- Langholz, B. and Clayton, D. (1994) Sampling strategies in nested case control studies. *Environmental Health Perspectives*, **102**, Suppl. 8, 47–51
- Langmuir, A.D. (1963) The surveillance of communicable diseases of national importance. *The New England Journal of Medicine*, **268**, 182–192
- Langmuir, A.D. (1965) Developing concepts in surveillance. *The Millbank Memorial Fund Quarterly*, **43** (2), 369–372
- Langmuir, A.D. (1971) Communicable disease surveillance. *Proceedings of the Royal Society of Medicine*, **64**, 681–684
- Langmuir, A.D. (1976) William Farr: founder of modern concepts of surveillance. *International Journal of Epidemiology*, **5**, 13–18
- Langois, J.P., Fahrig, L., Merriam, G. and Artsob, H. (2001) Landscape structure influences continental distribution of hantavirus in deer mice. *Landscape Ecology*, **16**, 255–266
- Langston, A.A., Mellersh, C.S., Wiegand, N.A., Acland, G. M., Ray, K. *et al.* (1999) Towards a framework linkage map of the canine genome. *Journal of Heredity*, **90**, 7–14
- Lankester, M.W. (2001) Extrapulmonary lungworms of cervids. In: *Parasitic Diseases of Wild Mammals*, 2nd edn. Eds Samuel, W.M., Lybus, M.J. and Kican, A.A., pp. 228–278. Iowa State University Press, Ames
- Lansdowne, Z.F. (1999) Risk matrix: an approach for prioritizing risks and tracking risk mitigation progress. In: *Proceedings of the 30th Annual Project Management Institute 1999 Seminars & Symposium*, Philadelphia, Pennsylvania, USA. Papers presented October 10 to 16, 1999 (unpaginated). Project Management Institute, Four Campus Boulevard, Newtown Square
- Lantz, C.A. and Nebenzahl, E. (1996) Behavior and interpretation of the kappa statistic: resolution of the two paradoxes. *Journal of Clinical Epidemiology*, **49**, 431–434
- Laplace, P.-S. (1814) *Essai Philosophique sur les Probabilités*. Translated as *A Philosophical Essay on Probabilities*. Truscott, F.W. and Emory, F.L. (translators) (1951). Dover Publications, New York
- Lara, L.J. and Rostagno, M.H. (2013) Impact of heat stress on poultry production. *Animals*, **3**, 356–369
- Laure, E., Alvarez, T., Cavagion, L. and Herrasti, A. (1992) Canine population dynamics in General Pico, Argentina, in the period 1986/1990. *Veterinaria Argentina*, **IX**, 536–542 (in Spanish; English abstract)
- Larsen, C.T., Domermuth, C.H., Sponenberg, D.P. and Gross, W.B. (1985) Colibacillosis of turkeys exacerbated by haemorrhagic enteritis virus – laboratory studies. *Avian Diseases*, **29**, 729–732

- Larsen, M. (1999) Biological control of helminths. *International Journal for Parasitology*, **29**, 139–146
- Lascalles, B.D., Cripps, P.J., Jones, A. and Waterman, A.E. (1997) Post-operative central hypersensitivity and pain: the pre-eruptive value of pethidine for ovariohysterectomy. *Pain*, **73**, 461–471
- Lasco, T.A., Bhagwat, J.G., Zou, K.H. and Ohno-Machado, L. (2005) The use of receiver operating characteristic curves in biomedical informatics. *Journal of Biomedical Informatics*, **38**, 404–415
- Last, J.M.. (1963) The iceberg 'completing the clinical picture' in general practice. *The Lancet*, **282**, 28–31
- Latour, B. (1999) *Pandora's Hope: Essays on the Reality of Science Studies*. Harvard University Press, Cambridge
- Laughlin, R.B. and Pines, D. (2000) The theory of everything. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 28–21
- Laurenson, K.L., Hoyle, D., Woolhouse, M. and Coleman, P. (2002) *Revised Assessment of the Risk of Importing Rabies if Pet Animals from North America were eligible for the Pet Travel Scheme (PETS)*. <http://www.defra.gov.uk/animalh/quarantine/pets/safety/edinburgh2002.PDF> (accessed 19 April 2006)
- Laut, P. (1986) Land evaluation for bovine tuberculosis eradication in Northern Australia. *Australian Geographical Studies*, **24**, 259–271
- Lave, L. (1996) Benefit-cost analysis: do the benefits exceed the costs? In: *Risks, Costs, and Lives Saved: Getting Better Results from Regulation*. Ed. Hahn, R.W., pp. 104–134. Oxford University Press, Oxford
- Lavie, C.J., Milani, R.V. and Ventura, H.O. (2009) Obesity and cardiovascular disease: risk factor, paradox and impact of weight loss. *Journal of the American College of Cardiology*, **53**, 1925–1932
- Lavrakas, P.J. (2009) Methods for sampling and interviewing in telephone surveys. In: *The SAGE Handbook of Applied Social Research Methods*, 2nd edn. Eds Bickman, L. and Rog, D.J., pp. 509–542. Sage Publications, Thousand Oaks
- Law, G.R. (2005) Invited commentary: do clusters of leukemia and lymphoma provide evidence for an infectious cause? *American Journal of Epidemiology*, **162**, 823–824
- Lawrence, K. (1998) Growth promoters in swine. In: *The 15th International Pig Veterinary Society Congress, Proceedings (Invited Papers)*, 5–9 July 1998. Eds Done, S., Thomson, J. and Varley, M., pp. 337–343. Nottingham University Press, Thrumpton
- Lawson, A.B. and Zhou, H. (2005) Spatial statistical modeling of disease outbreaks with particular reference to the UK foot and mouth disease (FMD) epidemic of 2001. *Preventive Veterinary Medicine*, **71**, 141–156
- Lazer, D., Kennedy, R., King, G. and Vespignani, A. (2014) The parable of Google Flu: traps in big data analysis. *Science*, **343**, 1203–1205
- Leach, K.A., Whay, H.R., Maggs, C.M., Barker, Z.E., Paul, E.S. *et al.* (2010a) Working towards a reduction in cattle lameness: 1. Understanding barriers to lameness control on dairy farms. *Research in Veterinary Science*, **89**, 311–317
- Leach, K.A., Whay, H.R., Maggs, C.M., Barker, Z.E., Paul, E.S. *et al.* (2010b) Working towards a reduction in cattle lameness: 2. Understanding dairy farmers' motivations. *Research in Veterinary Science*, **89**, 318–323
- Leavitt, J.L. (1996) *Typhoid Mary: Captive to the Public's Health*. Beacon Press, Boston
- Lebeau, A. (1953) L'âge du chien et celui de l'homme. Essai de statistique sur la mortalité canine. *Bulletin de l'Académie Vétérinaire de France*, **26**, 229–232
- Lecompte, L., Fichet-Calvet, E., Daffis, S., Koulémou, K., Sylla, O. *et al.* (2006) *Mastomys natalensis* and Lassa Fever, West Africa. *Emerging Infectious Diseases*, **12**, 1971–1974
- Lederberg, J., Shope, R.E. and Oaks, S.C. (1992) *Emerging Infections: Microbial Threats to Health in the United States*. National Academy Press, Washington DC
- Ledley, R.S. and Lusted, L.B. (1959) Reasoning foundations of medical diagnosis; symbolic logic, probability, and value theory aid our understanding of how physicians reason. *Science*, **130**, 9–21
- Lee, C.H., Dershaw, D.D., Kopans, D., Evans, P., Monsees, B. *et al.* (2010) Breast cancer screening with imaging: recommendations from the Society of Breast Imaging and the ACR on the use of mammography, breast MRI, breast ultrasound, and other technologies for the detection of clinically occult breast cancer. *Journal of the American College of Radiology*, **7**, 18–27
- Lee, E.T. (1992) *Statistical Methods for Survival Data Analysis*, 2nd edn. John Wiley, New York
- Lee, J.-A.L.M., More, S.J. and Cotiw-An, B.S. (1999) Problems translating a questionnaire in a cross-cultural setting. *Preventive Veterinary Medicine*, **41**, 187–194
- Lee, K. and Brumme, Z.L. (2013) Operationalizing the One Health approach: the global governance challenges. *Health Policy and Planning*, **28**, 778–785
- Lee, S.-M., Turula, V., Powell, B., Nisum, M., Mariagrazia Pizza, M. *et al.* (2015) Analytical control strategy of bacterial subunit vaccines. In: *Vaccine Analysis: Strategies, Principles, and Control*. Eds Nunnally, B.K., Turula, V.E. and Sitrin, R.D., pp. 187–270. Springer-Verlag, Berlin and Heidelberg
- Lee, W.M. and Yeilding, N.M. (2003) Molecular cell biology. In: *Oxford Textbook of Medicine*, Vol. 1. Eds

- Warrell, D.A., Cox, T.M., Firth, J.D. and Benz, E.J., pp. 90–107. Oxford University Press, Oxford
- Leech, A., Howarth, R.J., Thornton, I. and Lewis, G. (1982) Incidence of bovine copper deficiency in England and the Welsh borders. *Veterinary Record*, **111**, 203–204
- Leech, F.B. (1980). Relations between objectives and observations in epidemiological studies. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 7–11 May 1979. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 254–257. Australian Government Publishing Services, Canberra
- Leefang, M.M.G., Rutjes, A.W.S., Reitsma, J.B., Hooft, L. and Bossuyt, P.M.M. (2013) Variation of a test's sensitivity and specificity with disease prevalence. *Canadian Medical Association Journal*, **185**, E537–E544
- Leforban, Y. and Gerbier, G. (2003) Recent history and epidemiology of foot-and-mouth disease in Europe. In: *Foot-and-Mouth Disease: Control Strategies*. Symposium Proceedings, 2–5 June 2002, Lyons, France. Eds Dodet B. and Vicari, M., pp. 153–171. Elsevier, Paris
- Lehmann, E.L. and D'Abrera, H.J.M. (1975) *Nonparametrics*. Holden-Day, San Francisco
- Leighton, F.A. (1995) Surveillance of wild animal diseases in Europe. *Revue Scientifique et Technique, Office International des Epizooties*, **14**, 819–830
- Leighton, F.A. (2002) Health risk assessment of the translocation of wild animals. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 187–195
- Leimbacher, F. (1978) Experience with the 'Mt' system of forecasting fascioliasis in France. In: *Weather and Parasitic Animal Disease*. World Meteorological Organization Technical Note No. 159. Ed. Gibson, T. E., pp. 6–13. World Meteorological Organization, Geneva
- LeLorier, J., Grégoire, G., Benhaddad, A., Lapierre, J. and Derderian, F. (1997) Discrepancies between meta-analyses and subsequent large randomized, controlled trials. *The New England Journal of Medicine*, **337**, 536–542
- Lemeshow, S. and Hosmer, D.W. (1982) The use of goodness-of-fit statistics in the development of logistic regression models. *American Journal of Epidemiology*, **115**, 92–106
- Lemon, S.M., Sparling, P.F., Hamburg, M.A., Relman, D. A., Choffnes, E.R. *et al.* (Rapporteurs) (2008) *Vector-borne Diseases: Understanding the Environmental, Human Health, and Ecological Connections*. National Academies Press, Washington DC
- Lenartz, M.S., Nelson, M.E., Schrage, M.W. and Edwards, A.J. (2009) Temperature mediated moose survival in northeastern Minnesota. *Journal of Wildlife Management*, **73**, 503–510
- Lengerich, E.J., Teclaw, R.F., Mendlein, J.M., Mariolis, P. and Garbe, P.L. (1992) Pet populations in the catchment area of the Purdue Comparative Oncology Program. *Journal of the American Veterinary Medical Association*, **200**, 51–56
- Lennon, M.A. (2003) Water fluoridation: the public health benefits. *Biologist*, **50**, 200
- Lentner, C. (Ed.) (1982) *Geigy Scientific Tables*, 8th edn, Vol. 2. Ciba-Geigy, Basle
- Lepissier, H.E. and MacFarlane, I.M. (1966) Organisation for African Unity. Scientific, Technical and Research Commission. Joint Campaign against rinderpest. Phase I Final Report, November 1965. *Bulletin of Epizootic Diseases of Africa*, **14**, 193–224
- Leppänen, M., Paloheimo, A. and Saloniemi, H. (2000a) Attitudes of Finnish dog-owners about programs to control canine genetic diseases. *Preventive Veterinary Medicine*, **43**, 145–158
- Leppänen, M., Paloheimo, A. and Saloniemi, H. (2000b) Attitudes of Finnish dog-breeders about programs to control canine genetic diseases. *Preventive Veterinary Medicine*, **43**, 159–175
- Lequarré, A.-S., Andersson, L., André, C., Fredholm, M., Hitte, C. *et al.* (2011) LUPA: a European initiative taking advantage of the canine genome architecture for unravelling complex disorders in both human and dogs. *The Veterinary Journal*, **189**, 155–159
- Leslie, B.E., Meek, A.H., Kawash, G.F. and McKeown, D. B. (1994) An epidemiological investigation of pet ownership in south central Ontario. *Canadian Veterinary Journal*, **35**, 218–222
- Leslie, P.H. (1945) On the use of matrices in certain population mathematics. *Biometrika*, **33**, 183–212
- Leslie, P.H. (1948) Some further notes on the use of matrices in population mathematics. *Biometrika*, **35**, 213–245
- Leslie, W.D., O'Donnell, S., Jean, S., Legace, C., Walsh, P. *et al.* (2009) Trends in hip fracture rates in Canada. *Journal of the American Medical Association*, **302**, 883–889
- Lessard, P., L'Eplattenier, R., Norval, R.A.I., Kundert, K., Dolan, T.T. *et al.* (1990) Geographical Information Systems for studying the epidemiology of cattle disease caused by *Theileria parva*. *Veterinary Record*, **126**, 225–262
- Lessard, P., L'Eplattenier, R., Norval, R.A.I., Perry, B.D., Dolan, T.T. *et al.* (1988) The use of geographical information systems in estimating East Coast fever risk to African livestock. *Acta Veterinaria Scandinavica, Suppl.* **84**, 234–236
- Leutenegger, C.M., Pusterla, N., Wicki, R. and Lutz, H. (2003) New molecular tools in the diagnosis of tick-

- borne diseases. *The European Journal of Companion Animal Practice*, **13**, 185–191
- Levine, P.P. and Fabricant, J. (1950) A hitherto-undescribed virus disease of ducks in North America. *Cornell Veterinarian*, **40**, 71–86
- Levins, R. (1966) The strategy of model building in population biology. *American Scientist*, **54**, 421–431
- Lévi-Strauss, C. (1963) *Structural Anthropology*. Penguin Books, Harmondsworth
- Levy, J.K., Woods, J.E., Turick, S.L. and Etheridge, D.L. (2003) Number of unowned free-roaming cats in a college community in the southern United States and characteristics of community residents who feed them. *Journal of the American Veterinary Medical Association*, **223**, 202–205
- Levy, P.S. and Lemeshow, S. (2008) *Sampling of Populations: Methods and Applications*, 4th edn. John Wiley, New York
- Lewis, D. (1986) *Philosophical Papers*, Vol. 2. Oxford University Press, New York
- Lewis, T.W., Blott, S.C. and Woolliams, J.A. (2010) Genetic evaluation of hip score in UK Labrador retrievers. *PLoS ONE*, **5**, e12797; doi 10.1371/journal.pone.0012797
- Leyland, T., Lotira, R., Abebe, D., Bekele, G. and Catley A. (2014) *Community-based Animal Health Workers in the Horn of Africa: An Evaluation for the US Office for Foreign Disaster Assistance*. Feinstein International Center, Tufts University Africa Regional Office, Addis Ababa/Network UK, Great Holland
- Li, C.-R., Liao, C.-T. and Liu, J.-P. (2008) On the exact interval estimation for the difference in paired areas under the ROC curves. *Statistics in Medicine*, **27**, 224–242
- Liamputton, P. and Ezzy, D. (2005) *Qualitative Research Methods*, 2nd edn. Oxford University Press, Melbourne
- Liberati, A. (1995) Meta-analysis: statistical alchemy for the 21st century: discussion. A plea for a more balanced view of meta-analysis and systematic overviews of the effect of health care interventions. *Journal of Clinical Epidemiology*, **48**, 81–86
- Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gøtzsche, P.C. et al. (2009) The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Journal of Clinical Epidemiology*, **62**, e1–34; doi 10.1016/j.jclinepi.2009.06.006
- Liddell, S. and Bailey, D.V. (2001) Market opportunities and threats to the US pork industry posed by traceability systems. *International Food and Agribusiness Management Review*, **4**, 287–302
- Lienhardt, G. (1961) *Divinity and Experience, the Religion of the Dinka*. Oxford University Press, Oxford
- Liess, B. and Plowright, W. (1964) Studies on the pathogenesis of rinderpest in experimental cattle. I Correlations of clinical signs, viraemia and virus excretion by various routes. *Journal of Hygiene, Cambridge*, **62**, 81–100
- Lightner, D.V., Redman, R.M., Poulos, B.T., Nunan, L.M., Mari, J.L. et al. (1997) Risk of spread of pinead shrimp viruses in the Americas by the international movement of live and frozen shrimp. *Revue Scientifique et Technique, Office International des Epizooties*, **16**, 146–160
- Lightowers, M.W. (1996) Vaccination against cestode parasites. *International Journal for Parasitology*, **26**, 819–824
- Lijmer, J.G. and Bossuyt, P.M.M. (2009) Diagnostic testing and prognosis: the randomized controlled trial in test evaluation research. In: *The Evidence Base of Clinical Diagnosis: Theory and Methods of Diagnostic Research*, 2nd edn. Eds Knottnerus, J.A. and Buntinx, F., pp. 63–82. Blackwell Publishing, Oxford
- Lijmer, J.G., Mol, B.W., Heisterkamp, S., Bonsel, G.J., Prins, M.H. et al. (1999) Empirical evidence of design-related bias in studies of diagnostic tests. *Journal of the American Medical Association*, **282**, 1061–1066
- Likert, R. (1932) A technique for the measurement of attitudes. *Archives of Psychology*, **140**, 1–55
- Lilienfeld, A.M. and Lilienfeld, D.E. (1980) *Foundations of Epidemiology*, 2nd edn. Oxford University Press, New York
- Lilienfeld, D.E. (1978) Definitions of epidemiology. *American Journal of Epidemiology*, **107**, 87–90
- Lilienfeld, D.E. (1979) 'The greening of epidemiology': sanitary physicians and the London Epidemiological Society (1830–1870). *Bulletin of the History of Medicine*, **52**, 503–528
- Limmathurotsakul, D., Turner, E.L., Wuthiekanun, V., Thaipadungpanit, J., Suputtamongkol, Y. et al. (2012) Fool's gold: why imperfect reference tests are undermining the evaluation of novel diagnostics: a reevaluation of 5 diagnostic tests for leptospirosis. *Clinical Infectious Diseases*, **55**, 322–331
- Linard, C., Lamarque, P., Heyman, P., Ducoffre, G., Luyasu, V. et al. (2007a) Determinants of the geographic distribution of Puumala virus and Lyme borreliosis in Belgium. *International Journal of Health Geographics*, **6**, 15; doi 10.1186/1476-072X-6-15
- Linard, C., Ponçon, N., Fontenille, D. and Lambin, E.F. (2009) Risk of malaria re-emergence in southern France: testing scenarios with a multi-agent simulation model. *EcoHealth*, **6**, 135–147
- Linard, C., Tersago, K., Leirs, H. and Hamblin, E.F. (2007b) Environmental conditions and Puumala virus

- transmission in Belgium. *International Journal of Health Geographics*, **6**, 55; doi 10.1186/1476-072X-6-55
- Lincoln, F.C. (1930) Calculating waterfowl abundance on the basis of banding returns. In: *USDA Circular No. 118, May 1930*, pp. 1–4. United States Department of Agriculture, Washington DC
- Lind, J. (1753) *A Treatise of The Scurvy in Three Parts: Containing an Inquiry into the Nature, Causes and Cure of That Disease, Together with a Critical Chronological View of What has been Published on the Subject*. Murray and Cochran, Edinburgh
- Lindberg, A.L.E. (2003) Bovine viral diarrhoea virus infections and its control: a review. *Veterinary Quarterly*, **25**, 2–16
- Lindberg, A., Brownlie, J., Gunn, G.J., Houe, H., Moennig, V. *et al.* (2006) The control of bovine viral diarrhoea virus in Europe: today and in the future. *Revue Scientifique et Technique, Office International des Epizooties*, **25**, 961–979
- Lindblade, K.A., Walker, E.D., Onapa, A.W., Katungu, J. and Wilson, M.L. (2000) Land use change alters malaria transmission parameters by modifying temperature in a highland area of Uganda. *Tropical Medicine and International Health*, **5**, 263–274
- Lindblad-Toh, K., Wade, C.M., Mikkelsen, T.S., Karlsson, E.K., Jaffe, D.B. *et al.* (2005) Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature*, **438**, 803–819
- Linde, A., Rotzén-Östlund, M., Zweyberg-Wirgart, B., Rubinova, S. and Brytting, M. (2009) Does viral interference affect spread of influenza? *Eurosurveillance*, **14** (40), 2–4
- Lindemann, R.L. (1942) The trophic dynamic aspects of ecology. *Ecology*, **36**, 587–600
- Lindley, D.V. and Scott, W.F. (1984) *New Cambridge Elementary Statistical Tables*. Cambridge University Press, Cambridge
- Lindley, S. and Watson, P. (Eds) (2010) *BSAVA Manual of Canine and Feline Rehabilitation, Supportive and Palliative Care: Case Studies in Patient Management*. British Small Animal Veterinary Association, Gloucester
- Lindström, A. and Jaenson, G.T. (2003) Distribution of the common tick, *Ixodes ricinus* (Acari: Ixodidae), in different vegetation types in southern Sweden. *Journal of Medical Entomology*, **40**, 375–378
- Linthicum, K.J., Bailey, C.L., Tucker, C.J., Angleberger, D.R., Cannon, T. *et al.* (1991) Towards real-time prediction of Rift Valley fever epidemics in Africa. *Preventive Veterinary Medicine*, **11**, 325–334
- Lipscomb T.P., Schulman F.Y., Moffett D. and Kennedy S. (1994) Morbilliviral disease in Atlantic bottlenose dolphins (*Tursiops truncatus*) from the 1987–88 epizootic. *Journal of Wildlife Diseases*, **30**, 567–571
- Lipsey, M.W. and Hurley, S.M. (2009) Design sensitivity: statistical power for applied experimental research. In: *The Sage Handbook of Applied Social Research Methods*, 2nd edn. Eds Bickman, L. and Rog, D.J., pp. 44–76. Sage Publications, Thousand Oaks
- Lister, J. (1870) On the effects of the antiseptic system of treatment upon the salubrity of a surgical hospital. *The Lancet*, **95**, 4–6, 40–42
- Little, R.J.A. and Rubin, D.B. (2002) *Statistical Analysis with Missing Data*, 2nd edn. Wiley, New York
- Little, T.W.A., Swan, C., Thompson, H.V. and Wilesmith, J.W. (1982) Bovine tuberculosis in domestic and wild mammals in an area of Dorset. II. The badger population, its ecology and tuberculosis status. *Journal of Hygiene, Cambridge*, **89**, 211–224
- Liu, S.J., Xue, H.P., Pu, B.Q. and Qian, N.H. (1984) A new viral disease in rabbits. *Animal Husbandry and Veterinary Medicine (Xumu yu Shouyi)*, **16**, 253–255 (in Chinese)
- Lloyd-Smith, J.O., Cross, P.C., Briggs, C.J., Daugherty, M., Getz, W.M. *et al.* (2005) Should we expect population thresholds for wildlife diseases? *Trends in Ecology and Evolution*, **20**, 511–519
- Lobb, T. (1745) *Letters Relating to the Plague, and other Contagious Distempers*. Buckland, London
- Logan, E.F., McBeath, D.G. and Lowman, B.G. (1974) Quantitative studies on serum immunoglobulin levels in suckled calves from birth to five weeks. *Veterinary Record*, **94**, 367–370
- Logas, D. (2003) An approach to pruritus. In: *BSAVA Manual of Small Animal Dermatology*, 2nd edn. Eds Foster, A.P. and Foil, C.S., pp. 37–42. British Small Animal Veterinary Association, Gloucester
- LoGiudice, K., Ostfield, R.S., Schmidt, K.A. and Keeling, F. (2003) The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 567–571
- London, C.A. and Thamm, D.H. (2013) Mast cell tumours. In: *Withrow & MacEwen's Small Animal Clinical Oncology*, 5th edn. Eds Withrow, S.J., Vail, D. M. and Page, R.L., pp. 335–355. Elsevier Saunders, St Louis
- Longhi, A., Pasini, A., Cicognani, A., Baronio, F., Pellacani, A. *et al.* (2005) Height as a risk factor for osteosarcoma. *Journal of Pediatric Hematology/Oncology*, **27**, 314–318
- Longley, P.A., Goodchild, M.F., Maguire, D.J. and Rhind, D.W. (2005) *Geographic Information Systems and Science*, 2nd edn, Vol. 1. John Wiley and Sons, Chichester
- Loong, T-W. (2005) Understanding sensitivity and specificity with the right side of the brain. *British Medical Journal*, **327**, 716–719.

- López-Ratón, M., Rodríguez-Álvarez, M.X., Cadarso, Suárez, C.C. and Gude-Sampedro, F. (2014) OptimalCutpoints: an R package for selecting optimal cutpoints in diagnostic tests. *Journal of Statistical Software*, **61** (8), 1–35
- Lord, R.D. (1983) Ecological strategies for the prevention and control of health problems. *Bulletin of the Pan American Health Organization*, **17**, 19–34
- Lord, R.D., Calisher, C.H., Metzger, W.R. and Fischer, G. W. (1974) Urban St. Louis encephalitis surveillance through wild birds. *American Journal of Epidemiology*, **99**, 360–363
- Lorenz, K. (1977) *Behind the Mirror: A Search for the Natural History of Human Knowledge*. Methuen, London
- Lothrop, H.D., Lothrop, B. and Reisen, W.K. (2002) Nocturnal microhabitat distribution of adult *Culex tarsalis* (Diptera: Culicidae) impacts control effectiveness. *Journal of Medical Entomology*, **39**, 574–582
- Lotka, A.J. (1925) *Elements of Physical Biology*. Williams and Wilkins, Baltimore
- Louis, P.-C.-A. (1836) *Anatomical, Pathological and Therapeutic Researches upon the Disease Known under the Name of Gastro-Enterite Putrid, Adynamic, Ataxic, or Typhoid Fever, etc., Compared with the Most Common Acute Diseases*. Bowditch, H.I. (translator). Vol. 1 Hilliard Gray, Boston; Vol. 2 Isaac R. Butts, Boston
- Louis, T.A. (1991) Using empirical Bayes methods in biopharmaceutical research. *Statistics in Medicine*, **10**, 811–829
- Lovatt, F. (2004) Developing flock health plans. *In Practice*, **26**, 290–295
- Lovelock, J. (1979) *Gaia: A New Look at Life on Earth*. Oxford University Press, Oxford
- Loving, N.S. and Johnston, A.M. (1995) *Veterinary Manual for the Performance Horse*. Blackwell Science, Oxford
- Lowe, H.J. (1942) Rinderpest in Tanganyika Territory. *Empire Journal of Experimental Agriculture*, **10**, 189–203
- Lower, G.M. (1983) Systematic epidemiologic theory: conceptual foundations and axiomatic elements. *Medical Hypotheses*, **11**, 195–215
- Lower, G.M. and Kanarek, M.S. (1983) Conceptual/operational criteria of causality: relevance to systematic epidemiologic theory. *Medical Hypotheses*, **11**, 217–244
- Lowrance, W.W. (1976) *Of Acceptable Risk: Science and the Determination of Safety*. William Kaufmann, Los Altos
- Loya, Y., Sakai, K., Yamazato, K., Nakano, Y., Sambali, H. et al. (2001) Coral bleaching: the winners and the losers. *Ecology Letters*, **4**, 122–131
- Lubow, B.C. and Ransom, J.I. (2009) Validating aerial photographic mark–recapture for naturally marked feral horses. *Journal of Wildlife Management*, **73**, 1420–1429
- Ludi, A. and Rodruiguez, L. (2013) Novel approaches to foot-and-mouth disease vaccine development. In: *Vaccines and Diagnostics for Transboundary Animal Diseases*. Eds Roth, J.A., Richt, J.A. and Morozov, I.A., pp. 107–116. Karger, Basel
- Luis, A.D., Hayman, D.T.S., O'Shea, T.J., Cryan, P.M., Gilbert, A.T. et al. (2013) A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? *Proceedings of the Royal Society, Series B*, **280**, 20122753
- Lumeij, J.T., Endenburg, N. and Luyten, B.R.M. (1998) The percentage of feline, canine, avian and exotic animal consultations in veterinary practice in the Netherlands in 1994 and suggested consequences for the veterinary curriculum and residency programs. *Veterinary Quarterly*, **20**, 35–37
- Lund, E.M., Armstrong, P.J., Kirk, C.A., Kolar, L.M. and Klausner, J.S. (1999) Health status and population characteristics of dogs and cats examined at private veterinary practices in the United States. *Journal of the American Veterinary Medical Association*, **214**, 1336–1341
- Lund, E.M., James, K.M. and Neaton, J.D. (1998) Veterinary randomized clinical trial reporting: a review of the small animal literature. *Journal of Veterinary Internal Medicine*, **12**, 57–60
- Lunn, D.J., Thomas, A., Best, N., and Spiegelhalter, D. (2000) WinBUGS – a Bayesian modelling framework: concepts, structure, and extensibility. *Statistics and Computing*, **10**, 325–337
- Lust, G., Todhunter, R.J., Erb, H.N., Dykes, N.L., Williams, A. J. et al. (2001) Repeatability of dorsolateral subluxation scores in dogs and correlation with macroscopic appearance of hip osteoarthritis. *American Journal of Veterinary Research*, **62**, 1711–1715
- Lysons, R.E., Gibbens, J.C. and Smith, L.H. (2007) Progress with enhancing veterinary surveillance in the United Kingdom. *Veterinary Record*, **160**, 105–112
- Maarel, E. van der (1990) Ecotones and ecoclines are different. *Journal of Vegetation Science*, **1**, 135–138
- Mabiane, A., Byarugaba, D.K. and Schwabenbauer, K. (2009) *Influence of Awareness Campaigns Conducted in Uganda Regarding Prevention of HPAI in Poultry in Three Districts of Kampala, Lira and Kanungu*. AHBL – Promoting strategies for prevention and control of HPAI. Food and Agriculture Organization of the United Nations, Rome
- MacAllister, C.G., Andrews, F.M., Deegan, E., Ruoff, W. and Olovson, S.-G. (1997) A scoring system for gastric ulcers in the horse. *Equine Veterinary Journal*, **29**, 430–433

- MacBride, D. (1772) *A Methodical Introduction to the Theory and Practice of Physic*. W. Strahan and T. Cadell, London/A. Kincaid, W. Creech and J. Balfour, Edinburgh. Reprinted by Gale ECCO, New York, 2010
- MacDiarmid, S.C. (1987) A theoretical basis for the use of a skin test for brucellosis surveillance in extensively managed cattle. *Revue Scientifique et Technique, Office International des Epizooties*, **6**, 1029–1035
- MacDiarmid, S.C. (1991) Risk analysis and the importation of animals. *Surveillance*, **18** (5), 8–11
- MacDiarmid, S.C. (1993) Risk analysis and the importation of animals and their products. *Revue Scientifique et Technique, Office International des Epizooties*, **12**, 1093–1107
- MacDiarmid, S.C. (1994) *The Risk of Introducing Exotic Diseases of Fish into New Zealand through the Importation of Ocean-caught Pacific Salmon from Canada*. Ministry of Agriculture and Fisheries, Wellington
- MacDiarmid, S.C. and Corrin, K.C. (1998) Case study: the risk of introducing rabies through the importation of dogs. In: *Owning the Future: Integrated Risk Management in Practice*. Ed. Elms, D., pp. 221–226. Centre for Advanced Engineering, University of Canterbury, Christchurch
- MacDiarmid, S.C. and Thompson, E.J. (1997) The potential risks to animal health from imported sheep and goat meat. *Revue Scientifique et Technique, Office International des Epizooties*, **16**, 45–56
- Macdonald, D.W. and Bacon, P.J. (1980) To control rabies: vaccinate foxes. *New Scientist*, **87**, 640–645
- Macdonald, D.W., Riordan, P. and Mathews, F. (2006) Biological hurdles to the control of TB in cattle: a test of two hypotheses concerning wildlife to explain the failure of control. *Biological Conservation*, **131**, 268–286
- MacEwen, E.G. (1990) Spontaneous tumors in dogs and cats: models for the study of cancer biology and treatment. *Cancer and Metastasis Reviews*, **9**, 125–136
- Machado, M.A. (1969) *AFTOSA: A Historical Survey of Foot-and-Mouth Disease and Inter-American Relations*. State University of New York Press, Albany
- Machin, D., Campbell, M.J., Fayers, P.M. and Pinol, A.P.Y. (1997) *Sample Size Tables for Clinical Studies*, 2nd edn. Blackwell Scientific Publications, Oxford
- Mackay, D.K.J. (1996) Differentiating infection from vaccination in foot-and-mouth disease. *Veterinary Quarterly*, **20**, Suppl. 2, S2–S5
- Mackintosh, C.G., Schollum, L.M., Harris, R.E., Blackmore, D.K., Willis, A.F. *et al.* (1980) Epidemiology of leptospirosis in dairy farm workers in the Manawatu. Part I: A cross-sectional serological survey and associated occupational factors. *New Zealand Veterinary Journal*, **28**, 245–250
- MacLachlan, N.J. and Dubovi, E.J. (Eds) (2011) *Fenner's Veterinary Virology*, 4th edn. Academic Press, London
- Maclure, M. (1985) Popperian refutation in epidemiology. *American Journal of Epidemiology*, **121**, 343–350
- Maclure, M. and Schneeweiss, S. (2001) Causation of bias: the episcopo. *Epidemiology*, **12**, 114–122
- Maclure, M. and Willett, W.C. (1987) Misinterpretation and misuse of the kappa statistic. *American Journal of Epidemiology*, **126**, 161–169
- MacMahon, B. (1972) Concepts of multiple factors. In: *Multiple Factors in the Causation of Environmentally Induced Disease*. Fogarty International Center Proceedings No. 12. Eds Lee, D.H.K. and Kotin, P., pp. 1–12. Academic Press, New York and London
- MacMahon, B. and Trichopoulos, D. (1996) *Epidemiology: Principles and Methods*, 2nd edn. Little, Brown, Boston
- Macquarrie, J. (1978) *The Humility of God*. SCM Press, London
- Macquarrie, J. (1998) Mascall and Thomism. *Tufts Review*. May 1998, Issue 2, Vol. 1, 1–13
- MacVean, D.W., Monlux, A.W., Anderson, P.S., Silberg, S.L. and Roszel, J.F. (1978) Frequency of canine and feline tumours in a defined population. *Veterinary Pathology*, **15**, 700–715
- Madden, L. and Campbell, C. (1987) Potential effects of air pollutants on epidemics of plant diseases. *Agriculture, Ecosystems and Environment*, **18**, 251–262
- Maddicott, J.R. (1997) Plague in seventh-century England. *Past and Present*, **156**, 7–54
- Madzimure, J., Zander, K.K., Dzama, K. and Chimonyo, M. (2012) Farmer perceptions of classical swine fever outbreak in communal pig production systems of South Africa. *African Journal of Agricultural Research*, **7**, 5819–5826
- MAF (1977) *Brucellosis: A Veterinarian's Guide to the Literature*. Ministry of Agriculture and Fisheries, Government Printer, Wellington
- MAF (2000) *Import Risk Analysis: Chicken Meat and Chicken Meat Products; Bernard Matthews Foods Ltd Turkey Meat Preparations from the United Kingdom. Revised Quantitative Risk Assessments of Chicken Meat from the United States: Reassessment of Heat Treatments for Inactivation of Newcastle Disease Virus in Chicken Meat*. Ministry of Agriculture and Forestry, Wellington
- MAFF (1965) *Animal Health: A Centenary 1865–1965*. Her Majesty's Stationery Office, London
- MAFF (1981) *Enzootic Bovine Leukosis: Advice for Herd Owners*. Ministry of Agriculture, Fisheries and Food, Leaflet 773. Her Majesty's Stationery Office, Edinburgh

- MAFF (1983) *Brucellosis: A History of the Disease and its Eradication from Cattle in Great Britain*. Ministry of Agriculture, Fisheries and Food, London
- MAFF (1986) *Foot-and-Mouth Disease. Ageing of Lesions*. Reference Book 400. Her Majesty's Stationery Office, London
- MAFF (1987) *Warble Fly*. Ministry of Agriculture, Fisheries and Food, Alnwick
- MAFF (1998) *Quarantine and Rabies – A Reappraisal*. MAFF Publications, London
- MAFF (2001) Foot and mouth disease 2001 – epidemiological forecasts. MAFF News Release 112/01 23 March. <http://www.defra.gov.uk/news/newsrel/2001/010323a.htm> (accessed 21 October 2001)
- Magidson, J. and Vermunt, J.K. (2004) Latent class models. In: *The Sage Handbook of Quantitative Methodology for the Social Sciences*, Ed Kaplan, D., pp. 175–198. Sage Publications, Thousand Oaks
- Mahendran, S. and Bell, N. (2015) Lameness in cattle 2. Managing claw health through appropriate trimming techniques. *In Practice*, **37**, 231–242
- Maher, C.R. and Lott, D.F. (1995) Definitions of territoriality used in the study of variation in vertebrate spacing systems. *Animal Behaviour*, **49**, 1581–1597
- Mähl, P., Cliquet, F., Guiot, A.-L., Niin, E., Fournials, E. *et al.* (2014) Twenty year experience of the oral rabies vaccine SAG2 in wildlife: a global review. *Veterinary Research*, **45**, 77; doi 10.1186/s13567-014-0077-8
- Mahy, B.W.J. (Ed.) (2005) *Foot-and-Mouth Disease Virus*. Springer-Verlag, Berlin and Heidelberg
- Main, D.C.J., Clegg, J., Spatz, A. and Green, L.E. (2000) Repeatability of a lameness scoring system for finishing pigs. *Veterinary Record*, **147**, 574–576
- Makita, K., Tsuji, A., Iki, Y., Kurosawa, A., Kadowaki, H. *et al.* (2015) Mental and physical distress of field veterinarians during and soon after the 2010 foot and mouth disease outbreak in Miyazaki, Japan. *Revue Scientifique et Technique, Office International des Epizooties*, **34**, 699–712
- Malik, R., Hunt, G.B. and Allan, G.S. (1992) Prevalence of mitral valve insufficiency in cavalier King Charles spaniels. *Veterinary Record*, **130**, 302–303
- Malirat, V., De Mello, P.A., Tiraboschi, B., Beck, E., Gomes, I. *et al.* (1994) Genetic variation of foot-and-mouth disease virus during persistent infection in cattle. *Virus Research*, **34**, 31–48
- Malthus, T. R. (1798) *An Essay on the Principle of Population*, As It Affects the Future Improvement of Society, with Remarks on the Speculations of Mr. Godwin, M. Condorcet, and Other Writers. J. Johnson, London
- Manchester, K. (1984) Tuberculosis and leprosy in antiquity: an interpretation. *Medical History*, **28**, 162–173
- Mangili, A. and Gendreau, M.A. (2005) Transmission of infectious diseases during commercial air travel. *The Lancet*, **365**, 989–996
- Manly, F.J. (2005) *Multivariate Statistical Methods: A Primer*. Chapman and Hall/CRC, Boca Raton
- Mann, C.J. (2003) Observational research methods. Research design II: cohort, cross sectional and case-control studies. *Emergency Medicine Journal*, **20**, 54–60
- Mannelli, A., Mandola, M.L., Pedri, P., Tripoli, M. and Nebbia, P. (2001) Use of spatial statistics and GIS to study the distribution of seropositivity for *Rickettsia conorii* in dogs in Piedmonte (Italy). In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Noordwijkerhout, 28–30 March 2001. Eds Menzies, F.D. and Reid, S.W.J., pp. 92–99
- Mansley, L.M., Donaldson, A.I., Thrusfield, M.V. and Honhold, N. (2011) Destructive tension: mathematics versus experience – the progress and control of the 2001 foot and mouth disease epidemic in Great Britain. *Revue Scientifique et Technique, Office International des Epizooties*, **30**, 483–498
- Mansley, L.M., Dunlop, P.J., Whiteside, S.M. and Smith, R.G.H. (2003) Early dissemination of foot-and-mouth disease virus through sheep marketing in February 2001. *Veterinary Record*, **153**, 45–50
- Mantel, N. (1963) Chi-square tests with one degree of freedom: extension of the Mantel-Haenszel procedure. *Journal of the American Statistical Association*, **58**, 690–700
- Mantel, N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220
- Mantel, N. and Haenszel, W. (1959) Statistical aspects of the analysis of data from retrospective studies of disease. *Journal of the National Cancer Institute*, **22**, 719–748
- Mantovani, A. and Zanetti, R. (1993) Giovanni Maria Lancisi: de bovilla peste and stamping out. *Historia Medicinae Veterinariae*, **18**, 97–110
- Marabelli, R. (2003) The role of official veterinary services in dealing with new social challenges: animal health and protection, food safety and the environment. *Revue Scientifique et Technique, Office International des Epizooties*, **22**, 363–371
- Marante, R.P., Torres, E.B. and Valdez, C.A. (2007) Body weight estimation of local born thoroughbred horses (*Equus caballus*) using external body measurements. *Phillipine Journal of Veterinary Medicine*, **44**, 114–122
- Marathe, M. and Vullikanti A.K.S. (2013) Computational epidemiology. *Communications of the Association for Computing Machinery*, **56**, 88–96
- Marchevsky, N., Held, J.R. and Garcia-Carrillio, C. (1989) Probability of introducing diseases because of false

- negative test results. *American Journal of Epidemiology*, **130**, 611–614
- Marcos-López, M., Gale, P., Oidtmann, B.C. and Peeler, E.J. (2010) Assessing the impact of climate change on disease emergence in freshwater fish in the United Kingdom. *Transboundary and Emerging Diseases*, **57**, 293–304
- Marechal, F., Ribeiro, N., Lafaye, M. and Güell, A. (2008) Satellite imaging and vector-borne diseases: an approach of the French National Space Agency (CNES). *Geospatial Health*, **3**, 1–5
- Marek, J. (1907) Multiple Nervenetzündung (Polyneuritis) bei Hühnern. *Deutsch Tierärztliche Wochenschrift*, **15**, 417–421 (in German)
- Marennikova, S.S., Maltseva, N.N., Korneeva, V.I., and Garanina, N.M. (1977) Outbreak of pox disease among *Carnivore (Felidae)* and *Edentata*. *Journal of Infectious Diseases*, **135**, 358–366
- Margawani, K.R. and Robertson, I.D. (1995) A survey of urban pet ownership in Bali. *Veterinary Record*, **137**, 486–488
- María, G.A., Villarroel, M., Chacón, G. and Gebresenbet, G. (2004) Scoring system for evaluating stress to cattle of commercial loading and unloading. *Veterinary Record*, **154**, 818–821
- Mariner, J.C. (1996) The world without rinderpest: outreach to marginalized communities. In: *The World Without Rinderpest*. FAO Animal Health and Production Paper No. 129, pp. 97–107. Food and Agriculture Organization of the United Nations, Rome
- Mariner, J.C. (2000) *Manual on Participatory Epidemiology: Methods for the Collection of Action-Oriented Epidemiological Intelligence*. FAO Animal Health Manual No. 10. Food and Agriculture Organization of the United Nations, Rome
- Mariner, J.C. and Roeder, P.L. (2003) Use of participatory epidemiology in studies of lineage 2 rinderpest virus in East Africa. *Veterinary Record*, **152**, 641–647
- Mariner, J.C., Araba, A. and Makungu, S. (2002) Consultancy on the dynamics of CBPP endemism and the development of effective control/eradication strategies for pastoral communities. Pan African Programme for the Control of Epizootics, Community-based Animal Health Participatory Epidemiology Unit, Final Data Collection Report
- Mariner, J.C., Jones, B.A., Hendrickx, S., Masry, I.E., Jobre, Y. *et al.* (2014) Experiences in participatory surveillance and community-based reporting systems for H5N1 highly pathogenic avian influenza: a case study approach. *EcoHealth*, **11**, 22–35
- Mariner, J.C., McDermott, J., Heesterbeek, J.A.P., Thomson, G. and Martin, S.W. (2006) A model of contagious bovine pleuropneumonia transmission dynamics in East Africa. *Preventive Veterinary Medicine*, **73**, 55–74
- Markwell, D.D. and Shortridge, K.F. (1982) Possible waterborne transmission and maintenance of influenza viruses in domestic ducks. *Environmental Microbiology*, **43**, 110–115
- Marques, F.F.C., Buckland, S.T., Goffin, D., Dixon, C.E., Borchers, D.L. *et al.* (2001) Estimating deer abundance from line transect surveys of dung: sika deer in southern Scotland. *Journal of Applied Ecology*, **38**, 349–365
- Marr, C.M., Jeffcott, L.B., Mair, T.S. and Muir, W.W. (Eds) (2003) Evidence-based medicine: clinical evidence and the evolution of equine evidence-based medicine. *Equine Veterinary Journal*, **35**, 331–424
- Marsh, W. (1999) The economics of animal health in farmed livestock at the herd level. *Revue Scientifique et Technique, Office International des Epizooties*, **18**, 357–366
- Marsh, W.E., Damrongwatanapokin, T., Lartz, K. and Morrison, R.B. (1991) The use of a geographical information system in an epidemiological study of pseudorabies (Aujeszky's disease) in Minnesota swine herds. *Preventive Veterinary Medicine*, **11**, 249–254
- Marsh, W.E., Nimis, G.A., Jourdan, T.J., Landucci, A.M., Udomprasert, P. *et al.* (1993) DairyCHAMP: a computerized health and management program for use in epidemiological studies. *Preventive Veterinary Medicine*, **16**, 55–56
- Martikainen, P., Bartley, M. and Lahelma, E. (2002) Psychosocial determinants of health in social epidemiology. *International Journal of Epidemiology*, **31**, 1091–1093
- Martin, B., Mainland, D.D. and Green, M.A. (1982) VIRUS: a computer program for herd health and productivity. *Veterinary Record*, **110**, 446–448
- Martin, C.M. (1964) Interactions of hydrocarbon carcinogens with viruses and nucleic acids in vivo and in vitro. *Progress in Experimental Tumor Research*, **5**, 134–156
- Martin, M., Mathias, E. and McCorkle, C.M. (2001) *Ethnoveterinary Medicine*. ITDG Publishing, London
- Martin, P.M., Cotard, M., Mialott, J.-P., Andre, F. and Raynaud, J.-P. (1984) Animal models for hormone-dependent human breast cancer. Relationship between steroid receptor profiles in canine and feline mammary tumours and survival rate. *Cancer Chemotherapy and Pharmacology*, **12**, 13–17
- Martin, R.M., Marin, F.L.B. and Rivera, M. (1977) Demographic study of the canine population living in towns of less than 8,500 inhabitants in the province of Valdivia (Chile). *Archivos de Medicina Veterinaria*, **9**, 29–35 (in Spanish; English abstract)

- Martin, S.W. (1977) The evaluation of tests. *Canadian Journal of Comparative Medicine*, **41**, 19–25
- Martin, S.W. (1988) The interpretation of laboratory results. In: Lessard, P.R. and Perry, B.D. (Eds) Investigation of disease outbreaks and impaired productivity. *Veterinary Clinics of North America: Food Animal Practice*, **4**, 61–78
- Martin, S.W. and Bonnett, B. (1987) Clinical epidemiology. *Canadian Veterinary Journal*, **28**, 318–325
- Martin, S.W., Eves, J.A., Dolan, L.A., Hammond, R.F., Griffin, J.M. *et al.* (1997) The association between bovine tuberculosis status of herds in the East Offaly Project Area, and the distance to badger setts, 1988–1993. *Preventive Veterinary Medicine*, **31**, 113–125
- Martin, S.W., Lissemore, K. and Kelton, D. (1990) Animal health monitoring systems in Canada. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Belfast, 4–6 April 1990. Ed. Thrusfield, M.V., pp. 62–69
- Martin, S.W., Meek, A.H. and Willeberg, P. (1987) *Veterinary Epidemiology: Principles and Methods*. Iowa State University Press, Ames
- Martin, W.B., Martin, B., Hag, D., and Lauder, I.M. (1966) Bovine ulcerative mammillitis caused by a herpes virus. *Veterinary Record*, **78**, 494–497
- Martínez-López, B., Perez, A.M., De La Tour, A. and Rodriguez, J.M.S.-V. (2008) Quantitative risk assessment of foot-and-mouth disease introduction into Spain via importation of live animals. *Preventive Veterinary Medicine*, **86**, 43–56
- Martínez-López, B., Perez, A.M. and Sánchez-Vizcaíno, J. M. (2009) Social network analysis. Review of general concepts and use in preventive veterinary medicine. *Transboundary and Emerging Diseases*, **56**, 109–120
- Marubini, E. and Valsecchi, M.G. (1995) *Analysing Survival Data from Clinical Trials and Observational Studies*. John Wiley, Chichester
- Maslakov, V.I. (1968) On the problem of the elimination of communicable diseases. *Zeichft für Mikrobiologie, Epidemiologie und Immunologie*, **45**, 118–122
- Mason, I.S. (1995) Pruritus. In: *Handbook of Small Animal Dermatology*. Eds Moriello, K.A. and Mason, I. S., pp. 65–73. Pergamon, Oxford
- Mason, J.W. (1971) A re-evaluation of the concept of 'non-specificity' in stress theory. *Journal of Psychiatric Research*, **8**, 323–333
- Mason, J.W., Wool, M.S., Mougey, E.H., Wherry, F.E., Collins, D.R. *et al.* (1968) Psychological vs nutritional factors in the effects of 'fasting' on hormonal balance. *Psychosomatic Medicine*, **30**, 554–555
- Mateu-de-Antonio, E.M., Martin, M. and Soler, M. (1993) Use of indirect enzyme-linked immunosorbent assay with hot saline solution extracts of a variant (M-) strain of *Brucella canis* for diagnosis of brucellosis in dogs. *American Journal of Veterinary Research*, **54**, 1043–1046
- Mathijssen, A. (Ed.) (1997) *The Origins of Veterinary Schools in Europe – A Comparative Review*. Veterinair Historisch Genootschap, Utrecht
- Matičić, D., Stejskal, M., Pećin, M., Kreszinger, M., Pirkić, B. *et al.* (2010) Correlation of pain assessment parameters in dogs with cranial cruciate surgery. *Veterinarski Arhiv*, **80**, 597–609
- Matter, H.C., Wandeler, A.I., Neuenschwander, B.E., Harischandra, L.P.A. and Meslin, F.X. (2000) Study of the dog population and the rabies control activities in the Mirigama areas of Sri Lanka. *Acta Tropica*, **75**, 95–108
- Matthews, J.N.S., Altman, D.G., Campbell, M.J. and Royston, P. (1990) Analysis of serial measurements in medical research. *British Medical Journal*, **300**, 230–236
- Matthews, J.R. (1995) *Quantification and the Quest for Medical Certainty*. Princeton University Press, Princeton
- Matthews, S.A. (1989) GIS methodology and spatial analysis: the potential epidemiological study. In: First National Conference and Exhibition, the Association for Geographical Information, Birmingham, UK. Conference papers, Vol. 6 (4), pp. 1–6
- Mattin, M.J., Solano-Gallego, L., Dhollander, S., Afonso, A. and Brodbelt, D.C. (2014) The frequency and distribution of canine leishmaniosis diagnosed by veterinary practitioners in Europe. *The Veterinary Journal*, **200**, 410–419
- Maturana, H.R. and Varela, F.J. (1998) *The Tree of Knowledge: The Biological Roots of Human Understanding*, revised edn. Shambhala Publications, Boston
- Maudlin, I., Holmes, P.H. and Miles, M.A. (Eds) (2004) *The Trypanosomiases*. CAB Publishing, Wallingford
- Maxie, M.G. (Ed.) (2007) *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*, 5th edn, Vol. 1. Elsevier Saunders, Edinburgh and New York
- Maxwell, J.A. (1992) Understanding and validity in qualitative research. *Harvard Educational Review*, **62** (3), 279–300
- May, H.G. and Tittsler, R.P. (1925) Tracheo-laryngitis in poultry. *Journal of the American Veterinary Medical Association*, **67**, 229–231
- May, R.M. and Anderson, R.M. (1979) Population biology of infectious diseases. *Nature*, **280**, 455–461
- May, R.M. and Lloyd, A.L. (2001) Infection dynamics on scale-free networks. *Physical Review E*, **64**, 066112-1–066112-4

- May, R.M., Gupta, S. and McLean, A.R. (2001) Infectious disease dynamics: what characterizes a successful invader? *Philosophical Transactions of the Royal Society of London, Series B*, **356**, 901–910
- Mayer, S.J. (1992) Stratospheric ozone depletion and animal health. *Veterinary Record*, **131**, 120–122
- Mayle, B.A., Peace, A.J. and Gill, R.M.A. (1999) *How Many Deer? A Field Guide to Estimating Deer Population Size*. Forestry Commission, Edinburgh
- McCallum, H. (2008) Landscape structure, disturbance, and disease dynamics. In: *Infectious Disease Ecology*. Eds Ostfield, R.S., Keesing, F. and Eviner, V.T., pp. 100–122. Princeton University Press, Princeton and Oxford
- McCallum, H. (2012) Disease and the dynamics of extinction. *Philosophical Transactions of the Royal Society, Series B*, **367**, 2828–2839
- McCallum, H.I. and Dobson, A.P. (1995) Detecting disease and parasite threats to endangered species and ecosystems. *Trends in Ecology and Evolution*, **10**, 190–194
- McCallum, H., Jones, M., Hawkins, C., Hamede, R., Lachlisch, S. *et al.* (2009) Transmission dynamics of Tasmanian devil facial tumor disease may lead to disease induced extinction. *Ecology*, **90**, 3379–3392
- McCallum, H.I., Kuris, A., Harvell, C.D., Lafferty, K.D., Smith, G.W. *et al.* (2004). Does terrestrial epidemiology apply to marine systems? *Trends in Ecology and Evolution*, **19**, 585–591
- McCallum, H.I., Roshier, D.A., Tracey, J.P., Joseph, L. and Heinsohn, R. (2008) Will Wallace's Line save Australia from avian influenza? *Ecology and Society*, **13** (2), 41. <http://www.ecologyandsociety.org/vol13/iss2/art41/> (accessed 22 August 2016)
- McCallum, H.J.F. (1985) Stress and postcapture myopathy in red deer. In: *Biology of Deer Production*. Proceedings of an International Conference, Dunedin, New Zealand, 13–18 February 1983. Bulletin 22. Eds Fennessy, P.F. and Drew, K.R., pp. 65–72. The Royal Society of New Zealand, Wellington
- McCandless, L.C. (2012) Meta-analysis of observational studies with unmeasured confounders. *The International Journal of Biostatistics*, **8**, Issue 2, Article 5; doi 10.2202/1557-4679.1350
- McCarthy, H.E., Proudman, C.J. and French, N.P. (2001) Epidemiology of equine grass sickness: a literature review (1909–1999). *Veterinary Record*, **149**, 293–300
- McCarthy, N. and Giesecke, J. (1999) Case-case comparisons to study causation of common infectious diseases. *International Journal of Epidemiology*, **28**, 764–768
- McCaughey, W.J. (1992) Residues: sample selection. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Edinburgh, 1–3 April 1992. Ed. Thrusfield, M.V., pp. 13–23
- McCauley, E.H. (1985) Hog cholera in Honduras. Evaluation of the economic and technical aspects of the impact on production and consequences of vaccination programs; and report of 1985 owner survey results, February–April 1985. College of Veterinary Medicine, Department of Large Animal Clinical Sciences, University of Minnesota, St Paul
- McCauley, E.H., Majid, A.A., Tayeb, A. and Bushara, H.O. (1983a) Clinical diagnosis of schistosomiasis in Sudanese cattle. *Tropical Animal Health and Production*, **15**, 129–136
- McCauley, E.H., Tayeb, A. and Majid, A.A. (1983b) Owner survey of schistosomiasis mortality in Sudanese cattle. *Tropical Animal Health and Production*, **15**, 227–233
- McChesney, A.E., England, J.J., and Rich, L.J. (1973) Adenoviral infection in foals. *Journal of the American Veterinary Medical Association*, **162**, 545–549
- McClintock, P.M. (1988) *The Economic Contribution of the British Equine Industry*. British Horse Society, Stoneleigh
- McClure, J.J. (1988) Equine disease association studies: a clinician's perspective. *Animal Genetics*, **19**, 409–415
- McCluskey, B.J., Mumford, E.L., Salman, M.D. and Traub-Dargatz, J.J. (2002) Use of sentinel herds to study the epidemiology of vesicular stomatitis virus in the State of Colorado. In: *The Domestic Animal/Wildlife Interface: Issues for Disease Control, Conservation, Sustainable Food Production, and Emerging Diseases*. *Annals of the New York Academy of Sciences*, Vol. 969. Eds Gibbs, E.P.J. and Bokma, B.H., pp. 205–209. The New York Academy of Sciences, New York
- McConway, K.J., Jones, M.C. and Taylor, P.C. (2009) *Statistical Modelling using GENSTAT®*. John Wiley and Sons, Chichester
- McCorkle, C. M., Mathias, E. and Van Veen, T.W.S. (1996) *Ethnoveterinary Research and Development*. ITDG Publishing, London
- McCormick, J.B. (1998) Epidemiology of emerging/re-emerging antimicrobial-resistant bacterial pathogens. *Current Opinion in Microbiology*, **1**, 125–129
- McCracken, J.A., Pretty, J.N. and Conway, G.R. (1988) *An Introduction to Rapid Rural Appraisal for Agricultural Development*. International Institute for Environment and Development, London
- McCrea, C.T. and Head, K.W. (1978) Sheep tumours in north-east Yorkshire. I. Prevalence on seven moorland farms. *British Veterinary Journal*, **134**, 454–461
- McCrea, C.T. and Head, K.W. (1981) Sheep tumours in north-east Yorkshire. II. Experimental production of tumours. *British Veterinary Journal*, **137**, 21–30

- McDermott, J.J. and Schukken, Y.H. (1994) A review of methods to adjust for cluster effects in explanatory epidemiological studies of animal populations. *Preventive Veterinary Medicine*, **18**, 155–174
- McDougall, S., Heuer, C., Morton, J. and Brownlie, T. (2014) Use of herd management programmes to improve the reproductive performance of dairy cattle. *Animal*, **8**, Suppl. s1, 199–210
- McElvaine, M.D., McDowell, R.M., Fite, R.W. and Miller, L. (1993) An assessment of the risk of foreign animal disease introduction into the United States of America through garbage from Alaskan cruise ships. *Revue Scientifique et Technique, Office International des Epizooties*, **12**, 1065–1174
- McFerran, J.B., McNulty, M.S., McKillip, E., Conner, T.J., McCracken, R.M. *et al.* (1980) Isolation and serological studies with infectious bursal disease viruses from fowl, turkey and duck: demonstration of a second serotype. *Avian Pathology*, **9**, 395–404
- McGee, S. (2002) Simplifying likelihood ratios. *Journal of General Internal Medicine*, **17**, 647–650
- McGough, J.J. and Faraone, S.V. (2009) Estimating the size of treatment effects: moving beyond *P* values. *Psychiatry (Edgemont)*, **6** (10), 21–29
- McIlroy, S.G., Goodall, E.A., Rainey, J. and McMurray, C. H. (1988) A computerised management and disease information retrieval system for profitable broiler production. *Agricultural Systems*, **27**, 11–22
- McInerney, J.P. (1991) Economic aspects of the animal welfare issue. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, London, 17–19 April 1991. Ed. Thrusfield, M.V., pp. 83–91
- McInerney, J.P. (1996) Old economics for new problems – livestock disease: Presidential Address. *Journal of Agricultural Economics*, **47**, 295–314
- McInerney, J.P., Howe, K.S. and Schepers, J.A. (1992) A framework for the economic analysis of disease in farm livestock. *Preventive Veterinary Medicine*, **13**, 137–154
- McIntyre, K.M., Setzkorn, C., Baylis, M., Waret-Szkuta, A., Caminade, C. *et al.* (2010) Impact of climate change on human and animal health (Letter). *Veterinary Record*, **167**, 586
- McIntyre, K.M., Setzkorn, C., Wardeh, M., Hepworth, P. J., Radford, A.D. *et al.* (2013) What, where and weather? Integrating open-source taxonomic, spatial and climatological information into a comprehensive database of livestock infections. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Madrid, 20–22 March 2013. Eds Verheyen, K.L.P., Fourichon, C. and the SVEPM Executive Committee, pp. 143–159
- McKay, B., McCallum, S. and Morris, R.S. (1988) An expert system program for diagnosing reproductive problems in seasonal dairy herds. *Acta Veterinaria Scandinavica*, Suppl. 84, 480–482
- McKiernan, B.C., Smith, A.R. and Kissel, M. (1984) Bacterial isolates from the lower trachea of clinically healthy dogs. *Journal of the American Animal Hospital Association*, **20**, 139–142
- McKinnell, R.G. and Ellis, V.L. (1972) Epidemiology of the frog renal tumour and the significance of tumour nuclear transplantation studies to a viral aetiology of the tumour – a review. In: *Oncogenesis and Herpesviruses*. Proceedings of a symposium held at Christ's College, Cambridge, 20–25 June 1971. IARC Scientific Publications No. 2. Eds Biggs, P.M., de-The, G. and Payne, L.N., pp. 183–197. International Agency for Research on Cancer, Lyon
- McKnight, P.E., McKnight, K.M., Sidani, S. and Figueredo, A.J. (2007) *Missing Data: A Gentle Introduction*. Guilford Press, New York and London
- McLaren, L. and Hawe, P. (2005) Ecological perspectives in health research. *Journal of Epidemiology & Community Health*, **59**, 6–14
- McLauchlan, J.D. and Henderson, W.M. (1947) The occurrence of foot-and-mouth disease in the hedgehog under natural conditions. *Journal of Hygiene, Cambridge*, **45**, 474–479
- McLeod, K.S. (2000) Our sense of Snow: the myth of John Snow in medical geography. *Social Science & Medicine*, **50**, 923–935
- McManus, C., Paim, T.P., de Melo, C.B., Brasil, B.S.A.F. and Paiva, S.R. (2014) Selection methods for resistance to and tolerance of helminths in livestock. *Parasite*, **21**, 56; doi 10.1051/parasite/2014055
- McMichael, A.J., Campbell-Lendrum, D.H., Corvalán, C. F., Ebi, K.L., Githeko, A.K. *et al.* (Eds) (2003) *Climate Change and Human Health: Risks and Responses*. World Health Organization, Geneva
- McMillan, F.D. (2000) Quality of life in animals. *Journal of the American Veterinary Medical Association*, **216**, 1904–1910
- McNally, R.J.Q. and Eden, T.O.B. (2004) An infectious aetiology for childhood acute leukaemia: a review of the evidence. *British Journal of Haematology*, **127**, 243–263
- McNamara, T. (2007) The role of zoos in biosurveillance. *International Zoo Yearbook*, **41**, 12–15
- McNamee, R. (2003) Confounding and confounders. *Occupational and Environmental Medicine*, **60**, 227–234
- McNeil, P.H., Rhodes, A.P. and Willis, B.H. (1984) *A Flock Health and Productivity Service for New Zealand*. Report of a trial involving ten farms in the Dannevirke area, November 1979–June 1983. Veterinary Services Council, New Zealand
- McNeill, W.H. (1977) *Plagues and Peoples*. Basil Blackwell, Oxford

- McNurlin, B.C., Sprague, R.H. and Bui, T. (2009) *Information Systems Management in Practice*, 8th edn. Pearson Education, New Jersey
- McNutt, S.H., Murray, C., and Purwin, P. (1929) Swine pox. *Journal of the American Veterinary Medical Association*, **74**, 752–761
- McPherson, K., Vessey, M.P., Neil, A., Doll, R., Jones, L. *et al.* (1987) Early oral contraceptive use and breast cancer: results of another case-control study. *British Journal of Cancer*, **56**, 653–660
- McTaggart, H.S., Laing, A.H., Imlah, P., Head, K.W. and Brownlie, S.E. (1979) The genetics of hereditary lymphosarcoma in pigs. *Veterinary Record*, **105**, 36
- McVey, D.S. and Czuprynski, C. (2013) Pathogenicity and virulence. In: *Veterinary Microbiology*, 3rd edn. Eds McVey, D.S., Kennedy, M. and Chengappa, M.M., pp. 3–6. Wiley-Blackwell, Ames
- Mead, G.C. (2000) Prospects for ‘competitive exclusion’ treatment to control Salmonellas and other foodborne pathogens in poultry. *The Veterinary Journal*, **159**, 111–123
- Meagher, M. (1989) Evaluation of boundary control for bison of Yellowstone National Park. *Wildlife Society Bulletin*, **17**, 15–19
- Meah, M.N. and Lewis, G.A. (2000) *Veterinary Surveillance in England and Wales – A Review*. Ministry of Agriculture, Fisheries and Food Publications, London
- Mebus, C.A., House, C., Gonzalvo, F.R., Pineda, J.M., Tapiador, J. *et al.* (1993) Survival of foot-and-mouth disease, African swine fever, and hog cholera viruses in Spanish serrano cured hams and Iberian cured hams, shoulders and loins, *Food Microbiology*, **10**, 133–143
- Mech, L.D. (2012) Is science in danger of sanctifying the wolf? *Biological Conservation*, **150**, 143–149
- MECIDS (2015) *Middle East Consortium on Infectious Disease Surveillance*. <http://www.mecidsnetwork.org/> (accessed 14 August 2015)
- Medawar, P.B. (1969) *Induction and Intuition in Scientific Thought*. Methuen, London
- Medawar, P.B. (1979) *Advice to a Young Scientist*. Basic Books, New York
- Medley, G.F. (2003) The design of test and clearance programmes. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Warwick, 31 March–2 April 2003. Eds Reid, S.W.J. and Menzies, F.D., pp. 60–71
- Medlock, J.M. and Leach, S.A. (2015) Effect of climate change on vector-borne disease risk in the UK. *The Lancet Infectious Diseases*, **15**, 721–730
- Medlock, J.M. and Vaux, A.G.C. (2015) Impacts of the creation, expansion and management of English wetlands on mosquito presence and abundance – developing strategies for future disease mitigation. *Parasites & Vectors*, **8**, 142; doi 10.1186/s13071-015-0751-3
- Medlock, J.M., Avenell, D., Barrass, I. and Leach, S. (2006) Analysis of the potential for survival and seasonal activity of *Aedes albopictus* (Diptera: Culicidae) in the United Kingdom. *Journal of Vector Ecology*, **31**, 292–304
- Mee, J.F. (2007) The role of the veterinarian in bovine fertility management on modern dairy farms. *Theriogenology*, **68S**, S257–S265
- Meeusen, E.N.T. and Maddox, J.F. (1999) Progress and expectations for helminth vaccines. *Advances in Veterinary Medicine*, **41**, 241–256
- Mehta, C.R. and Patel, N.R. (1983) A network algorithm for performing Fisher’s exact test in $r \times c$ contingency tables. *Journal of the American Statistical Association*, **78**, 327–434
- Mehta, C.R., Patel, N.R. and Gray, R. (1985) Computing an exact confidence interval for the common odds ratio in several 2×2 contingency tables. *Journal of the American Statistical Association*, **80**, 969–973
- Meinert, C.L. (1989) Meta-analysis: science or religion? *Controlled Clinical Trials*, **10**, 257S–263S
- Meinert, C.L. (2012) *Clinical Trials, Design, Conduct, and Analysis*, 2nd edn. Oxford University Press, New York
- Meischke, H.R.C., Ramsey, W.R. and Shaw, F.D. (1974) The effect of horns on bruising in cattle. *Australian Veterinary Journal*, **50**, 432–434
- Meisinger, J.L., Pendell, D.L., Morris, D.L., Belk, K.E. and Smith, G.C. (2008) Swine traceability systems in selected countries outside of North America. *The Professional Animal Scientist*, **24**, 295–301
- Mellaart, J.C. (1967) *Catal Huyuk*. McGraw-Hill, New York
- Mellor, D.J. and Bayvel, A.C.D. (Eds) (2014) Animal welfare: focusing on the future. *Revue Scientifique et Technique, Office International des Epizooties*, **33**, 1–358
- Mellor, D.J., Innocent, G.T., McEwen, S.A., Reilly, W.J., Taylor, D.J. *et al.*, (2004) A semi-quantitative risk assessment model for contamination of beef carcasses with *Escherichia coli* O157. In: *Food Safety Assurance and Veterinary Public Health. Volume 2. Food Safety Assurance and Veterinary Public Health*. Eds Smulders, F.J.M. and Collins, J.D., pp. 386–388. Wageningen Academic, Wageningen
- Mellor, D.J., Love, S., Gettinby, G. and Reid, S.W.J. (1999) Demographic characteristics of the equine population of northern Britain. *Veterinary Record*, **145**, 299–304
- Mellor, P.S. and Hamblin, C. (2004) African horse sickness. *Veterinary Research*, **35**, 445–466
- Mellor, P.S., Jennings, D.M., Wilkinson, P.J. and Boorman, J.P.T. (1985) *Culicoides imicola*: a

- bluetongue virus vector in Spain and Portugal. *Veterinary Record*, **116**, 589–590
- Melzack, R. (1983) *Pain Measurement and Assessment*. Raven Press, New York
- Melzack, R. (1987) The short-form McGill pain questionnaire. *Pain*, **30**, 191–197
- Mengeling, W.L. (2006) Porcine parvovirus. In: *Diseases of Swine*, 9th edn. Eds Straw, B.E., Zimmerman, J.J., D'Allaire, S. and Taylor, D.J., pp. 373–385. Blackwell Publishing, Ames
- Menzies, F.D., Goodall, E.A., McLoughlin, M.F. and Wheatley, S. (1992) Computerised monitoring of disease and production in farmed Atlantic salmon (*Salmo salar*). In: Society for Veterinary Epidemiology and Preventive Medicine, Denary Proceedings, Edinburgh, 1–3 April 1992. Ed. Thrusfield, M.V., pp. 138–147
- Mepharm, B. (2001) Foot and mouth disease and British agriculture: ethics in a crisis. *Journal of Agricultural and Environmental Ethics*, **14**, 339–347
- Merle, R., Hajek, P., Käsbohrer, A., Hegger-Gravenhorst, C., Mollenhauer, Y. *et al.* (2012) Monitoring of antibiotic consumption in livestock: a German feasibility study. *Preventive Veterinary Medicine*, **104**, 34–43
- Merlo, D.F., Rossi, L., Pellegrino, C., Ceppi, M., Cardellino, U. *et al.* (2008) Cancer incidence in pet dogs: findings of the Animal Tumor Registry of Genoa, Italy. *Journal of Veterinary Internal Medicine*, **22**, 976–984
- Merriam, C.H. (1893) The Geographic Distribution of Life in North America. *Smithsonian Institute Annual Report*, pp. 365–415
- Merry, D.L. and Kolar, J.R. (1984) A comparative study of four rabies vaccines. *Veterinary Medicine and Small Animal Clinician*, **79**, 661–664
- Meselson, M., Guillemin, J., Hugh-Jones, M., Langmuir, A., Popova, I. *et al.* (1994) The Sverdlovsk anthrax outbreak of 1979. *Science*, **266**, 1202–1208
- Messam, L.L. McV., Branscum, A.J., Collins, M.T. and Gardner, I.A. (2008) Frequentist and Bayesian approaches to prevalence estimation using examples from Johne's disease. *Animal Health Research Reviews*, **9**, 1–23
- Messenger, A.M., Barnes, A.N. and Gray, G.C. (2014) Reverse zoonotic disease transmission (zooanthroponosis): a systematic review of seldom-documented human biological threats to animals. *PLoS ONE*, **9**, 2, e89055; doi 10.1371/journal.pone.0089055
- Metcalf, C.J.E., Hampson, K., Tatem, A.J., Grenfell, B.T. and Bjørnstad, O.N. (2013) Persistence in epidemic metapopulations: quantifying the rescue effects for measles, mumps, rubella and whooping cough. *PLoS ONE*, **8**, 9, e74696; doi 10.1371/journal.pone.0074696
- Mettenleiter, T.C. (2005) *Qualitative Risk Assessment of the Introduction of Avian Influenza virus to Germany: Outbreaks Caused by the Highly Pathogenic Avian Influenza (HPAI) Virus in Turkey, Romania and Russia as well as Influenza H5 Antibody Detection in Greece*. Friedrich Loeffler Institute, Federal Research Institute for Animal Health. Insel Riems. http://www.germanembassy.org.au/en/economy/Risikobewertung_Influenza_19-10-05-en.pdf (accessed 24 January 2006)
- Meuten, D.J. (Ed.) (2002) *Tumors in Domestic Animals*, 4th edn. Iowa State Press, Ames
- Mi, E., Mi, E. and Jeggo, M. (2016) Where to now for One Health and Ecohealth? *EcoHealth*, **13**, 12–17
- Michel, J.-F., Dray, S., de La Rocque, S., Desquesnes, M., Solano, P. *et al.* (2002) Modelling bovine trypanosomosis spatial distribution by GIS in an agro-pastoral zone of Burkina Faso. *Preventive Veterinary Medicine*, **56**, 5–18
- Michell, A.R. (Ed.) (1993) *The Advancement of Veterinary Science. The Bicentenary Symposium Series. Volume 1: Veterinary Medicine Beyond 2000*. CAB International, Wallingford
- Michell, A.R. (1999) Longevity of British breeds of dog and its relationships with sex, size, cardiovascular variables and disease. *Veterinary Record*, **145**, 625–629
- Miettinen, O.S. (1970) Matching and design efficiency in retrospective studies. *American Journal of Epidemiology*, **91**, 111–118
- Miettinen, O.S. (1972) Components of the crude risk ratio. *American Journal of Epidemiology*, **96**, 168–172
- Miettinen, O.S. (1974) Confounding and effect-modification. *American Journal of Epidemiology*, **100**, 350–353
- Miettinen, O.S. (1976) Estimability and estimation in case-referent studies. *American Journal of Epidemiology*, **103**, 226–235
- Mikkelsen, B. (2005) *Methods for Development Work and Research*, 2nd edn. Sage Publications India Pvt, New Delhi
- Miles, J.A.R. (1964) Some ecological aspects of the problem of arthropod-borne animal viruses in the Western Pacific and South-East Asia regions. *Bulletin of the World Health Organization*, **30**, 197–210
- Milgrom, J. (1992) Food and faith: the ethical foundations of the Biblical diet laws. *Bible Review*, December 1992, 5–10
- Mill, J.S. (1868) *A System of Logic, Ratiocinative and Inductive: Being a Connected View of the Principles of Evidence and the Methods of Scientific Investigation*, 7th edn. Longmans, Green, Reader and Dyer, London
- Millar, R. and Francis, J. (1974) The relation of clinical and bacteriological findings to fertility in thoroughbred mares. *Australian Veterinary Journal*, **50**, 351–355

- Miller, D.R., Rosenberg, L., Kaufman, D.W., Stolley, P., Washauer, M.F. *et al.* (1989) Breast cancer before age 45 and oral contraceptives. *American Journal of Epidemiology*, **129**, 269–280
- Miller, N.J. (1955) Infectious necrotic rhinotracheitis of cattle. *Journal of the American Veterinary Medical Association*, **126**, 463–467
- Miller, P.G. and Koch, G. (2013) Newcastle disease. In: *Diseases of Poultry*, 13th edn. Ed. Swayne, D.E., pp. 89–107. Wiley-Blackwell, Ames
- Miller, R.S., Farnsworth, M.L. and Malmberg, J.L. (2013) Diseases at the livestock-wildlife interface: status, challenges and opportunities in the United States. *Preventive Veterinary Medicine*, **110**, 119–132
- Miller, W.C. (1998) Bias in discrepant analysis: when two wrongs don't make a right. *Journal of Clinical Epidemiology*, **51**, 219–231
- Miller, W.M. (1976) A state-transition model of epidemic foot and mouth disease. In: *New Techniques in Veterinary Epidemiology and Economics*. Proceedings of a Symposium, Reading, 12–17 July 1976. Eds Ellis, P.R., Shaw, A.P.M. and Stephens, A.J., pp. 56–60
- Mills, D., Kunoli, H., Keen, N.T. and Mayama, S. (Eds) (1995) *Molecular Aspects of Pathogenicity and Resistance*. APS Press, St Paul
- Milne, A. (1950) The ecology of the sheep tick, *Ixodes ricinus* (L.). Spatial distribution. *Parasitology*, **41**, 189–207
- Milton, G. (1999) *Nathaniel's Nutmeg: How One Man's Courage Changed the Course of History*. Hodder and Stoughton, London
- Mims, C.A., Nash, A. and Stephen, J. (2000) *Mims' Pathogenesis of Infectious Disease*, 5th edn. Academic Press, London
- Mintiens, K., Balavarca, Y., Verloo, D. and Aerts, M. (2007) Sample sizes for declaring disease freedom with uncertain diagnostic test performance and prevalence. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Espoo, 28–30 March 2007. Eds Mellor, D.J. and Newton, J.R., pp. 29–37
- Misdorp, W. (1987) The impact of pathology on the study and treatment of cancer. In: *Veterinary Cancer Medicine*, 2nd edn. Eds Theilen, G.H. and Madewell, B. R., pp. 53–70. Lea and Febiger, Philadelphia
- Misdorp, W. (1991) Progestagens and mammary tumours in dogs and cats. *Acta Endocrinologica*, **125**, Suppl. 1, 27–31
- Mishan, E.J. and Quah, E. (2007) *Cost-Benefit Analysis*, 5th edn. Routledge, Abingdon
- MISSINGDATA (2015) Missingdata.org.uk. <http://www.missingdata.org.uk/> (accessed 14 April 2015)
- Mitchell, A., Bourn, D., Mawdsley, J., Wint, W., Clifton-Handley, R. *et al.* (2005) Characteristics of cattle movement in Britain – an analysis of records from the Cattle Tracing System. *Animal Science*, **80**, 265–273
- Mittleman, M.A., Maclure, M., Tofler, G.H., Sherwood, J. B., Goldberg, R.J. *et al.* (1993) Triggering of acute myocardial infarction by heavy physical exertion. *The New England Journal of Medicine*, **329**, 1677–1683
- Miura, Y., Hayashi, S., Ishihara, T., Inaba, Y., Omori, T. *et al.* (1974) Neutralizing antibody against Akabane virus in precolostral sera from calves with congenital arthrogryposis – hydranencephaly syndrome. Brief report. *Archiv für die Gesamte Virusforschung*, **46**, 377–380
- Miura, Y., Kubo, M., Goto, Y. and Kono, K. (1990) Hydranencephaly-cerebellar hypoplasia in a newborn calf after infection of its dam with Chuzon virus. *Japanese Journal of Veterinary Science*, **52**, 689–694
- Moberg, G.P. (Ed.) (1985) *Animal Stress*. American Physiological Society, Bethesda
- Moberg, G.P. and Mench, J.A. (2000) *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare*. CABI Publishing, Wallingford
- Mochabo, K.O.M., Kitale, P.M., Gathura, P.B., Ogara, W.O., Catley, A. *et al.*, (2005) Community perceptions of important camel diseases in Lapur Division of Turkana District, Kenya. *Tropical Animal Health and Production*, **37**, 187–204
- Mockford, K. (2004) Web services architecture. *BT Technology Journal*, **22**, 19–26
- Moe, L., Bredal, W.P. and Glatte, E. (2001) *Hundetelling I Norge (Census of dogs in Norway)*. Norwegian School of Veterinary Science, Oslo (in Norwegian and English)
- Moennig, V., Floegel-Niesmann, G. and Greiser-Wilke, I. (2003) Clinical signs and epidemiology of classical swine fever: a review of new knowledge. *Veterinary Journal*, **165**, 11–20
- Mohammed, H.O., Divers, T.J., Summers, B.A. and de Lahunta, A. (2007) Vitamin E deficiency and risk of motor neuron disease. *Acta Veterinaria Scandinavica*, **49**, 17; doi 10.1186/1751-0147-49-17
- Moher, D., Hopewell, S., Schulz, K.F., Montori, V., Gotzsche, P.C. *et al.* (2010) CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *Journal of Clinical Epidemiology*, **63**, e1–37
- Moher, D., Jones, A., Lepage, L. and CONSORT Group (Consolidated Standards for Reporting of Trials) (2001) Use of the CONSORT statement and quality of reports of randomized trials: a comparative before-and-after evaluation. *Journal of the American Medical Association*, **285**, 1992–1995
- Moher, D., Liberati, A., Tetzlaff, J. and Altman, D.G. (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Journal of Clinical Epidemiology*, **62**, 1006–1012

- Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A. *et al.* (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews*, **4**, 1; doi 10.1186/2046-4053-4-1
- Mollison, D. (1984) Simplifying epidemic models. *Nature*, **310**, 224–225
- Molony, V. (1997) Comments on Anand and Craig (Letter). *Pain*, **70**, 293
- Molony, V., Kent, J.E. and McKendrick, I.M. (2002) Validation of a method for assessment of an acute pain in lambs. *Applied Animal Behaviour Science*, **76**, 215–238
- Monaghan, M.L.M. and Hannan, J. (1983) Abattoir survey of bovine kidney disease. *Veterinary Record*, **113**, 55–57
- Money, N.P. (2007) *The Triumph of the Fungi: A Rotten History*. Oxford University Press, New York
- Mongeau, J.D., Truscott, R.B., Ferguson, A.E. and Connell, M.C. (1959) Virus hepatitis in turkeys. *Avian Diseases*, **4**, 388–396
- Montgomery, R.D., Stein, G., Stotts, V.D. and Settle, F.H. (1979) The 1978 epornitic of avian cholera on the Chesapeake Bay. *Avian Diseases*, **23**, 966–978
- Montgomery, R.E. (1917) On a tick-borne gastro-enteritis of sheep and goats occurring in British East Africa. *Journal of Comparative Pathology and Therapeutics*, **30**, 28–57
- Montgomery, R.E. (1921) On a form of swine fever occurring in British East Africa (Kenya Colony). *Journal of Comparative Pathology and Therapeutics*, **34**, 159–191, 247–262
- Moolgavkar, S.H. (1986) Carcinogenesis modelling: from molecular biology to epidemiology. *Annual Review of Public Health*, **7**, 151–169
- Moore, B. (1957) Observations pointing to the conjunctiva as a portal of entry in salmonella infections of guinea-pigs. *Journal of Hygiene, Cambridge*, **55**, 414–433
- Moore, G.E., Frana, T.S., Guptill, L.F., Ward, M.P., Lewis, H.B. *et al.* (2006) Post-marketing surveillance for dog and cat vaccines: new resources in changing times. *Journal of the American Veterinary Medical Association*, **227**, 1066–1069
- Moorhouse, P.D. and Hugh-Jones, M.E. (1981) Serum banks. *Veterinary Bulletin*, **51**, 277–290
- Moorhouse, P.D. and Hugh-Jones, M.E. (1983) The results of retesting bovine sera stored at –18oC for twenty years, with lessons for future serum banks. In: *Proceedings of the Third International Symposium on Veterinary Epidemiology and Economics*. Arlington, Virginia, 6–10 September 1982, pp. 44–51. Veterinary Medicine Publishing Company, Edwardsville
- Morabia, A. (2013) Hume, Mill, Hill, and the sui generis epidemiological approach to causal inference. *American Journal of Epidemiology*, **178**, 1526–1532
- Morales, M.A., Varas, C. and Ibarra, L. (2009) Demographic characterization of the dog population in Viña del Mar, Chile. *Archivos de Medicina Veterinaria*, **41**, 89–95 (in Spanish; English abstract)
- Moran, P.A.P. (1953) The statistical analysis of the Canadian lynx cycle. II. Synchronization and meteorology. *Australian Journal of Zoology*, **1**, 291–298
- Morand, S. (1996) Biodiversity of parasites in relation to their life-cycles. In: *Aspects of the Genesis and Maintenance of Biological Diversity*. Eds Hochberg, M. E., Clobert, J. and Barbault, R., pp. 243–260. Oxford University Press, Oxford
- Morand, S., Owers, K.A., Waret-Szkuta, A., McIntyre, K. M. and Baylis, M. (2013) Climate variability and outbreaks of infectious diseases in Europe. *Scientific Reports*, **3**, 1774; doi 10.1038/srep01774
- Morand, S., Robert, F., and Connors, V.A. (1995) Complexity in parasite life cycles: population biology of cestodes in fish. *Journal of Animal Ecology*, **64**, 256–264
- Morant, S.V. (1984) Factors affecting reproductive performance including the importance and effect of problem cows. In: *Dairy Cow Fertility*. Proceedings of a joint British Veterinary Association and British Society of Animal Production conference, Bristol University, 28–28 June 1984. Eds Eddy, R.G. and Ducker, M.J., pp. 15–23. British Veterinary Association, London
- More, S.J., Aznar, I., Bailey, D.C., Larkin, J.F., Leadon, D. P., Lenihan, P. *et al.* (2008) An outbreak of equine infectious anemia (EIA) in Ireland during 2006: the investigation methodology, the initial source of infection, diagnosis and clinical presentation, the modes of transmission and spread in the Meath cluster. *Equine Veterinary Journal*, **40**, 706–708
- Moreno, S.G., Sutton, A.G., Turner, E.H., Abrams, K.R., Cooper, N.J. *et al.* (2009) Novel methods to deal with publication biases: secondary analysis of antidepressant trials in the FDA trial registry database and related journal publications. *British Medical Journal*, **339**, b2981; doi 10.1136/bmj.b2981
- Morens, D.M. and Fauci, A.S. (2013) Emerging infectious diseases: threats to human health and global stability. *PLoS Pathogens*, **9**, 7, e1003467; doi 10.1371/journal.ppat.1003467
- Moresco, A. and Agnew, D.W. (2013) Reproductive health surveillance in zoo and wildlife medicine. *Journal of Zoo and Wildlife Medicine*, **44**, S26–S33
- Morgan, J.P., Wind, A. and Davidson, A.P. (2000) *Hereditary Bone and Joint Diseases in the Dog*. Schlütersche GmbH and Co, Hanover
- Morgan, K.L. and Tuppen, A.T.A. (1988) Sheep health, productivity, and the IBM PC. In: *The Research and Academic Users' Guide to the IBM Personal Computer*.

- Ed. Barnetson, P., pp. 147–153. Oxford University Press, Oxford
- Morgan, M. (2008) Methicillin-resistant *Staphylococcus aureus* and animals: zoonosis or humanosis? *Journal of Antimicrobial Chemotherapy*, **62**, 1181–1187
- Morgan, M.G. and Henrion, M. (1990) *Uncertainty: A Guide to Dealing with Uncertainty in Quantitative Risk and Policy Analysis*. Cambridge University Press, Cambridge
- Morgan, N. (2001) *Repercussions of BSE on International Meat Trade: Global Market Analysis*. Commodities and Trade Division, Food and Agriculture Organization of the United Nations, Rome. <http://www.fao.org/livestock/agap/frg/feedsafety/ffsp6.htm> (accessed 10 March 2016)
- Morita, K., Nabeshima, T. and Buerano, C.C. (2015) Japanese encephalitis. *Revue Scientifique et Technique, Office International des Epizooties*, **34**, 441–452
- Mörk, M.J., Wolff, C., Lindberg, A., Vågsholm, I. and Egenvall, A. (2010) Validation of a national disease recording system for dairy cattle against veterinary practice records. *Preventive Veterinary Medicine*, **93**, 183–192
- Morley, F.H.W., Watt, B.R., Grant, I.McL. and Galloway, D.B. (1983) A flock health and production program for sheep. In: *Proceedings of the Third International Symposium on Veterinary Epidemiology and Economics*. Arlington, Virginia, 6–10 September 1982, pp. 186–194. Veterinary Medical Publishing Company, Edwardsville
- Morley, R.S. (1988) National Animal Health Information System, Agriculture Canada. *Revue Scientifique et Technique, Office International des Epizooties*, **7**, 577–581
- Morley, R.S. (Coordinator) (1993a) Risk analysis, animal health and trade. *Revue Scientifique et Technique, Office International des Epizooties*, **12**, 1001–1362
- Morley, R.S. (1993b) A model for the assessment of the animal disease risks associated with the importation of animals and animal products. *Revue Scientifique et Technique, Office International des Epizooties*, **12**, 1055–1092
- Mörner, T., Obendorf, D.L., Artois, M. and Woodford, M. H. (2002) Surveillance and monitoring of wildlife. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 67–76
- Morocoima, A., Chique, J., Zavala-Jaspe, R., Díaz-Bello, Z., Ferrer, E. *et al.* (2010) Commercial coconut palm as an ecotope of Chagas disease vectors in north-eastern Venezuela. *Journal of Vector Borne Diseases*, **47**, 76–84
- Morris, D.D., Moore, J.N. and Ward, S. (1989) Comparison of age, sex, breed, history and management in 229 horses with colic. *Equine Veterinary Journal, Suppl. 7*, 129–132
- Morris, J. (Ed.) (1976) *Domesday Book*. 34 vols. Phillimore, Chichester. (Originally compiled at Winchester in 1086 as the *Liber de Wintonia*)
- Morris, R.S. (1982) New techniques in veterinary epidemiology – providing workable answers to complex problems. In: *Epidemiology in Animal Health*. Proceedings of a symposium held at the British Veterinary Association's Centenary Congress, Reading, 22–25 September 1982, pp. 1–16. Society for Veterinary Epidemiology and Preventive Medicine
- Morris, R.S. (1999) The application of economics in animal health programmes: a practical guide. *Revue Scientifique et Technique, Office International des Epizooties*, **18**, 305–314
- Morris, R.S., Sanson, R.L., McKenzie, J.S. and Marsh, W. E. (1993) Decision support systems in animal health. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings*, Exeter, 31 March–2 April 1993. Ed. Thrusfield, M.V., pp. 188–199
- Morris, R.S., Sanson, R.L. and Stern, M.W. (1992) EpiMAN – a decision support system for managing a foot-and-mouth disease epidemic. In: *Dutch Society for Veterinary Epidemiology and Economy, Proceedings*, 5. Wageningen, December 9, 1992. Eds Frankena, K. and van der Hoofd, C.M., pp. 1–35
- Morris, R.S., Wilesmith, J.W., Stern, M.W., Sanson, R.L. and Stevenson, M.A. (2001) Predictive spatial modelling of alternative control strategies for the foot-and-mouth disease epidemic in Great Britain, 2001. *Veterinary Record*, **149**, 137–144
- Morrow, D.A. (1980) Examination schedule for a dairy reproductive health programme. In: *Current Therapy in Theriogenology*. Ed. Morrow, D.A., pp. 549–562. W. B. Saunders Company, Philadelphia, London and Toronto
- Morse, S.S. (1995) Factors in the emergence of infectious diseases. *Emerging Infectious Diseases*, **1**, 7–15
- Mort, M., Convery, I., Baxter, J. and Bailey, C. (2005) Psychosocial effects of the 2001 UK foot and mouth disease epidemic in a rural population: qualitative diary based study. *British Medical Journal*, **331**, 1234–1237
- Mort, M., Convery, I., Baxter, J. and Bailey, C. (2008) Animal disease and human trauma: the psychosocial implications of the 2001 UK foot and mouth disease disaster. *Journal of Applied Animal Welfare Science*, **11**, 133–148
- Mortensen, S. and Madsen, K. (1992) The occurrence of PRRS in Denmark. *American Association of Swine Practitioners Newsletter*, **4** (4), 48
- Mortimer, P.P. (2008) Ridding London of smallpox: the aerial transmission debate and the evolution of a precautionary approach. *Epidemiology and Infection*, **136**, 1297–1305

- Morton, D. (1992) Docking of dogs: practical and ethical aspects. *Veterinary Record*, **131**, 301–306
- Morton, N.E. (1982) *Outline of Genetic Epidemiology*. Karger, New York
- Morton, S.C., Adams, J.L., Suttorp, M.J. and Shekelle, P.G. (2004) *Meta-regression Approaches: What, Why, When, and How?* Agency for Healthcare Research and Quality (US), Rockville
- Moss, R. (Coordinator) (1994) Animal welfare and veterinary services. *Revue Scientifique et Technique, Office International des Epizooties*, **13**, 1–302
- Mossel, D.A.A. (1989) Adequate protection of the public against food-transmitted diseases of microbial aetiology. Achievements and challenges, half a century after the introduction of the Prescott-Meyer-Wilson strategy of active intervention. *International Journal of Food Microbiology*, **9**, 271–294
- Mossman, K. (2011) *Viruses and Interferon*. Caister Academic Press, Norfolk
- Moulder, J.W. (1974) Intracellular parasitism: life in an extreme environment. *Journal of Infectious Diseases*, **130**, 300–306
- Mousing, J. (1988) The Danish Pig Health Scheme: a questionnaire survey and analysis of swine producers' attitudes and willingness to pay. *Preventive Veterinary Medicine*, **6**, 157–170
- Moutou, F., Dufour, B. and Ivanov, Y. (2001) A qualitative assessment of the risk of introducing foot and mouth disease into Russia and Europe from Georgia, Armenia and Azerbaijan. *Revue Scientifique et Technique, Office International des Epizooties*, **20**, 723–730
- MRC (1938) *Epidemics in Schools. An Analysis of the Data Collected during the First Five Years of a Statistical Inquiry by the Schools Epidemic Committee*. Medical Research Council Special Report Series No. 227. His Majesty's Stationery Office, London
- Muench, H. (1959) *Catalytic Models in Epidemiology*. Harvard University Press, Cambridge
- Mughini-Gras, L., Enserink, R., Friesema, I., Heck, M., van Duynhoven, Y. *et al.* (2014) Risk factors for human salmonellosis originating from pigs, cattle, broiler chickens and egg laying hens: a combined case-control and source attribution analysis. *PLoS ONE*, **4**, e87933; doi 10.1371/journal.pone.0087933
- Mughini Gras, L., Smid, J., Wagenaar, J.A., de Boer, A.G., Havellar, A.H. *et al.* (2012) Risk factors for campylobacteriosis of chicken, ruminant, and environmental origin: a combined case-control and source attribution analysis. *PLoS ONE*, **7**, e42599; doi 10.1371/journal.pone.0042599
- Mugunieri, G.L., Irungu, P. and Omiti, J.M. (2004) Performance of community-based animal health workers in the delivery of livestock health services. *Tropical Animal Health and Production*, **36**, 523–535
- Muirhead, M.R. (1976) Veterinary problems of intensive pig husbandry. *Veterinary Record*, **99**, 288–292
- Muirhead, M.R. (1978). Intensive pig production: studies in preventive medicine. Fellowship Thesis, Royal College of Veterinary Surgeons
- Muirhead, M.R. (1980) The pig advisory visit in preventive medicine. *Veterinary Record*, **106**, 170–173
- Muirhead, M.R., Alexander, T.J.L. and Carr, J. (2013) *Managing Pig Health: A Reference for the Farm*, 2nd edn. 5M Publishing, Sheffield
- Mukhebi, A.W., Wathanga, J., Perry, B.D., Irvin, A.D. and Morzaria, S.P. (1989) Financial analysis of East Coast fever control on beef production under farm conditions. *Veterinary Record*, **125**, 456–459
- Mukherjee, N. (1993) *Participatory Rural Appraisal: Methodology and Applications*. Concept Publishing Company, New Dehli
- Mulaik, S.A., Raju, N.S. and Harshman, R.A. (1997) There is a time and place for significance testing. In: *What if There Were No Significance Tests?* Eds Harlow, L.L., Mulaik, S.A. and Steiger, J.H., pp. 65–115. Lawrence Erlbaum Associates, New Jersey
- Mulatti, P., Ferrè, N., Patregnani, T., Bonfanti, L. and Marangon, S. (2011) Geographical information systems in the management of the 2009–2010 emergency oral anti-rabies vaccination of foxes in north-eastern Italy. *Geospatial Health*, **5**, 217–226
- Mulder, C.A.T., Bonnett, B.N., Martin, S.W., Lissemore, K. and Page, P.D. (1994) The usefulness of the computerized medical records of one practice for research into pregnancy loss in dairy cows. *Preventive Veterinary Medicine*, **21**, 43–63
- Mulhern, F.J., Mott, L.O., Shahan, M.S. and Anderson, R. G. (1962) Eradication of animal diseases. In: *After a Hundred Years, The Yearbook of Agriculture 1962*, pp. 313–320. The US Government Printing Office, Washington DC
- Müller, W.W. (1991) New areas of oral fox vaccination in Europe, 1991. *Rabies Bulletin Europe*, **15** (3), 11–12. WHO Collaborating Centre for Rabies Surveillance and Research, Tübingen
- Mullner, P., Shadbolt, T., Collins-Emerson, J.M., Midwinter, A.C., Spencer, S.E. *et al.* (2010) Molecular and spatial epidemiology of human campylobacteriosis: source association and genotype-related risk factors. *Epidemiology and Infection*, **138**, 1372–1383
- Muma, J.B., Toft, N., Oloya, J., Lund, A., Nielsen, K. *et al.* (2007) Evaluation of three serological tests for brucellosis in naturally infected cattle using latent class analysis. *Veterinary Microbiology*, **125**, 187–192
- Mumford, L. (1970) *The Pentagon of Power*. Harcourt Brace Jovanovich, New York

- Münch, R. (2003) Robert Koch. *Microbes and Infection*, **5**, 69–74
- Munoz, R.M. del, Montano, M.F., Renteria, T.B., Sanchez, E., Moreno, J.F. *et al.* (2007) Assessment of the economic impact of a brucellosis control program in a dairy herd using the partial budget method. *Journal of Animal and Veterinary Advances*, **6**, 146–151
- Munro, H.M.C. and Thrusfield, M.V. (2001a) 'Battered pets': features that raise suspicion of non-accidental injury. *Journal of Small Animal Practice*, **42**, 218–226
- Munro, H.M.C. and Thrusfield, M.V. (2001b) 'Battered pets': non-accidental physical injuries found in dogs and cats. *Journal of Small Animal Practice*, **42**, 279–290
- Munro, H.M.C. and Thrusfield, M.V. (2001c) 'Battered pets': Munchausen syndrome by proxy (factitious illness by proxy). *Journal of Small Animal Practice*, **42**, 385–389
- Munro, H.M.C. and Thrusfield, M.V. (2001d) 'Battered pets': sexual abuse. *Journal of Small Animal Practice*, **42**, 333–337
- Munro, H.M.C. and Thrusfield, M.V. (2005) 'Battered pets': sexual abuse. In: *Bestiality and Zoophilia: Sexual Relations with Animals*. Eds Beetz, A. M. and Podberscek, A. L., pp. 71–81. Purdue University Press, West Lafayette
- Munro, H.M.C. and Thrusfield, M.V. (2015) Abuse. In: *Clinical Veterinary Advisor: Dogs and Cats*, 3rd edn. Ed. Côté, E., pp. 10–11. Mosby/Elsevier, St Louis
- Munson, L. and Cook, R.A. (1993) Monitoring, investigation and surveillance of diseases in captive wildlife. *Journal of Zoo and Wildlife Medicine*, **24**, 281–290
- Munson, L., Terio, K.A., Kock, R., Mlengeya, T., Roelke, M.E. *et al.* (2008) Climate extremes promote fatal co-infections during canine distemper epidemics in African lions. *PLoS ONE*, **3**, 6, e2545; doi 10.1371/journal.pone.0002545
- Murchie, L., Xia, B., Madden, R., Whyte, P. and Kelly, L. (2008) Qualitative exposure assessment for *Salmonella* spp. in shell eggs produced on the island of Ireland. *International Journal of Food Microbiology*, **125**, 308–319
- Muriuki, J., Thaiyah, A., Mbugua, S. and Kitaa, J. (2016) Dog populations and dog-keeping practices in relation to rabies control in Kisumu and Siaya counties, Kenya. *International Journal of Agricultural Sciences and Veterinary Medicine*, **4**, 90–100
- Murphy, B.R. and Webster, R.G. (1990) Orthomyxoviruses. In: *Virology*, Vol. 1. Eds Fields, B.N. and Knipe, D.M., pp. 1091–1152. Raven Press, New York
- Murphy, C.P., Reid-Smith, R.J., Boerlin, P., Weese, J.S., Prescott, J.F. *et al.* (2012) Out-patient antimicrobial drug use in dogs and cats for new disease events from community companion animal practices in Ontario. *Canadian Veterinary Journal*, **53**, 291–298
- Murphy, R.G.L., Pendell, D.L., Morris, D.L., Scanga, J.A., Belk, K.E. *et al.* (2008) Animal identification systems in North America. *The Professional Animal Scientist*, **24**, 277–286
- Murray, A., Moriarty, K.M. and Scott, D.B. (1989) A cloned DNA probe for the detection of *Mycobacterium paratuberculosis*. *New Zealand Veterinary Journal*, **37**, 47–50
- Murray, D.L., Cox, E.W., Ballard, W.B., Whitlaw, H.A., Lenarz, M.S. *et al.* (2006) Pathogens, nutritional deficiency, and climate influences on a declining moose population. *Wildlife Monographs*, **166**, 1–30
- Murray, D.M., Varnell, S.P. and Blitstein, J.L. (2004a) Design and analysis of group-randomized trials: a review of recent methodological developments. *American Journal of Public Health*, **94**, 423–432
- Murray, J. (1968) Some aspects of ovicaprid and pig breeding in neolithic Europe. In: *Studies in Ancient Europe. Essays Presented to Stuart Piggott*. Eds Coles, J. M. and Simpson, D.D.A., pp. 71–81. Leicester University Press, Leicester
- Murray, J.K. and Gruffydd-Jones, T.J. (2012) Proportion of pet cats registered with a veterinary practice and factors influencing registration in the UK. *Veterinary Journal*, **192**, 461–466
- Murray, J.K., Browne, W.J., Roberts, M.A., Whitmarsh, A. and Gruffydd-Jones, T.J. (2010a) Number and ownership profiles of cats and dogs in the UK. *Veterinary Record*, **166**, 163–168
- Murray, J.K., Gruffydd-Jones, T.J., Roberts, M.A. and Browne, W.J. (2015) Assessing changes in the UK pet cat and dog populations: numbers and household ownership. *Veterinary Record*, **177**, 259; doi 10.1136/vr.103223
- Murray, J.K., Habacher, G. and Gruffydd-Jones, T.J. (2012) Differences between samples of vet-registered and vet-visiting cats. Proceedings of the 13th International Symposium on Veterinary Epidemiology and Economics, Maastricht, p. 59. Wageningen Academic Publishers, Wageningen
- Murray, J.K., Singer, E.R., Morgan, K.L., Proudman, C.J. and French, N.P. (2004b) Memory decay and performance-related information bias in the reporting of scores by event riders. *Preventive Veterinary Medicine*, **63**, 173–182
- Murray, K., Selleck, P., Hooper, P., Hyatt, A., Gould, A. *et al.* (1995) A morbillivirus that caused fatal disease in horses and humans. *Science*, **268**, 94–97
- Murray, M.J., Schusser, G.F., Pipers, F.S. and Gross, S.J. (1996) Factors associated with gastric lesions in thoroughbred racehorses. *Equine Veterinary Journal*, **28**, 368–374

- Murray, N. (2002) *Import Risk Analysis: Animals and Animal Products*. Ministry of Agriculture and Forestry, Wellington
- Murray, N., MacDiarmid, S.C., Wooldridge, M., Gummow, B., Morley, R.S. *et al.* (2010b) *Handbook on Import Risk Analysis for Animals and Animal Products, Volume I. Introduction and Qualitative Risk Analysis*, 2nd edn. Office International des Epizooties, Paris
- Murray, N., MacDiarmid, S.C., Wooldridge, M., Gummow, B., Morley, R.S. *et al.* (2004c) *Handbook on Import Risk Analysis for Animals and Animal Products, Volume II. Quantitative Risk Analysis*. Office International des Epizooties, Paris
- Murray, P.K. and Eaton, B.T. (1996) Vaccines for bluetongue. *Australian Veterinary Journal*, **73**, 207–210
- Mussman, H.C., McCallon, W.R. and Otte, E. (1980) Planning and implementation of animal disease control programs in developing countries. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 4–11 May 1979. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 551–557. Australian Government Publishing Service, Canberra
- Musso, D. (2015) Zika virus transmission from French Polynesia to Brazil (Letter). *Emerging Infectious Diseases*, **21**, 1887; doi 10.3201/eid2110.151125
- Mutondo, J.E., Brorsen, B.W. and Henneberry, S.R. (2009) Welfare impacts of BSE-driven trade bans. *Agricultural and Resource Economics Review*, **38**, 324–329
- Mwangi, W., de Figueiredo, P. and Criscitiello, M.F. (2016) One Health: addressing global challenges at the nexus of human, animal, and environmental health. *PLOS Pathogens*, **12**, 9, e1005731; doi 10.1371/journal.ppat.1005731
- Myers, J.H. (1988) Can a general hypothesis explain population cycles of forest Lepidoptera? *Advances in Ecological Research*, Vol. 18. Eds Begon, M., Fitter, A. H., Ford, E.D. and Macfadyen, A., pp. 179–242. Academic Press, London
- Myles, P.S. and Ciu, J. (2007) Using the Bland-Altman method to measure agreement with repeated measures. *British Journal of Anaesthesia*, **99**, 309–311
- NADIS (2015) *National Animal Disease Information Service*. NADIS, Haverfordwest, <http://www.nadis.org.uk/> (accessed 23 July 2015)
- NAHIS (2014) *National Animal Health Information System*. Animal Health Australia, Deakin ACT. http://nahis.animalhealthaustralia.com.au/public.php?page=pub_home&program=1 (accessed 27 October 2014)
- NAHMS (2014) *National Animal Health Monitoring System*. United States Department of Agriculture, Animal and Plant Health Inspection Service. http://www.aphis.usda.gov/animal_health/nahms/ (accessed 27 October 2014)
- Naikan, V.N.A. (2008) Statistical process control. In: *Handbook of Performability Engineering*. Ed. Misra, K. B., pp. 187–201. Springer-Verlag, London
- Nakayachi, K. (1998) How do people evaluate risk reduction when they are told zero risk is impossible? *Risk Analysis*, **18**, 235–242
- Nam, J. (1995) Confidence limits for the ratio of two binomial proportions based on likelihood scores: non-iterative method. *Biometrics Journal*, **37**, 375–379
- NAMP (2015) National Arbovirus Monitoring Program. <http://www.animalhealthaustralia.com.au/programs/disease-surveillance/national-arbovirus-monitoring-program/> (accessed 13 July 2015)
- Nardelli, L., Lodetti, E., Gualandi, G.L., Burrows, R., Goodridge, D. *et al.* (1968) A foot-and-mouth disease syndrome in pigs caused by an enterovirus. *Nature*, **219**, 1275–1276
- Nash, D., Mostashari, F., Fine, A., Miller, J.D., O'Leary, D. *et al.* (2001) The outbreak of West Nile virus infection in the New York City area in 1999. *The New England Journal of Medicine*, **344**, 1807–1814
- Nasser, R. and Mosier, J.E. (1980) Canine population dynamics: a study of the Manhattan, Kansas, canine population. *American Journal of Veterinary Research*, **41**, 1798–1803
- Nasser, R. and Mosier, J.E. (1982) Feline population dynamics: a study of the Manhattan, Kansas, feline population. *American Journal of Veterinary Research*, **43**, 167–170
- Nasser, R., Mosier, J.E. and Williams, L.W. (1984) Study of the canine and feline populations in the Greater Las Vegas area. *American Journal of Veterinary Research*, **45**, 282–287
- Nath, M., Woolliams, J.A. and Bishop, S.C. (2008) Assessment of the dynamics of microparasite infections in genetically homogenous and heterogeneous populations using a stochastic epidemic model. *Journal of Animal Science*, **86**, 1747–1757
- National Audit Office (2002) *The 2001 Outbreak of Foot and Mouth Disease. Report by the Comptroller and Auditor General*. HC 939 Session 2001–2002: 21 June 2002. The Stationery Office, London
- Navarro, J.-C., Medina, G., Vasquez, C., Coffey, L.L., Wang, E. *et al.* (2005) Postepizootic persistence of Venezuelan equine encephalitis virus, Venezuela. *Emerging Infectious Diseases*, **11**, 1907–1915
- Naveh, Z. and Lieberman, A.S. (1994) *Landscape Ecology: Theory and Application*, 2nd edn. Springer-Verlag, New York
- Naylor, C.D. (1989) Meta-analysis of controlled clinical trials. *Journal of Rheumatology*, **16**, 424–426

- NCAHP (2004) National Center for Animal Health Programs: Swine Brucellosis Surveillance Standards. <http://www.aphis.usda.gov/vs/naahps/surveillance/brucella-suis.html> (accessed 7 November 2004)
- NCBI (2017a) *The NCBI Nucleotide database homepage*. US National Library of Medicine, Bethesda. <http://www.ncbi.nlm.nih.gov/nuccore> (accessed 25 July 2017)
- NCBI (2017b) *PubMed*. US National Library of Medicine, Bethesda. <http://www.ncbi.nlm.nih.gov/pubmed/> (accessed 25 July 2017)
- Neaton, J.D., Duchene, A.G., Svendsen, K.H. and Wentworth, D. (1990) Examination of the efficiency of some quality assurance methods commonly employed in clinical trials. *Statistics in Medicine*, **9**, 115–124
- Neave, F.K., Dodd, F.H., Kingwill, R.G. and Westgarth, D. R. (1969) Control of mastitis in the dairy herd by hygiene and management. *Journal of Dairy Science*, **52**, 696–707
- Nebbia, C. (2001) Biotransformation enzymes as determinants of xenobiotic toxicity in domestic animals. *The Veterinary Journal*, **161**, 238–252
- Neitz, W.O. (1948) Immunological studies on bluetongue in sheep. *Onderstepoort Journal of Veterinary Science*, **23**, 93–135
- Nelder, J.A. and Wedderburn, R.W.M. (1972) Generalized linear models. *Journal of the Royal Statistical Society, Series A*, **135** (3), 370–384
- Nelson, A.W. and White, L.K. (1990) Medical records. In: *Clinical Textbook for Veterinary Technicians*, 2nd edn. Ed. McCurnin, D.M., pp. 43–55. W.B. Saunders, Philadelphia
- New, J.C., Salman M.D., King, M., Scarlett, J.M., Kass, P.H. *et al.* (2000) Characteristics of shelter-relinquished animals and their owners compared with animals and their owners in US pet-owning households. *Journal of Applied Animal Welfare Science*, **3**, 179–201
- Newcombe, H.B. (1964) Session VII discussion In: *Second International Conference on Congenital Malformations*. Papers and Discussions Presented at the Second International Conference on Congenital Malformations, New York City, July 14–19, 1963, pp. 345–349 International Medical Congress, New York
- Newcombe, R.G. (2013) *Confidence Intervals for Proportions and Related Measures of Effect Size*. CRC Press, Boca Raton
- Newman, M.E.J. (2003) The structure and function of complex networks. *SIAM Review*, **45**, 167–256
- Newson, S.E., Evans, K.L., Noble, D.G., Greenwood, J.J.D. and Gaston, K.J. (2008) Use of distance sampling to improve national population sizes for common and widespread breeding birds in the UK. *Journal of Applied Ecology*, **45**, 1330–1338
- Newson, S.E., Massimino, D., Johnston, A., Baillie, S.R. and Pearce-Higgins, J.W. (2013) Should we account for detectability in population trends? *Bird Study*, **60**, 384–390
- Ngategize, P.K., Kaneene, J.B., Harsh, S.B., Bartlett, P.C. and Mather, E.L. (1986) Decision analysis in animal health programs: merits and limitations. *Preventive Veterinary Medicine*, **4**, 187–197
- Nicholas, F.W. (2010) *Introduction to Veterinary Genetics*, 3rd edn. Wiley-Blackwell, Chichester
- Nicholls, N. (1986) A method for predicting Murray Valley encephalitis in southeast Australia using the Southern Oscillation. *Australian Journal of Experimental Biology and Medical Science*, **64**, 587–594
- Nicholls, T.J., Barton, M.G. and Anderson, B.P. (1981) An outbreak of mastitis in dairy cows due to *Pseudomonas aeruginosa* contamination of dry-cow therapy at manufacture. *Veterinary Record*, **108**, 93–96
- Nicholson, M.C. and Mather, T.N. (1996) Methods for evaluating Lyme disease risks using Geographic Information Systems and Geospatial Analysis. *Journal of Medical Entomology*, **33**, 711–720
- Nielsen, C.F., Kidd, S., Sillah, A.R.M., Davis, E., Mermin, J. *et al.* (2015) Improving burial practices and cemetery management during an Ebola virus disease epidemic – Sierra Leone, 2014. *Morbidity and Mortality Weekly Report*, **64** (1), 20–27
- Niessen, S.J.M., Powney, S., Guitian, J., Niessen, A.P.M., Pion, P.D. *et al.* (2010) Evaluation of a quality-of-life tool for cats with diabetes mellitus. *Journal of Veterinary Internal Medicine*, **24**, 1098–1105
- Niezen, J.H., Charleston, W.A.G., Hodgson, J., Mackay, A. D. and Leatherwick, D.M. (1996) Controlling internal parasites in grazing ruminants without recourse to anthelmintics: approaches, experiences and prospects. *International Journal for Parasitology*, **26**, 983–992
- Niles, D., Eicker, S. and Stewear, S. (2001) Using pregnancy rate to monitor reproductive performance. Proceedings of the 5th Western Dairy Management Conference, April 4–6, 2001, Las Vegas, Nevada, pp. 117–121. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, Kansas
- Nir, I., Kedzierski, W., Chen, J. and Travis, G.H. (2000) Expression of Bcl-2 protects against photoreceptor degeneration in retinal degeneration slow (rds) mice. *Journal of Neuroscience*, **20**, 2150–2154
- Nishiura, H. (2007) Early efforts in modeling the incubation period of infectious diseases with an acute course of illness. *Emerging Themes in Epidemiology*, **4**, 2; doi 10.1186/1742-7622-4-2
- Nishiura, H. and Chowell, G. (2009) The effective reproduction number as a prelude to statistical

- estimation of time-dependent epidemic trends. In: *Mathematical and Statistical Estimation Approaches in Epidemiology*. Eds Chowell, G., Hyman, J.M., Bettencourt, L.M.A. and Castillo-Chavez, C., pp. 103–121. Springer, Dordrecht
- Niskanen, M. and Thrusfield, M.V. (1998) Associations between age, parity, hormonal therapy, breed, and pyometra in Finnish Dogs. *Veterinary Record*, **143**, 493–498
- NISO (2004) *Understanding Metadata*. National Information Standards Organization. NISO Press, Bethesda
- Nizeyi, J.B., Cranfield, M.R. and Graczyk, T.K. (2002) Cattle near the Bwindi Impenetrable National Park, Uganda, as a reservoir of *Cryptosporidium parvum* and *Giardia duodenalis* for a local community of free-ranging gorillas. *Parasitology Research*, **88**, 380–385
- NMR (2014) *InterHerd*. National Milk Records, Chippenham. <http://www.nmr.co.uk/interherd/> (accessed 27 October 2014)
- Noack, H. (1987) Concepts of health and health promotion. In: *Measurement in Health Promotion and Protection*. Eds Abelin, T., Brzezinski, Z.J. and Carstairs, V.D.L., pp. 5–28. World Health Organization Regional Office for Europe, Copenhagen
- Nokes, J.D. (1992) Microparasites: viruses and bacteria. In: *Natural Enemies: the Population Biology of Predators, Parasites and Diseases*. Ed. Crawley, M.J., pp. 349–374. Blackwell Scientific Publications, Oxford
- Noordhuizen, J.P.T.M. (2000) Preventive medicine practice and quality assurance through HACCP. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings*, Edinburgh, 29–31 March 2000. Eds Thrusfield, M.V. and Goodall, E.A., pp. 37–45
- Noordhuizen, J.P.T.M. and Buurman, J. (1984) Veterinary automated management and production control programme for dairy herds (VAMPP). The application of MUMPS for data processing. *Veterinary Quarterly*, **6**, 62–77
- Noordhuizen, J.P.T.M. and Dufour, B. (2001) Monitoring and surveillance systems (MOSS): design and operationalization. In: *Application of Quantitative Methods in Veterinary Epidemiology*, revised reprint. Noordhuizen, J.P.T.M., Frankena, K., Thrusfield, M.V. and Graat, E.A.M., pp. 367–381. Wageningen Pers, Wageningen
- Noordhuizen, J.P.T.M. and Frankena, K. (1999) Epidemiology and quality assurance: applications at farm level. *Preventive Veterinary Medicine*, **39**, 93–110
- Noordhuizen, J.P.T.M., Cannas da Silva, J., Boersema J.S. C. and Vieira, A. (Eds) (2008) *Applying HACCP-based Quality Risk Management on Dairy Farms*. Wageningen Academic Publishers, Wageningen
- Noordhuizen, J.P.T.M., Frankena, K., Ploeger, H. and Nell, T. (Eds) (1993) *Field Trial and Error*. Proceedings of the international seminar with workshops on the design, conduct and interpretation of field trials, Berg en Dal, Netherlands, 27–28 April 1993. Epidecon, Wageningen
- Noordman, J.W.J. and Endenburg, N. (2008) Terugblik op de MKZ-crisis door betrokkenen. De gevolgen van de MKZ-crisis bij dierenartsen. [The foot and mouth disease outbreak 6 years later: consequences for veterinarians.] *Tijdschrift voor Diergeneeskunde*, **133**, 1042–1045 (in Dutch)
- Nordenfelt, L. (2006) *Animal and Human Health and Welfare: A Comparative Philosophical Analysis*. CABI, Wallingford
- Nordenstam, T. and Tornebohm, H. (1979) Research, ethics and development. *Zeitschrift für Allgemeine Wissenschaftstheorie*, **10**, 54–66
- Nordgaard, A. and Rasmusson, B. (2012) The likelihood ratio as value of evidence – more than a question of numbers. *Law, Probability and Risk*, **11**, 303–315
- Norman, G. (2010) Likert scales, levels of measurement and the 'laws' of statistics. *Advances in Health Science Education*, **15**, 625–632
- Norman, G., Monteiro, S. and Salama, S. (2012) Sample size calculations: should the emperor's clothes be off the peg or made to measure? *British Medical Journal*, **345**, e5278; doi 10.1136/bmj.e5278 (erratum: **349**, g5341; doi 10.1136/bmj.g5341; comments: **345**, e6585; doi 10.1136/bmj.e6585; **345**, e6586; doi 10.1136/bmj.e6586)
- Nørrung, B. (2006) Risk assessment of *Campylobacter* in poultry. In: *Integrated Food Safety and Veterinary Public Health*. Ed. Buncic, A., pp. 365–370. CABI, Wallingford
- Norton-Griffiths, M. (1978) *Counting Animals*, 2nd edn. African Wildlife Leadership Foundation, Nairobi
- Notturmo, M. (1999) *Science and the Open Society: The Future of Karl Popper's Philosophy*. Central European University Press, Budapest
- Novakowski, N.S., Cousineau, J.G., Kolenosky, G.B., Wilton, G.S. and Choquette, L.P.E. (1963) Parasites and diseases of bison in Canada. II. Anthrax epizooty in the Northwest Territories. *Transactions on the North American Wildlife and Natural Resources Conference*, **28**, 233–239
- Novick, G. (2008) Is there a bias against telephone interviews in qualitative research? *Research in Nursing & Health*, **31**, 391–398
- Nurmi, E. (1988) Modern methods of public health practice: exclusion of food-borne pathogens. *Acta Veterinaria Scandinavica, Suppl.* **84**, 49–56
- Nunn, F., Cave, T.A., Knottenbelt, C. and Poxton, I.R. (2004) Association between Key-Gaskell syndrome and infection by *Clostridium botulinum* type C/D. *Veterinary Record*, **155**, 111–115

- Nurminen, M. (1995) To use or not to use the odds ratio in epidemiologic studies? *European Journal of Epidemiology*, **11**, 365–371
- Nusbaum, K.E., Wenzel, J.G.W. and Everly, G.S. (2007) Psychologic first aid and veterinarians in rural communities undergoing livestock depopulation. *Journal of the American Veterinary Medical Association*, **231**, 692–694
- Nusser, S.M., Clark, W.R., Otis, D.L. and Huang, L. (2008) Sampling considerations for disease surveillance in wildlife populations. *Journal of Wildlife Management*, **72**, 52–60
- NVCR (2015) *National Veterinary Cancer Registry*. <http://nationalveterinarycancerregistry.org/> (accessed 27 July 2015)
- Nye, E.R. and Gibson, M.E. (1997) *Ronald Ross: Malarialogist and Polymath*. St Martin's Press, New York
- O'Brien, D.J., Kaneene, J.B., Getis, A., Lloyd, J.W., Rip, M. R. *et al.* (1999) Spatial and temporal distribution of selected canine cancers in Michigan, USA, 1964–1994. *Preventive Veterinary Medicine*, **42**, 1–15
- O'Brien, P.J. and Ball, R. (2006) Porcine stress syndrome. In: *Diseases of Swine*, 9th edn, Eds Straw, B.E., Zimmerman, J.J., D'Allaire, S. and Taylor, D.J., pp. 945–963. Blackwell Publishing, Oxford
- Obuchowski, N.A. (1998) Sample size calculations in studies of test accuracy. *Statistics in Medicine*, **7**, 371–392
- Ocaña, J., Sánchez, M.P.O., Sánchez, A. and Carrasco, J.L. (2008) On equivalence and bioequivalence testing. *SORT (Statistics and Operations Research Transactions)*, **32**, 151–176
- O'Connor, A. (2010) Reporting guidelines for primary research: saying what you did. *Preventive Veterinary Medicine*, **97**, 144–149
- O'Connor, A.M. (2013) Interpretation of odds and risk ratios. *Journal of Veterinary Internal Medicine*, **27**, 600–603
- O'Connor, A.M., Anderson, K.M., Goodell, C.K. and Sargeant, J.M. (2014) Conducting systematic reviews on intervention questions I: writing the review protocol, formulating the question and searching the literature. *Zoonoses and Public Health*, **61**, Suppl. 1, 28–38
- O'Connor, A.M., Sargeant, J.M., Gardner, I.A., Dickson, J. S., Torrence, M.E. *et al.* (2010) The REFLECT Statement: methods and processes of creating reporting guidelines for randomized controlled trials for livestock and food safety. *Journal of Food Protection*, **73**, 132–139; *Journal of Veterinary Internal Medicine*, **24**, 57–64
- Odend'hal, S. (1983) *The Geographical Distribution of Animal Viral Diseases*. Academic Press, New York and London
- O'Driscoll, J.G. Troy, P.T. and Geoghegan, F.J. (1977) An epidemic of venereal infection in Thoroughbreds. *Veterinary Record*, **101**, 359–360
- OECD (2012) *Livestock Diseases: Prevention, Control and Compensation Schemes*. OECD Publications, Paris
- Ogundipe, G.A.T., Oluokun, S.B. and Esuroso, G.G. (1989) The development and efficiency of the national animal health information system in Nigeria. *Preventive Veterinary Medicine*, **7**, 121–135
- Oidtman, B., Peeler, E., Lyngstad, T., Brun, E., Jensen, B. B. *et al.* (2013) Risk-based methods for fish and terrestrial animal disease surveillance. *Preventive Veterinary Medicine*, **112**, 12–26
- OIE (1987) *Procedures for Immediate and Monthly Reporting of Significant Disease Outbreaks to the Office International des Epizooties. Guidelines*. Office International des Epizooties, Paris
- OIE (1993) Biotechnology applied to the diagnosis of animal diseases. *Revue Scientifique et Technique, Office International des Epizooties*, **12**, 1–672
- OIE (1997) *International Animal Health Code. Special Edition*. Office International des Epizooties, Paris
- OIE (1998) Genetic resistance to animal diseases. *Revue Scientifique et Technique, Office International des Epizooties*, **17**, 1–329
- OIE (2003) *Rinderpest in Kenya*. Office International des Epizooties, Disease Information, Vol. 16, No. 43. Office International des Epizooties, Paris
- OIE (2010) *Training Manual on Wildlife Diseases and Surveillance, First Cycle: Workshop for OIE National Focal Points for Wildlife*. Office International des Epizooties, Paris
- OIE (2012) *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees)*, 7th edn, Vol. 1. Office International des Epizooties, Paris
- OIE (2013) Import risk analysis. In: *Terrestrial Animal Health Code*, 22nd edn, Section 2. World Organisation for Animal Health, Paris
- OIE (2014) *OIE-Listed Diseases, Infections and Infestations in Force in 2014*. <http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2014/> (accessed 28 October 2014)
- OIE (2015a) *Terrestrial Animal Health Code*, 24th edn. Office International des Epizooties, Paris
- OIE (2015b) *World Animal Health Information System*. <http://www.oie.int/animal-health-in-the-world/the-world-animal-health-information-system/the-oie-data-system/> (accessed 14 August 2015)
- OIE (2015c) *Training Manual on Surveillance and International Reporting of Diseases in Wild Animals, Second Cycle: Workshop for OIE National Focal Points for Wildlife*. Office International des Epizooties, Paris
- OIE (2015d) *OIE Standards, Guidelines and Resolution on Antimicrobial Resistance and the Use of*

- Antimicrobial Agents*. World Organisation for Animal Health, Paris
- Okamoto, K.W. and Amarasekare, P. (2012) The biological control of disease vectors. *Journal of Theoretical Biology*, **309**, 47–57
- Okello, A.L., Gibbs, E.P.J., Vandersmissen, A. and Welburn, S.C. (2011) One Health and the neglected zoonoses: turning rhetoric into reality. *Veterinary Record*, **169**, 281–285
- Okello-Onen, J., Mukhebi, A.W., Tukahirwa, E.M., Musisi, G., Bode, E. *et al.* (1998) Financial analysis of dipping strategies of indigenous cattle under ranch conditions in Uganda. *Preventive Veterinary Medicine*, **33**, 241–250
- Okoh, A.E.J. (1988) Dog population census: Jos, Plateau State, Nigeria. *Tropical Veterinarian*, **6**, 89–94
- Okuyama, H. (Ed.) (2007) *Prevention of Coronary Heart Disease: From the Cholesterol Hypothesis to ω 3/ ω 6 Balance*. Karger, Basel
- Olabode, O.K., Kazeem, H.M., Raji, M.A. and Ibrahim, N. D. (2014) Participatory appraisal of foot and mouth disease outbreaks in Ilesha Baruba, Kwara State, Nigeria. *Alexandria Journal of Veterinary Sciences*, **40**, 132–138
- Olafson, P., MacCallum, A.D., and Fox, F.H. (1946) An apparently new transmissible disease of cattle. *Cornell Veterinarian*, **36**, 205–213
- Oldstone, M.B. (1998) Viral persistence: mechanisms and consequences. *Current Opinion in Microbiology*, **1**, 438–441
- Ollerenshaw, C.B. (1966) The approach to forecasting the incidence of fascioliasis over England and Wales, 1958–1962. *Agricultural Meteorology*, **3** (1/2), 35–54
- Ollerenshaw, C.B. and Rowlands, W.T. (1959) A method of forecasting the incidence of fascioliasis in Anglesey. *Veterinary Record*, **71**, 591–598
- Olshansky, S.J. (2010) The Law of Mortality revisited: interspecies comparisons of mortality. *Journal of Comparative Pathology*, **142**, S4–S9
- Olson, K.D. (2004) *Farm Management: Principles and Strategies*. Iowa State Press, Ames
- Olson, N.O. (1950) A respiratory disease (bronchitis) of quail caused by a virus. *Proceedings of the United States Livestock Sanitary Association*, **54**, 171–174
- Olsson, H., Moller, T.R. and Ronstan, J. (1989) Oral contraceptive use and breast cancer among premenopausal women: final report from a study in Southern Sweden. *Journal of the National Cancer Institute*, **81**, 1000–1004
- Omori, T., Inaba, Y., Morimoto, T., Tanaka, Y., Ishitani, R. *et al.* (1969) Ibaraki virus, an agent of epizootic disease of cattle resembling bluetongue. I. Epidemiologic, clinical and pathologic observations and experimental transmission to calves. *Japanese Journal of Microbiology*, **13**, 139–157
- Omurtag, A. and Fenton, A.A. (2012) Assessing diagnostic tests: how to correct for the combined effects of interpretation and reference standard. *PLoS ONE*, **7**, 12, e52221; doi 10.1371/journal.pone.0052221
- One Health Global Network (2011) *One Health Global Network Webportal*. <http://www.onehealthglobal.net> (accessed 9 May 2016)
- One Health Initiative (2006) *One Health Initiative*. <http://www.onehealthinitiative.com> (accessed 9 May 2016)
- One Health Initiative Task Force (2008) *One Health: A New Professional Imperative*. https://www.avma.org/KB/Resources/Reports/Documents/onehealth_final.pdf (accessed 16 August 2013)
- O'Neill, D.G., Church, D.B., McGreevy, P.D., Thomson, P.C. and Brodbelt, D.C. (2013) Longevity and mortality of owned dogs in England. *The Veterinary Journal*, **198**, 638–643
- O'Neill, D.G., Church, D.B., McGreevy, P.D., Thomson, P.C. and Brodbelt, D. (2014) Approaches to canine health surveillance. *Canine Genetics and Epidemiology*, **1**, 1–13
- O'Neill, D.G., Elliott, J., Church, D.B., McGreevy, P.D., Thomson, P.C. and Brodbelt, D.C. (2013) Chronic kidney disease in dogs in UK veterinary practices: prevalence, risk factors, and survival. *Journal of Veterinary Internal Medicine*, **27**, 814–821
- O'Neill, D.G., Hendricks, A., Summers, J. and Brodbelt, D.C. (2012) Primary care veterinary usage of systemic glucocorticoids in cats and dogs in three UK practices. *Journal of Small Animal Practice*, **53**, 217–222
- Onuigbo, W.I.B. (1975) Some nineteenth century ideas on links between tuberculous and cancerous diseases of the lung. *British Journal of Diseases of the Chest*, **69**, 207–210
- Opdenakker, R. (2006) Advantages and disadvantages of four interview techniques in qualitative research. *Forum Qualitative Sozialforschung/Forum: Qualitative Social Research*, **7** (4), Article 11. <http://www.qualitative-research.net/index.php/fqs/article/view/175/391> (accessed 21 October 2014)
- Opit, L.J. (1987) How should information on health care be generated and used? *World Health Forum*, **8**, 409–417 (discussion: 417–438)
- Oppenheim, A.N. (1992) *Questionnaire Design, Interviewing and Attitude Measurement*. Pinter, London
- Orkin, M. (1967) Animal models (spontaneous) for human disease. Experiments of nature. *Archives of Dermatology*, **95**, 524–531
- Ortega-Pacheco, A., Rodriguez-Buenfil, J.G., Bolio-Gonzalez, M.E., Sauri-Arceo, C.H., Jiménez-Coello, M. *et al.* (2007) A survey of dog populations in urban and

- rural areas of Yucatan, Mexico. *Anthrozoös*, **20**, 261–274
- Ortiz-Pelaez, A., Kelly, L. and Adkin, A. (2012) The risk of introducing scrapie from restocking goats in Great Britain. *Preventive Veterinary Medicine*, **107**, 222–230
- Ortiz-Pelaez, A., Thompson, C.E. and Dawson, M. (2014) The impact of the National Scrapie Plan on the PRNP genotype distribution on the British national flock, 2002–2012. *Veterinary Record*, **174**, 530; doi 10.1136/vr.102255
- Oscar, T. (1998) The development of a risk assessment model for use in the poultry industry. *Journal of Food Safety*, **18**, 371–381
- Osenberg, C.W., Sarnelle, O., Cooper, S.D. and Holt, R.D. (1999) Resolving ecological questions through meta-analysis: goals, metrics, and models. *Ecology*, **80**, 1105–1117
- Osgood, C.E. (1953) *Method and Theory in Experimental Psychology*. Oxford University Press, New York
- Osgood, C.E., Suci, G.J. and Tannenbaum, P.H. (1957) *The Measurement of Meaning*. University of Illinois
- O'Shea, T.J., Cryan, P.M., Cunningham, A.A., Fooks, A.R., Hayman, D.T.S. *et al.* (2014) Bat flight and zoonotic viruses. *Emerging Infectious Diseases*, **20**, 741–745
- Osterhaus, A.D.M.E. and Vedder, E.J. (1988) Identification of virus causing recent seal deaths. *Nature*, **335**, 20
- Ostrander, E. and Ruvinsky, A. (2010) *The Genetics of the Dog*, 2nd edn. CABI, Wallingford
- Ostrander, E.A., Galibert, F. and Patterson, D.F. (2000) Canine genetics comes of age. *Trends in Genetics*, **16**, 117–124
- Ostrowski, S.R. (1990) Sentinel animals (dogs) as predictors of childhood exposure to environmental lead contamination: observations and preliminary results. In: *In Situ Evaluation of Biological Hazards of Environmental Pollutants*. Eds Sandhu, S.S., Lower, W. R., de Serres, F.J., Suk, W.A. and Tice, R.R., pp. 145–150. Plenum Press, New York and London.
- Otake, S., Dee, S.A., Rossow, K.D., Joo, H.S., Deen, J. *et al.* (2002) Transmission of porcine reproductive and respiratory syndrome virus by needles. *Veterinary Record*, **150**, 114–115
- O'Toole, D.O. (2010) Monitoring and investigating natural disease by veterinary pathologists in diagnostic laboratories. *Veterinary Pathology*, **47**, 40–44
- Otte, J., Costales, A., Dijkman, J., Pica-Ciamarra, U., Robinson, T. *et al.* (2012) *Livestock Sector Development for Poverty Reduction: An Economic and Policy Perspective – Livestock's Many Virtues*. Food and Agriculture Organization of the United Nations, Rome
- Otte, M.J. and Chilonda, P. (2011) *Animal Health Economics: An Introduction*. Animal Information, Sector Analysis and Policy Branch, Animal Production and Health Division, Food and Agricultural Organization of the United Nations, Rome, <ftp://ftp.fao.org/docrep/fao/010/ag275e/ag275e.pdf> (accessed 8 September 2017)
- Otte, M.J. and Gumm, I.D. (1997) Intra-cluster correlation coefficients of 20 infections calculated from the results of cluster-sample surveys. *Preventive Veterinary Medicine*, **31**, 147–150
- Otte, M.J., Nugent, R. and McLeod, A. (2004) *Transboundary Animal Diseases: Assessment of Socio-economic Impacts and Institutional Responses*. Livestock Policy Discussion Paper No. 9. Food and Agriculture Organization of the United Nations, Rome
- Ouagal, M., Hendriks, P., Saegerman, C. and Berkvens, D. (2010) Comparison between active and passive surveillance within the network of epidemiological surveillance of animal diseases in Chad. *Acta Tropica*, **116**, 147–151
- Ouelett, B.L., Romeder, J.M. and Lance, J.M. (1979) Premature mortality attributable to smoking and hazardous drinking in Canada. *American Journal of Epidemiology*, **109**, 451–463
- Overballe-Petersen, S., Harms, K., Orlando, L.A.A., Mayar, V.M., Rasmussen, S. *et al.* (2013) Bacterial natural transformation by highly fragmented and damaged DNA. *Proceedings of the National Academy of Sciences of the United States of America*, **10**, 19860–19865
- Overgaauw, P.A.M. (1996) Effect of a government educational campaign in the Netherlands on awareness of *Toxocara* and toxocarosis. *Preventive Veterinary Medicine*, **28**, 165–174
- Owen, J.M. (1985). Preventive medicine in stud and stable. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Reading, 27–29 March 1985. Ed. Thrusfield, M.V., pp. 56–60
- Owen, L.N. (1979) A comparative study of canine and human breast cancer. *Investigative & Cell Pathology*, **2**, 257–275
- Owen, M. (1989) *SPC and Continuous Improvement*. IFS Publications/Springer-Verlag, Berlin
- Oxford, J.S. (1985) Biochemical techniques for the genetic and phenotypic analysis of viruses: 'Molecular Epidemiology'. *Journal of Hygiene, Cambridge*, **94**, 1–7
- Oxford English Dictionary* (1971) *The Compact Edition of the Oxford English Dictionary*, 2 vols. Oxford University Press, Oxford
- Oxman, A.D. and Guyatt, G.H. (1993) The science of reviewing research. *Annals of the New York Academy of Sciences*, **703**, 125–134
- Padmawati, S. and Nichter, M. (2008) Community response to avian flu in Central Java, Indonesia. *Anthropology & Medicine*, **15**, 31–51

- Page, E.S. (1961) Cumulative sum charts. *Technometrics*, **3**, 1–9
- Page, S., Davenport, M. and Hewitt, A. (1991) *The GATT Uruguay Round: Effects on Developing Countries*. Overseas Development Institute, London
- Paiba, G.A., Roberts, S.R., Houston, C.W., Williams, E.C., Smith, L.H. *et al.* (2007) UK surveillance: provision of quality assured information for combined datasets. *Preventive Veterinary Medicine*, **81**, 117–134
- Palatnik-de-Sousa, C.B. and Day, M.J. (2011) One Health: the global challenge of epidemic and endemic leishmaniasis. *Parasites & Vectors*, **4**, 197; doi 10.1186/1756-3305-4-197
- Palmer, L.J., Burton, P.R. and Smith, G.D. (2011) *An Introduction to Genetic Epidemiology*. The Policy Press, Bristol
- Palmer, S., Brown, D. and Morgan, D. (2005) Early qualitative risk assessment of the emerging zoonotic potential of animal diseases. *British Medical Journal*, **331**, 1256–1260
- Palmieri, T.L., Enkhbaatar, P., Bayliss, R., Traber, L.D., Cox, R.A. *et al.* (2006) Continuous nebulized albuterol attenuates acute lung injury in an ovine model of combined burn and smoke inhalation. *Critical Care Medicine*, **34**, 1719–1724
- Panagiotou, D. (2008) Trade bans, imperfect competition, and welfare: BSE and the US beef industry. Dissertation presented to the Faculty of the Graduate College at the University of Nebraska in partial fulfillment of requirements for the Degree of Doctor of Philosophy
- Pang, J., Modlin, J. and Yolken, R. (1992) Use of modified nucleotides and uracil-DNA glycosylase (UNG) for the control of contamination in the PCR-based amplification of RNA. *Molecular and Cellular Probes*, **6**, 251–256
- Panthier, R. (1968) Épidémiologie du virus West Nile. étude d'un foyer en Camargue. I. Introduction. *Annales de l'Institut Pasteur*, **114**, 518–520
- Paoloni, M.C. and Chanda, C.K. (2007) Comparative oncology today. *Veterinary Clinics of North America: Small Animal Practice*, **37**, 1023–1032
- Pappaioanou, M. (2004) Veterinary medicine protecting and promoting the public's health and well-being. *Preventive Veterinary Medicine*, **62**, 153–163
- Pappaioanou, M., Schwabe, C.W. and Polydorou, K. (1984) Epidemiological analysis of the Cyprus anti-echinococcosis campaign I. The prevalence of *Echinococcus granulosus* in Cypriot village dogs, the first dog-test period of the campaign, June–December, 1972. *Preventive Veterinary Medicine*, **3**, 159–180
- Parida, S. (2009) Vaccination against foot-and-mouth disease virus: strategies and effectiveness. *Expert Review of Vaccines*, **8**, 347–365
- Parish, H.J. (1965) *A History of Immunization*. Livingston, Edinburgh and London
- Park, P. (1989) What is participatory research? A theoretical and methodological perspective. In: *Voices of Change: Participatory Research in the United States and Canada*. Eds Park, P., Brydon-Miller, M., Hall, B. and Jackson, T., pp. 1–19. Bergin and Garvey, Westport
- Parker, G.A., Stedham, M.A. and Van Dellen, A. (1977) Myocarditis of probable viral origin in chickens. *Avian Diseases*, **21**, 123–132
- Parker, H.G., Shearin, A.L. and Ostrander, E.A. (2010) Man's best friend becomes biology's best show: genome analyses in the domestic dog. *Annual Review of Genetics*, **44**, 309–336
- Parker, J.E., Knowler, S.P., Rusbridge, C., Noorman, E. and Jeffery, N.D. (2011) Prevalence of asymptomatic syringomyelia in Cavalier King Charles spaniels. *Veterinary Record*, **168**, 667; doi 10.1136/vr.d1726
- Parkes, J.L., Slatin, S.L., Pardo, S. and Ginsberg, B.H. (2000) A new consensus error grid to evaluate the clinical significance of inaccuracies in the measurement of blood glucose. *Diabetes Care*, **23**, 1143–1148
- Parkin, T.D.H., Clegg, P.D., French, N.P., Proudman, C.J., Riggs, C.M. *et al.* (2005) Risk factors for fatal lateral condylar fracture of the third metacarpus/metatarsus in UK racing. *Equine Veterinary Journal*, **37**, 192–199
- Parrish, J.M. and Hunter, B.F. (1969) Waterfowl botulism in the southern San Joaquin Valley, 1967–68. *California Fish and Game*, **55**, 265–272
- Parsonnet, J. (1995) Bacterial infection as a cause of cancer. *Environmental Health Perspectives*, **103**, Suppl. 8, 263–268
- Parsons, J.H. (1982) Funding of control measures. In: *The Control of Infectious Diseases in Farm Animals*. British Veterinary Association Trust Project on the Future of Animal Health Control, London, pp. 58–62
- Parsons, T.D., Smith, G. and Galligan, D.T. (1986) Economics of porcine parvovirus vaccination assessed by decision analysis. *Preventive Veterinary Medicine*, **4**, 199–204
- Paston-Williams, S. (1993) *The Art of Dining. A History of Cooking and Eating*. The National Trust, London
- Pastoret, P.P. and Brochier, B. (1998) Epidemiology and elimination of rabies in Western Europe. *The Veterinary Journal*, **156**, 83–90
- Pastoret, P.-P., Blancou, J., Vannier, P. and Verschueren, C. (Eds) (1997) *Veterinary Vaccinology*. Elsevier, Amsterdam
- Pastorok, R.A. and Bilyard, G.R. (1985) Effects of sewage pollution on coral-reef communities. *Marine Ecology Progress Series*, **21**, 175–189

- Paterson, A.D. (1995) Problems encountered in the practical implementation of geographical information systems (GIS) in veterinary epidemiology. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Reading, 29–31 March 1995. Ed. Goodall, E.A., pp. 162–171
- Paterson, A.D., Otte, M.J., Slingenbergh, J., Wint, W. and Rogers, D. (2000) The application of GIS and remote sensing based modelling techniques, for use in the economic and epidemiological assessment of disease control interventions, at a regional or national level. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Edinburgh, 29–31 March 2000. Eds Thrusfield, M.V. and Goodall, E.A., pp. 172–182
- Paton, D.J., Füßel, A.-E., Vosloo, W., Dekker, A. and De Clercq, K. (2014) The use of serosurveys following emergency vaccination, to recover the status of 'foot-and-mouth disease free where vaccination is not practised'. *Vaccine*, **32**, 7050–7056
- Paton, G. and Gettinby, G. (1983) The control of a parasitic nematode population in sheep represented by a discrete time network with stochastic inputs. *Proceedings of the Royal Irish Academy*, **83B**, 267–280
- Paton, G. and Gettinby, G. (1985) Comparing control strategies for parasitic gastro-enteritis in lambs grazed on previously contaminated pasture: a network modelling approach. *Preventive Veterinary Medicine*, **3**, 301–310
- Paton, G., Thomas, R.J. and Waller, P.J. (1984) A prediction model for parasitic gastro-enteritis in lambs. *International Journal of Parasitology*, **14**, 439–445
- Paton, L.A., Milne, C.E. and Robers, D.J. (2015) What have we done to animal health planning? In: 20th International Farm Management Congress, Laval University, Québec City, Canada, 12–17 July 2015, pp. 402–409
- Patronek, G.J., Beck, A.M. and Glickman, L.E. (1997) Dynamics of dog and cat populations in a community. *Journal of the American Veterinary Medical Association*, **210**, 637–642
- Patterson, D.F. (1968) Epidemiologic and genetic studies of congenital heart disease in the dog. *Circulation Research*, **23**, 171–202
- Patterson, D.F. (1980) A catalog of genetic disorders of the dog. In: *Current Veterinary Therapy VII. Small Animal Practice*. Ed. Kirk, R.W., pp. 82–103. W.B. Saunders Company, Philadelphia
- Patterson, D.F. (1993) Understanding and controlling inherited diseases in dogs and cats. *Tijdschrift voor Diergeneeskunde*, **118**, Suppl. 1, 23S–27S
- Patterson, D.F. (2000) Companion animal medicine in the age of medical genetics. *Journal of Veterinary Internal Medicine*, **14**, 1–9
- Patterson, D.F. and Medway, W. (1966) Hereditary diseases of the dog. *Journal of the American Veterinary Medical Association*, **149**, 1741–1754
- Patterson, D.F., Haskins, M.E. and Jezyk, P.F. (1982) Models of human genetic disease in domestic animals. In: *Advances in Human Genetics*, Vol. 12. Eds Harris, H. and Hirschhorn, K., pp. 263–339. Plenum Press, New York and London
- Patterson, D.F., Pyle, R.L., Buchanan, J.W., Trautveter, E. and Abt, D.A. (1971) Hereditary patent ductus arteriosus and its sequellae in the dog. *Circulation Research*, **29**, 1–13
- Pattnaik, P. (2006) Kyasanur forest disease: an epidemiological view in India. *Reviews in Medical Virology*, **16**, 151–165
- Patz, J.A., Campbell-Lendrum, D., Holloway, T. and Foley, J.A. (2005) Impact of regional climate on human health. *Nature*, **438**, 310–317
- Paul, J.R. (1965) Serological epidemiology and the function of serum banks. *Archiv für die gesamte Virusforschung*, **17**, 465–471
- Paul, J.R. and White, C. (Eds) (1973) *Serological Epidemiology*. Academic Press, New York and London
- Paul, S.R. and Donner, A. (1989) A comparison of tests of homogeneity of odds ratios in $K \times 2$ tables. *Statistics in Medicine*, **8**, 1455–1468
- Pavlin, D., Cemazar, M., Sersa, G. and Tozon, N. (2012) IL-12 based gene therapy in veterinary medicine. *Journal of Translational Medicine*, **10**, 234; doi 10.1186/1479-5876-10-234
- Pavlovsky, E.N. (1964) *Prirodnaya Ochagovost' Transmissivnykh Bolezney v Svyazi s Landshaftnoy Epidemiologiyey Zooantroponozo*. Translated as *Natural Nidality of Transmissible Disease with Special Reference to the Landscape Epidemiology of Zooanthroponoses*. F.K. Plous (translator), N.D. Levine (Ed.) (1966). University of Illinois Press, Urbana
- Payne, A.M. (1963) Basic concepts of eradication. *American Review of Respiratory Diseases*, **88**, 449–455
- Payne, J.M., Dew, S.M., Manston, R. and Faulks, M. (1970) The use of a metabolic profile test in dairy herds. *Veterinary Record*, **87**, 150–158
- Payne, L.N. (1982) Breeding resistant stock. In: *The Control of Infectious Diseases in Farm Animals*. British Veterinary Association Trust Project on the Future of Animal Health Control, London, pp. 37–42
- Payton, M.E., Greenstone, M.H. and Schenker, N. (2003) Overlapping confidence intervals or standard error intervals: what do they mean in terms of statistical significance? *Journal of Insect Science*, **3** (34), 1–6; doi 10.1673/031.003.3401
- Pearce, D.W. and Sturme, S.G. (1966) Private and social costs and benefits: a note on terminology. *Economic Journal*, March **1966**, 152–158

- Pearce, N. (2012) Classification of epidemiological study designs. *International Journal of Epidemiology*, **41**, 393–397
- Pearce, N. and Douwes, J. (2013) Research interface between human and veterinary health. *Preventive Veterinary Medicine*, **111**, 187–193
- Pearl, J. (2000) *Causality: Models, Reasoning and Inference*. Cambridge University Press, New York
- Pearl, J. (2009a) *Causality: Models, Reasoning, and Inference*, 2nd edn. Cambridge University Press, New York
- Pearl, J. (2009b) Causal inference in statistics: an overview. *Statistics Surveys*, **3**, 96–146
- Pearson, K. (1904) Report on certain enteric fever inoculations statistics. *British Medical Journal*, **3**, 1243–1246
- Pearson, K. (1911) *The Grammar of Science. Part 1. Physical*, revised 3rd edn. Adam and Charles Black, London
- Pearson, R.A. (1983) Prevention of foot lesions in broiler breeder hens kept in individual cages. *British Poultry Science*, **24**, 183–190
- Pease, M. (1947) How long do poultry breeding stock live? *Agriculture*, **54**, 263–266
- Pedersen, A.B., Jones, K.E., Nunn, C.L. and Altizer, S. (2007) Infectious diseases and extinction risk in wild mammals. *Conservation Biology*, **21**, 1269–1279
- Pedgley, D.E. (1983) Windborne spread of insect-transmitted diseases of animals and man. *Philosophical Transactions of the Royal Society of London, Series B*, **302**, 463–470
- Peeler, E.J., Reese, R.A. and Thrush, M.A. (2015) Animal disease import risk analysis – a review of current methods and practice. *Transboundary and Emerging Diseases*, **62**, 480–490
- Pelander, L., Ljungvall, I., Egenvall, A., Syme, H., Elliott, J. *et al.* (2015) Incidence of and mortality from kidney disease in over 600,000 insured Swedish dogs. *Veterinary Record*, **176**, 656; doi 10.1136/vr.103059
- Pencina, M.J., D'Agostino Sr, R.B., D'Agostino Jr, R.B. and Vasan, R.S. (2008) Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Statistics in Medicine*, **27**, 157–172 (commentaries: 173–206)
- Pencina, M.J., D'Agostino, R.B., Larson, M.G., Massaro, J. M. and Vasan, R.S. (2009) Predicting the 30-year risk of cardiovascular disease: the Framingham Heart Study. *Circulation*, **119**, 3078–3084
- Penell, J.C., Egenvall, A., Bonnett, B.N. Olson, P. and Pringle, J. (2007) Validation of computerised Swedish horse insurance data against veterinary clinical records. *Preventive Veterinary Medicine*, **82**, 236–251
- Penny, R.H.C., Cameron, R.D.A., Johnson, S., Kenyon, P. J., Smith, H.A. *et al.* (1980) Foot rot of pigs: the influence of biotin supplementation on foot lesions in sows. *Veterinary Record*, **107**, 350–351
- Penrose, R. (2004) *The Road to Reality: A Complete Guide to the Laws of the Universe*. Jonathan Cape, London
- Pepe, M.S. (2003) *The Statistical Evaluation of Medical Tests for Classification and Prediction*. Oxford University Press, New York
- Peralta, E.A., Carpenter, T.E. and Farver, T.B. (1982) The application of time series analysis to determine the pattern of foot-and-mouth disease in cattle in Paraguay. *Preventive Veterinary Medicine*, **1**, 27–36
- Perel, P., Roberts, I., Sena, E., Wheble, P., Briscoe, C. *et al.* (2007) Comparison of treatment effects between animal experiments and clinical trials: systematic review. *British Medical Journal*, **334**, 197–200
- Perera, F.P. and Weinstein, I.B. (1982) Molecular epidemiology and carcinogen-DNA adduct detection: new approaches to studies of human cancer causation. *Journal of Chronic Diseases*, **35**, 581–600
- Perez, A.M., Ward, M.P. and Ritacco, V. (2011) Modelling the feasibility of bovine tuberculosis eradication in Argentina. *Revue Scientifique et Technique, Office International des Epizooties*, **30**, 635–643
- Perneger, T.V. (1998) What's wrong with Bonferroni adjustments? *British Medical Journal*, **316**, 1236–1238
- Perrin, T. (2009) The Business of Urban Animals Survey: the facts and statistics on companion animals in Canada. *Canadian Veterinary Journal*, **50**, 48–52
- Perry, B. and Grace, D. (2009) The impacts of livestock diseases and their control on growth and development processes that are pro-poor. *Philosophical Transactions of the Royal Society of London, Series B*, **364**, 2643–2655
- Perry, B.D. and McCauley, E.H. (1984) Owner interview surveys as a basis for estimating animal productivity and disease impact in developing countries. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Edinburgh, 10–11 July 1984. Ed. Thrusfield, M.V., pp. 54–62
- Perry, B.D., Lessard, P., Norval, R.A.I., Kundert, K. and Kruska, R. (1990) Climate, vegetation and the distribution of *Rhipicephalus appendiculatus* in Africa. *Parasitology Today*, **6**, 100–104
- Perry, B.D., Matthewman, R.W., Eicher, E. and Snacken, M. (1983) An evaluation of the efficacy of a questionnaire survey by comparison with data from sentinel herds. In: Proceedings of the Third International Symposium on Veterinary Epidemiology and Economics. Arlington, Virginia, 6–10 September 1982, p. 667. Veterinary Medical Publishing Company, Edwardsville
- Perry, B.D., Mwanauma, B., Schels, H.F., Eicher, E. and Zaman, M.R. (1984) A study of health and productivity

- in traditionally managed cattle in Zambia. *Preventive Veterinary Medicine*, **2**, 633–653
- Perry, R.N. (2012) Understanding the survival strategies of nematodes. In: *Animal Science Reviews 2011*. Ed. Hemming, D., pp. 99–103. CABI, Wallingford
- Persing, D.H., Smith, T.F., Tenover, F.C. and White, T.J. (Eds) (1993) *Diagnostic Molecular Microbiology. Principles and Applications*. American Society for Microbiology, Washington DC
- Peters, A.R., Martinez, T.A. and Cook, A.J.C. (2000) A meta-analysis of studies of the effect of GnRH 11–14 days after insemination on pregnancy rates in cattle. *Theriogenology*, **54**, 1317–1326
- Peters, J.L. and Mengersen, K.L. (2008) Meta-analysis of repeated measures study designs. *Journal of Evaluation in Clinical Practice*, **14**, 941–950
- Peterse, D.J. and Antonisse, W. (1981) Genetic aspects of feet soundness in cattle. *Livestock Production Science*, **8**, 253–261
- Petersen, G.R., Weis, D. and Van Der Valk, P.C. (1992) Vaccinations against *Mycoplasma hyopneumoniae* in swine. *Pig Veterinary Journal*, **28**, 35–39
- Petersen, G.V. (1983) The effect of swimming lambs and subsequent resting periods on the ultimate pH of meat. *Meat Science*, **9**, 237–246
- Peterson, E.H. and Hymas, T.A. (1951) Antibiotics in the treatment of an unfamiliar turkey disease. *Poultry Science*, **30**, 466–468
- Petitti, D.B. (1991) Associations are not effects. *American Journal of Epidemiology*, **133**, 101–102
- Peto, R. (1978) Clinical trial methodology. *Biomedicine Special*, **28**, 24–36
- Peto, R. (1984) The need for ignorance in cancer research. In: *The Encyclopedia of Medical Ignorance*. Eds. Duncan, R. and Weston-Smith, M., pp. 129–133. Pergamon Press, Oxford
- Petrie, A. and Watson, P. (2013) *Statistics for Veterinary and Animal Science*, 3rd edn. Wiley-Blackwell, Chichester
- Petrovski, K.R., Heuer, C., Parkinson, T.J. and Williamson, N.B. (2009) The incidence and aetiology of clinical bovine mastitis on 14 farms in Northland, New Zealand. *New Zealand Veterinary Journal*, **57**, 109–115
- Petters, R.M. and Sommer, J.R. (2000) Transgenic animals as models for human disease. *Transgenic Research*, **9**, 347–351
- Petticrew, M.P., Sowden, A.J., Lister-Sharp, D. and Wright, K. (2000) False-negative results in screening programmes: systematic review of impact and implications. *Health Technology Assessment*, **4** (5), 1–60; doi 10.3310/hta4050
- Pfeiffer, G. and Behera, D.K. (1997) *Contemporary Society: Tribal Studies. Volume 1. Structure and Process*. Concept Publishing Company, New Delhi
- Pfeiffer, D.U. (1996) Survey data collection methods – questionnaires and interviews. In: *Primary Animal Health Activities in Southern Africa*. Proceedings of an International Seminar held in Mzuzu, Malawi, February 26 to March 8, 1996. Eds Zimmerman, W., Pfeiffer, D.U. and Zessin, K.-H., pp. 159–169. Deutsche Stiftung für internationale Entwicklung (DSE) and Zentralstelle für Ernährung Landwirtschaft (ZEL), Feldafing
- Pfeiffer, D.U. (2000) Spatial analysis – a new challenge for veterinary epidemiologists. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings*, Edinburgh, 29–31 March 2000. Eds Thrusfield, M.V. and Goodall, E.A., pp. 86–106
- Pfeiffer, D. (2004) Science, epidemiological models and decision making. *The Veterinary Journal*, **167**, 123–124
- Pfeiffer, D.U. and Hugh-Jones, M.E. (2002) Geographical information systems as a tool in epidemiological assessment and wildlife disease management. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 91–99
- Pfeiffer, D.U., Robinson, T.P., Stevenson, M., Stevens, K. B., Rogers, D.J. et al. (2008) *Spatial Analysis in Epidemiology*. Oxford University Press, Oxford.
- PFMA (2015) *Pet Food Manufacturers' Association, Statistics*. <http://www.pfma.org.uk/statistics/> (accessed 11 August 2015)
- Pharo, H.J. (1983) DAISY – health and fertility monitoring for dairy herds. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings*, Southampton, 12–13 April 1983. Ed. Thrusfield, M.V., pp. 37–44
- Pharo, H.J. (1999) *Import Risk Analysis. Imported Seropositive Animals: Assurance Provided by Serological Tests*. Ministry of Agriculture and Forestry, Wellington
- Pharo, H.J. (2001) *Import Risk Analysis. Avian Paramyxovirus Type 1 in Hens' Hatching Eggs*. Ministry of Agriculture and Forestry, Wellington
- Pharo, H.J. (2002a) Foot-and-mouth disease: an assessment of the risks facing New Zealand. *New Zealand Veterinary Journal*, **50**, 46–55
- Pharo, H.J. (2002b) Determination of the acceptable level of risk of introduction of FMD virus in passenger luggage following the UK outbreak in 2001. In: *Proceedings of Australian College of Veterinary Scientists (Veterinary Epidemiology chapter)*, College Science Week, Gold Coast Australia, 1–6 July 2002. The Australian College of Veterinary Scientists, Eight Mile Plains. <http://www.biosecurity.govt.nz/pests-diseases/animals/risk/paper-acceptable-risk-fmd.pdf> (accessed 29 June 2006)

- Pharo, H.J. (2004) Acceptable risk in animal biosecurity import risk analysis – the limits of rationalism. *Public Sector*, **27** (4), 8–14
- Pharo, H.J., Esslemont, R.J. and Putt, S.N.H. (1984) Assessing the benefits and costs of a computerised information system in dairy herd management. In: Society for Veterinary Epidemiology and Preventive Medicine Proceedings, Edinburgh 10–11 July 1984. Ed. Thrusfield, M.V., pp. 80–89
- PHE (2015) *Risk Assessment of the Ebola Virus Disease Outbreak in West Africa: Updated 24 June 2015*. PHE (Public Health England) Publications Gateway No. 2014578, published (v7): 24 June 2015. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/437990/PHE_Risk_Assessment_Update_7_FINAL.pdf (accessed 2 November 2015)
- Philander, S.G.H. (1990) *El Niño, La Niña and the Southern Oscillation*. Academic Press, San Diego
- Phillipson, D.W. and Reynolds, A. (1996) B.I.E.A. excavation at Axum, northern Ethiopia 1995. *Azania*, **31**, 99–147
- Pià, L.M. (2007) Review of mathematical models for sow herd management. *Livestock Science*, **106**, 107–119
- Piantadosi, S., Byar, D.P. and Green, S.B. (1988) The ecological fallacy. *American Journal of Epidemiology*, **127**, 893–904
- Pierrepont, C.G., Thomas, S. and Eaton, C.L. (1984) Hormones and cancer. In: *Progress in Cancer Research and Therapy*, Vol. 31. Eds Bresciani, F., King, R.J.B., Lippman, M.E., Namer, M. and Raynaud, J.-P., pp. 357–365. Raven Press, New York
- PigCHAMP (2013) *Summary of the 2012 Benchmarking Data, US*. http://benchmark.farms.com/2013_Summary_of_the_2012_Benchmark_data.html# (accessed 23 April 2014)
- PigCHAMP (2017) *PigCHAMP Knowledge Software*. PigCHAMP, Ames. <http://www.pigchamp.com/> (accessed 25 July 2017)
- Pigott, T.D. (2001) A review of methods for missing data. *Educational Research and Evaluation*, **7**, 353–383
- Pike, M.C. and Casagrande, J.T. (1979) Re: cost considerations and sample size requirements in cohort and case-control studies (Letter). *American Journal of Epidemiology*, **110**, 100–102
- Pike, M.C. and Smith, P.G. (1968) Disease clustering: a generalization of Knox's approach to the detection of space-time interactions. *Biometrics*, **24**, 541–556
- Pilchard, E.I. (1985) Standardized nomenclature of veterinary medicine: taking the next step. *Journal of the American Veterinary Medical Association*, **187**, 798–799
- Pilcher, N. (2010) Population structure and growth of immature green turtles at Mantanani, Sabah, Malaysia. *Journal of Herpetology*, **44**, 168–171
- Pinchbeck, G.L., Clegg, P.D., Proudman, C.J., Morgan, K.L. and French, N.P. (2004a) A prospective cohort study to investigate risk factors for horse falls in UK hurdle and steeplechase racing. *Equine Veterinary Journal*, **36**, 595–601
- Pinchbeck, G.L., Clegg, P.D., Proudman, C.J., Stirk, A., Morgan, K.L. *et al.* (2004b) Horse injuries and racing practices in National Hunt racehorses in the UK: the results of a prospective cohort study. *Veterinary Journal*, **167**, 45–52
- Pinho, S.S., Carvalho, S., Cabral, J., Reis, C.A. and Gärtner, F. (2012) Canine tumors: a spontaneous animal model of human carcinogenesis. *Translational Research*, **159**, 165–172
- Pinney, M.E. (1981) How I learned to live with the computer. *Veterinary Record*, **109**, 431–432
- Pinto, R.C. and Blair, J.A. (1993) *Reasoning: A Practical Guide*. Prentice Hall, Engelwood Cliffs
- PIRSA (2015) *Enhanced Abattoir Surveillance Program*. Primary Industries and Regions SA. http://pir.sa.gov.au/biosecurity/animal_health/sheep/sheep_health_and_diseases/enhanced_abattoir_surveillance_program (accessed 24 July 2015)
- Pisano, R.G. and Storer, T.I. (1948) Burrows and feeding of the Norway rat. *Journal of Mammalogy*, **29**, 374–383
- Pitkänen, A., Buckmaster, P.S., Galanopoulou, A.S. and Moshé, S.L. (Eds) (2017) *Models of Seizures and Epilepsy*, 2nd edn. Academic Press, London
- Pivnick, H. and Nurmi, E. (1982) The Nurmi concept and its role in the control of *Salmonellae* in poultry. In: *Developments in Food Microbiology – 1*. Ed. Davies, R., pp. 41–70. Applied Science Publishers, Barking
- Plackett, P. and Alton, G.G. (1975) A mechanism for prozone formation in the complement fixation test for bovine brucellosis. *Australian Veterinary Journal*, **52**, 136–140
- Plant, J.D. (2007) Repeatability and reproducibility of numerical rating scales and visual analogue scales for canine pruritus severity scoring. *Veterinary Dermatology*, **18**, 294–300
- Plavšić, B., Nedić, D., Mićović, Z., Tešić, M., Stanojević, S. *et al.* (2009) Veterinary information management system (VIMS) in the process of notification and management of animal diseases. *Acta Veterinaria (Beograd)*, **59**, 99–108
- Pleguezuelo, R.H., Pérez, J.G. and Rambaud, S.C. (2003) A note on the reasonableness of PERT hypotheses. *Operations Research Letters*, **31**, 60–62
- Plowright, R.C. and Paloheimo, J.E. (1977) A theoretical study of population dynamics in the sheep tick. *Theoretical Population Biology*, **12**, 286–297
- Plowright, W. (1998) *Professional Commitment to the Success of the Global Rinderpest Eradication*

- Campaign*. Technical Consultation on the Global Rinderpest Eradication Programme, FAO Rome, 28–30 September 1998
- Plowright, W., Ferris, R.D. and Scott, G.R. (1960) Blue wildebeest and the aetiological agent of bovine malignant catarrhal fever. *Nature, London*, **188**, 1167–1169
- Plowright, W., Perry, C.T. and Greig, A. (1974) Sexual transmission of African swine fever virus in the tick, *Ornithodoros moubata porcinus*, Walton. *Research in Veterinary Science*, **17**, 106–113
- Plumiers, F.H., Akkerman, A.M., van der Wal, P., Dekker, A. and Bianchi, A. (2002) Lessons from the foot and mouth disease outbreak in the Netherlands in 2001. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 711–721
- Plutynski, A. (2011) Four problems of abduction: a brief history. *HOPPOS*, **1**, 227–248
- Pocock, S.J. (1977) Group sequential methods in the design and analysis of clinical trials. *Biometrika*, **64**, 191–199
- Pocock, S.J. (1983) *Clinical Trials: A Practical Approach*. John Wiley and Sons, Chichester and New York
- Pocock, S.J. and Hughes, M.D. (1990) Estimation issues in clinical trials and overviews. *Statistics in Medicine*, **9**, 657–671
- Pocock, S.J. and Simon, R. (1975) Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. *Biometrics*, **31**, 101–115
- Pointon, A. and Hueston, W.D. (1990) The National Animal Health Monitoring System (NAHMS): evolution of an animal health information database system in the USA. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Belfast, 4–6 April 1990. Ed. Thrusfield, M.V., pp. 70–82
- Poisson, S.-D. (1837) *Probabilité des jugements en matière criminelle et en matière civile, précédées des règles générales du calcul des probabilités*. Bachelier, Paris
- Polack, E.W., King, J.M., Cummings, J.F., Mohammed, H. O., Birch, M. *et al.* (2000) Concentrations of trace minerals in the spinal cord of horses with equine motor neuron disease. *American Journal of Veterinary Research*, **61**, 609–611
- Pollard, J.C., Littlejohn, R.P., Johnstone, P., Laas, F.J., Corson, I.D. *et al.* (1992) Behavioural and heart rate responses to velvet antler removal in red deer. *New Zealand Veterinary Journal*, **40**, 65–61
- Pollari, F.L., Bonnett, B.N., Allen, D.G., Bamsey, S.C. and Martin, S.W. (1996) Quality of computerized medical record abstract data at a veterinary teaching hospital. *Preventive Veterinary Medicine*, **27**, 141–154
- Pollitzer, R. and Meyer, K.F. (1961) The ecology of plague. In: *Studies in Disease Ecology*. Ed. May, J.M., pp. 433–510. Hafner Publishing, New York
- Polydorou, K. (1980) The epizootiology, diagnosis and control of bluetongue. *Bulletin de l'Office International des Epizooties*, **92**, 557–565
- Polzin, D.J., Lund, E., Walter, P. and Klausner, J. (2000) From journal to patient: evidence-based medicine. In: *Kirk's Current Veterinary Therapy XIII*. Ed. Bonagura, J.D., pp. 2–8. W.B. Saunders, Philadelphia
- Pomeroy, B.S. and Fenstermacher, R. (1937) Haemorrhagic enteritis in turkeys. *Poultry Science*, **16**, 378–382
- Pommier, P., Keita, A., Pagot, E. and Flochlay, A. (2000) Field efficacy of a *Mycoplasma hyopneumoniae* vaccine in the control of enzootic pneumonia in swine. *Revue de Médecine Vétérinaire*, **151**, 835–840
- Pong, A. and Chow, S.-C. (Eds) (2010) *Handbook of Adaptive Designs in Pharmaceutical and Clinical Development*. CRC Press, Boca Raton
- Poppensiek, G.C., Budd, D.E. and Scholtens, R.G. (1966) *A Historical Survey of Animal-Disease Morbidity and Mortality Reporting*. Publication No. 1346. National Academy of Sciences, Washington DC
- Popper, K.R. (1959) *Logik der Forschung*. Translated as *The Logic of Scientific Discovery*. Popper, K.R., Freed, J. and Freed, L. (translators). Hutchison, London
- Porphyre, T., Stevenson, M.A. and McKenzie, J. (2008) Risk factors for bovine tuberculosis in New Zealand cattle farms and their relationship with possum control strategies. *Preventive Veterinary Medicine*, **86**, 93–106
- Porrello, A., Cardelli, P. and Spugnini, E.P. (2006) Oncology of companion animals as a model for humans. An overview of tumor histotypes. *Journal of Experimental & Clinical Cancer Research*, **25**, 97–105
- Porta, M. (2014) *A Dictionary of Epidemiology*, 6th edn. Oxford University Press, Oxford
- Porter, P. (1979) Hazards of recycling enteropathogens (Letter). *Veterinary Record*, **105**, 515–516
- Porter, R. (1997) *The Greatest Benefit to Mankind: A Medical History of Humanity from Antiquity to the Present*. Harper Collins, London
- Porter, R., Norman, R.A. and Gilbert, L. (2013) An alternative to killing? Treatment of reservoir hosts to control a vector pathogen in a susceptible species. *Parasitology*, **140**, 247–257
- Porter, T.M. (1995) *Trust in Numbers: the Pursuit of Objectivity in Science and Public Life*. Princeton University Press, Princeton
- Potter, K., Kniss, J., Riesenfeld, R. and Johnson C. (2010) Visualizing summary statistics and uncertainty. In: *Eurographics/IEEE-VGTC Symposium on Visualization 2010*, Vol. 29 (3). Eds Melançon, G., Munzer, T. and Weiskopf, D., unpaginated. Blackwell Publishing, Oxford
- Pouillot, R., Gerbier, G. and Gardner, I.A. (2002) 'TAGS', a program for the evaluation of test accuracy in the

- absence of a gold standard. *Preventive Veterinary Medicine*, **53**, 67–81
- Poulin, R. (1994) Meta-analysis of parasite-induced behavioural changes. *Animal Behaviour*, **48**, 137–146
- Poulin, R. (1995) 'Adaptive' changes in behaviour of parasitized animals: a critical review. *International Journal of Parasitology*, **25**, 1371–1383
- Poulin, R. (1998) *Evolutionary Ecology of Parasites*. Chapman and Hall, London
- Poulin, R. and Chappell, L.H. (Eds) (2002) Parasites in marine systems. *Parasitology*, **124**, Suppl. 2002, 81–207
- Poulin, R. and Forbes, M.R. (2012) Meta-analysis and research on host–parasite interactions: past and future. *Evolutionary Ecology*, **26**, 1169–1185
- Power, A.P. and Harris, S.A. (1973) A cost-benefit evaluation of alternative control policies for foot-and-mouth disease in Great Britain. *Journal of Agricultural Economics*, **24**, 573–597 (discussion: 598–600)
- Prabhakaran, P., Soman, M., Iyer, R.P. and Abraham, J. (1980) Common disease conditions among cattle slaughtered in Trichur municipality slaughterhouse, a preliminary study. *Kerala Journal of Veterinary Science*, **11**, 159–163
- Pratt, D.J., Greenway, P.J. and Gwynne, M.D. (1966) A classification of East African rangeland, with an appendix on terminology. *Journal of Applied Ecology*, **3**, 369–382
- Presi, P., Stärk, K.D.C., Knopf, L., Breidenbach, E., Sanaa, M. *et al.* (2008) Risk-based sampling versus random sampling for monitoring tetracycline residues in slaughtered calves in Switzerland. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Liverpool, 26–28 March 2008. Eds Peeler, E.J., Alban, L. and Russell, A., pp. 273–281
- Pretty, J.N. (1995) Participatory learning for sustainable agriculture. *World Development*, **23**, 1247–1263
- Price, P.V. (1979) *The Penguin Book of Spirits and Liqueurs*. Allen Lane, London
- Priester, W.A. (1964) *Standard Nomenclature of Veterinary Diseases and Operations*. USHEW/PHS National Cancer Institute, Bethesda. 1st edition revised 1966; 2nd (abridged) edition 1975
- Priester, W.A. (1971) *Coding Supplement to Standard Nomenclature of Veterinary Diseases and Operations*. USHEW/PHS National Cancer Institute, Bethesda. Revised 1977
- Priester, W.A. (1975) Collecting and using veterinary clinical data. In: *Animal Disease Monitoring*. Eds Ingram, D.G., Mitchell, W.F. and Martin, S.W., pp. 119–128. Charles C. Thomas, Springfield
- Pritchard, D.G., Carpenter, G.A., Morzaria, S.P., Harkness, J.W., Richards, M.S. *et al.* (1981) Effect of air filtration on respiratory disease in intensively housed veal calves. *Veterinary Record*, **109**, 5–9
- Pritchard, D.G., Little, T.W.A., Wrathall, A.E. and Jones, P. (1985) Epidemiology of leptospirosis in relation to reproductive disease in pigs. *The Pig Veterinary Society Proceedings*, **12**, 65–82
- Pritchard, G., Dennis, I. and Waddilove, J. (2005) Biosecurity: reducing disease risks to pig breeding herds. *In Practice*, **27**, 230–237
- Pritchard, W.R. (1986) Veterinary education for the 21st century. *Journal of the American Veterinary Medical Association*, **189**, 172–177
- Pritchard, W.R. (Ed.) (1989) *Current Status and Future Directions for Veterinary Medicine*. Pew National Veterinary Education Program Report. Duke University, Durham
- Pritchett, J., Thilmany, D., and Johnson, K. (2005) Animal disease economic impacts: a survey of literature and typology of research approaches. *International Food and Agribusiness Management Review*, **8**, 23–45
- Prothro, R.M. (1977) Disease and mobility: a neglected factor in epidemiology. *International Journal of Epidemiology*, **6**, 259–267
- Proudman, C.J. and Edwards, G.B. (1992) Validation of a centrifugation/flotation technique for the diagnosis of equine cestodiasis. *Veterinary Record*, **131**, 71–72
- Puffer, S., Torgerson, D.J. and Watson, J. (2005) Cluster randomized trials. *Journal of Evaluation in Clinical Practice*, **11**, 479–483
- Pugh, C.A., Bronsvoort, M. de C., Handel, I.G., Summers, K.M. and Clements, D.N. (2014) What can cohort studies in the dog tell us? *Canine Genetics and Epidemiology*, **1**, 5; doi 10.1186/2052-6687-1-5
- Pugh, C.A., Bronsvoort, B.M. de C., Handel, I.G., Querry, D., Rose, E. *et al.* (2016) Cumulative incidence and risk factors for limber tail in the Dogslife Labrador retriever cohort. *Veterinary Record*, **179**, 275; doi 10.1136/vr.103729
- Pugh, C.A., Summers, K.M., Bronsvoort, B.M.C., Handel, I.G. and Clements, D.N. (2015) Validity of Internet-based longitudinal study data: the elephant in the virtual room. *Journal of Medical Internet Research*, **17**, 4, e96; doi 10.2196/jmir.3530
- Pugsley, S.L. (1981) The veterinary computer system at London zoo. In: *A Computerised Veterinary Clinical Data Base*. Proceedings of a Symposium, Edinburgh, 30 September 1981. Ed. Thrusfield, M.V., p. 55. Department of Animal Health, Royal (Dick) School of Veterinary Studies, Edinburgh
- Purdey, M. (2000) Ecosystems supporting clusters of sporadic TSEs demonstrate excesses of the radical-generating divalent cation manganese and deficiencies of antioxidant co-factors Co, Se, Fe, Zn – does a foreign

- cation substitution at prion protein's Cu domain initiate TSE? *Medical Hypotheses*, **54**, 278–306
- Purse, B.V., Mellor, P.S., Rogers, D.J., Samuel, A.R., Mertens, P.P.C. *et al.* (2005) Climate change and the recent emergence of bluetongue in Europe. *Nature Reviews Microbiology*, **3**, 171–181
- QMMS (2017) *TotalVet, Dairy Herd Management and Production Software*. Quality Milk Management Services, Bath. <http://www.total-vet.co.uk> (accessed 25 July 2017)
- Quesenberry, C.P. and Hurst, D.C. (1964) Large sample simultaneous confidence intervals for multinomial proportions. *Technometrics*, **6**, 191–195
- Quetelet, L.A.J. (1835) *Sur l'homme et le développement de ses facultés*. Translated as *A Treatise on Man and the Development of His Faculties*. Knox, R. (translator) (1962). Burt Franklin, New York
- Quinn, M.M., Keuler, N.S., Lu, Y., Faria, M.L., Muir, P. *et al.* (2007) Evaluation of agreement between numerical rating scales, visual analogue scoring scales, and force plate gait analysis in dogs. *Veterinary Surgery*, **36**, 360–367
- Quinn, P.J. (1991) Disinfection and disease prevention in veterinary medicine. In: *Disinfection, Sterilization and Preservation*, 4th edn. Ed. Block, S.S., pp. 846–870. Lea and Febiger, London and Philadelphia
- Quinn, P.J., Markey, B.K., Leonard, F.C., FitzPatrick, E.S., Fanning, A. *et al.* (2011) *Veterinary Microbiology and Microbial Disease*, 2nd edn. Wiley-Blackwell, Chichester
- Råberg, L., Grahn, M., Hasselquist, D. and Svensson, E. (1998) On the adaptive significance of stress-induced immunosuppression. *Proceedings of the Royal Society of London, Series B*, **265**, 1637–1641
- Rabinowitz, P.M., Gordon, Z., Holmes, R., Taylor, B., Wilcox, M. *et al.* (2005) Animals as sentinels of human environmental health hazards: an evidence-based analysis. *EcoHealth*, **2**, 26–37
- Rabinowitz, P., Scotch, M. and Conti, L. (2009) Human and animal sentinels for shared health risks. *Veterinaria Italiana*, **45**, 23–24
- Rabinowitz, P., Wiley, J., Odofoin, L., Wilcox, M. and Dein, J. (2008) Animals as sentinels of chemical terrorism agents: an evidence-based review. *Clinical Toxicology*, **46**, 93–100
- Racine, R. and Winslow, G.M. (2009) IgM in microbial infections: taken for granted? *Immunology Letters*, **125**, 79–85
- Racloz, V., Hadhorn, D., Schwermer, H.P., Griot, C., Cagienard, A. *et al.* (2008) Bluetongue surveillance in Switzerland: from research to policy. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Liverpool, 26–28 March 2008. Eds Peeler, E.J., Alban, L. and Russell, A., pp. 45–53
- Radford, A.D., Noble, P.J., Coyne, K.P., Gaskell, R.M., Jones, P.H. *et al.* (2011) Antibacterial prescribing patterns in small animal veterinary practice identified through SAVSNET: the Small Animal Veterinary Surveillance Network. *Veterinary Record*, **169**, 310; doi 10.1136/vr.d5062
- Radford, A., Tierney, Á., Coyne, K.P., Gaskell, R.M., Noble, P.J. *et al.* (2010) Developing a network for small animal disease surveillance. *Veterinary Record*, **167**, 472–474
- Radostits, O.M., Gay, C.C., Blood, D.C. and Hinchcliff, K. W. (1999) *Veterinary Medicine*, 9th edn W.B. Saunders, London
- Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. (2007) *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats*, 10th edn. W.B. Saunders, Philadelphia
- Ragan, V.E. (2002) The Animal and Plant Inspection Service (APHIS) brucellosis eradication program in the United States. *Veterinary Microbiology*, **90**, 11–18
- Ragland, W.L., Keown, G.H. and Spencer, G.R. (1970) Equine sarcoid. *Equine Veterinary Journal*, **2**, 2–11
- Rahmann, G. and Seip, H. (2007) Alternative management strategies to prevent and control endo-parasite diseases in sheep and goat farming systems – a review of recent scientific knowledge. *Landbauforschung Völknerode*, **57**, 75–88
- RAIZO (2015) *Réseau d'alerte et d'information zoosanitaire (RAIZO)*. <http://www.mapaq.gouv.qc.ca/fr/Productions/santeanimale/maladies/RAIZO/Pages/raizo.aspx> (accessed 14 August 2015)
- Rajoriya, R., Rajoriya, S. and Kumar, N. (2013) Transgenic animals: prospects for improving livestock productivity. *Journal of Bio Information*, **2**, 240–259
- Ramnial, V., Kosmider, R., Aylan, O., Freuling, C., Müller, T. *et al.* (2010) Quantitative risk assessment to compare the risk of rabies entering the UK from Turkey via quarantine, the Pet Travel Scheme and the EU Pet Movement Policy. *Epidemiology and Infection*, **138**, 1114–1125
- Ramsey, D.S.L. and Efford, M.G. (2010) Management of bovine tuberculosis in brushtail possums in New Zealand: predictions from a spatially explicit, individual-based model. *Journal of Applied Ecology*, **47**, 911–919
- Ramsey, F.K. and Chivers, W.H. (1953) Mucosal disease of cattle. *North American Veterinarian*, **34**, 629–633
- Rand, E.K. (1928) *Founders of the Middle Ages*. Harvard University Press, Cambridge
- Rand, M.S. (2008) Selection of biomedical animal models. In: *Sourcebook of Models for Biomedical Research*. Ed. Conn, P.M., pp. 9–15. Humana Press, Totowa
- Randall, A., Hillier, A., Cole, L.K., Kwochka, K.W., Needham, G. *et al.* (2003) Quantitation of house dust

- mites and house dust mite allergens in the microenvironment of dogs. *American Journal of Veterinary Research*, **64**, 1580–1588
- Randolph, S. (1994) Population dynamics and density-dependent seasonal mortality indices of the tick *Rhipicephalus appendiculatus* in eastern and southern Africa. *Medical and Veterinary Entomology*, **8**, 351–368
- Randolph, S.E. (2009) Tick-borne disease systems emerge from the shadows: the beauty lies in molecular detail, the message in epidemiology. *Parasitology*, **136**, 1403–1413
- Rangel, M.F.C., Cárendas Lara, J. and De Aluja, A.S. (1981) The canine population of Mexico City: an estimative study. *Animal Regulation Studies*, **3**, 281–290
- Ransohoff, D.F. and Feinstein, A.R. (1978) Problems of spectrum bias in evaluating the efficacy of diagnostic tests. *The New England Journal of Medicine*, **299**, 926–930
- Ranta, E., Kaitala, V., Lindstrom, J. and Linden, H. (1995) Synchrony in population dynamics. *Proceedings of the Royal Society of London, Series B*, **262**, 113–118
- Rapnicki, P., Eicker, S. and Stewart, S. (2012) DairyCOMP 305: utilizzo gestionale dei dati riproduttivi, *Large Animal Review*, **18**, 327–333 (in Italian)
- Rapoport, E. and Shimshony, A. (1997) Health hazards to the small ruminant population of the Middle East posed by the trade of sheep and goat meat. *Revue Scientifique et Technique, Office International des Epizooties*, **16**, 57–64
- Rapport, D.J., Costanza, R. and McMichael, A.K. (1998) Assessing ecosystem health. *Trends in Ecology and Evolution*, **13**, 397–402
- Ratcliffe, H. (1850) *Observations on the Mortality and Sickness Existing Among Friendly Societies: particularised for various trades, occupations, and localities, with a series of tables, shewing the value of annuities, sick gift, assurance for death, and contributions to be paid equivalent thereto: calculated from the experience of the members composing the Manchester Unity of the Independent Order of Odd Fellows*. George Falkener, Manchester. Reprinted in *Mortality in Mid 19th Century Britain*. Introduced by Wall, R. (1974). Gregg International, Amersham
- Ratcliffe, P.R. (1987) Red deer population changes and the independent assessment of population size. *Symposia of the Zoological Society of London*, **58**, 153–165
- Rawlings, P., Pro, M.J., Pena, I., Ortega, M.D. and Capela, R. (1997) Spatial and seasonal distribution of *Culicoides imicola* in Iberia in relation to the transmission of African horse sickness virus. *Medical and Veterinary Entomology*, **11**, 49–57
- Read, R.A., Wood, J.L.N. and Lakhani, K.H. (1998) Pectinate ligament dysplasia (PLD) and glaucoma in flat coated retrievers. I. Objectives, technique and results of a PLD survey. *Veterinary Ophthalmology*, **1**, 85–90
- Reaser, J.K., Gentz, E.J. and Clark, E.E. (2002) Wildlife health and environmental security: new challenges and opportunities. In: *Conservation Medicine: Ecological Health in Practice*. Eds Aguire, A.A., Ostfield, R.S., Tabor, G.M., House, C. and Pearl, M.C., pp. 383–395. Oxford University Press, New York
- Red Tractor Farm Assurance Dairy Scheme (2013) Home page. <http://assurance.redtractor.org.uk/rtassurance/global/home.eb> (accessed 25 November, 2013)
- Redmill, F. (2002) Risk analysis – a subjective process. *Engineering Management Journal*, **12**, 91–96
- Reece, R.L. and Beddome, V.D. (1983) Causes of culling and mortality in three flocks of broiler chickens in Victoria during 1979. *Veterinary Record*, **112**, 450–452
- Rees, W.G.H. and Davies, G. (1992) Legislation for health. In: *Livestock Health and Welfare*. Ed. Moss, R., pp. 118–159. Longman Scientific and Technical, Harlow
- Reeve-Johnson, L. (1998) Use of disease models in the development and evaluation of therapeutic trials. *Veterinary Record*, **142**, 638–642
- Reeves, M.J., Gay, J.M., Hilbert, B.J. and Morris, R.S. (1989) Association of age, sex and breed factors in acute equine colic: a retrospective study of 320 cases admitted to a veterinary teaching hospital in the USA. *Preventive Veterinary Medicine*, **7**, 149–160
- Reeves, M.J., Salman, M.D. and Smith G. (1996a) Risk factors for equine acute abdominal disease (colic): results from a multi-center case-control study. *Preventive Veterinary Medicine*, **26**, 285–301
- Reeves, M.J., Salman, M.D. and Smith, G. (1996b) Repeatability of equine health and management information obtained via a telephone questionnaire. *Preventive Veterinary Medicine*, **26**, 347–351
- Refaai, W., Van Aert, M., Abn El-Aal, A.M., Behery, A.E. and Opsomer, G. (2013) Infectious diseases causing lameness in cattle with a main emphasis on digital dermatitis (Mortellaro disease). *Livestock Science*, **156**, 53–63
- Regula, G., Torriani, K., Gassner, B., Stucki, F. and Müntener, C.R. (2009) Prescription patterns of antimicrobials in veterinary practices in Switzerland. *Journal of Antimicrobial Chemotherapy*, **63**, 805–811
- Reiczigel, J., Földi, J. and Ózsvári, L. (2010) Exact confidence limits for prevalence of a disease with an imperfect diagnostic test. *Preventive Veterinary Medicine*, **138**, 1674–1678

- Reid, H.W. (2002) FMD in a parturient sheep flock (Letter). *Veterinary Record*, **150**, 791
- Reid, J. and Nolan, A.M. (1991) A comparison of the post-operative analgesic and sedative effects of flunixin and papaveretum in the dog. *Journal of Small Animal Practice*, **32**, 603–608
- Reid, J., Scott, M., Nolan, A. and Wiseman-Orr, L. (2013) Pain assessment in animals. *In Practice*, **35**, 51–56
- Reid, R.S., Kruska, R.L., Deichmann, U., Thornton, P.K., and Leak, S.G.A. (2000) Human population growth and the extinction of the tsetse fly. *Agriculture Ecosystems and Environment*, **77**, 227–236
- Reid, S.W.J. and Peterson, M.M. (2000) Methods of estimating canine longevity. *Veterinary Record*, **147**, 630–631
- Reif, J.S. (1976) Seasonality, natality and herd immunity in feline panleukopenia. *American Journal of Epidemiology*, **103**, 81–87
- Reif, J.S. (1983) Ecologic factors and disease. In: *Textbook of Veterinary Internal Medicine. Volume 1. Diseases of the Dog and Cat*, 2nd edn. Ed. Ettinger, S.J., pp. 147–173. W.B. Saunders, Philadelphia
- Reif, J.S. (1986) Animal models for environmental epidemiology. In: *Practices in Veterinary Public Health and Preventive Medicine in the United States*. Ed. Woods, G.T., pp. 273–290. Iowa State University Press, Ames
- Reif, J.S. (2011) Animal sentinels for environmental and public health. *Public Health Reports*, **126**, Suppl. 1, 50–57
- Reif, J.S. and Cohen, D. (1970) Canine pulmonary disease. II. Retrospective radiographic analysis of pulmonary disease in rural and urban dogs. *Archives of Environmental Health*, **20**, 684–689
- Reif, J.S. and Cohen, D. (1971) The environmental distribution of canine respiratory tract neoplasms. *Archives of Environmental Health*, **22**, 136–140
- Reif, J.S. and Cohen, D. (1979) Canine pulmonary disease: a spontaneous model for environmental epidemiology In: *Animals as Monitors of Environmental Pollutants*, pp. 241–250. National Academy of Sciences, Washington DC
- Reif, J.S., Maguire, T.G., Kenney, R.M. and Brodey, R.S. (1979) A cohort study of canine testicular neoplasia. *Journal of the American Veterinary Medical Association*, **175**, 719–723
- Reif, J.S., Schweitzer, D.J., Ferguson, S.W. and Benjamin, S.A. (1983) Canine neoplasia and exposure to uranium mill tailings in Mesa County, Colorado. In: *Epidemiology Applied to Health Physics*. Proceedings of the 16th Midyear Topical Meeting of the Health Physics Society, Albuquerque, New Mexico, 9–13 January 1983, pp. 461–469. Rio Grande Chapter of the Health Physics Society
- Reijniers, J., Begon, M., Ageyev, V.S. and Leirs, H. (2014) Plague epizootic cycles in Central Asia. *Biology Letters*, **10**, 20140302; doi 10.1098/rsbl.2014.0302
- Reijniers, J., Davis, S., Begon, M., Heesterbeek, J.A.P., Ageyev, V.S. and Leirs, H. (2012) A curve of thresholds governs plague epizootics in Central Asia. *Ecology Letters*, **15**, 554–560
- Reilly, B.K. and van der Vyver, F. (2000) Helicopter-based censusing of domestic dogs in Gauteng Province, South Africa. *Journal of the South African Veterinary Association*, **71**, 187–191
- Reilly, W.J. (1996) Verotoxigenic *Escherichia coli* O157 in Scotland. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Glasgow, 27–29 March 1996. Eds Thrusfield, M.V. and Goodall, E.A., pp. 60–71
- Reinemeyer, C.R. and Nielsen, M.K. (2013) *Handbook of Equine Parasite Control*. Wiley-Blackwell, Ames
- Reingold, A. (2000) Outbreak investigation – a perspective. *Epidemiological Bulletin*, **21**, 1–7
- Reingold, A. (2003) If syndromic surveillance is the answer, what is the question? *Biosecurity and Bioterrorism*, **1**, 77–81
- Reinharz, S. (1983) Quoted by Kelly, L. (1988) *Surviving Sexual Violence*. Polity Press, Cambridge
- Renn, O. (1992) Concepts of risk: a classification. In: *Social Theories of Risk*. Eds. Krinsky, S. and Golding, D., pp. 53–79. Praeger, Westlort
- Report (1983) *Risk Assessment: A Study Group Report*. The Royal Society, London
- Report (1990) Potential use of live viral and bacterial vectors for vaccines. *Vaccine*, **8**, 425–437
- Report (1992) *Risk Analysis, Perception and Management. Report of a Royal Society Study Group*. The Royal Society, London
- Report (2000) *An Epidemiological Investigation into Bovine Tuberculosis*. Second Report of the Independent Scientific Group on Cattle TB. MAFF Publications, London
- Report (2007) *Bovine TB: The Scientific Evidence. A Science Base for a Sustainable Policy to Control TB in Cattle, An Epidemiological Investigation into Bovine Tuberculosis*. Final Report of the Independent Scientific Group on Cattle TB. Department for Environment, Food and Rural Affairs, London
- Resnick, M.P., Grunenwald, P., Blackmar, D., Hailey, C., Bueno, R. et al. (2008) Juvenile dogs as potential sentinels for West Nile virus. *Zoonoses and Public Health*, **55**, 443–447
- Reutlinger, S. (1970) Techniques for Project Appraisal Under Uncertainty. World Bank Staff Occasional Paper No. 10, Washington DC

- RevMan (2014) *Review Manager (RevMan) Version 5.3*. The Cochrane Collaboration, The Nordic Cochrane Centre, Copenhagen
- Rexstad, E. and Burnham, K.P. (1991) *User's Guide for Interactive Program CAPTURE*. Colorado Cooperative Fish and Wildlife Research Unit, Colorado State University, Fort Collins
- Reynal, J. (1873) *Traité de Police Sanitaire des Animaux Domestiques*. Asselin, Paris
- Reynolds, D. (2006) A review of tuberculosis science policy in Britain. *Veterinary Microbiology*, **112**, 119–126
- Ribble, C.S. (1989) Principles of implementing and marketing bovine health management programs. In: *Proceedings of the American Association of Bovine Practitioners*, **21**, 27–33
- Ribble, C.S., Janzen, E.D. and Campbell, J. (1998) Disease outbreak investigation in the beef herd: defining the problem. *Proceedings of the Annual Convention of the American Association of Bovine Practitioners*, **31**, 121–127
- Ribó, O., Korn, C., Meloni, U., Cropper, M., De Winne, P. *et al.* (2001) IDEA: a large-scale project on electronic identification of livestock. *Revue Scientifique et Technique, Office International des Epizooties*, **20**, 426–436
- Rice, D., Hancock, D. and Besser, T.E. (2003) Faecal culture of wild animals for *Escherichia coli* O157:H7. *Veterinary Record*, **152**, 82–83
- Rich, K.M. and Perry, B.D. (2011) The economic and poverty impacts of animal diseases in developing countries: new roles, new demands for economics and epidemiology. *Preventive Veterinary Medicine*, **101**, 133–147
- Richards, M. (1954) *The Laws of Hywel Dda (The Book of Blegywryd)*. The University of Liverpool Press, Liverpool
- Richardson, L.L. (2016) *Aurantimonas coralicida* – White Plague Type II. In: *Diseases of Coral*. Eds Woodley, C.M., Downs, C.A., Bruckner, A.W., Porter, J.W. and Galloway, S.B., pp. 231–235. John Wiley and Sons, Hoboken
- Richardson, W.S., Wilson, M.C., Nishikawa, J. and Hayward, R.S.A. (1995) The well-built clinical question: a key to evidence-based decisions. *ACP Journal Club*, **123**, A-12
- Rideout, B.A., Rotstein, D., Oaks, J.L., Sandfort, C., Hall, C. *et al.* (1997) An adenovirus outbreak in captive Aplomado falcons (*Falca femoralis septentrionalis*) causing high morbidity and mortality. *Proceedings of the American Association of Zoo Veterinarians, Annual Conference, Houston, Texas, October 29–30, 1997*, pp. 45–46
- Ridpath, J. (2010) The contribution of infections with bovine viral diarrhoea viruses to bovine respiratory disease. *Veterinary Clinics of North America: Food Animal Practice*, **26**, 335–348
- Rifkin, S.B., Llewondo-Hundt, G. and Draper, A.K. (1998) *Review of Participatory Approaches in Health Promotion and Planning*. Health Education Council/London School of Hygiene and Tropical Medicine, London
- Riggenbach, C. (1988) National animal health information system in Switzerland. *Revue Scientifique et Technique, Office International des Epizooties*, **7**, 503–507
- Rikihisa, Y. and Perry, B.D. (1985) Causative ehrlichial organisms in Potomac horse fever. *Infection and Immunity*, **49**, 513–517
- Riley, L.W. (2004) *Molecular Epidemiology of Infectious Diseases – Principles and Practices*. ASM Press, Washington DC
- Rinaldi, L., Fusco, G., Musella, V., Veneziano, V., Guarino, A. *et al.* (2005) *Neospora caninum* in pastured cattle: determination of climatic, environmental, farm management and individual animal risk factors using remote sensing and geographical information systems. *Veterinary Parasitology*, **128**, 219–230
- Rinaldi, L., Musella, V., Biggeri, A. and Cringoli, G. (2006) New insights into the application of geographical information systems and remote sensing in veterinary parasitology. *Geospatial Health*, **1**, 33–47
- Ripple, W.J., Painter, L.E., Beschta, R.L. and Gates, C.C. (2010) Wolves, elk, bison and secondary trophic cascades in Yellowstone National Park. *The Open Ecology Journal*, **3**, 31–37
- Risser, P.R. (1995) The status of the science examining ecotones – a dynamic aspect of landscape is the area of steep gradients between more homogeneous vegetation associations. *Bioscience*, **45**, 318–325
- Ristow, L.E., Silva, L.G.C., Fóscolo, C.B. and Silva, A.F. (2002) Comparison of *M. hyopneumoniae* vaccines and the influence of maternal antibodies. In: *Proceedings of the 17th Congress of the International Pig Veterinary Society Congress, June 2 – 5, 2002, Ames, Iowa. Volume 2: Poster Presentations*, p. 330
- Rivers, T.M. (1937) Viruses and Koch's postulates. *Journal of Bacteriology*, **33**, 1–12
- Robert, F., Renaud, F., Mathieu, F. and Gabrion, C. (1988) Importance of the paratenic host in the biology of *Bothriocephalus gregarious* (Cestoda, Psuedophyllidea), a parasite of turbot. *International Journal for Parasitology*, **18**, 611–621
- Roberts, D.F. (1985) A definition of genetic epidemiology. In: *Diseases of Complex Aetiology in Small Populations: Ethnic Differences and Research Approaches*. *Proceedings of a Symposium on Genetic Epidemiology in an Anthropological Context*, Victoria, British

- Columbia, August 18 and 19, 1983. Eds Chakraborty, R. and Szathmary, E.J.E., pp. 9–20. Alan R. Liss, New York
- Roberts, K., Smith, C.F., Snelling, A.M., Kerr, K.G., Banfield, K.R. *et al.* (2008) Aerial dissemination of *Clostridium difficile* spores. *BMC Infectious Diseases*, **8**, 7; doi 10.1186/1471-2334-8-7
- Roberts, L., Lawson, G.H.K., Rowland, A.C. and Laing, A. H. (1979) Porcine intestinal adenomatosis and its detection in a closed pig herd. *Veterinary Record*, **104**, 366–368
- Roberts, R.S., Spitzer, W.O., Delmore, T. and Sackett, D.S. (1978) An empirical demonstration of Berkson's bias. *Journal of Chronic Disease*, **31**, 119–128
- Robertson, F.J. (1971) Brucellosis: a possible symptomless carrier. *Veterinary Record*, **88**, 313–314
- Robin, C.A., Wylie, C.E., Wood, J.L.N. and Newton, J.R. (2011) Making use of equine population demography for disease control purposes: Preliminary observations on the difficulties of counting and locating horses in Great Britain. *Equine Veterinary Journal*, **43**, 372–375
- Robinson, L.E., Miranda, M.E., Miranda, N.L. and Childs, J.E. (1996) Evaluation of a canine rabies vaccination campaign and characterization of owned-dog populations in the Philippines. *Southeast Asian Journal of Tropical Medicine and Public Health*, **27**, 250–256
- Robinson, S.E. and Christley, R.M. (2007) Exploring the role of auction markets in cattle movements within Great Britain. *Preventive Veterinary Medicine*, **81**, 21–37
- Robinson, S.E., Everett, M.G. and Christley, R.M. (2007) Recent network evolution increases the potential for large epidemics in the British cattle population. *Journal of the Royal Society Interface*, **4**, 669–674
- Robinson, W.S. (1950) Ecological correlations and the behavior of individuals. *American Sociological Review*, **15**, 351–357
- Roble, G.S., Gillespie, V. and Lipman, N.S. (2012) Infectious disease survey of *Mus musculus* from pet stores in New York City. *Journal of the American Association for Laboratory Animal Science*, **51**, 37–41
- Robotham, J. and Green, L.E. (2004) Pilot study to investigate the feasibility of surveillance of small animals in the UK. *Journal of Small Animal Practice*, **45**, 213–218
- Robyn, M., Priyono, W.B., Kim, L.M. and Brum, E. (2012) Diagnostic sensitivity and specificity of a participatory disease surveillance method for highly pathogenic avian influenza in household chicken flocks in Indonesia. *Avian Diseases*, **56**, 377–380
- Rock, M., Buntain, B.J., Hatfield, J.M. and Hallgrímsson, B. (2009) Animal-human connections, 'One Health', and the syndemic approach to prevention. *Social Science & Medicine*, **68**, 991–995
- Roder, J.D. (2001) *Veterinary Toxicology*. Butterworth-Heinemann, Boston
- Rodrigues, C.A., Gardner, I.A. and Carpenter, T.E. (1990) Financial analysis of pseudorabies control and eradication in swine. *Journal of the American Veterinary Medical Association*, **197**, 1316–1323
- Rodrigues, L. and Kirkwood, B.R. (1990) Case-control designs in the study of common diseases: updates on the demise of the rare disease assumption and the choice of sampling schemes for controls. *International Journal of Epidemiology*, **19**, 205–213
- Roe, R.T. (1980) Features of the Australian National Animal Disease Information System. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 7–11 May 1979. Eds. Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 26–34. Australian Government Publishing Service, Canberra
- Roeder, P. and Rich, K. (2009) Conquering the cattle plague: The global effort to eradicate rinderpest. In: *Millions Fed: Proven Successes in Agricultural Development*. Eds Spielman, D.J. and Pandya-Lorch, R., pp. 109–116. IFPRI, Washington DC. <http://www.ifpri.org/sites/default/files/publications/oc64ch16.pdf> (accessed 25 September 2013)
- Roeder, P.L. and Taylor, W.P. (2002) Rinderpest. *Veterinary Clinics of North America: Food Animal Practice*, **18**, 515–547
- Roeder, P., Mariner, J. and Kock, R. (2013) Rinderpest: the veterinary perspective on eradication. *Philosophical Transactions of the Royal Society, Series B*, **368**, 20120139; doi 10.1098/rstb.2012.0139
- Rogan, W.J. and Gladen, B. (1978) Estimating prevalence from the results of a screening test. *American Journal of Epidemiology*, **107**, 71–76
- Rogers, D.J. (2000) Satellites, space, time and the African trypanosomiasis. *Advances in Parasitology*, **47**, 129–171
- Rogers, D.J. and Randolph, S.E. (2006) Climate change and vector-borne diseases. *Advances in Parasitology*, **62**, 345–381
- Rogowitz, B.E. and Treinish, L.A. (1998) Data visualization: the end of the rainbow. *IEEE Spectrum*, **35**, 52–59
- Rohani, P., Green, C.J., Mantilla-Beniers, N.B. and Grenfell, B.T. (2003) Ecological interference between fatal diseases. *Nature*, **422**, 885–888
- Rohlf, V. and Bennett, P. (2005) Perpetration-induced traumatic stress in persons who euthanize nonhuman animals in surgeries, animal shelters, and laboratories. *Society & Animals*, **13**, 201–219
- Rohr, J.R., Dobson, A.P., Johnson, P.T.J., Kilpatrick, A.M., Paull, S.H. *et al.* (2011) Frontiers in climate change – disease research. *Trends in Ecology and Evolution*, **26**, 270–277

- Rolfe, D.C. (1986) The development of an animal disease information system. In: Proceedings of the 4th International Symposium on Veterinary Epidemiology and Economics, Singapore, 18–22 November 1985, pp. 362–364. Singapore Veterinary Association, Singapore
- Rolin, A.I., Berrang-Ford, L. and Kulkarni, M.A. (2013) The risk of Rift Valley fever virus introduction and establishment in the United States and European Union. *Emerging Microbes and Infections*, **2** (12), e81; doi 10.1038/emi.2013.81
- Romagnano, A. (1999) Examination and preventive medicine protocols in psittacines. *Veterinary Clinics of North America: Exotic Animal Practice*, **2**, 333–355
- Roos, L.L., Sharp, S.M. and Wajda, A. (1989) Assessing data quality: a computerized approach. *Social Science & Medicine*, **28**, 175–182
- Roper, T.J., Garnett, B.T. and Delahay, R.J. (2003) Visits to farm buildings and cattle troughs by badgers (*Meles meles*); a potential route for transmission of bovine tuberculosis (*Mycobacterium bovis*) between badgers and cattle. *Cattle Practice*, **11**, 9–12
- Rose, A., Sinka, K., Watson, J.M., Mortimer, J.Y. and Charlett, A. (2002) An estimate of the contribution of HIV infection to the recent rise in tuberculosis in England and Wales. *Thorax*, **57**, 442–445
- Rose, G. (1985) Sick individuals and sick populations. *International Journal of Epidemiology*, **14**, 32–38
- Rosell, J.M. and de la Fuente, L.F. (2009) Culling and mortality in breeding rabbits, *Preventive Veterinary Medicine*, **88**, 120–127
- Rosell, J.M. and de la Fuente, L.F. (2016) Causes of mortality in breeding rabbits. *Preventive Veterinary Medicine*, **127**, 56–63
- Rosen, G. (Translator) (1938) *Jacob Henle: On Miasmata and Contagia. (Van den Miasmen und Kontagien)*. The Johns Hopkins Press, Baltimore. (Reprinted from the *Bulletin of the Institute of the History of Medicine*, **VI** (8), October 1938)
- Rosenberg, E. and Ben-Haim, Y. (2002) Microbial diseases of corals and global warming. *Environmental Microbiology*, **4**, 318–326
- Rosenberg, F.J., Astudillo, V.M. and Goic, R. (1980) Regional strategies for the control of foot-and-mouth disease – an ecological outlook. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 7–11 May 1979. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 587–596. Australian Government Publishing Service, Canberra
- Rosenberger, W.F. and Lachlin, J.M. (2002) *Randomization in Clinical Trials: Theory and Practice*. John Wiley and Sons, New York
- Rosenquist, H., Nielsen, N.L., Sommer, H.M., Nørrung, B. and Christensen, B.B. (2003) Quantitative risk assessment of human campylobacteriosis associated with thermophilic *Campylobacter* species in chickens. *International Journal of Food Microbiology*, **83**, 87–103
- Rosenthal R.C. (2004) Evidence-based medicine concepts. *Veterinary Clinics of North America: Small Animal Practice*, **34**, 1–6
- Roslow, S. and Roslow, L. (1972) Unlisted phone subscribers are different. *Journal of Advertising Research*, **12**, 35–38
- Rosner, F., Zarrabi, M.H., Benach, J.L. and Habicht, G.S. (1984) Babesiosis in splenectomized adults: review of 22 reported cases. *American Journal of Medicine*, **76**, 696–701
- Rosqvist, R., Skurnik, M. and Wolf-Watz, H. (1988) Increased virulence of *Yersinia pseudotuberculosis* by independent mutations. *Nature*, **334**, 522–526
- Ross, J., Tittensor, A.M., Fox, A.P. and Sanders, M.F. (1989) Myxomatosis in farmland rabbit populations in England and Wales. *Epidemiology and Infection*, **103**, 333–357
- Ross, J.A., Severson, R.K., Swensen, A.R., Pollock, B.H., Gurney, J.G. et al. (1999) Seasonal variations in the diagnosis of childhood cancer in the United States. *British Journal of Cancer*, **81**, 549–553
- Ross, J.G. (1978) Stormont 'wet-day' fluke forecasting. In: *Weather and Parasitic Animal Disease*. Ed. Gibson, T. E., pp. 4–20. World Meteorological Organization Technical Note No. 159, World Meteorological Organization, Geneva
- Roth, H.H., Keymer, I.F. and Appleby, E.C. (1973) Computerised data recording from captive and free-living wild animals. *International Zoo Yearbook*, **13**, 252–257
- Rothman, K.J. (1975) A pictorial representation of confounding in epidemiologic studies. *Journal of Chronic Diseases*, **28**, 101–108
- Rothman, K.J. (1976) Causes. *American Journal of Epidemiology*, **104**, 587–592
- Rothman, K.J. (1990) A sobering start for the cluster busters' conference. *American Journal of Epidemiology*, **132**, Suppl. 1, S6–S13
- Rothman, K.J. (2012) *Epidemiology: An Introduction*. Oxford University Press, New York
- Rothman, K.J. and Greenland, S. (1998) *Modern Epidemiology*, 2nd edn. Lippincott-Raven, Philadelphia
- Rothman, K.J., Greenland, S. and Lash, L.T. (2008) *Modern Epidemiology*, 3rd edn. Lippincott Williams and Wilkins, Philadelphia
- Rothman, N., Hainaut, P., Schulte, P., Smith, M., Boffetta, P. et al. (2012) *Molecular Epidemiology: Principles and*

- Practices*, IARC Scientific Publication No. 163. International Agency for Research on Cancer, Lyon
- Rothstein, H.R., Sutton, A.J. and Borenstein, M. (Eds) (2005) *Publication Bias in Meta-Analysis: Prevention, Assessment and Adjustments*. John Wiley and Sons, Chichester
- Roudebush, P. (1984) Canine cough knowledge coupler. In: *Proceedings of the Second Symposium on Computer Applications in Veterinary Medicine*, College of Veterinary Medicine, Mississippi State University, 23–25 May 1984, pp. 127–129
- Roueché, B. (1991) *The Medical Detectives*. Truman Talley Books/Plume, New York
- Roughgarden, J., May, R.M. and Levin, S.A. (Eds) (1989) *Perspectives in Ecological Theory*. Princeton University Press, Princeton
- Rougoor, C.W., Dijkhuizen, A.A., Barkema, H.W. and Schukken, Y.H. (1994) The economics of caesarean section in dairy cattle. *Preventive Veterinary Medicine*, **19**, 27–37
- Rowe, W.D. (1977) *An Anatomy of Risk*. John Wiley, New York
- Roy, J.H.B. (1980) *The Calf. Studies in the Agricultural and Food Sciences*, 4th edn. Butterworths, London and Boston
- Royama, T. (1992) *Analytical Population Dynamics*. Chapman and Hall, London
- Royston P. and Altman D.G. (1994) Regression using fractional polynomials of continuous covariates: parsimonious parametric modelling (with Discussion). *Applied Statistics*, **43**, 429–467
- Ru, G., Terracini, B. and Glickman, L. (1998) Host related risk factors for canine osteosarcoma. *The Veterinary Journal*, **156**, 31–39
- Rubarth, S. (1947) An acute virus disease with liver lesion in dogs (Hepatitis contagiosa canis) a pathologico-anatomical and etiological investigation. *Acta Pathologica et Microbiologica Scandinavica*, **69**, Suppl., 1–222
- Rubin, D.B. (2008) For objective causal inference, design trumps analysis. *The Annals of Applied Statistics*, **2**, 808–840
- Rubin, H. (2011) The early history of tumor virology: Rous, RIF, and RAV. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 14389–14396
- Rubio, D.M., Schoenbaum, E.E., Lee, L.S., Schteingart, D. E., Marantz, P.R. *et al.* (2010) Defining translational research: implications for training. *Academic Medicine*, **85**, 470–475
- Ruble, R.P. and Hird, D.W. (1993) Congenital abnormalities in immature dogs from a pet store: 253 cases (1987–1988). *Journal of the American Veterinary Medicine Association*, **202**, 633–636
- Ruch, P., Gobeill, J., Lovis, C. and Geissbühler, A. (2008) Automatic medical encoding with SNOMED categories. *BMC Medical Informatics and Decision Making*, **8**, Suppl. 1, S6; doi 10.1186/1472-6947-8-S1-S6
- Rudman, R. and Keiper, R.R. (1991) The body condition score of feral ponies on Assateague Island. *Equine Veterinary Journal*, **23**, 453–456
- Rueff, L. (2000) Diagnostic approaches to reproductive failure in pigs. *Swine Health and Production*, **8**, 285–207
- Ruegg, P.L. (2001) Health and production management in dairy herds. In: *Herd Health: Food Animal Production Medicine*, 3rd edn. Ed. Radostits, O.M., pp. 211–254. W.B. Saunders, Philadelphia
- Rüegg, S.R., McMahon, B.J., Häslar, B.J., Esposito, R., Nielsen, L.R. *et al.* (2017) A blueprint to evaluate One Health. *Frontiers in Public Health*, **5**, 20; doi 10.3389/fpubh.2017.00020
- Ruiz-Garcia, L. and Lunadei, L. (2011) The role of RFID in agriculture: applications, limitations and challenges. *Computers and Electronics in Agriculture*, **79**, 42–50
- Rupp, R. and Boichard, D. (2003) Genetics of resistance to mastitis in dairy cattle. *Veterinary Research*, **34**, 671–688
- Ruppanner, R. (1972) Measurement of disease in animal populations based on interviews. *Journal of the American Veterinary Medical Association*, **161**, 1033–1038
- Rushen, J. (1986) Some problems with the physiological concept of ‘stress’. *Australian Veterinary Journal*, **63**, 359–361
- Rushton, J. (2009) *The Economics of Animal Health and Production*. CAB International, Wallingford.
- Rushton, P. (2005) Cholera and its impact on nineteenth-century Mormon migration. *Brigham Young University Studies*, **44**, 123–144
- Russel, A. (1985) Nutrition of the pregnant ewe. In *Practice*, **7**, 23–28
- Russel, A. (1991) Body condition scoring of sheep. In: *Sheep and Goat Practice*. Ed. Boden, E., pp. 3–10. Baillière Tindall, London
- Russell, A.M. and Rowlands, G.J. (1983) COSREEL: computerised recording system for herd health information management. *Veterinary Record*, **112**, 189–193
- Russell, A.M., Rowlands, G.J., Shaw, S.R. and Weaver, A. D. (1982) Survey of lameness in British dairy cattle. *Veterinary Record*, **111**, 155–160
- Russell, C.A., Real, L.A. and Smith, D.L. (2006) Spatial control of rabies on heterogeneous landscapes. *PLoS ONE*, **1** (1), e27; doi 10.1371/journal.pone.0000027
- Ruston, A., Shorthall, O., Green, M., Brennan, M., Wapenaar, W. *et al.* (2016) Challenges facing the farm

- animal veterinary profession in England: a qualitative study of veterinarians' perceptions and responses. *Preventive Veterinary Medicine*, **127**, 84–93
- Rutjes, A.W.S., Reitsma, J.B., Coomarasamy, A., Khan, K. S. and Bossuyt, P.M.M. (2007) Evaluation of diagnostic tests when there is no gold standard. A review of methods. *Health Technology Assessment*, **11** (50) 1–72
- Rutstein, D.D., Mullan, R.J., Frazier, T.M., Halperin, W.E., Melius, J.M. *et al.* (1983) Sentinel health events (occupational): a basis for physician recognition and public health surveillance. *American Journal of Public Health*, **73**, 1054–1062
- Rutter, J.M. (1982) Diseases caused by mixed agents and factors. In: *The Control of Infectious Diseases in Farm Animals*, pp. 5–10. British Veterinary Association Trust Project on the Future of Animal Health Control, London
- Rweyemamu, M., Roeder, P., Mackay, D., Sumption, K., Brownlie, J. *et al.* (2008) Epidemiological patterns of foot-and-mouth disease worldwide. *Transboundary and Emerging Diseases*, **55**, 57–72
- Ryan, T.J. and Yates, E.M. (1994) The New Zealand national livestock database. In: Proceedings of the 7th International Symposium on Veterinary Epidemiology and Economics, Nairobi, 15–19 August 1994. Eds Rowlands, G.J., Kyule, M.N. and Perry, B.D. *The Kenya Veterinarian*, **18** (2), 325–327
- Saah, A.J. and Hoover, D.R. (1997) 'Sensitivity' and 'specificity' reconsidered: the meaning of these terms in analytical and diagnostic settings. *Annals of Internal Medicine*, **126**, 91–94
- SAC (2014) SAC Consulting. *Premium Sheep and Goat Health Schemes*. http://www.sruc.ac.uk/info/120113/premium_sheep_and_goat_health_schemes (accessed 23 April, 2014)
- Sack, D.A., Lyke, C., McLaughlin, C. and Suwanvanichkij, C. (2001) *Antimicrobial Resistance in Shigellosis, Cholera and Campylobacteriosis*. WHO/CDS/CSR/DRS/2001.8. World Health Organization, Geneva
- Sackett, D.L. (1979) Bias in analytic research. *Journal of Chronic Diseases*, **32**, 51–63
- Sackett, D.L., Straus, S.E., Richardson, W.S., Rosenberg, W. and Haynes, R.B. (2000) *Evidence-Based Medicine: How to Practice and Teach EBM*, 2nd edn. Churchill Livingstone, Edinburgh
- Sacks, H.S., Berrier, J., Reitman, D., Ancone-Berk, V.A. and Chalmers, T.C. (1987) Meta-analysis of controlled clinical trials. *The New England Journal of Medicine*, **316**, 450–455
- Sadooghi-Alvandi, S.M. (1988) A note on the efficiency of proportional stratification. *Australian Journal of Statistics*, **30**, 196–199
- Saegerman, C., Berkvens, D. and Mellor, P.S. (2008) Bluetongue epidemiology in the European Union. *Emerging Infectious Diseases*, **14**, 539–544
- Sahai, H. and Kurshid, A. (1995) *Statistics in Epidemiology, Methods and Applications*. CRC Press, Boca Raton
- Sakamoto, K. and Yoshida, K. (2002) Recent outbreaks of foot and mouth disease in countries of east Asia. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 459–463
- Sakharkar, K.R., Sakharkar, M.K. and Chandra, R. (Eds) (2015) *Post-genomic Approaches in Cancer and Nano Medicine*. River Publishers, Aalborg
- Salak-Johnson, J.L. and McGlone, J.J. (1991) Making sense of apparently conflicting data: stress and immunity in swine and cattle. *Journal of Animal Science*, **85**, E81–E88
- Sallander, M., Hedhammar, Å., Rindgren, M. and Lindberg, J.E. (2001) Demographic data of a population of insured Swedish dogs measured in a questionnaire study. *Acta Veterinaria Scandinavica*, **42**, 71–80
- Salman, M.D. (2004) Controlling emerging diseases in the 21st century. *Preventive Veterinary Medicine*, **62**, 177–184
- Salman, M.D., Reif, J.S., Rupp, L. and Aaronson, M.J. (1990) Chlorinated hydrocarbon insecticides in Colorado beef cattle – a pilot environmental monitoring system. *Journal of Toxicology and Environmental Health*, **31**, 125–132
- Salt, J. (1994) The epidemiological significance of foot and mouth disease virus carriers: a review. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Belfast, 13–15 April 1994. Ed. Thrusfield, M.V., pp. 71–84
- Saltelli, A., Ratto, M., Andres, T., Campolongo, F., Cariboni, J. *et al.* (2008) *Global Sensitivity Analysis: The Primer*. John Wiley and Sons, Chichester
- Samia, N.I., Kausrud, K.L., Heesterbeek, H., Ageyev, V., Begon, M. *et al.* (2011) Dynamics of the plague-wildlife-human system in Central Asia are controlled by two epidemiological thresholds. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 14527–14532
- Samsa, G.P. (1996) Sampling distributions of p_{pos} and p_{neg} . *Journal of Clinical Epidemiology*, **49**, 917–919
- Samuel, A.T. and Knowles, N.J. (2001) Foot-and-mouth disease type O viruses exhibit genetically and geographically distinct evolutionary lineages (topotypes). *Journal of General Virology*, **82**, 609–621
- Samuels, M.L., Witmer, J.A. and Schaffner, A. (2012) *Statistics for the Life Sciences*, 4th edn. Pearson Education, Boston
- Sanchez, M.M. and Chen, X. (2006) Choosing the analysis population in non-inferiority studies: per protocol or intent-to-treat. *Statistics in Medicine*, **25**, 1169–1181
- Sanchez-Vazquez, M.J., Gunn, G.J., Strachan, W.D., Armstrong, D. and Nielsen, M. (2011) The British pig

- health schemes: integrated systems for large-scale pig abattoir lesion monitoring. *Veterinary Record*, **169**, 413; doi 10.1136/vr.d4814
- Sánchez-Vizcaíno, F., Jones, P.H., Menacere, T., Heayns, B., Wardeh, M. *et al.*, (2015) Small animal disease surveillance. *Veterinary Record*, **177**, 591–594
- Sandman, P.M. (1987) Risk communication: facing public outrage. *EPA Journal*, **13**, (9) 21–22
- Sandøe, P., Corr, S. and Palmer, C. (2016) *Companion Animal Ethics*. John Wiley and Sons, Chichester
- Sangaré, O., Bastos, A.D.S., Ventner, E.H. and Vosloo, W. (2004) A first molecular epidemiological study of SAT-2 type foot-and-mouth disease viruses in West Africa. *Epidemiology and Infection*, **132**, 525–532
- Sänger, H.L. (1968) Characteristics of tobacco rattle virus. *Molecular and General Genetics*, **17**, 346–367
- Sanson, R.L. (1994) The epidemiology of foot-and-mouth disease: implications for New Zealand. *New Zealand Veterinary Journal*, **42**, 41–53
- Sanson, R.L. and Pearson, A. (1997) AgriBase – a national spatial farm database. In: Proceedings of the 8th International Symposium on Veterinary Epidemiology and Economics, Paris, July 1997. *Epidémiologie et Santé Animale*, **31–32** (2), 12.16.1–12.16.3
- Sanson, R.L., Brooks, H.V. and Horner, G.W. (2000) An epidemiological study of the spread of rabbit haemorrhagic disease virus amongst previously non-exposed rabbit populations in the North Island of New Zealand. *New Zealand Veterinary Journal*, **48**, 105–110
- Sanson, R.L., Liberona, H. and Morris, R.S. (1991a) The use of a geographical information system in the management of a foot-and-mouth disease epidemic. *Preventive Veterinary Medicine*, **11**, 309–313
- Sanson, R.L., Morris, R.S. and Stern, M.W. (1999) EpiMAN-FMD a decision support system for managing epidemics of vesicular disease. *Revue Scientifique et Technique*, **18**, 593–605
- Sanson, R.L., Pfeiffer, D.U. and Morris, R.S. (1991b) Geographic information systems: their applications in animal disease control. *Revue Scientifique et Technique, Office International des Epizooties*, **10**, 179–195
- Santman-Berends, I.G.M.A., Brouwer-Middelesch, H., Van Wuijckhuise, L., de Bont-Smolenaars, A.J.G. and Van Schalk, G. (2016) Surveillance of cattle health in the Netherlands: monitoring trends and developments using routinely collected cattle census data. *Preventive Veterinary Medicine*, **134**, 103–112
- Sard, D.M. (1979) *Dealing with Data: The Practical Use of Numerical Information*. British Veterinary Association Publications, London
- Sargeant, A.B., Allen, S.H. and Hastings, J.O. (1987) Spatial relations between sympatric coyotes and red foxes in North Dakota. *Journal of Wildlife Management*, **51**, 285–293
- Sargeant, J.M. and O'Connor A.M. (2014) Introduction to systematic reviews in animal agriculture and veterinary medicine. *Zoonoses and Public Health*, **61**, Suppl. 1, 3–9
- Sargeant, J.M., O'Connor A.M., Gardner, I.A., Dickson, J. S., Torrence, M.E. *et al.* (2010) The REFLECT Statement: reporting guidelines for randomized controlled trials in livestock and food safety: explanation and elaboration. *Zoonoses and Public Health*, **57**, 105–136
- Sargent, J., Connolly, D.J., Watts, V., Mötsküla, P., Volk, H.A. *et al.* (2015) Assessment of mitral regurgitation in dogs: comparison of results of echocardiography with magnetic resonance imaging. *Journal of Small Animal Practice*, **56**, 641–650
- Sargent, R.P., Shephard, R.M. and Glantz, S.A. (2004) Reduced incidence of admissions for myocardial infarction associated with public smoking ban: before and after study. *British Medical Journal*, **328**, 977–983
- Sargison, N. (2008) *Sheep Flock Health: A Planned Approach*. Blackwell Publishing, Oxford
- Saris, W.E. (1991) *Computer-Assisted Interviewing*. Sage Publications, Newbury Park
- Sartwell, P.E. (1950) The distribution of incubation periods of infectious disease. *American Journal of Hygiene*, **51**, 310–318
- Sartwell, P.E. (1966) The incubation period and the dynamics of infectious disease. *American Journal of Epidemiology*, **83**, 204–216
- Sartwell, P.E. (1973) *Preventive Medicine and Public Health*. Appleton, Century Crofts, New York
- Sasaki, M. (1991) Eradication of rinderpest – a review. In: *South Asia Rinderpest Eradication Campaign*. Proceedings of the Regional Expert Consultation on Rinderpest Eradication in South Asia, Bangkok, Thailand, 5–8 June 1990. RAPA Publication 1991/8, pp. 67–77. Food and Agriculture Organization, Bangkok
- Satake, A. and Koizumi, I. (2008) Population synchrony in ecological systems. *Population Ecology*, **50**, 325–327
- Sato, Y., Bourne, D. and Willis, B. (2009) Dynamics of seasonal outbreaks of black band disease in an assemblage of *Montipora* species at Pelorus Island (Great Barrier Reef, Australia). *Proceedings of the Royal Society, Series B*, **276**, 2795–2803
- Saunders, L.Z. (2000) Virchow's contribution to veterinary medicine: celebrated then, forgotten now. *Veterinary Pathology*, **37**, 199–207
- Savage, M., Devine, F., Cunningham, N., Taylor, M., Li, Y. *et al.* (2013) A new model of social class? Findings from the BBC's Great British class survey. *Sociology*, **47**, 219–250

- Savitz, D.A. (1994) In defense of black box epidemiology. *Epidemiology*, **5**, 550–552
- Sayers, I. (2010) Approach to preventive health care and welfare in rabbits. *In Practice*, **32**, 190–198
- Scanga, J.A., Hoffman, T., Picanso, J., Rajopadhye, S.V., Kim, D.G. *et al.* (2007) Development of computational models for the purpose of conducting individual livestock premises traceback investigations utilizing National Animal Identification System-compliant data. *Journal of Animal Science*, **85**, 503–511
- Scantlebury, C.E., Zerfu, A., Pinchbeck, G.P., Reed, K., Gebreab, F. *et al.* (2015) Participatory appraisal of the impact of epizootic lymphangitis in Ethiopia. *Preventive Veterinary Medicine*, **120**, 265–276
- Scarff, D.H. and Lloyd, D.H. (1992) Double blind, placebo-controlled, crossover study of evening primrose oil in the treatment of canine atopy. *Veterinary Record*, **131**, 97–99
- Scarlett, J.M. (2008) Interface of epidemiology, pet population issues and policy. *Preventive Veterinary Medicine*, **86**, 188–197
- Schaberg, D.R., Power, G., Betzold, J. and Forbes, B.A. (1985) Conjugative R plasmids in antimicrobial resistance of *Staphylococcus aureus* causing nosocomial infections. *Journal of Infectious Diseases*, **152**, 43–49
- Schachter, J., Sugg, N. and Sung, M. (1978) Psittacosis: the reservoir persists. *Journal of Infectious Diseases*, **137**, 44–49
- Schaid, D.J., Ingle, J.N., Wieand, S. and Ahmann, D.L. (1988) A design for Phase II testing of anticancer agents within a Phase III clinical trial. *Controlled Clinical Trials*, **9**, 107–118
- Schalk, A.F. and Hawn, M.C. (1931) An apparently new respiratory disease of baby chicks. *Journal of the American Veterinary Medical Association*, **78**, 413–422
- Schardt, C., Adams, M.B., Owens, T., Keitz, S. and Fontelo, P. (2007) Utilization of the PICO framework to improve searching PubMed for clinical questions. *BMC Medical Informatics and Decision Making*, **7**, 6; doi 10.1186/1472-6947-7-16
- Schaumburg, F., Mugisha, L., Peck, B., Becker, K., Gillespie, T.R. *et al.* (2012) Drug-resistant human *Staphylococcus aureus* in sanctuary apes pose a threat to endangered wild ape populations. *American Journal of Primatology*, **74**, 1071–1075
- Scheaffer, R.L., Mendenhall, W., Ott, R.L. and Gerow, K. G. (2012) *Elementary Survey Sampling*, 7th edn. Brooks/Cole, Boston
- Schenker, N. and Gentleman, J.F. (2001) On judging the significance of differences by examining the overlap between confidence intervals. *The American Statistician*, **55**, 182–186
- Schepers, J.A. (1990) Data requirements and objectives for economic analyses of disease in farm livestock. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Belfast, 4–6 April 1990. Ed. Thrusfield, M.V., pp. 120–132
- Scherer, A. and McLean, A. (2002) Mathematical models of vaccination. *British Medical Bulletin*, **62**, 187–199
- Scherer, R.W., Dickersin, K. and Langenberg, P. (1994) Full publication of results initially presented in abstracts. A meta-analysis. *Journal of the American Veterinary Medical Association*, **272**, 158–162
- Scherer, R.W., Langenberg, P. and von Elm, E. (2007) Full publication of results initially presented in abstracts. *Cochrane Database of Systematic Reviews*, Issue 2. Article No. MR000005; doi 10.1002/14651858.MR000005.pub3
- Schimmer, B., Ter Schegget, R., Wegdam, M., Zuchner, L., de Bruin, A. *et al.* (2010) The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. *BMC Infectious Diseases*, **10**, 69
- Schlesselman, J.J. (1982) *Case-Control Studies: Design, Conduct, Analysis*. Oxford University Press, New York
- Schmelzer, L.L. and Tabershaw, I.R. (1968) Exposure factors in occupational coccidioidomycosis. *American Journal of Public Health*, **58**, 107–113
- Schmid, B.V., Büntgen, U., Easterday, W.R., Ginzler, C., Walløe, L. *et al.* (2015) Climate-driven introduction of the Black Death and successive plague reintroductions into Europe. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 3020–3025
- Schmidt, F.L. and Hunter, J.E. (2015) *Methods of Meta-Analysis: Correcting Error and Bias in Research Findings*, 3rd edn. Sage Publications, Thousand Oaks
- Schmidt, P.L. (2009) Companion animals as sentinels for public health. *Veterinary Clinics of North America: Small Animal Practice*, **39**, 241–250
- Schmitz, O.J. and Nudds, T.D. (1993) Parasite-mediated competition in deer and moose: how strong is the effect of a meningeal worm on moose? *Ecological Applications*, **4**, 91–103
- Schneider, R. (1970) Comparison of age, sex, and incidence rates in human and canine breast cancer. *Cancer*, **26**, 419–426
- Schneider, R. (1975) Observations on overpopulation of dogs and cats. *Journal of the American Veterinary Medical Association*, **167**, 281–284
- Schneider, R. (1976) Epidemiologic studies of cancer in man and animals sharing the same environment. In: *Prevention and Detection of Cancer. Volume 2. Part I Etiology: Prevention Methods*. Proceedings of the 3rd International Symposium on the Detection and Prevention of Cancer, New York, April 26–May 1,

1976. Ed. Nieburgs, H.E., pp. 1377–1387. Marcel Dekker, New York
- Schneider, R. and Vaida, M.L. (1975) Survey of canine and feline populations: Alameda and Contra Costa Counties, California, 1970. *Journal of the American Veterinary Medical Association*, **166**, 481–486
- Schneider, R., Dorn, C.R. and Taylor, D.O.N. (1969) Factors influencing canine mammary cancer development and postsurgical survival. *Journal of the National Cancer Institute*, **43**, 1249–1261
- Schneiderman, M.A. (1980) The uncertain risks we run: hazardous materials. In: *Societal Risk Assessment: How Safe is Enough?*. Eds Schwing, R.C. and Albers, A., pp. 19–37 (discussion: pp. 38–41). Plenum Press, New York
- Schnurrenberger, P.R., Kangilaski, E., Berg, L.E. and Bashe, W.J. (1961) Characteristics of a rural Ohio dog population. *Veterinary Medicine*, **56**, 519–523
- Schofield, F.W. (1949) Virus enteritis in mink. *North American Veterinarian*, **30**, 651–654
- Schonlau, M., Fricker, R.D. and Elliott, M.N. (2002) *Conducting Research Surveys via e-mail and the Web*. RAND, San Monica
- Schork, N.J. (2015) Time for one-person trials. *Nature*, **520**, 609–611
- Schrader-Frechette, K.S. (1991) *Risk and Rationality: Philosophical Foundations for Populist Reforms*. University of California Press, Berkeley
- Schrag, S.J. and Wiener, P. (1995) Emerging infectious diseases: what are the relative roles of ecology and evolution? *Trends in Ecology and Evolution*, **10**, 319–324
- Schriger, D.L. (2005) Suggestions for improving the reporting of clinical research: the role of narrative. *Annals of Emergency Medicine*, **45**, 437–443
- Schuh, J.C. (2004) Trials, tribulations, and trends in tumor modeling in mice. *Toxicologic Pathology*, **32**, 53–66
- Schuurmann, D.J. (1987) A comparison of the two one-sided tests procedure and the power approach for assessing equivalence of average bioavailability. *Journal of Pharmacokinetics and Biopharmaceutics*, **15**, 657–680
- Schukken, Y.H., Erb, H.N., White, M.E., Schwager, S.J. and Scholl, D.T. (1990) A review of statistical techniques to evaluate space-time clustering in disease occurrence. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings*, Belfast, 4–6 April 1990. Ed. Thrusfield, M.V., pp. 167–178
- Schukken, Y.H., Grohn, Y.T., McDermott, B. and McDermott, J.J. (2003) Analysis of correlated discrete observations: background, examples and solutions. *Preventive Veterinary Medicine*, **59**, 223–240
- Schulte, P.A. and Perera (Eds) (1993) *Molecular Epidemiology: Principles and Practices*. Academic Press, San Diego
- Schultz, M. (2008) Rudolph Virchow. *Emerging Infectious Diseases*, **14**, 1480–1481
- Schultz, R.D. (Ed.) (1999) *Veterinary Vaccines and Diagnostics. Advances in Veterinary Medicine, Volume 41*. Academic Press, San Diego
- Schulz, K.F., Altman, D.G. and Moher, D. (2010) CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. *Obstetrics and Gynecology*, **115**, 1063–1070
- Schwabe, C.W. (1980a) Animal disease control. Part I. Developments prior to 1960. *Sudan Journal of Veterinary Science and Animal Husbandry*, **21**, 43–54
- Schwabe, C.W. (1980b) Animal disease control. Part II. Newer methods, with possibility for their application in the Sudan. *Sudan Journal of Veterinary Science and Animal Husbandry*, **21**, 55–65
- Schwabe, C. (1982) The current epidemiological revolution in veterinary medicine. Part 1. *Preventive Veterinary Medicine*, **1**, 5–15
- Schwabe, C.W. (1984) *Veterinary Medicine and Human Health*, 3rd edn. Williams and Wilkins, Baltimore and London
- Schwabe, C.W., Riemann, H. and Franti, C. (1977) *Epidemiology in Veterinary Practice*. Lea and Feibiger, Philadelphia
- Schwartz, G. (1978) Estimating the dimensions of a model. *The Annals of Statistics*, **6**, 461–464
- Schwartz, S. (1994) The fallacy of the ecological fallacy: the potential misuse of a concept and the consequences. *American Journal of Public Health*, **84**, 819–824
- Schwartz-Cornil, I., Mertens, P.P.C., Contreras, V., Hemati, B., Pascale, F. *et al.* (2008) Bluetongue virus: virology, pathogenesis and immunity. *Veterinary Research*, **39**, 46; doi 10.1051/vetres:2008023
- Schwarzer, G., Carpenter, J. and Rucker, G. (2010) Empirical evaluation suggests Copas selection model preferable to trim-and-fill method for selection bias in meta-analysis. *Journal of Clinical Epidemiology*, **63**, 282–288
- Sciarretta, A., Girma, M., Tikubet, G., Belayehun, L., Ballo, S. *et al.* (2005) Development of an adaptive tsetse population management scheme for the Luke community, Ethiopia. *Journal of Medical Entomology*, **42**, 1006–1019
- Scotch, M., Odofoin, L. and Rabinowitz, P. (2009) Linkages between animal and human health sentinel data. *BMC Veterinary Research*, **5**, 15; doi 10.1186/1746-6148-5-15
- Scott, G.R. (1964) Rinderpest. *Advances in Veterinary Science*, **9**, 113–224

- Scott, G.R. (1996) The history of rinderpest in Britain: Part one: 809–1799. *State Veterinary Journal*, **6** (4), 8–10
- Scott, G.R. (1997) The history of rinderpest in Britain: Part two: 1800 to date. *State Veterinary Journal*, **7** (1), 9–13
- Scott, H.M., Soskolne, C.L., Lissemore, K.D., Martin, S.W., Shoukri, M.M. *et al.* (2003a) Associations between air emissions from sour gas processing plants and indices of cow retainment and survival in dairy herds in Alberta. *Canadian Journal of Veterinary Research*, **67**, 1–11
- Scott, H.M., Soskolne, C.L., Martin, S.W., Basarab, J.A., Coppock, R.W. *et al.* (2003b) Lack of associations between air emissions from sour-gas processing plants and beef cow-calf herd health and productivity in Alberta, Canada. *Preventive Veterinary Medicine*, **57**, 35–68
- Scott, N.W., McPherson, G.C., Ramsay, C.R. and Campbell, M.K. (2002) The method of minimization for allocation to clinical trials: review. *Controlled Clinical Trials*, **23**, 662–674
- Scott, P.R. (2001) Health and production management in sheep flocks. In: *Herd Health: Food Animal Production Medicine*, 3rd edn. Ed. Radostits, O.M., pp. 765–844. W.B. Saunders, Philadelphia
- Scott, P.R., Sargison, N.D. and Wilson, D.J. (2007) The potential for improving welfare standards and productivity in United Kingdom sheep flocks using veterinary flock health plans. *The Veterinary Journal*, **173**, 522–531
- Scott, R.J. (1981) Zoonoses in the abattoir. In: *Advances in Veterinary Public Health*, Vol. 2. Ed. Senevirata, P., pp. 68–82. Australian College of Veterinary Scientists, Brisbane
- Scudamore, J.M. (1984) The international cattle trade problems and control of disease. *British Veterinary Journal*, **140**, 413–429
- Scudamore, J.M. (1995) GATT and the principles of risk assessment as they apply to trade. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Reading, 29–31 March 1995. Ed. Goodall, E.A., pp. 34–39
- Scudamore, J.M. (2000) Surveillance – past, present and future. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Edinburgh, 29–31 March 2000. Eds Thrusfield, M.V. and Goodall, E.A., pp. xi–xx
- Scudamore, J.M. (2004) FMD – differentiating vaccinated from infected animals. *The Veterinary Journal*, **167**, 3–4
- Sebei, P.J., McCrindle, C.M. and Webb, E.C. (2004) An economic analysis of communal goat production. *Journal of the South African Veterinary Association*, **75**, 19–23
- Seber, G.A.F. (1973) *The Estimation of Animal Abundance*. Hafner Press, New York
- Seibold, H.R., Bailey, W.S., Hoerlein, B.F., Jordan, E.M. and Schwabe, C.W. (1955) Observations on the possible relation of malignant esophageal tumors and *Spirocerca lupi* lesions in the dog. *American Journal of Veterinary Research*, **16**, 5–14
- Seifter, A., Schwartzwalder, A., Geis, K. and Aucott, J. (2010) The utility of ‘Google Trends’ for epidemiological research: Lyme disease as an example. *Geospatial Health*, **4**, 135–137
- Selby, L.A., Corwin, R.M. and Hayes, H.M. (1980) Risk factors associated with canine heartworm infection. *Journal of the American Veterinary Medical Association*, **176**, 33–35
- Selby, L.A., Edmonds, L.D. and Hyde, L.D. (1976) Epidemiological field studies of animal populations. *Canadian Journal of Comparative Medicine*, **40**, 135–141
- Selby, L.A., Edmonds, L.D., Parke, D.W., Stewart, R.W., Marienfeld, C.J. *et al.* (1973) Use of mailed questionnaire data in a study of swine congenital malformations. *Canadian Journal of Comparative Medicine*, **37**, 413–417
- Selikoff, I.J., Hammond, E.C. and Seidman, H. (1979) Mortality experience of insulation workers in the United States and Canada, 1943–1976. *Annals of the New York Academy of Sciences*, **330**, 91–116
- Sellers, R.F. (1971) Quantitative aspects of the spread of foot and mouth disease. *Veterinary Bulletin*, **41**, 431–439
- Sellers, R.F. (1982) Eradication – local and national. In: *The Control of Infectious Diseases in Farm Animals*, pp. 18–21. British Veterinary Association Trust Project on the Future of Animal Health Control, London.
- Sellers, R.F. and Forman, A.J. (1973) The Hampshire epidemic of foot and mouth disease, 1967. *Journal of Hygiene, Cambridge*, **71**, 15–34
- Sellers, R.F. and Gloster, J. (1980) The Northumberland epidemic of foot-and-mouth disease, 1966. *Journal of Hygiene, Cambridge*, **85**, 129–140
- Sellers, R.F. and Maarouf, A.R. (1990) Trajectory analysis of winds and vesicular stomatitis virus in North America, 1982–85. *Epidemiology and Infection*, **104**, 313–328
- Sellers, R.F., Herniman, K.A. and Donaldson, A.I. (1971) The effects of killing or removal of animals affected with foot-and-mouth disease on the amounts of airborne virus present in looseboxes. *British Veterinary Journal*, **127**, 358–365
- Sellers, R.F., Pedgley, D.E. and Tucker, M.R. (1977) Possible spread of African horse sickness on the wind. *Journal of Hygiene, Cambridge*, **79**, 279–298

- Sellers, R.F., Pedgley, D.E. and Tucker, M.R. (1978) Possible windborne spread of bluetongue to Portugal, June–July, 1956. *Journal of Hygiene, Cambridge*, **81**, 189–196
- Selvin, H.C. (1958) Durkheim's 'Suicide' and problems of empirical research. *American Journal of Sociology*, **63**, 607–619
- Selye, H. (1946) The general adaptation syndrome and the diseases of adaptation. *Journal of Clinical Endocrinology*, **6**, 117–230
- Semizarov, D. and Blomme, E. (2008) *Genomics in Drug Discovery and Development*. John Wiley and Sons, Hoboken
- Simmelweis, I.P. (1861) *Die Aetiologie, der Begriff und die Prophylaxis des Kindbettfiebers*. C.A. Hartleben's Verlags-Expedition, Pest, Wein and Leipzig. Reprinted 1966 (with introduction by A.F. Guttmacher) by Johnson Reprint Corporation, New York and London
- Senn, S. (2001) Individual therapy: new dawn or false dawn? *Drugs Information Journal*, **35**, 1479–1494
- Senn, S. (2002) *Cross-over Trials in Clinical Research*, 2nd edn. John Wiley, Chichester
- Serafini, C.A.V., Rosa, G.A., Guimaraes, A.M.S., De Moraes, H.A. and Biondo, A.W. (2008) Survey of owned feline and canine populations in apartments from a neighbourhood in Curitiba, Brazil. *Zoonoses and Public Health*, **55**, 402–405
- Sergeant, E.S.G. (2015) *Epitools Epidemiological Calculators*. AusVet Animal Health Services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease. <http://epitools.ausvet.com.au> (accessed 7 January 2015)
- Sergeant, E. and Perkins, N. (2015) *Epidemiology for Field Veterinarians: An Introduction*. CABI, Wallingford
- Sergeant, E.S.G., Marshall, D.J., Eamens, G.J., Kearns, C. and Whittington, R.J. (2003) Evaluation of an absorbed ELISA and an agar-gel immuno-diffusion test for ovine paratuberculosis in sheep in Australia. *Preventive Veterinary Medicine*, **61**, 235–248
- Service, M.W. (1989) Irrigation: boon or bane. In: *Demography and Vector-Borne Diseases*. Ed. Service, M.W., pp. 237–254. CRC Press, Boca Raton
- Setbon, M., Rande, J., Fischler, C. and Flahault, A. (2005) Risk perception of the 'Mad Cow Disease' in France: determinants and consequences. *Risk Analysis*, **25**, 813–826
- Sficas, N., Greenhalgh, D. and Lewis, F. (2007) The basic reproduction number and the vaccination coverage required to eliminate rubella from England and Wales. *Mathematical Population Studies*, **14**, 3–29
- Shafer, G. (1996) *The Art of Causal Conjecture*. MIT Press, Cambridge, MA
- Shaffer, J.P. (1995) Multiple hypothesis testing. *Annual Review of Psychology*, **46**, 561–584
- Shahan, M. (1953) The control of foot and mouth disease in Mexico. *Canadian Journal of Comparative Medicine*, **17**, 369–374
- Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A. et al. (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA –P) 2015: elaboration and explanation. *British Medical Journal*, **349**, g7647; doi 10.1136/bmj.g7647 (erratum: **349**, i4086; doi 10.1136/bmj.i4086)
- Shanahan, C., Kernan, B., Ayalew, G., McDonnell, K., Butler, F. and Ward, S. (2009) A framework for beef traceability from farm to slaughter using global standards: an Irish perspective. *Computers and Electronics in Agriculture*, **66**, 62–69
- Shane, S.M., Gyimah, J.E., Harrington, K.S. and Snider, T. G. (1985) Etiology and pathogenesis of necrotic enteritis. *Veterinary Research Communications*, **9**, 269–287
- Shanks, N., Greek, R. and Greek, J. (2009) Are animal models predictive for humans? *Philosophy, Ethics and Humanities in Medicine*, **4**, 2; doi 10.1186/1747-5341-4-2
- Shapiro, S.H. (1997) Confounding by indication? *Epidemiology*, **8**, 110–111
- Sharma, K., Shashni, B., Sakharkar, M.K., Sakharkar, K.R. and Chandra, R. (2015) Animal model of cancer and infection. In: *Post-genomic Approaches in Cancer and Nano Medicine*. Eds Sakharkar, K.R., Sakharkar, M.K. and Chandra, R., pp. 85–100. River Publishers, Aalborg
- Sharma, S.K. (1978) Studies on FMD virus in sheep with special reference to distribution of the virus and carrier status. *Veterinary Research Bulletin*, **1**, 156–157
- Shaw, A.P.M., Wint, G.R.W., Cecchi, G., Torr, S.J., Mattioli, R.C. et al. (2015) Mapping the benefit-cost ratios of interventions against bovine trypanosomosis in Eastern Africa. *Preventive Veterinary Medicine*, **122**, 406–416
- Shearer, T.S. (Ed.) (2011) Palliative medicine and hospice care. *Veterinary Clinics of North America: Small Animal Practice*, **41**, 477–702
- Sheriff, G. and Osgood, D. (2010) Disease forecasts and livestock health disclosure: a shepherd's dilemma. *American Journal of Agricultural Economics*, **92**, 776–788
- Shewhart, W.A. (1931) *The Economic Control of the Quality of the Manufactured Product*. Macmillan and Company, New York
- Shimozako, H.J. and de Barros Couto, E. (2010) *Photographic Capture-Recapture for Estimation of Stray Dog Population: A Methodological Evaluation and an Ecological Study*. VDM Verlag Dr Müller Aktiengesellschaft and Co., Saarbrücken
- Shinn, E.A., Smith, G.W., Prospero, J.M., Betzer, P., Hayes, M.L. et al. (2000) African dust and the demise

- of coral reefs. *Geophysical Research Letters*, **27**, 3029–3032
- Shope, R.E. (1931) Swine influenza. III. Filtration experiment and etiology. *Journal of Experimental Medicine*, **54**, 373–385
- Short, C.E. (Ed.) (1987) *Principles and Practice of Veterinary Anesthesia*. Williams and Wilkins, Baltimore
- Short, N. (2002) The use of information and communication technology in veterinary education. *Research in Veterinary Science*, **72**, 1–6
- Shorthall, O.K. and Kaler, J. (2016) Exploring expert opinion on the practicality and effectiveness of biosecurity measures on dairy farms using best worst case scenario choice analysis. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Elsinore, 16–18 March 2016. Eds Lindberg, A. and Thulke, H.-H., pp. 158–165
- Shorthall, O., Ruston, A., Green, M., Brennan, M., Wapenaar, W. *et al.* (2016) Broken biosecurity? Veterinarians' framing of biosecurity on dairy farms in England. *Preventive Veterinary Medicine*, **132**, 20–31
- Shott, S. (1985) Statistics in veterinary research *Journal of the American Veterinary Medical Association*, **187**, 138–141
- Shtark, O.Y., Borisov, A.Y., Zhukov, V.A., Provorov, N.A. and Tikhonovich, I.A. (2010) Intimate associations of beneficial soil microbes with plant hosts. In: *Soil Microbiology and Sustainable Crop Production*. Eds Dixon, G.R. and Tilson, E.L., pp. 119–196. Springer, Dordrecht
- Shumway, R.H. and Stoffer, D.S. (2011) *Time Series Analysis and Its Applications*, 3rd edn. Springer, New York
- Shuster, J. (1992) *Practical Handbook of Sample Size Guidelines for Clinical Trials*. CRC Press, Boca Raton
- Sibley, L.D. (2004) Intracellular parasite invasion strategies. *Science*, **304**, 248–253
- Siegel, S. and Castellan, N.J. (1988) *Nonparametric Statistics for the Behavioral Sciences*, 2nd edn. McGraw-Hill, New York
- Siembieda, J.L., Kock, R.A., McCracken, T.A. and Newman, S.H. (2011) The role of wildlife in transboundary animal diseases. *Animal Health Research Reviews*, **12**, 95–111
- Sigurdsson, B. (1954) Maedi, a slow progressive pneumonia of sheep: an epizootiological and pathological study. *British Veterinary Journal*, **110**, 255–270
- Sim, J. and Wright, C.C. (2005) The kappa statistic in reliability studies: use, interpretation, and sample size requirements. *Physical Therapy*, **85**, 257–268
- Simel, D.L., Samsa, G.P. and Matchar, D.B. (1991) Likelihood ratios with confidence: sample size estimation for diagnostic test studies. *Journal of Clinical Epidemiology*, **44**, 763–760
- Simmons, A. and Cuthbertson, J.C. (1985) Time series analysis of ovine pneumonia using Scottish slaughterhouse data. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Reading, 27–29 March 1985. Ed. Thrusfield, M.V., pp. 130–141
- Simonsohn, U., Nelson, L.D. and Simmons, J.P. (2014) *p*-curve and effect size: correcting for publication bias using only significant results. *Perspectives on Psychological Science*, **9**, 666–681
- Simpson, B.H. and Wright, D.F. (1980) The use of questionnaires to assess the importance of clinical disease in sheep. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 7–11 May 1979. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 97–105. Australian Government Publishing Service, Canberra
- Simpson, G.G. (1951) *Horses: The Story of the Horse Family in the Modern World Through Sixty Million Years of History*. Oxford University Press, New York
- Sinclair, I.J., Tarry, D.W. and Wassall, D.A. (1989) Serological survey of the incidence of *Hypoderma bovis* in cattle in 1988. *Veterinary Record*, **124**, 243–244
- Singer, M. (2009) *Introduction to Syndemics: A Critical Systems Approach to Public and Community Health*. Jossey-Bass, San Francisco
- Singer, M. and Clair, S. (2003) Syndemics and public health: reconceptualizing disease in a bio-social context. *Medical Anthropology Quarterly*, **17**, 423–441
- Singer, P. (2000) *Writings on an Ethical Life*. Fourth Estate, London
- Singer, R.S., Case, J.T., Carpenter, T.E., Walker, R.L. and Hirsh, D.C. (1998) Assessment of spatial and temporal clustering of ampicillin and tetracyclin-resistant strains of *Pasteurella multocida* and *P. haemolytica* isolated from cattle in California. *Journal of the American Veterinary Medical Association*, **212**, 1001–1005
- Singh, K.P.R., Khorshed, M.P. and Anderson, C.R. (1964) Transmission of Kyasanur Forest disease virus by *Haemaphysalis turturis*, *Haemaphysalis papuana kinneari* and *Haemaphysalis minuta*. *Indian Journal of Medical Research*, **52**, 566–573
- Singleton, W.B. (1993) Companion animal medicine: future outlook. In: *The Advancement of Veterinary Science. The Bicentenary Symposium Series. Volume 1: Veterinary Medicine Beyond 2000*. Ed. Michell, A.R., pp. 249–263. CAB International, Wallingford
- Sinnecker, H. (1976) *General Epidemiology*. Walker, N. (translator). John Wiley, London
- Siraj, A.S., Santos-Vega, M., Bouma, M.J., Yadeta, D., Carrascal, D.R. *et al.* (2014) Altitudinal changes in

- malaria incidence in highlands of Ethiopia and Colombia. *Science*, **343**, 1154–1158
- Sison, C.P. and Glaz, J. (1995) Simultaneous confidence intervals and sample size determination for multinomial proportions. *Journal of the American Statistical Association*, **90**, 366–369
- Sjöberg, L. (2001) Political decisions and public risk perception. *Reliability Engineering and System Safety*, **72**, 115–123
- Slater, M. (1997) How good are data from questionnaires: a review of measuring reliability. In: Proceedings of the 8th International Symposium on Veterinary Epidemiology and Economics, Paris, July 1997. *Epidémiologie et Santé Animale*, **31–32** (2), 12.09.1–12.09.3
- Slater, M.R. (1996a) Methods and issues in conducting breed-specific canine health surveys. *Preventive Veterinary Medicine*, **28**, 69–79
- Slater, M.R. (1996b) Nutritional epidemiology in small animal medicine. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Glasgow, 27–29 March 1996. Eds Thrusfield, M.V. and Goodall, E.A., pp. 117–126
- Slater, M.R. (2001) The role of veterinary epidemiology in the study of free-roaming dogs and cats. *Preventive Veterinary Medicine*, **48**, 273–286
- Slater, M.R., Di Nardo, A., Pediconi, O., Dalla Valla, P., Candeloro, L. *et al.* (2008) Cat and dog ownership and management patterns in central Italy. *Preventive Veterinary Medicine*, **85**, 267–294
- Slater, M.R., Scarlett, J.M. and Erb, H.N. (1991) A comparison of five strategies for selecting control groups: a canine osteochondritis dissecans example. *Preventive Veterinary Medicine*, **10**, 319–326
- Sleeper, M.M., Bish, L.T. and Sweeney, H.L. (2009) Gene therapy in large animal models of human cardiovascular disease. *ILAR Journal*, **50**, 199–205
- Slim, H. and Thomson, P. (1994) Ways of listening: the art of collecting testimony. In: *Listening For A Change*. Eds Bennet, O. and Cross, N., pp. 61–94. New Society Publishers, Philadelphia
- Slocombe, J.O.D. (1975) Surveillance for parasitism in domestic animals. In: *Animal Disease Monitoring*. Eds Ingram, D.G., Mitchell, W.F. and Martin, S.W., pp. 129–135. Charles C. Thomas, Springfield
- Slorach, S.A. (Ed.) (2013) Coordinating surveillance policies in animal health and food safety ‘from farm to fork’. *Revue Scientifique et Technique, Office International des Epizooties*, **32**, 299–593
- Slovic, P. (2000) *The Perception of Risk*. Earthscan, London
- Sluyter, F.J.H. (2001) Traceability of Equidae: a population in motion. *Revue Scientifique et Technique, Office International des Epizooties*, **20**, 500–509
- Smallman-Raynor, M. and Cliff, A. (2012) *Atlas of Epidemic Britain: A Twentieth Century Picture*. Oxford University Press, Oxford
- Smith, A. (1776) *An Inquiry into the Nature and Causes of the Wealth of Nations*. W. Strahan and T. Cadell, London
- Smith, A.H. and Bates, M.N. (1992) Confidence limit analyses should replace power calculations in the interpretation of epidemiologic studies. *Epidemiology*, **3**, 449–452
- Smith, A.H., Pearce, N.E. and Callas, P.W. (1988) Cancer case-control studies with other cancers as controls. *International Journal of Epidemiology*, **17**, 298–306
- Smith, C.V. (1964) Some evidence for the windborne spread of fowl pest. *Meteorological Magazine*, **93**, 257–263
- Smith, E.J., Kelly, J.M. and Whitaker, D.A. (1983) Health and fertility data recorded by members of the Dairy Herd Health and Productivity Service. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Southampton, 12–13 April 1983. Ed. Thrusfield, M.V., pp. 8–12.
- Smith, G. (1984) Density-dependent mechanisms in the regulation of *Fasciola hepatica* populations in sheep. *Parasitology*, **88**, 449–461
- Smith, G. (2011) Models of macroparasitic infections in domestic ruminants: a conceptual review and critique. *Revue Scientifique et Technique, Office International des Epizooties*, **30**, 447–456
- Smith, I. and Wang, L.F. (2013) Bats and their virome: an important source of emerging viruses capable of infecting humans. *Current Opinion in Virology*, **3**, 84–91
- Smith, K.G.V. (Ed.) (1973) *Insects and Other Arthropods of Medical Importance*. The Trustees of the British Museum (Natural History), London
- Smith, L.P. and Hugh-Jones, M.E. (1969) The weather factor in foot-and-mouth disease epidemics. *Nature*, **223**, 712–715
- Smith, R.A., Stokka, G.L., Radostits, O.M. and Griffin, D. (2001) Health and production management in beef feedlots. In: *Herd Health: Food Animal Production Medicine*, 3rd edn. Ed. Radostits, O.M., pp. 581–633
- Smith, R.D. (1988) Veterinary clinical research: a survey of study designs and clinical issues appearing in a practice journal. *Journal of Veterinary Medical Education*, **15** (1), 2–7
- Smith, R.D. (2006) *Veterinary Clinical Epidemiology: A Problem-Oriented Approach*, 3rd edn. CRC Press, Boca Raton
- Smith, T. and Kilbourne, F.L. (1893) *Investigation into the Nature, Causation and Prevention of Texas or Southern Cattle Fever. Bulletin 1*. Bureau of Animal Industries, US Department of Agriculture, Washington DC

- Smith, W.D. (1999) Prospects for vaccines of helminth parasites for grazing ruminants. *International Journal of Parasitology*, **29**, 17–24
- Smith, W.J. and Straw, B.E. (2006) Prolapses. In: *Diseases of Swine*, 9th edn. Eds Straw, B.E., Zimmerman, J.J., D'Allaire, S. and Taylor, D.J., pp. 965–970. Blackwell Publishing, Oxford
- Smithcors, J.F. (1957) *Evolution of the Veterinary Art*. Veterinary Medicine Publishing Company, Kansas
- Smolin L. (2006) *The Trouble with Physics*. Penguin Books, London
- Smulders, F.J.M. and Collins, J.D. (Eds) (2002) *Food Safety Assurance and Veterinary Public Health. Volume 1. Food Safety Assurance in the Pre-harvest Phase*. Wageningen Academic, Wageningen
- Smulders, F.J.M. and Collins, J.D. (Eds) (2004) *Food Safety Assurance and Veterinary Public Health. Volume 2. Safety Assurance During Food Processing*. Wageningen Academic, Wageningen
- Snary, E.L., Kelly, L.A., Davison, H.C., Teale, C.J. and Wooldridge, M. (2004) Antimicrobial resistance: a microbial risk assessment perspective. *Journal of Antimicrobial Chemotherapy*, **53**, 906–917
- Snary, E. L., Ramnial, V., Breed, A.C., Stephenson, B., Field, H.E. *et al.* (2012) Qualitative release assessment to estimate the likelihood of henipavirus entering the United Kingdom. *PLoS ONE*, **7**, e27918; doi 10.1371/journal.pone.0027918
- Sneddon, G. (2014) Who was ... Sir John Lawes? *The Biologist*, **61** (4), 24–27
- Snedecor, G.W. and Cochran, W.G. (1989) *Statistical Methods*, 8th edn. Iowa State University Press, Ames
- Snedeker, K.G., Campbell, M., Totton, S.C., Guthrie, A. and Sargeant, J.M. (2010a) Comparison of outcomes and other variables between conference abstracts and subsequent peer-reviewed papers involving pre-harvest or abattoir-level interventions against foodborne pathogens. *Preventive Veterinary Medicine*, **97**, 67–76
- Snedeker, K.G., Totton, S.C. and Sargeant, J.M. (2010b) Analysis of trends in the full publication of papers from conference abstracts involving pre-harvest or abattoir-level interventions against foodborne pathogens. *Preventive Veterinary Medicine*, **95**, 1–9
- Snell, J.L. (1987) *Introduction to Probability*. Random House, New York
- Snieszko, S.F. (1974) The effects of environmental stress on outbreaks of infectious diseases of fishes. *Journal of Fish Biology*, **6**, 197–208
- Snijders, T.A.B. and Bosker, R (2012) *Multilevel Analysis*, 2nd edn. Sage Publications, London
- Snodgrass, D.R. (1974) Studies on bovine petechial fever and tick-borne fever. PhD Thesis, University of Edinburgh.
- SNOMED (2004) *Systematized Nomenclature of Human and Veterinary Medicine*. <http://www.snomed.com> (accessed 5 February 2004)
- Snow, J. (1855) *On the Mode of Communication of Cholera*, 2nd edn. Churchill, London. Reproduced in *Snow on Cholera*. Commonwealth Fund, New York, 1936. Reprinted by Hafner, New York, 1965
- Snowdon, S.K. and Stewart-Brown, S.L. (1997) Preschool vision screening. *Health Technology Assessment*, **1**, 1–85
- Snyder, L.J. (2011) *The Philosophical Breakfast Club*. Broadway Books, New York
- Soegiarto (2011) Animal health information systems in Indonesia. In: *Challenges of Animal Health Information Systems and Surveillance for Animal Diseases and Zoonoses*. Proceedings of the International Workshop organized by FAO, 23–26 November 2010, Rome, Italy. Animal Production and Health Proceedings No. 14, pp. 25–28. Food and Agriculture Organization of the United Nations, Rome
- Soll, M.D. and Knight, D.H. (1995) Proceedings of the Heartworm Symposium '95, Auburn, Alabama, March 31–April 2, 1995 (Endpaper). American Heartworm Society, Batavia
- Solymosi, N., Reiczigel, J., Berke, O., Harnos, A., Szigeti, S. *et al.* (2004) Spatial risk assessment of herd serostatus of Aujeszky's disease in a county in Hungary. *Preventive Veterinary Medicine*, **65**, 9–16
- Somvanshi, R. (1991) Horn cancer in Indian cattle. *Veterinary Bulletin*, **61**, 901–911
- Song, F., Parekh, S., Hooper, L., Loke, Y.K., Ryder, J. *et al.* (2010) Dissemination and publication of research findings: an updated review of related biases. *Health Technology Assessment*, **14**, 1–193
- Sonnenschein, E.G., Glickman, L.T., Goldschmidt, M.H. and McKee, L.J. (1991) Body conformation, diet, and risk of breast cancer in pet dogs: a case-control study. *American Journal of Epidemiology*, **133**, 694–703
- Sørensen, H.T., Sabroe, S. and Olsen, J. (1996) A framework for evaluation of secondary data sources for epidemiological research. *International Journal of Epidemiology*, **25**, 435–442
- Sørensen, J.H., Mackay, D.J., Jensen, C.O. and Donaldson, A.I. (2000) An integrated model to predict the atmospheric spread of foot-and-mouth disease virus. *Epidemiology and Infection*, **124**, 577–590
- Sørensen, J.T. and Enevoldsen, C. (1992) Modelling the dynamics of the health-production complex in livestock herds: a review. *Preventive Veterinary Medicine*, **13**, 287–297
- Sørensen, K.J., Madsen, K.G., Madsen, E.S., Salt, J.S., Nqindi, J. *et al.* (1998) Differentiation of infection from vaccination in foot-and-mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB

- and 3ABC in ELISA using antigens expressed in baculovirus. *Archives of Virology*, **143**, 1461–1476
- Sorensen, M.A., Buss, M.S. and Tyler, J.W. (1995) Accuracy of microchip identification in dogs and cats. *Journal of the American Veterinary Medical Association*, **207**, 766–767
- Sotomayor, G. (2011) Progress and challenges in official information systems for disease surveillance in Chile. In: *Challenges of Animal Health Information Systems and Surveillance for Animal Diseases and Zoonoses*. Proceedings of the International Workshop organized by FAO, 23–26 November 2010, Rome, Italy. Animal Production and Health Proceedings No. 14, pp. 23–24. Food and Agriculture Organization of the United Nations, Rome
- Soulsby, E.J.L. (1982) *Helminths, Arthropods and Protozoa of Domestic Animals*, 7th edn. Baillière Tindall, London
- Sousa, C.A. (2013) Diagnostic approach to the itchy dog. In *Practice FOCUS*, November 2013, 2–5
- Southwood, T.R.E. (1978) *Ecological Methods with Particular Reference to the Study of Insect Populations*, 2nd edn. Chapman and Hall, London
- Spain, C.V., Scarlett, J.M. and Houpt, K.A. (2004a) Long-term risks and benefits of early-age gonadectomy in cats. *Journal of the American Veterinary Medical Association*, **224**, 372–379
- Spain, C.V., Scarlett, J.M. and Houpt, K.A. (2004b) Long-term risks and benefits of early-age gonadectomy in dogs. *Journal of the American Veterinary Medical Association*, **224**, 380–387
- Speare, D.J., MacNair, N. and Hammell, K.L. (1995) Demonstration of tank effect on growth indices of juvenile rainbow trout (*Oncorhynchus mykiss*) during an ad libitum feed trial. *American Journal of Veterinary Research*, **56**, 1372–1379
- Spearman, C. (1908) The method of 'right and wrong' cases ('constant stimuli') without Gauss's formulae. *British Journal of Psychology*, **2**, 227–242
- Spedding, C. (2000) *Animal Welfare*. Earthscan Publications, London
- Spedding, C.R.W. (1988) *An Introduction to Agricultural Systems*, 2nd edn. Elsevier Applied Science, London
- Spedding, R.N., Hindson, J.C. and Earl, J.A. (2007) Flock health programmes. In: *Diseases of Sheep*, 4th edn. Ed. Aitken, I.D., pp. 537–544. Blackwell Publishing, Oxford
- Speiser, D. von (1982) Daniel Bernoulli (1700–1982). *Helvetica Physica Acta*, **55**, 504–523
- Spencer, J.E. and Thomas, W.L. (1978) *Introducing Cultural Geography*. John Wiley, New York
- Spinage, C.A. (2003) *Cattle Plague: A History*. Kluwer Academic/Plenum Publishers, New York
- Spinu, I. and Biberi-Moroianu, S. (1969) Theoretical and practical problems concerning the eradication of communicable diseases. *Archives Roumaines de Pathologie Experimentale et de Microbiologie*, **28**, 725–742
- Spire, M.F. (1990) Cow/calf production records: justification, gathering and interpretation. In: Proceedings of the 23rd Annual Convention of the American Association of Bovine Practitioners, Indianapolis, Indiana, 13–16 September 1990, pp. 93–95
- Spodnick, G.J., Berg, J., Rand, W.M., Schelling, S.H., Couto, G. *et al.* (1992) Prognosis for dogs with appendicular osteosarcoma treated by amputation alone: 162 cases (1978–1988) *Journal of the American Veterinary Medical Association*, **200**, 995–999
- Sprecher, D.J., Hostetler, D.E. and Kaneene, J.B. (1997) Locomotion scoring of dairy cattle. *Theriogenology*, **47**, 1178–1187
- Sprijt, B.M., van den Bos, R. and Pijlman, F.T.A. (2001) A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. *Applied Animal Behaviour Science*, **72**, 145–171
- Srinand, S., Ames, T.R., Maheswaran, S.K. and King, V.L. (1995) Efficacy of various vaccines against pneumonic pasteurellosis in cattle: a meta-analysis. *Preventive Veterinary Medicine*, **25**, 7–17
- St Leger-Gordon, R.E. (1994) *The Witchcraft and Folklore of Dartmoor*. Peninsula Press, Newton Abbot
- Stahl, S. (2006) The evolution of the Normal distribution. *Mathematics Magazine*, **79**, 96–113
- Stallbaumer, M.F. (1983) The prevalence of *Cysticercus tenuicollis* in slaughtered sheep in Britain. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Southampton, 12–13 April 1983. Ed. Thrusfield, M.V., pp. 124–130
- Stamp, L.D. (1964) *A Geography of Life and Death*. Cornell University Press, Ithaca
- Stanford, K., Stitt, J., Kellar, J.A. and McAllister, T.A. (2001) Traceability in cattle and small ruminants. *Revue Scientifique et Technique, Office International des Epizooties*, **20**, 510–522
- Stangle, D.K. and Berry, D.A. (Eds) (2000) *Meta-analysis in Medicine and Health Policy*. Marcel Dekker, New York
- Stansfield, S.A. (2002) Commentary: the problem with stress: minds, hearts and disease. *International Journal of Epidemiology*, **31**, 1113–1116
- Stapp, P., Antolin, M.F. and Ball, M. (2004) Patterns of extinction in prairie dog metapopulations: plague outbreaks follow El Niño events. *Frontiers in Ecology and the Environment*, **2**, 235–240

- Staquet, M., Rozenzweig, M., Lee, Y.J. and Muggia, F.M. (1981) Methodology for the assessment of new dichotomous diagnostic tests. *Journal of Chronic Diseases*, **34**, 599–610
- Stare, J. and Maucourt-Boulch, D. (2016) Odds ratio, hazard ratio and relative risk. *Metodološki Zvezki (Advances in Methodology and Statistics)*, **13**, 59–67
- Stärk, K.D.C. (2000) The epidemiological investigation of environmental risk factors for respiratory diseases in pigs – a literature review. *The Veterinary Journal*, **159**, 37–56
- Stärk, K.D.C. and Nevel, A. (2009) Strengths, weaknesses, opportunities and threats of the pig health monitoring systems used in England. *Veterinary Record*, **165**, 461–465
- Stärk, K.D.C., Frei, P.P., Frei-Stäheli, C., Pfeiffer, D.U. and Audigé, L. (1996) A health profile of Swiss dairy cows. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Glasgow, 27–29 March 1996. Eds Thrusfield, M.V. and Goodall, E.A., pp. 86–93
- Stärk, K.D.C., Morris, R.S., Benard, H.J. and Stern, M.W. (1998) EpiMAN-SF: a decision support system for managing swine fever epidemics. *Revue Scientifique et Technique, Office International des Epizooties*, **17**, 682–690
- Stärk, K.D.C., Regula, G., Hernandez, J., Knopf, L., Fuchs, K. *et al.* (2006) Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health: review of current approaches. *BMC Health Services Research*, **6**, 20; doi 10.1186/1472-6963-6-20
- Stärk, K.D.C., Vicari, A. and Nicolet, J. (1994) Contagious bovine pleuropneumonia (CBPP) surveillance in Switzerland: a pilot study. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Belfast, 13–15 April 1994. Ed. Thrusfield, M.V., pp. 96–103
- Starr, C. (1969) Social benefit versus technological risk. *Science*, **165**, 1232–1238
- Starr, C. (2003) The precautionary principle versus risk analysis. *Risk Analysis*, **23**, 1–3
- Statham, J. (2011a) Cattle health schemes: 1. single-agent infectious diseases. In *Practice*, **33**, 210–217
- Statham, J. (2011b) Cattle health schemes: 2. multifactorial or management diseases. In *Practice*, **33**, 282–285
- Stationery Office (1997) *The Pennington Group. Report on the Circumstances Leading to the 1996 Outbreak of Infection with E. coli O157 in Central Scotland, The Implications for Food Safety and Lessons to be Learned*. The Stationery Office, Edinburgh
- Stationery Office (2009) *The Horse Passports Regulations 2009, Statutory Instrument 2009 No. 1611, Animals, England*. The Stationery Office, London
- Staubach, C., Klöß, D., Kowalczyk, S., Kramer, M., Kranz, P. *et al.* (2011) The German animal disease notification and information system. In: *Challenges of Animal Health Information Systems and Surveillance for Animal Diseases and Zoonoses*. Proceedings of the International Workshop organized by FAO, 23–26 November 2010, Rome, Italy. Animal Production and Health Proceedings No. 14, pp. 49–51. Food and Agriculture Organization of the United Nations, Rome
- Staubach, C., Tackmann, K., Löschner, U., Mix, H., Busse, W. *et al.* (1998) Geographic information system-aided analysis of factors potentially influencing *Echinococcus multilocularis* infections in foxes. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Ennis, 25–27 March 1998. Eds Thrusfield, M.V. and Goodall, E.A., pp. 40–47
- Stavisky, J., Brennan, M.L., Downes, M. and Dean, R. (2012) Demographics and economic burden of un-owned cats and dogs in the UK: results of a 2010 census. *BMC Veterinary Research*, **8**, 163; doi 10.1186/1746-6148-8-163
- Stedman, T.L. (1989) *Stedman's Medical Dictionary*, 25th edn. Williams and Wilkins, Baltimore
- Steel, J.W. and Wardhaugh, K.G. (2002) Ecological impact of macrolytic lactones on dung fauna. In: *Macrolytic Lactones in Antiparasitic Therapy*. Eds Vercruysse, J. and Rew, R.S., pp. 141–162. CABI Publishing, Wallingford
- SteelFisher, G.K., Blendon, R.J. and Lasala-Blanco, N. (2015) Ebola in the United States – public reactions and implications. *The New England Journal of Medicine*, **373**, 789–791
- Steere, A.C., Malawista, S.E., Snyderman, D.R., Shope, R.E., Andiman, W.A. *et al.* (1977) Lyme arthritis. An epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. *Arthritis and Rheumatism*, **20**, 7–17
- Steffan, J., Favrot, C. and Mueller, R. (2006) A systematic review and meta-analysis of the efficacy and safety of cyclosporin for the treatment of atopic dermatitis in dogs. *Veterinary Dermatology*, **17**, 3–16
- Stege, H., Bager, F., Jacobsen, E. and Thougard, A. (2003) VETSTAT – the Danish system for surveillance of the veterinary use of drugs for production animals. *Preventive Veterinary Medicine*, **57**, 105–115
- Stein, T.E. (1988) Problem-oriented population medicine in swine breeding herds. *Compendium on Continuing Education for the Practicing Veterinarian*, **10**, 871–879

- Stein, T., Molitor, T., Joo, H.S. and Leman, A.D. (1982) Porcine parvovirus infection and its control. *The Pig Veterinary Society Proceedings*, **9**, 158–167
- Stenseth, N.C., Samia, N.I., Viljugrein, H., Kausrud, K.L., Begon, M. *et al.* (2006) Plague dynamics are driven by climate variation. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 13110–13115
- Stephen, L.E. (1980) An index for coding and recording veterinary diseases and pathological states, according to body system(s) involved and aetiology or indicated cause. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 7–11 May 1979. Eds Geering, W.A., Roe, R. T. and Chapman, L.A., pp. 247–249. Australian Government Publishing Service, Canberra
- Stern, A.M. and Markel, H. (2005) The history of vaccines and immunization: familiar patterns, new challenges. *Health Affairs*, **24**, 611–621
- Sternbach, G. (2003) The history of anthrax. *Journal of Emergency Medicine*, **24**, 463–467
- Sterne, J.A.C., Hernán, M.A., Reeves, B.C., Savović, J., Berkman, N.D. *et al.* (2016) ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *British Medical Journal*, **355**, i4919; doi 10.1136/bmj.i4919
- Sterne, J.A.C., Sutton, A.J., Ioannidis, J.P.A., Terrin, N., Jones, D.R. *et al.* (2011) Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *British Medical Journal*, **343**, d4002; doi 10.1136/bmj.d4002
- Sterne, J.A.C., White, I.R., Carlin, J.B., Spratt, M., Royston, P. *et al.* (2009) Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *British Medical Journal*, **338**, b2393
- Sterner, R.T., Meltzer, M.I., Shwiff, S.A. and Slate, D. (2009) Tactics and economics of wildlife oral rabies vaccination, Canada and the United States. *Emerging Infectious Diseases*, **15**, 1176–1184
- Stevens, S.S. (1946) On the theory of scales and measurement. *Science*, **103**, 667–680
- Stewart, G.T. (1968) Limitations of the germ theory. *The Lancet*, **291**, 1077–1081
- Stewart, I. (1992) Risky business. In: *The New Scientist: Inside Science*. Ed. Fifield, R., pp. 329–342. Penguin Books, Harmondsworth
- Stigler, S.M. (1986) *The History of Statistics: The Measurement of Uncertainty Before 1900*. Belknap Press of Harvard University Press, Cambridge
- Stiglitz, J.E. (2006) *Making Globalization Work*. Allen Lane, London
- Stirling, A. (2001) The precautionary principle in science and technology. In: *Reinterpreting the Precautionary Principle*. Eds O'Riordan, T., Cameron, J. and Jordan, A., pp. 61–94. Cameron May, London
- Stirling, A. (2010) Keep it complex. *Nature*, **468**, 1029–1031
- Stites, D.P., Rodgers, R.P.C., Folds, J.D. and Schmitz, J. (1997) Clinical laboratory methods for detection of antigens and antibodies. In: *Medical Immunology*, 9th edn. Eds Stites, D.P., Terr, A.I. and Parslow, T.G., pp. 211–253. Appleton and Lange, Stamford
- Stites, D.P., Stobo, J.D., Fundenberg, H.H. and Wells, J.V. (1982) *Basic and Clinical Immunology*, 4th edn. Lange Medical Publications, Los Altos
- Stock, J.M. and Holmes, J.C. (1988) Functional relationships and microhabitat distributions of helminths of grebes (Podicipedidae): the evidence for interactive communities. *Journal of Parasitology*, **74**, 214–227
- Stolwijk, A.M., Straatman, H. and Zielhuis, G.A. (1999) Studying seasonality by using sine and cosine functions in regression analysis. *Journal of Epidemiology & Community Health*, **53**, 235–238
- Stone, D.J.W. and Thrusfield, M.V. (1989) A small animal clinical and epidemiological relational database. *Preventive Veterinary Medicine*, **7**, 289–302
- Storer, B.E. (1990) A sequential phase II/III trial for binary outcomes. *Statistics in Medicine*, **9**, 229–235
- Storrs, M.J., Courvalin, P. and Foster, T.J. (1988) Genetic analysis of gentamycin resistance in methicillin and gentamycin resistant strains of *Staphylococcus aureus* isolated in Dublin hospitals. *Antimicrobial Agents and Chemotherapy*, **32**, 1174–1181
- Stott, A.W., Lloyd, J., Humphry, R.W. and Gunn, G.J. (2003) A linear programming approach to estimate the economic impact of bovine viral diarrhoea (BVD) at the whole-farm level in Scotland. *Preventive Veterinary Medicine*, **59**, 51–66
- Strauss, S.E., Glasziou, P., Richardson, W.S. and Haynes, R.B. (2011) *Evidence-Based Medicine: How to Practice and Teach It*, 4th edn. Churchill Livingstone Elsevier, Edinburgh
- Straw, B.E., Zimmerman, J.J., D'Allaire, S. and Taylor, D.J. (Eds) (2006) *Diseases of Swine*, 9th edn. Blackwell Publishing, Ames
- Strawson, G. (1989) *The Secret Connexion: Causation Realism and David Hume*. Clarendon Press, Oxford
- Streiner, D.L. and Norman, G.R. (2008) *Health Measurement Scales: A Practical Guide to their Development and Use*, 4th edn. Oxford University Press, Oxford
- Stringer, A.P., Bell, C.E., Christley, R.M., Gebreab, F., Tefera, G. *et al.* (2011) A cluster-randomised trial to compare the effectiveness of different knowledge-

- transfer interventions for rural working equid users in Ethiopia. *Preventive Veterinary Medicine*, **100**, 90–99
- Stringer, L. and Thrusfield, M. (2001) *Foot-and-Mouth Disease Scotland 2001. Serosurveillance to Demonstrate Freedom from Disease*. Report of the Ayr Animal Health Office Epidemiology Unit, September 2001. MAFF/DEFRA (unpublished)
- Strogatz, S.H. (2001) Exploring complex networks. *Nature*, **410**, 268–276
- Stroup, D.F., Berlin, J.A., Morton, S.C., Olkin, I., Williamson, D.G. *et al.* (2000) Meta-analysis of observational studies in epidemiology: a proposal for reporting. *Journal of the American Medical Association*, **283**, 2008–2012
- Stuart, R.D. (1946) Canine leptospirosis in Glasgow. *Veterinary Record*, **58**, 131–132
- Stuart-Harris, C.H. and Schild, G.C. (1976) *Influenza, the Viruses and the Disease*. Edward Arnold, London
- Suarez, D.L. (2000) Evolution of avian influenza viruses. *Veterinary Microbiology*, **74**, 15–27
- Suazo, F.M., Escalera, A.M.A. and Torres, R.M.G. (2003) A review of *M. bovis* BCG protection against TB in cattle and other animal species. *Preventive Veterinary Medicine*, **58**, 1–13
- Subasinghe, R.P., McGladerry, S.E. and Hill, B.J. (Eds) (2004) *Surveillance and Zoning for Aquatic Animal Diseases*. FAO Fisheries Technical Paper No. 451. Food and Agriculture Organization of the United Nations, Rome
- Subbaraj, R., Chandrasekaran, N., Rajkumar, E. and Ramajayam, S. (1992) Dog population in Madurai City – random sample survey – 1987. *Livestock Adviser*, **XVII** (2), 31–33
- Summers, M. (1961) *The Vampire in Europe*. University Books, New York
- Summers, M. (1994) *The History of Witchcraft*. Senate, London
- Sundrum, A. (2001) Organic livestock farming: a critical review. *Livestock Production Science*, **67**, 207–215
- Sung, H.-T. (1992) Disease reporting systems in Taiwan, R.O.C. *Office International des Epizooties Quarterly Epidemiology Report (Asian and Pacific Region)*, **1**, 40–44
- Susser, M. (1977) Judgment and causal inference: criteria in epidemiologic studies. *American Journal of Epidemiology*, **105**, 1–15
- Susser, M. and Adelstein, A. (1975) An introduction to the work of William Farr. *American Journal of Epidemiology*, **101**, 469–476
- Susser, M. and Susser, E. (1996) Choosing a future for epidemiology: II. from black box to Chinese boxes and eco-epidemiology. *American Journal of Public Health*, **86**, 674–677
- Sutmoller, P. and Gaggero, A. (1965) Foot and mouth disease carriers. *Veterinary Record*, **77**, 968–969
- Sutmoller, P. and Olascoaga, R.C. (2002) Unapparent foot and mouth disease infection (sub-clinical infection and carriers): implications for control. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 519–529
- Sutmoller, P. and Wrathall, A.E. (1995) *Quantitative Assessment of the Risk of Disease Transmission by Bovine Embryo Transfer*. Pan American Foot-and-Mouth Disease Centre, Scientific and Technical Monograph Series No. 17. Pan American Health Organization, Brazil
- Sutmoller, P. and Wrathall, A.E. (1997) The risks of disease transmission by embryo transfer in cattle. *Revue Scientifique et Technique, Office International des Epizooties*, **16**, 226–239
- Sutmoller, P., Barteling, S.S., Olascoaga, R.C. and Sumption, K.J. (2003) Control and eradication of foot-and-mouth disease. *Virus Research*, **91**, 101–144
- Sutmoller, P., McVicar, J.W. and Cottral, G.E. (1968) The epizootiological importance of foot-and-mouth disease carriers. *Archiv für die gesamte Virusforschung*, **23**, 227–235
- Svensson, E. (2001) Guidelines to statistical evaluation of data from rating scales and questionnaires. *Journal of Rehabilitation Medicine*, **33**, 47–48
- Svobodova, M., Vorisek, P., Votypka, J. and Weidinger, K. (2004) Heteroxenous coccidian (Apicomplexa: Sarcocystidae) in the populations of their final and intermediate hosts: European buzzard and small mammals. *Acta Protozoologica*, **43**, 251–260
- SVS (1982) Effect of the drought of 1976 on the health of cattle, sheep and other farm livestock in England and Wales. *Veterinary Record*, **111**, 407–411
- Swenson, L., Audell, L. and Hedhammar, Å. (1997a) Prevalence and inheritance of and selection for elbow arthrosis in Bernese Mountain dogs and Rottweilers in Sweden and benefit:cost analysis of a screening and control program. *Journal of the American Veterinary Medical Association*, **210**, 207–214
- Swenson, L., Audell, L. and Hedhammar, Å. (1997b) Prevalence and inheritance of and selection for hip dysplasia in seven breeds of dogs in Sweden and benefit:cost analysis of a screening and control program. *Journal of the American Veterinary Medical Association*, **210**, 215–221
- Swift, J. (1988) *Major Issues in Pastoral Development with Special Emphasis on Selected African Countries*. Food and Agriculture Organization of the United Nations, Rome
- Swift, J., Toulmin, C. and Chatting, S. (1990) *Providing Services for Nomadic People: A Review of the Literature and Annotated Bibliography*. UNICEF Staff Working Paper No. 8. UNICEF, New York

- Swinkels, J.M., Rooijendijk, J.G.A., Zadoks, R.N. and Hogeveen, H. (2005) Use of partial budgeting to determine the economic benefits of antibiotic treatment of chronic subclinical mastitis caused by *Streptococcus uberis* or *Streptococcus dysgalactiae*. *Journal of Dairy Research*, **72**, 75–85
- Swinton, J., Harwood, J., Grenfell, B.T. and Gilligan, C.A. (1998) Persistence thresholds for phocine distemper virus infection in harbour seal *Phoca vitulina* metapopulations. *Journal of Animal Ecology*, **67**, 54–68
- Sykes, A.R. and Coop, R.L. (2001) Interaction between nutrition and gastrointestinal parasitism in sheep. *New Zealand Veterinary Journal*, **49**, 222–226
- Symeonakis, E., Robinson, T. and Drake, N. (2007) GIS and multiple-criteria evaluation for the optimisation of tsetse fly eradication programmes. *Environmental Monitoring and Assessment*, **124**, 89–103
- Szmant, A.M. (2002) Nutrient enrichment on coral reefs: is it a major cause of coral reef decline? *Estuaries*, **25**, 743–766
- Tabachnick, W.J. (2010) Challenges in predicting climate and environmental effects on vector-borne disease epistemics in a changing world. *The Journal of Experimental Biology*, **213**, 946–954
- Tam, D. (2009) A theoretical analysis of cumulative sum slope (CUSUM-slope) statistic for detecting signal onset (begin) and offset (end) trends from background noise level. *The Open Statistics and Probability Journal*, **1**, 43–51
- Tambi, E.N., Maina, O.W., Mukhebi, A.W. and Randolph, T.F. (1999) Economic impact assessment of rinderpest control in Africa. *Revue Scientifique et Technique, Office International des Epizooties*, **18**, 458–477
- Tanaka, J. (1992) Outline of animal health information processing in Japan. *Office International des Epizooties Quarterly Epidemiology Report (Asian and Pacific Region)*, **1**, 36–39
- Tang, Y.W. (2013) Promoting translational research in human and veterinary medical virology. *Veterinary Microbiology*, **165**, 2–6
- Tangle, D.K. and Berry, D.A. (Eds) (2000) *Meta-Analysis in Medicine and Health Policy*. Marcel Dekker, New York
- Tannahill, R. (1968) *The Fine Art of Food*. The Folio Society, London
- Tansley, A.G. (1935) The use and abuse of vegetational concepts and terms. *Ecology*, **16**, 284–307
- Tatem, A.J., Capela, R., Pena, I., Mellor, P.S., Baylis, M. et al. (2003) Prediction of *Culicoides*-borne disease risk in Europe and North Africa using satellite imagery. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Warwick, 31 March–2 April 2003. Eds Reid, S.W.J. and Menzies, F.D., pp. 253–264
- Tatem, A.J., Rogers, D.J. and Hay, S.I. (2006) Global transport networks and infectious disease spread. *Advances in Parasitology*, **62**, 293–343
- Tater, K.C. (2012) An approach to pruritus. In: *BSAVA Manual of Canine and Feline Dermatology*, 3rd edn. Eds Jackson, H. and Marsella, R., pp. 37–45. British Small Animal Veterinary Association, Quedgeley
- Taves, D.R. (2010) The use of minimization in clinical trials. *Contemporary Clinical Trials*, **31**, 180–184
- Taylor, K.C. (2002) Environmental impacts of the foot and mouth disease outbreak in Great Britain in 2001: the use of risk analysis to manage the risks in the countryside. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 797–813
- Taylor, N. (2003) *Review of the Use of Models in Informing Disease Control Policy Development and Adjustment. A Report to DEFRA*. <http://www.defra.gov.uk/science/Publications/2003/UseofModelsInDiseaseControlPolicy.pdf> (accessed 20 August 2003)
- Taylor, R. (1967) Causation. In: *The Encyclopedia of Philosophy*, Vol. 2. Ed. Edwards, P., pp. 56–66. The Macmillan Company and The Free Press, New York
- Taylor, W.P. and Marshall, I.E. (1975) Adaptation studies with Ross River virus: retention of field level virulence. *Journal of General Virology*, **28**, 73–83
- Tchangai, C.P., Mehta, P., Hendrickx, S. and Kaboré, S. (2010) *Training Course for Participatory Epidemiology Trainers. PENAPH, 25–29 January 2010, Lomé, Togo* [Report]. ILRI, Nairobi. <http://hdl.handle.net/10568/1106> (accessed 1 July 2015)
- Teal, C.J. (2002) Antimicrobial resistance and the food chain. *Journal of Applied Microbiology Symposium Supplement*, **92**, 85S–89S
- Teclaw, R., Mendlein, J., Garbe, P. and Mariolis, P. (1992) Characteristics of pet populations and households in the Purdue Comparative Oncology Program catchment area, 1988. *Journal of the American Veterinary Medical Association*, **201**, 1725–1729
- Tedor, J. and Reif, J.S. (1978) Natal patterns among registered dogs in the United States. *Journal of the American Veterinary Medical Association*, **172**, 1179–1185
- Tefera, M. (2004) Recent evidence of animal exploitation in the Axumite epoch, 1st–5th centuries AD. *Tropical Animal Health and Production*, **36**, 105–116
- Teich, N. (1982) Taxonomy of retroviruses. In: *Molecular Biology of Tumor Viruses: RNA Tumor Viruses*, 2nd edn. Eds Weiss, R., Teich, N., Varmus, H. and Coffin, J., pp. 25–207. Cold Spring Harbor Laboratory, New York
- Tenzin, D.N.K., Dorjee, J. and Ward, M.P. (2011) Re-emerging rabies in dogs and other domestic animals in eastern Bhutan, 2005–2007. *Epidemiology and Infection*, **139**, 220–225

- Terborgh, J. and Estes, J.A. (Eds) (2010) *Trophic Cascades: Predators, Prey, and the Changing Dynamics of Nature*. Island Press, Washington DC
- Thacker, S.B. and Birkhead, G.S. (2008) Surveillance. In: *Field Epidemiology*, 3rd edn. Ed. Gregg, M.B., pp. 38–64. Oxford University Press, New York
- The Economist* (2002) The race to computerise biology. *The Economist Technology Quarterly*, 16 December 16, 20–22
- The Royal Society (1992) *Risk: Analysis, Perception and Management*. The Royal Society, London
- The SURE Collaboration* (2015) *SURE Guides for Preparing and Using Evidence-Based Policy Briefs*. The SURE Collaboration, World Health Organization, Geneva. <http://www.who.int/evidence/sure/guides/en/#> (accessed 22 September 2015)
- The Times* (1983) Running dogs banned. *The Times*, 13 October, 6
- Theis, J.H., Kass, P.H. and Stevens, F. (1998) Effects of drought and chemoprophylaxis on heartworm transmission in domestic dogs in California (1983–1991). In: *Recent Advances in Heartworm Disease: Symposium '98, Tampa, Florida, May 1–3, 1998*. Ed. Seward, R.L., pp. 37–50. American Heartworm Society, Batavia
- Theodorou, M.K. and France, J. (Eds) (2000) *Feeding Systems and Feed Evaluation Models*. CAB Publishing, Wallingford
- Thiermann, A.B. (2005) Globalization, international trade and animal health: the new roles of OIE. *Preventive Veterinary Medicine*, **67**, 101–108
- Thomas, A., O'Hara, B., Ligges, U. and Sturtz, S. (2006.) Making BUGS Open. *R News*, **6**, 12–17
- Thomas, A.D. and Maré, C.V.E. (1945) Knopvelsiekte. *Journal of the South African Veterinary Medical Association*, **16**, 36–43
- Thomas, A.D. and Reid, N. R. (1944). Rinderpest in game. A description of an outbreak and an attempt at limiting its spread by means of a bush fence. *Onderstepoort Journal of Veterinary Science and Animal Industry*, **20**, 7–20
- Thomas, L.F., de Glanville, W.A., Cook, E.A. and Fevre E. M. (2013) The spatial ecology of free-ranging domestic pigs (*Sus scrofa*) in western Kenya. *BMC Veterinary Research*, **9**, **46**; doi 10. 1186/1746-6148-9-46
- Thomas, R.J. (2013) Particle size and pathogenicity in the respiratory tract. *Virulence*, **4**, 847–858
- Thompson, D., Muriel, P., Russell, D., Osborne, P., Bromley, A. *et al.* (2002) Economic costs of the foot and mouth disease outbreak in the United Kingdom in 2001. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 675–687
- Thompson, E.T. and Hayden, A.C. (1961) *Standard Nomenclature of Diseases and Operations*, 5th edn. McGraw-Hill, New York
- Thompson, W.D. (1991) Effect modification and the limits of biological inference. *Journal of Clinical Epidemiology*, **44**, 221–232
- Thomsen, P.T. (2008) Rapid screening method for lameness in dairy cows. *Veterinary Record*, **164**, 689–690
- Thomson, G.R. (1996) The role of carriers in the transmission of foot and mouth disease. In: Conference Proceedings of the 64th General Session of Office International des Epizooties, pp. 87–103
- Thomson, G.R., Vosloo, W. and Bastos, A.D.S. (2003) Foot and mouth disease in wildlife. *Virus Research*, **91**, 145–161
- Thor, J., Lundberg, J., Ask, J., Olsson, J., Carli, C. *et al.* (2007) Application of statistical process control in healthcare improvement: systematic review. *Quality and Safety in Health Care*, **16**, 387–399
- Thorns, C.J. and Morris, J.A. (1983) The immune spectrum of *Mycobacterium bovis* infection in some mammalian species: a review. *Veterinary Bulletin*, **53**, 543–550
- Thornton, P.D. and Waterman-Pearson, A.E. (1999) Quantification of the pain and distress responses to castration in young lambs *Research in Veterinary Science*, **66**, 107–118
- Thornton, P.K. (2010) Livestock production: recent trends, future prospects. *Philosophical Transactions of the Royal Society, Series B*, **365**, 2853–2867
- Thrusfield, M.V. (1985a) How to use a knowledge of epidemiology in practice. *The Pig Veterinary Society Proceedings*, **12**, 25–37
- Thrusfield, M.V. (1985b) Data recording in general practice. *In Practice*, **7**, 128–138
- Thrusfield, M.V. (1989) Demographic characteristics of the canine and feline populations of the UK in 1986. *Journal of Small Animal Practice*, **30**, 76–80
- Thrusfield, M. (1991) A computerised small animal practice health management system with a centralised data-analysis facility. In: Proceedings of the 6th International Symposium on Veterinary Epidemiology and Economics, Ottawa, 12–16 August 1991. Ed. Martin, S.W., pp. 459–461
- Thrusfield, M.V. (1992) Canine kennel cough: a review. In: *The Veterinary Annual*, Vol. 32. Eds Raw, M.-E. and Parkinson, T.J., pp. 1–12. Wright, London
- Thrusfield, M. (1993) Quantitative approaches to veterinary epidemiology. In: *The Advancement of Veterinary Science. The Bicentenary Symposium Series. Volume 1: Veterinary Medicine Beyond 2000*. Ed. Michell, A.R., pp. 121–142. CAB International, Wallingford
- Thrusfield, M. (2001) Introduction to epidemiology. In: *Application of Quantitative Methods in Veterinary Epidemiology*, revised reprint. Eds Noordhuizen, J.P.T.

- M., Frankena, K., Thrusfield, M.V. and Graat, E.A.M., pp. 3–11. Wageningen Pers, Wageningen
- Thrusfield, M. (2006) *Potential Biological Health Risks in the Handling and Processing of Animal Carcasses for Taxidermy: A Qualitative Risk Assessment*. Department of Natural Sciences, National Museums of Scotland, Edinburgh
- Thrusfield, M. (2016a) Etiology. In: *Diseases of Coral*. Eds Woodley, C.M., Downs, C.A., Bruckner, A.W., Porter, J.W. and Galloway, S.B., pp. 16–27. John Wiley and Sons, Hoboken
- Thrusfield, M. (2016b) Epidemiology. In: *Diseases of Coral*. Eds Woodley, C.M., Downs, C.A., Bruckner, A.W., Porter, J.W. and Galloway, S.B., pp. 28–51. John Wiley and Sons, Hoboken
- Thrusfield, M.V., Aitken, C.G.G. and Darke, P.G.G. (1985) Observations on breed and sex in relation to canine heart valve incompetence. *Journal of Small Animal Practice*, **26**, 709–717
- Thrusfield, M.V., Holt, P.E. and Muirhead, R.H. (1998) Acquired urinary incontinence in bitches: its incidence and relationship to neutering practices. *Journal of Small Animal Practice*, **39**, 559–566
- Thrusfield, M., Mansley, L., Dunlop, P., Pawson, A. and Taylor, J. (2005a) The foot-and-mouth disease epidemic in Dumfries and Galloway, 2001. 2: Serosurveillance, and efficiency and effectiveness of control procedures after the national ban on animal movements. *Veterinary Record*, **156**, 269–278
- Thrusfield, M., Mansley, L., Dunlop, P., Taylor, J. Pawson, A. *et al.* (2005b) The foot-and-mouth disease epidemic in Dumfries and Galloway, 2001. 1: Characteristics and control. *Veterinary Record*, **156**, 229–252
- Thulke, H.-H., Grimm, V., Müller, M.S., Staubach, C., Tischendorf, L. *et al.* (1999) From pattern to practice: a scaling-down strategy for spatially explicit modelling illustrated by the spread and control of rabies. *Ecological Modelling*, **117**, 179–202
- Thurber, E.T., Bass, E.P. and Beckenhauer, W.H. (1977) Field trial evaluation of a reo-coronavirus calf diarrhea vaccine. *Canadian Journal of Comparative Medicine*, **41**, 131–146
- Thygesen, L.C., Andersen, G.S. and Andersen, H. (2005) A philosophical analysis of the Hill criteria. *Journal of Epidemiology & Community Health*, **59**, 512–516
- Tiira, K. and Lohi, H. (2014) Reliability and validity of a questionnaire survey in canine anxiety research. *Applied Animal Behaviour Science*, **155**, 82–92
- Timbs, D.V. (1980) The New Zealand national bovine serum bank. In: *Veterinary Epidemiology and Economics*, Proceedings of the Second International Symposium, Canberra, 7–11 May 1979. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 76–80. Australian Government Publishing Service, Canberra
- Timoney, J.F., Gillespie, J.H., Scott, F.W. and Balough, J.E. (1988) *Hagan and Bruner's Microbiology and Infectious Diseases of Domesticated Animals*, 8th edn. Comstock Publishing Associates, Ithaca
- Timoney, P.J. (2000) Factors influencing the international spread of equine diseases. *Veterinary Clinics of North America: Equine Practice*, **16**, 537–551
- Tinline, R. (1970) Lee wave hypothesis for the initial pattern of spread during the 1967–68 foot-and-mouth disease epizootic. *Nature*, **227**, 860–862
- Tinline, R. (1972) A simulation study of the 1967–68 foot-and-mouth epizootic in Great Britain. PhD Thesis, University of Bristol
- Tizard, I. (1990) Risks associated with use of live vaccines. *Journal of the American Veterinary Medical Association*, **196**, 1851–1858
- Tizard, I.R. (2013) *Veterinary Immunology*, 9th edn. Elsevier Saunders, St Louis
- Tjalma, R.A. (1968) Implications of animal cancers to human neoplasia: epidemiologic considerations. *International Journal of Cancer*, **3**, 1–6
- Tjalma, R.A., Priester, W.A., Adelstein, E.H. and Vander Zwaag, R. (1964) Clinical records systems and data retrieval function in veterinary medicine: a proposal for system data programming. *Journal of the American Veterinary Medical Association*, **145**, 1189–1197
- Toft, N., Åkerstedt, J., Tharaldsen, J. and Hopp, P. (2007) Evaluation of three serological tests for diagnosis of maedi-visna virus infection using latent class analysis. *Veterinary Microbiology*, **120**, 77–86
- Toker, M., Sen, Y., Kaya, M., Boztok, B., Bumin, A. *et al.* (2011) Mitral valve prolapse (MVP) in dogs: a 12 year long retrospective study by echocardiography. *Revue de Médecine Vétérinaire*, **162**, 352–357
- Toma, B., Moutou, F., Dufour, B. and Durand, B. (2002) Ring vaccination against foot and mouth disease. *Comparative Immunology, Microbiology and Infectious Diseases*, **25**, 365–372
- Toma, B., Vaillancourt, J.-P., Dufour, B., Eloit, M., Moutou, F. *et al.* (Eds) (1999) *Dictionary of Veterinary Epidemiology*. Iowa State University Press, Ames
- Toma, L., Stott, A.W., Heffernan, C., Ringrose, S. and Gunn, G.J. (2013) Determinants of biosecurity behaviour of British cattle and sheep farmers – a behavioural economics analysis. *Preventive Veterinary Medicine*, **108**, 321–333
- Tomasetti, C. and Vogelstein, B. (2015) Variation in cancer risk among tissues can be explained by the number of stem cell divisions. *Science*, **347**, 78–81
- Topley, W.W.C. (1942) The biology of epidemics. *Proceedings of the Royal Society of London, Series B*, **130**, 337–359

- Torgerson, P. and Claxton, J. (1999) Epidemiology and control. In: *Fasciolosis*. Ed. Dalton, J.P., pp. 113–149. CABI Publishing, Wallingford
- Toribio, J.-A.L.M.L. and Rushton, J. (2012) Participatory epidemiology: the emergence of a sub-discipline. *The Veterinary Journal*, **191**, 145–146
- Toribio, J.-A.L.M., Norris, J.M., White, J.D., Dhand, N. K., Hamilton, S.A. *et al.* (2009) Demographics and husbandry of pet cats living in Sydney, Australia: results of a cross-sectional survey of pet ownership. *Journal of Feline Medicine and Surgery*, **11**, 449–461
- Tornor, W. (1927) Eine veterinärpolizeiliche Verordnung Friedrich Wilhelms I von Preußen aus dem Jahr 1716 zur Bekämpfung des ‘Sterbens unter dem Horne-Viehe’. *Tierärztliche Rundschau*, **33**, 345–346 (in German)
- Torres-Anjel, M.J. and Tshikuka, J.G. (1988) The microepidemiology of the animal acquired immunodeficiency syndromes (AIDS) in the context of human AIDS (HAIDS). In: *Environment and Animal Health*. Proceedings of the 6th International Congress on Animal Hygiene, Skara, Sweden, Vol. 2. Ed. Ekesbo, I., pp. 745–749. Swedish University of Agricultural Sciences, Report No. 21
- Toth, T. (1969) Studies of an agent causing mortality among ducklings immune to duck virus hepatitis. *Avian Diseases*, **13**, 834–846
- Toutain, P.-L., Ferran, A. and Bousquet-Mélou, A. (2010) Species differences in pharmacokinetics and pharmacodynamics. In: *Comparative and Veterinary Pharmacology*. Handbook of Experimental Pharmacology No. 199. Eds Cunningham, F., Elliott, J. and Lees, P., pp. 19–48. Springer, Heidelberg
- Townley, J. (1827) *The Reasons of the Laws of Moses: From the ‘More Nevochim’ of Maimonides, with Notes, Dissertations, and a Life of the Author*. Longman, Rees, Orme, Brown and Green, London
- Traas, A.M., Casal, M., Haskins, M. and Henthorn, P. (2006) Genetic counseling in the era of molecular diagnostics. *Theriogenology*, **66**, 599–605
- Trainor, B.C., Sweeney, C. and Cardiff, R. (2009) Isolating the effects of social interactions on cancer biology. *Cancer Prevention Research*, **2**, 843–846
- Tran, A., Gaidet, N., L’Ambert, G., Balenghein, T., Balanca, G. *et al.* (2007) The use of remote sensing for the ecological description of multi-host disease systems: a case study of West Nile virus in southern France. *Veterinaria Italiana*, **43**, 687–697
- Traum, J. (1936) Vesicular exanthema of swine. *Journal of the American Veterinary Medical Association*, **88**, 316–334
- Travis, C.C. (1987) Interspecies extrapolations in risk analysis. *Toxicology*, **47**, 3–13
- Trees, A.J., Campbell, M.R.L.H., Fishwick, J., O’Rourke, D., Herrtage, M.E. *et al.* (2013) *Ethical Review for Practice-Based Research: A Report of a Joint RCVS/BVA Working Party*. <http://www.rcvs.org.uk/publications/ethical-review-for-practice-based-research/> (accessed 27 January 2015)
- Trenkle, A. (2006) *Evaluation of Rumen Boluses as an Electronic Identification System for Cattle in an Automated Data Collection System*. Iowa State University Animal Industries Report 2006, A.S. Leaflet R2073. <http://www.ans.iastate.edu/report/air/2006pdf/R2073.pdf> (accessed 16 July 2015)
- Trevisi, E., Zecconi, A., Cogrossi, S., Razzuoli, E., Grossi, P. *et al.* (2014) Strategies for reduced antibiotic usage in dairy cattle farms. *Research in Veterinary Science*, **96**, 229–233
- Trewhella, W.R. and Anderson, R.M. (1983) Modelling bovine tuberculosis in badgers. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Southampton, 12–13 April 1983. Ed. Thrusfield, M.V., pp.78–84
- Tribe, H.T. (1980) Prospects for the biological control of plant-parasitic nematodes. *Parasitology*, **81**, 619–639
- Tricco, A.C., Pham, B., Brehaut, J., Tetroe, J., Capelli, M. *et al.* (2009) An international survey indicated that unpublished systematic reviews exist. *Journal of Clinical Epidemiology*, **62**, 617–623
- Trotter, W. (1930) Observation and experiment and their use in the medical sciences. *British Medical Journal*, **ii**, 129–134
- Trotz-Williams, L.A. and Trees, A.J. (2003) Systematic review of the distribution of the major vector-borne parasitic infections in dogs and cats in Europe. *Veterinary Record*, **152**, 97–105
- Trouwborst, A. (2002) *Evolution and Status of the Precautionary Principle in International Law*. Kluwer Law International, The Hague
- Tryfos, P. (1996) *Sampling Methods for Applied Research*. John Wiley, New York
- Tsetsarkin, K.A., Vanlandingham, D.L., McGee, C.E. and Higgs, S. (2007) A single mutation in Chikungunya virus affects vector specificity and epidemic potential. *PLoS Pathogens*, **3**, 12, e201; doi 10.1371/journal.ppat.0030201
- Tugwell, P. and Knottnerus, A.J. (2015) The new paradigm: from ‘bench to bedside’ to translational research ‘valleys of death’. *Journal of Clinical Epidemiology*, **68**, 725–726
- Tukey, J.W. (1977) *Exploratory Data Analysis*. Addison-Wesley Publishing, Philippines
- Tuohimaa, P., Tenkanen, L., Ahonen, M., Lumme, S., Jellum, E. *et al.* (2004) Both high and low levels of blood vitamin D are associated with a higher prostate cancer risk: a longitudinal, nested case-control study in the

- Nordic countries. *International Journal of Cancer*, **108**, 104–108
- Turkson, P.K. (2003) Profile of veterinarians and veterinary practice in Ghana. *Tropical Animal Health and Production*, **35**, 321–340
- Turkson, P.K. and Brownie, C.F. (1999) Perceived constraints to privatization of delivery of veterinary services in Ghana. *Tropical Animal Health and Production*, **31**, 103–114
- Turner, A.S. (2002) The sheep as a model for osteoporosis in humans. *The Veterinary Journal*, **163**, 232–239
- Turner, J., Bowers, R.G., Clancy, D., Behnke, M.C. and Christley, R.M. (2008) A network model of *E.coli* O157 transmission within a typical UK dairy herd: the effect of heterogeneity and clustering on the prevalence of infection. *Journal of Theoretical Biology*, **254**, 45–54
- Turner, T.A., Kneller, S.K., Badertscher, R.R. and Stowater, J.L. (1987) Radiographic changes in the navicular bones of normal horses. In: Proceedings of the 32nd Annual Convention of the American Association of Equine Practitioners, Nashville, 29 November–3 December 1986, pp. 309–314
- Tuytens, F.A.M., Long, B., Fawcett, T., Skinner, A., Brown, J.A. *et al.* (2001) Estimating group size and population density of Eurasian badgers *Meles meles* by quantifying latrine use. *Journal of Applied Ecology*, **38**, 1114–1121
- Tversky, A. and Kahneman, D. (1974) Judgement under uncertainty: heuristics and biases. *Science*, **185**, 1124–1131
- Tversky, A. and Kahneman, D. (1981) The framing of decisions and the psychology of choice. *Science*, **211**, 453–458
- Twisk, J.W.R. (2006) *Applied Multilevel Analysis*. Cambridge University Press, Cambridge
- Tyler, L. (1991) PARC sero-monitoring: survey design, implementation and the analysis, presentation and use of results. In: *The Sero-monitoring of Rinderpest Throughout Africa. Phase One*. Proceedings of a Final Research Co-ordination Meeting of the FAO/IAEA/SIDA/OAU/BAR/PARC Co-ordinated Research Programme, Bingerville, Cote d'Ivoire, 19–23 November 1990. IAEA-TECDOC-623, pp. 67–79. International Atomic Energy Agency, Vienna
- Tyndale-Biscoe, H. (1994) The CRC for biological control of vertebrate pest populations: fertility control of wildlife for conservation. *Pacific Conservation Biology*, **1**, 160–162
- Tyokumbur, E.T. (2014) Review of ecological perspectives on climate change in Nigeria. *American Journal of Environmental Engineering and Science*, **1**, 36–49
- Tyson, J.D. (2002) FMD in a parturient sheep flock (Letter). *Veterinary Record*, **151**, 127
- Ubbink, G.J., van de Broek, J., Hazewinkel, H.A.W. and Rothuizen, J. (1998a) Cluster analysis of the genetic heterogeneity and disease distributions in purebred dog populations. *Veterinary Record*, **142**, 209–213
- Ubbink, G.J., van de Broek, J., Hazewinkel, H.A.W. and Rothuizen, J. (1998b) Risk estimates for dichotomous genetic disease traits based on a study of relatedness in purebred dog populations. *Veterinary Record*, **142**, 328–331
- Udomprasert, P. and Williamson, N.B. (1990) The DairyCHAMP program: a computerised recording system for dairy herds. *Veterinary Record*, **127**, 256–262
- Uhaa, I.J., Mandel, E.J., Whiteway, R. and Fishbein, D.B. (1992) Rabies surveillance in the United States during 1990. *Journal of the American Veterinary Medical Association*, **200**, 920–929
- Uilenberg, G. (1998) *A Field Guide for the Diagnosis, Treatment and Prevention of African Animal Trypanosomosis*. Food and Agriculture Organization of the United Nations, Rome
- UK National Case-Control Study Group (1989) Oral contraceptive use and breast cancer risk in young women. *The Lancet*, **333**, 974–982
- UKILGRA (1996) *United Kingdom Interdepartmental Liaison Group on Risk Assessment, Use of Risk Assessment Within Government Departments*. (Quoted by Redmill, 2002)
- Uphoff, N. (1992) *Learning from Gal Oya: Possibilities for Participatory Development and Post-Newtonian Science*. Cornell University Press, New York
- Upjohn, M., Church, D., Jasani, S. and Brodbelt, D. (2008) Establishing an Electronic Patient Record (EPR) in first opinion veterinary practice: challenges to overcome (Poster). In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Liverpool, 26–28 March 2008. Eds Peeler, E.J., Alban, L., Russell, A. *et al.* <http://www.svepm.org.uk/posters/2008/Establishing%20an%20Electronic%20Patient%20Record%20%28EPR%29%20in%20first%20opinion%20practice.pdf> (accessed 30 October 2014)
- Uriarte, A. and Saiz, I.M. (2016) Canine versus human epilepsy: are we up to date? *Journal of Small Animal Practice*, **57**, 115–121
- Urquhart, G.M. (1980) Application of immunity in the control of parasitic disease. *Veterinary Parasitology*, **6**, 217–239
- USDA (1998) Composition of the US equine population. Centers for Epidemiology and Animal Health, United States Department of Agriculture, Fort Collins. <http://www.aphis.usda.gov/vs/ceah/cahm/Equine/eq98comp.pdf> (accessed 14 March 2003)

- USDA (2006) *Equine 2005, Part I: Baseline Reference of Equine Health and Management, 2005*. USDA:APHIS: VS, CEAH. Fort Collins
- USDA (2007) *National Animal Identification System (NAIS) – A User Guide and Additional Information Resources*, Version 2.0. United States Department of Agriculture, Washington DC
- USDA (2011) *Guidelines for Surveillance of Bovine Tuberculosis in Wildlife*, Version 1.0. United States Department of Agriculture, Washington. http://www.aphis.usda.gov/animal_health/animal_diseases/tuberculosis/downloads/wildlife_tb_surv_manual.pdf (accessed 3 June 2015)
- USDA (2013) *Risk Analysis: Foot-and-Mouth Disease (FMD) Risk from Importation of Fresh (Chilled or Frozen), Maturated, Deboned Beef from a Region in Brazil into the United States*. Animal and Plant Health Inspection Service, Veterinary Services National Center for Import and Export, United States Department of Agriculture, Washington DC
- USDHHS (2000) *Healthy People 2010*. United States Department of Health and Human Services, Public Health Service, Washington DC
- USDHHS (2011) *Report on Carcinogens*, 12th edn. United States Department of Health and Human Services, Washington DC
- USGS (2015) *US Geological Survey. West Nile Virus Maps*. <http://diseasemaps.usgs.gov/mapviewer/> (accessed 13 July 2015)
- Uttenthal, Å., Parida, S., Rassmusen, T.B., Paton, D.J., Haas, B. *et al.* (2010) Strategies for differentiating infection in vaccinated animals (DIVA) for foot-and-mouth disease, classical swine fever and avian influenza. *Expert Review of Vaccines*, **9**, 73–87
- Vach, W. and Blettner, M. (1991) Biased estimation of the odds ratio in case-control studies due to the use of ad hoc methods of correcting for missing values of confounding variables. *American Journal of Epidemiology*, **134**, 895–907 (correction: **140**, 789)
- Vail, D.M. and MacEwen, E.G. (2000) Spontaneously occurring tumors of companion animals as models for human cancer. *Cancer Investigation*, **18**, 781–792
- Vaillancourt, J.-P., Martineau, G., Morrow, M., Marsh, W. and Robinson, A. (1991) Construction of questionnaires and their use in veterinary medicine. In: *Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, London, 17–19 April 1991*. Ed. Thrusfield, M.V., pp. 94–106
- Valensin, A. (1972) Satan in the Old Testament. In: *Soundings in Satanism*. Ed. Sheed, F.J., pp. 105–120. Mowbrays, Oxford
- Vallat, B. (2001) Traceability of animals and animal products. *Revue Scientifique et Technique, Office International des Epizooties*, **20**, 359–361
- Valle, P.S., Skjerve, E., Martin, S.W., Larssen, R.B., Østerås, O. *et al.* (2000) A cost benefit evaluation of the Norwegian bovine virus diarrhoea control and eradication program. *Proceedings of the 9th International Symposium on Veterinary Epidemiology and Economics, Breckenbridge, Colorado*. Abstract ID 511
- Van Arendonk, J.A.M. (1985) Studies on the replacement policies in dairy cattle. PhD Thesis, Wageningen Agricultural University
- van Baalen, M. (2000) Parent-to-offspring infection and the struggle for transmission. In: *Evolutionary Biology of Host–Parasite Relationships: Theory Meets Reality*. Eds Poulin, R., Moran, S. and Skorping, A., pp. 97–116. Elsevier, Amsterdam
- van Bakkum, J.G. (1973) The carrier state and foot-and-mouth disease. In: *Proceedings of the Second International Conference on Foot-and-Mouth Disease*. Ed. Pollard, M., pp. 45–50. Gustav Stern Foundation, New York
- van Boven, M., Kretzschmar, M., Wallinga, J., O'Neill, P. D., Wichmann, O. *et al.* (2010) Estimation of measles vaccine efficacy and critical vaccination coverage in a highly vaccinated population. *Journal of the Royal Society Interface*, **7**, 1537–1544
- van Boven, M., Ruijs, W.L.M., Wallinga, J., O'Neill, P.D. and Hahné, S. (2013) Estimation of vaccine efficacy and critical vaccination coverage in partially observed outbreaks. *PLoS Computational Biology*, **9**, 5, e1003061; doi 10.1371/journal.pcbi.1003061
- van de Braak, A.E., van't Klooster, A.Th. and Malestein, A. (1987) Influence of a deficient supply of magnesium during the dry period on the rate of calcium mobilisation by dairy cows at parturition, *Research in Veterinary Science*, **42**, 101–108
- Van den Bergh, J.C.J.M., Button, K.J., Nijkamp, P., Pepping, G.C., Baaijens, S.R. *et al.* (1997) *Meta-Analysis in Environmental Economics*. Kluwer Academic Publishers, Dordrecht
- van den Broek, A.H.M., Thrusfield, M.V., Dobbie, G.R. and Ellis, W.A. (1991) A serological and bacteriological survey of leptospiral infection in dogs in Edinburgh and Glasgow. *Journal of Small Animal Practice*, **32**, 118–124
- Van Der Fels-Klerx, I.H.J., Goossens, L.H.J., Saatkamp, H. W. and Horst, S.H.S. (2002) Elicitation of quantitative data from a heterogeneous expert panel: formal process and application in animal health. *Risk Analysis*, **22**, 67–81
- Van der Kamp, G., Dijkhuizen, A.A. and Peterse, D.J. (1990) Simulation of leptospirosis control in Dutch dairy herds. *Preventive Veterinary Medicine*, **9**, 9–26
- van der Worp, H.B., Howells, D.W., Sena, E.S., Porritt, M. J., Rewell, S. *et al.* (2010) Can animal models of disease

- reliably inform human studies? *PLoS Medicine*, **7**, 3, e1000245; doi 10.1371/journal.pmed.1000245
- van Dijk, J. (2013) Towards risk-based test protocols: estimating the contribution of intensive testing to the UK bovine tuberculosis problem. *PLoS ONE*, **8**, 5, e63961; doi 10.1371/journal.pone.0063961
- Van Eck, J.H.H., Davelaar, F.G., Van den Heuvel-Plesman, T.A.M., Van Kol, N., Kouwenhoven, B. *et al.* (1976) Dropped egg production, soft shelled and shell-less eggs associated with appearance of precipitins to adenovirus in flocks of laying fowl. *Avian Pathology*, **5**, 261–272
- van Hagen, M.A.E., Ducro, B.J., van den Broek, J. and Knol, B.W. (2005) Incidence, risk factors, and heritability estimates of hind limb lameness caused by hip dysplasia in a birth cohort of Boxers. *American Journal of Veterinary Research*, **66**, 307–312
- van Heuveln, B. (2000) A preferred treatment of Mill's methods: some misinterpretations by modern textbooks. *Informal Logic*, **20**, 19–42
- van Houwelingen, H.C., Arends, L.R. and Stijnen, T. (2002) Advanced methods in meta-analysis: multivariate approach and meta-regression. *Statistics in Medicine*, **21**, 589–624
- Van Kooten, G.C. and Bulte, E.H. (2000) *The Economics of Nature: Managing Biological Assets*. Blackwell Publishers, Oxford
- Van Nes, A., De Jong, M.C.M., Buijtsels, J.A.A.M. and Verheijden, J.H.M. (1998) Implications derived from a mathematical model for eradication of pseudorabies virus. *Preventive Veterinary Medicine*, **33**, 39–58
- Van Regenmortel, M.H. (2004) Reductionism and complexity in molecular biology. *EMBO Reports*, **5**, 1016–1020
- Van Schaik, G., Nielen, M. and Dijkhuizen, A.A. (2001) Biosecurity on dairy farms: the economic benefits. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Noordwijkerhout, 28–30 March, 2001. Eds Menzies, F.D. and Reid, S.W.J., pp. 175–184
- van Sluijs, F.J. and van Nes, J.J. (2009) Medical records. In: *Medical History and Physical Examination in Companion Animals*, 2nd edn. Eds Rijnberk, A. and Sluijs, F.J., pp. 27–39. Bohn Stafleu van Loghum, Houten/Saunders Elsevier, Philadelphia
- van Steenbeek, F.G., Leegwater, P.A.J., van Sluijs, F.J., Heuven, H.C.M. and Rothuizen, J. (2009) Evidence of inheritance of intrahepatic portosystemic shunts in Irish wolfhounds. *Journal of Veterinary Internal Medicine*, **23**, 950–952
- Van Velden, D.J.J., Meyer, J.D., Oliver, J., Gear, J.H.S. and McIntosh, B. (1977) Rift Valley fever affecting humans in South Africa. *South African Medical Journal*, **51**, 867–871
- Van Welzen, P.C., Parnell, J.A.N. and Slik, J.W.F. (2011) Wallace's Line and plant distributions: two or three phytogeographical areas and where to group Java? *Biological Journal of the Linnean Society*, **103**, 531–545
- Vandenbrouke, J.P. and Pearce, N. (2012) Case-control studies: basic concepts. *International Journal of Epidemiology*, **41**, 1480–1489
- VanderWeele, T.J. (2009) On the distinction between interaction and effect modification. *Epidemiology*, **20**, 863–871
- Vanhamme, L., Pays, E., McCulloch, R. and Barry, J.D. (2001) An update on antigenic variation in African trypanosomes. *Trends in Parasitology*, **17**, 338–343
- Vanopdenbosch, E. and Wellemans, G. (1990) Bovine birna type virus: a new etiological agent of neonatal calf diarrhoea? *Vlaams Diergeneeskundig Tijdschrift*, **59**, 137–140
- Vanwambeke, S.O., Šumilo, D., Bormane, A., Lambin, E. F. and Randolph, S.E. (2010) Landscape predictors of tick-borne encephalitis in Latvia: land cover, land use, and land ownership. *Vector-Borne and Zoonotic Diseases*, **10**, 497–506
- VAS (2017) *Valley Agricultural Software, DairyComp 305*. <http://web.vas.com/en/Products/Detail/4570> (accessed 25 July 2017)
- Vascellari, M., Baioni, E., Ru, G., Carminato, A. and Mutinelli, F. (2009) Animal tumour registry of two provinces in northern Italy: incidence of spontaneous tumours in dogs and cats. *BMC Veterinary Research*, **5**, 39; doi 10.1186/1746-6148/5/39
- Vecchio, T.J. (1966) Predictive value of a single diagnostic test in unselected populations. *The New England Journal of Medicine*, **274**, 1171–1173
- Velasova, M., Drewe, J.A., Gibbons, J., Green, M. and Guitian, J. (2015) Evaluation of health and production recording of the dairy cattle population in Great Britain. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Ghent, 25–27 March 2015. Eds Thulke, H.-H. and Verheyen, K., pp. 222–229
- Veldhuis, A., Brouwer-Middelesch, H., Marceau, A., Madouasse, A., Van der Stede, Y. *et al.* (2016) Application of syndromic surveillance on routinely collected cattle reproduction and milk production data for the early detection of outbreaks of Bluetongue and Schmallenberg viruses. *Preventive Veterinary Medicine*, **124**, 15–24
- VeNom Coding Group (2014) *VeNom Veterinary Nomenclature*. <http://www.venomcoding.org> (accessed 25 July 2017)
- Verberne, L.R.M. and Mirck, M.H. (1976) A practical health programme for prevention of parasitic and infectious diseases in horses and ponies. *Equine Veterinary Journal*, **8**, 123–125

- Verdier, K. de, Öhagen, P. and Alenius, S. (2003) No effect of a homeopathic preparation on neonatal calf diarrhoea in a randomised double-blind, placebo-controlled trial. *Acta Veterinaria Scandinavica*, **44**, 97–101
- Verge, J. and Cristoforoni, N. (1928) La gastro-énerite infectieuse des chats est elle due à un virus filtrable? *Compte Rendus des Seance de la Societe de Biologie*, **99**, 312–314 (in French)
- Vermeulen, C., Lejeune, P., Lisein, J., Sawadogo, P. and Bouché, P. (2013) Unmanned aerial survey of elephants. *PLoS ONE* **8**, 2, e54700; doi 10.1371/journal.pone.0054700
- Vermunt, J.J. and Greenough, P.R. (1994) Predisposing factors of laminitis in cattle. *British Veterinary Journal*, **150**, 151–164
- Vermunt, J.K. and Magidson, J. (2004) Latent class analysis. In: *The Sage Encyclopedia of Social Sciences Research Methods*. Eds Lewis-Beck, M., Bryman, A. and Lao, T.F., pp. 549–553. Sage Publications, Thousand Oaks
- Vermunt, J.K. and Magidson, J. (2005) *Technical Guide for Latent Gold Choice 4.0: Basic and Advanced*. Statistical Innovations, Belmont
- Verster, A., Imes, G.D.J. and Smit, J.P.J. (1975) Helminths recovered from the Bontbok. *Damaliscus dorcas dorcas* (Pallas, 1766). *Onderstepoort Journal of Veterinary Research*, **42**, 29–32
- VetCompass (2014) *VetCompass*. The Royal Veterinary College, University of London. <http://www.rvc.ac.uk/vetcompass> (accessed 10 October 2014)
- VIDA (2013) *Veterinary Investigation Surveillance Report 2013 and 2006–2013*. Department for Environment, Food and Rural Affairs, London/ National Assembly for Wales Agriculture Department, Cardiff/Scottish Executive Environment and Rural Affairs Department, Edinburgh. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/356643/vida13.pdf (accessed 23 July 2015)
- Vilà, C., Savolainen, P., Maldonado, J.E., Amorim, I.R., Rice, J.E. *et al.* (1997) Multiple and ancient origins of the domestic dog. *Science*, **276**, 1687–1689
- Villafuerte, R., Calvete, C., Gortzar, C. and Moreno, S. (1994) First epizootic of rabbit hemorrhagic disease in free living populations of *Oryctolagus cuniculus* at Doñana National Park, Spain. *Journal of Wildlife Diseases*, **30**, 176–179
- Villarroel, A. (2015) *Practical Clinical Epidemiology for the Veterinarian*. John Wiley and Sons, Ames
- Visser, I.J.R., Ingh, T.S.G.A.M., van den Kruijf, J.M., Tielen, M.J.M., Urlings, H.A.P. *et al.* (1988) Atrofische rhinitis: beoordeling van de lengtedoorsnede van varkenskoppen aan de slachtlijn ter bepaling van voorkomen en mate van concha-atrofie. [Atrophic rhinitis: the use of longitudinal sections of pigs' heads in the diagnosis of atrophy of the turbinate bones at the slaughter line.] *Tijdschrift voor Diergeneeskunde*, **113**, 1345–1355 (in Dutch)
- Vizard, A.L., Anderson, G.A. and Gasser, R.B. (1990) Determination of the optimum cut-off value of a diagnostic test. *Preventive Veterinary Medicine*, **10**, 137–143
- VMDB (2014) *The Veterinary Medical Databases*. <http://www.vmdb.org> (accessed 27 October 2014)
- Vögeli, P., Kuhn, B., Kühne, R., Obrist, R., Stranziger, G. *et al.* (1992) Evidence for linkage between the swine L blood group and the loci specifying the receptors mediating adhesion of K88 *Escherichia coli* pilus antigens. *Animal Genetics*, **23**, 19–29
- Voigt, D.R. and Earle, B.D. (1983) Avoidance of coyotes by red fox families. *Journal of Wildlife Management*, **47**, 852–857
- Voisin, C., Tonnel, A.B., Lahoute, C., Robin, H., Lebas, J. *et al.* (1983) Bird fanciers lung: studies of bronchiolar lavage and correlations with inhalation provocation tests. *Lung*, **159**, 17–22
- Volkova, V.V., Howey, R., Savill, N.J. and Woolhouse, M. E. J. (2010) Sheep movement networks and the transmission of infectious diseases. *PLoS ONE*, **5**, e11185; doi 10.1371/journal.pone.0011185
- Vollmer, F. (1999) *Agent Causality*. Kluwer Academic Publishers, Dordrecht
- von Elm, E., Altman, D.G., Egger, M., Pocock, S.J., Gøtzsche, P.C. *et al.* (2007) The Strengthening the reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational epidemiology. *Preventive Medicine*, **45**, 247–251
- Vordermeier, H.M., Cockle, P.J., Whelan, A.O., Rhodes, S., Chambers, M.A. *et al.* (2000) Effective DNA vaccination of cattle with mycobacterial antigens MPB83 and MBP70 does not compromise the specificity of the comparative intradermal tuberculin skin test. *Vaccine*, **19**, 1246–1255
- Vordermeier, H.M., Lowrie, D.B. and Hewinson, R.G. (2003) Improved immunogenicity of DNA vaccination with mycobacterial HSP65 against bovine tuberculosis by protein boosting. *Veterinary Microbiology*, **93**, 349–359
- Vose, D. (2008) *Risk Analysis: A Quantitative Guide*, 3rd edn. John Wiley, Chichester
- Vosloo, W., Boshoff, K., Dwarka, R. and Bastos, A. (2002) The possible role that buffalo played in recent outbreaks of foot-and-mouth disease in South Africa. *Annals of the New York Academy of Sciences*, **969**, 187–190
- VSO (2009) *Participatory Approaches: a Facilitator's Guide*. Voluntary Service Overseas, Kingston upon

- Thames. <http://community.eldis.org/.59c6ec19/> (accessed 6 July 2015)
- VTCHIP (2003) *Vermont Cattle Health Improvement Project*. Vermont Department of Agriculture, Montpelier. <http://www.vermontagriculture.com/vtchip.htm> (accessed 4 July 2006)
- Wachendörfer, G. and Frost, J.W. (1992) Epidemiology of red fox rabies: a review. In: *Wildlife Rabies Control*. Eds Bögel, K., Meslin, F.X. and Kaplan, M., pp. 19–31. Wells Medical, Tunbridge Wells
- Wacholder, S. (2009) Bias in full cohort and nested case-control studies? *Epidemiology*, **20**, 339–340
- Wacholder, S., Dosemeci, M. and Lubin, J.H. (1991) Blind assessment of exposure does not always prevent differential misclassification. *American Journal of Epidemiology*, **134**, 433–437
- Wacholder, S., McLaughlin, J.K., Silverman, D.T. and Mandel, J.S. (1992a) Selection of controls in case-control studies, I. Principles. *American Journal of Epidemiology*, **135**, 1019–1028
- Wacholder, S., Silverman, D.T., McLaughlin, J.K. and Mandel, J.S. (1992b) Selection of controls in case-control studies, II. Types of controls. *American Journal of Epidemiology*, **135**, 1029–1041
- Wagner, B. and Salman, M.D. (2004) Strategies for two-stage sampling designs for estimating herd-level prevalence. *Preventive Veterinary Medicine*, **66**, 1–17
- Wahlgren, J. (2011) Influenza A viruses: an ecology review. *Infection Ecology & Epidemiology*, **1**, 6004; doi 10.3402/iee.v1i0.6004
- Wahlström, H., Elvander, M., Engvall, A. and Vågsholm, I. (2002) Risk of introduction of BSE into Sweden by import of cattle from the United Kingdom. *Preventive Veterinary Medicine*, **54**, 131–140
- Wakefield, E.G. and Dellinger, S.C. (1939) Possible reasons for trephining the skull in the past. *Ciba Symposia*, **1**, 166–169. Ciba Pharmaceutical Products, Summit
- Waksberg, J. (1978) Sampling methods for random digit dialing. *Journal of the American Statistical Association*, **73**, 40–46
- Wald, A. (1943) Tests of statistical hypotheses concerning several parameters when the number of observations is large. *Transactions of the American Mathematical Society*, **54**, 426–482
- Walden, A. (2003) Dr Louis Pasteur. *Primary Care Update for Ob/Gyns*, **10**, 68–70
- Waldner, C.L. and Campbell, J.R. (2006) Disease outbreak investigation in food animal practice. *Veterinary Clinics of North America: Food Animal Practice*, **22**, 75–101
- Waldner, C.L., Henderson, J., Wu, J.T.Y., Breker, K. and Chow, E.Y.W. (2001a) Reproductive performance of a cow-calf herd following a *Neospora caninum*-associated abortion epidemic. *Canadian Veterinary Journal*, **42**, 355–360
- Waldner, C.L., Janzen, E.D., Henderson, J. and Haines, D. M. (1999) Outbreak of abortion associated with *Neospora caninum* infection in a beef herd. *Journal of the American Veterinary Medical Association*, **215**, 1485–1490
- Waldner, C.L., Ribble, C.S., Janzen, E.D. and Campbell, J. R. (2001b) Associations between oil- and gas-well sites, processing facilities, flaring, and beef cattle reproduction and calf mortality in western Canada. *Preventive Veterinary Medicine*, **50**, 1–17
- Waldner, C.L., Ribble, C.S., Janzen, E.D. and Campbell, J. R. (2001c) Associations between total sulfation, hydrogen sulfide deposition, and beef-cattle breeding outcomes in western Canada. *Preventive Veterinary Medicine*, **50**, 19–33
- Wallace, A.R. (1860) On the zoological geography of the Malay archipelago. *Journal of the Linnean Society of London*, **4**, 172–184
- Wallace, A.R. (2017) *The Malay Archipelago*. The Folio Society, London
- Wallace, R.R. and Norton, B.G. (1992) Policy implications of Gaian theory. *Ecological Economics*, **6**, 103–118
- Waller, P.J. (1992) Prospects for biological control of nematode parasites of ruminants. *New Zealand Veterinary Journal*, **40**, 1–3
- Waller, P.J. (1997) Sustainable helminth control of ruminants in developing countries. *Veterinary Parasitology*, **71**, 195–207
- Wallgren, P., Löfstedt, M. and Heldmer, E. (1998) Strategic vaccination against *Mycoplasma hyopneumoniae* to avoid marketing contagious animals from multiplying herds. In: Proceedings of the 15th Congress of the International Pig Veterinary Society, 5th–9th July, 1998. Volume II: Oral Presentations, p. 149
- Walter, M.J. and Hone, J. (2003) A comparison of 3 aerial survey techniques to estimate wild horse abundance in the Australian Alps. *Wildlife Society Bulletin*, **31**, 1138–1149
- Walter, S.D. (1977) Determination of significant relative risks and optimal sampling procedures in prospective and retrospective comparative studies of various sizes. *American Journal of Epidemiology*, **105**, 387–397
- Walters, J.R. (1978) Problems associated with the movement and marketing of weaners. *The Pig Veterinary Society Proceedings*, **3**, 37–44
- Walther, B.A. and Ewald, P.W. (2004) Pathogen survival in the external environment and the evolution of virulence. *Biological Reviews*, **79**, 849–869
- Waltner-Toews, D. (1983) Questionnaire design and administration. In: Proceedings of the Third International Symposium on Veterinary Epidemiology

- and Economics. Arlington, Virginia, 6–10 September 1982, pp. 31–37. Veterinary Medical Publishing Company, Edwardsville
- Waltner-Toews, D., Martin, S.W. and Meek, A.H. (1986) Estimating disease prevalence using a test with no false positives. In: Proceedings of the 4th International Symposium on Veterinary Epidemiology and Economics, Singapore, 18–22 November 1985, pp. 414–415. Singapore Veterinary Association, Singapore
- Wang, C.Y., Chiu, C.W., Pamukcu, A.M. and Bryan, G.T. (1976) Identification of carcinogenic tannin isolated from bracken fern (*Pteridium aquilinum*). *Journal of the National Cancer Institute*, **56**, 33–36
- Wang, L.L., Watts, A.S., Anderson, R.A. and Little, T.D. (2013) Common fallacies in quantitative research methodology. In: *The Oxford Handbook of Quantitative Methods. Volume 2: Statistical Analysis*. Ed. Little, T.D., pp. 718–758. Oxford University Press, New York
- Wang, S., Linkletter, C., Maclure, M., Dore, D., Mor, V. *et al.* (2011) Future-cases as present controls to adjust for exposure-trend bias in case-only studies. *Epidemiology*, **22**, 568–574
- Wang, X. (2012) Mathematical models of schistosomiasis transmission, morbidity and control with applications to endemic communities in coastal Kenya. PhD Thesis, Case Western Reserve University
- Wang, Y.H. (2000) Fiducial intervals: what are they? *The American Statistician*, **54**, 105–111
- Wansbrough, D. (2004) Deciding what the biosecurity system should deliver. *Biosecurity, Issue* **54**, 4–6
- Warble, A. (1994) Veterinary Medical Data Base (VMDB) update. *American Veterinary Computer Society Newsletter*, September–October, 8–10
- Ward, G., Hastie, T., Barry, S., Elith, J. and Leathwick, J.R. (2009) Presence-only data and the EM algorithm. *Biometrics*, **65**, 554–563
- Ward, K.A., Nancarrow, C.D., Byrne, C.R., Shanahan, C. M., Murray, J.D. *et al.* (1990) The potential of transgenic animals for improved agricultural productivity. *Revue Scientifique et Technique, Office International des Epizooties*, **9**, 847–864
- Ward, M.P. (2000) Forecasting blowfly strike in Queensland sheep flocks. *Veterinary Parasitology*, **92**, 309–317
- Ward, M.P. (2002) Seasonality of canine leptospirosis in the United States and Canada and its association with rainfall. *Preventive Veterinary Medicine*, **56**, 203–213
- Ward, M.P. (2014) Rabies in the Dutch East Indies a century ago: a spatio-temporal case study in disease emergence. *Preventive Veterinary Medicine*, **114**, 11–20
- Ward, M.P. and Armstrong, T.F. (2000) Time-space clustering of reported blowfly strike in Queensland sheep flocks. *Preventive Veterinary Medicine*, **41**, 195–202
- Ward, M.P. and Carpenter, T.E. (2000a) Analysis of time-space clustering in veterinary epidemiology. *Preventive Veterinary Medicine*, **43**, 225–237
- Ward, M.P. and Carpenter, T.E. (2000b) Techniques for analysis of disease clustering in space and in time in veterinary epidemiology. *Preventive Veterinary Medicine*, **45**, 257–284
- Ward, M.P. and Kelman, M. (2011) Companion animal disease surveillance: a new solution to an old problem? *Spatial and Spatiotemporal Epidemiology*, **2**, 147–157
- Wardeh, M., Risley, C., McIntyre, M.K., Setzkorn, C. and Baylis, M. (2015) Database of host-pathogen and related species interactions, and their global distribution. *Scientific Data*, **2**, 150049; doi 10.1038/sdata.2015.49
- Warren, F. (1931) *The Dance of Death*. Early English Text Society, Oxford. Reprinted 1971 by Kraus Reprint Co., New York
- Wassell, T.R. and Esslemont, R.J. (1992) Survey of the operation of dairy herd health schemes by veterinary practices in the United Kingdom. *Veterinary Record*, **130**, 260–263
- Wasserman, S. and Faust, K. (1993) *Social Network Analysis: Methods and Applications*. Cambridge University Press, Cambridge
- Wasserstein, R.L. and Lazar, N.A. (2016) The ASA's statement on *p*-values: context, process, and purpose. *The American Statistician*, **70**, 129–133
- Waters, D.J., Patronek, G.J., Bostwick, D.G. and Glickman, L.T. (1996) Comparing the age at prostate cancer diagnosis in humans and dogs. *Journal of the National Cancer Institute*, **88**, 1686–1687
- Wathes, C.M. (1987) Airborne microorganisms in pig and poultry houses. In: *Environmental Aspects of Respiratory Disease in Intensive Pig And Poultry Houses, Including Implications for Human Health*. Proceedings of a Meeting, Aberdeen, 29–30 October 1986. Eds Bruce, J.M. and Sommer, M., pp. 57–71. Commission of the European Communities, Luxembourg. Report EUR 10820 EN
- Wathes, C.M. (1989) Ventilation of stables. *Farm Buildings and Engineering*, **6**, 21–25
- Wathes, C.M. (1994) Air and surface hygiene. In: *Livestock Housing*. Eds Wathes, C.M. and Charles, D.R., pp. 123–148. CAB International, Wallingford
- Wathes, C.M., Jones, C.D.R. and Webster, A.J.F. (1983) Ventilation, air hygiene and animal health. *Veterinary Record*, **113**, 554–559
- Wathes, C.M., Zaidan, W.A.R., Pearson, G.R., Hinton, M. and Todd, J.N. (1988) Aerosol infection of calves and mice with *Salmonella typhimurium*. *Veterinary Record*, **123**, 590–594
- Watson, J.C. (1982) Food hygiene. Methods of linking meat inspection findings with disease on the farm and

- in human populations. In: *Epidemiology in Animal Health*. Proceedings of a symposium held at the British Veterinary Association's Centenary Congress, Reading 22–25 September 1982, pp. 131–140. Society for Veterinary Epidemiology and Preventive Medicine
- Watt, G.E.L. (1980) An approach to determining the prevalence of liver fluke in a large region. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 7–11 May 1979. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 152–155. Australian Government Publishing Service, Canberra
- Wauters, E. and Rojo-Gimeno, C. (2014) Socio-psychological veterinary epidemiology: a new discipline for an old problem. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Dublin, 26–28 March 2014. Eds Mintiens, K. and Parkin, T.D.H., pp. 133–151
- Way, M.J. (1977) Pest and disease status in mixed stands vs. monocultures; the relevance of ecosystem stability. In: *Origins of Pest, Parasite, Disease and Weed Problems*. 18th Symposium of the British Ecological Society, Bangor, 12–14 April 1976. Eds Cherrett, J.M. and Sagar, G.R., pp. 127–138. Blackwell Scientific Publications, Oxford
- Weatherall, J.A.C. and Haskey, J.C. (1976) Surveillance of malformations. *British Medical Bulletin*, **32**, 39–44
- Weaver, A.D., St Jean, G. and Steiner, A. (2005) *Bovine Surgery and Lameness*, 2nd edn. Blackwell Publishing, Oxford
- Webb, C.R. and Sauter-Louis, C. (2002) Investigations into the contact structure of the British sheep population. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Cambridge, 3–5 April 2002. Eds Menzies, F.D. and Reid, S.W.J., pp. 10–20
- Webb, J.S., Thornton, I., Thompson, M., Howarth, R.J. and Lowenstein, P.L. (1978) *The Wolfston Geochemical Atlas of England and Wales*. Oxford University Press, London
- Webber, L.M., and Ison, R.L. (1995) Participatory rural appraisal design: conceptual and process issues. *Agricultural Systems*, **47**, 107–131
- Weber, E., Van Bouwel, J. and De Vreese, L. (2013) *Scientific Explanation*. Springer, Dordrecht
- Weber, M. (1958) *Gesammelte Politischen Schriften*, 2nd edn. J.C.B. Mohr, Tübingen
- Weber, M.F., van Roermund, H.J.W., Assink, H.B.J. and Stegman, J.A. (2004) Rate and structure of cattle transfers between cattle herds considered to be free of paratuberculosis. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Martigny, 24–28 March 2004. Eds Reid, S.J.W., Menzies, F.D. and Russell, A.M., pp. 216–225
- Webster, A.J.F. (1981) Weather and infectious disease in cattle. *Veterinary Record*, **108**, 183–187
- Webster, A.J.F. (2001) Farm animal welfare: the five freedoms and the free Market. *The Veterinary Journal*, **161**, 229–237
- Webster, J. (Ed.) (2011) *Management and Welfare of Farm Animals: The UFAW Farm Handbook*, 5th edn. Wiley-Blackwell, Oxford
- Webster, R.G. (1993) Influenza. In: *Emerging Viruses*, Ed. Morse, S.S., pp. 37–45. Oxford University Press, New York
- Webster, R.G., Bean, W.J., Gorman, O.T., Chambers, T. M. and Kawaoka, Y. (1992) Evolution and ecology of influenza A viruses. *Microbiological Reviews*, **56** (1), 152–179
- Webster, R.G., Laver, W.G., Air, G.M. and Schild, G.C. (1982) Molecular mechanisms of variation in influenza viruses. *Nature*, **296**, 115–121
- Weed, D.L. (1986) On the logic of causal inference. *American Journal of Epidemiology*, **123**, 965–979
- Weed, D.L. (1998) Beyond black box epidemiology. *American Journal of Public Health*, **88**, 12–14
- Weigl, R. (2005) *Longevity of Mammals in Captivity; from the Living Collections of the World*. Kleine Senckenberg-Reihe, Stuttgart
- Weijer, K. and Hart, A.A.M. (1983) Prognostic factors in feline mammary carcinoma. *Journal of the National Cancer Institute*, **70**, 709–716
- Weinberg, C.R. (1993) Toward a clearer definition of confounding. *American Journal of Epidemiology*, **137**, 1–8
- Weinberg, R.A. (1983) A molecular basis of cancer. *Scientific American*, **249** (5), 102–116
- Weingart, P. (1999) Scientific expertise and political accountability: paradoxes of science in politics. *Science and Public Policy*, **26**, 151–161
- Weinstein, M.C. and Fineberg, H.V. (1980) *Clinical Decision Analysis*. W.B. Saunders, Philadelphia
- Weiss, B.L. and Aksoy, S. (2014) Tsetse paratransgenesis: a novel strategy for reducing the spread of African trypanosomiasis. In: *Transgenic Insects: Techniques and Applications*. Ed. Benedict, M.Q., pp. 250–262. CABI, Wallingford
- Weiss, R.A. and Esparza, J. (2015) The prevention of smallpox: a commentary on Sloane (1755) 'An account of inoculation'. *Philosophical Transactions of the Royal Society, Series B*, **370**, 20140378; doi 10.1098/rstb.2014.0378
- Weiss, R.A. and McMichael, A.J. (2004) Social and environmental risk factors in the emergence of infectious diseases. *Nature Medicine*, **10**, S70–S76
- Weiss, R.A. and Vogt, P.K. (2011) 100 years of Rous sarcoma virus. *Journal of Experimental Medicine*, **208**, 2351–2355

- Wekesa, S.N., Sangula, A.K., Belsham, G.J., Tjornehoj, K., Muwanika, V.B. *et al.* (2015) Characterisation of recent foot-and-mouth disease viruses from African buffalo (*Syncerus caffer*) and cattle in Kenya is consistent with independent virus populations. *BMC Veterinary Research*, **11**, 17; doi 10.1186/s12917-015-0333-9
- Wells, D.J. and Wells, K.E. (2002) Gene transfer studies in animals: what do they really tell us about the prospects for gene therapy in DMD? *Neuromuscular Disorders*, **12**, S11–S22
- Wells, G.A.H., Scott, A.C., Johnson, C.T., Cuning, R.F., Hancock, R.D. *et al.* (1987) A novel progressive spongiform encephalopathy in cattle. *Veterinary Record*, **121**, 419–420
- Welsh, E.M., Gettinby, G. and Nolan, A.M. (1993) Comparison of a visual analogue scale and a numerical rating scale for assessment of lameness, using sheep as a model. *American Journal of Veterinary Research*, **54**, 976–983
- Wesseling, I., Uychiaoco, A.J., Aliño, P.M., Aurin, T. and Vermaat, J.E. (1999) Damage and recovery of four Philippine corals from short-term sediment burial. *Marine Ecology Progress Series*, **176**, 11–15
- West, D.M., Bruere, A.N. and Ridler, A.L. (2009) *The Sheep: Health, Disease and Production*, 3rd edn. New Zealand Veterinary Association, Palmerston North
- West, G.P. (1972) *Rabies in Animals and Man*. David and Charles, Newton Abbot
- West, G.P. (Ed.) (1995) *Black's Veterinary Dictionary*, 18th edn. A and C Black, London
- Westgarth, C., Heron, J., Ness, A.R., Bundred, P., Gaskell, R.M. *et al.* (2010a) Family pet ownership during childhood: findings from a UK birth cohort and implications for public health research. *International Journal of Environmental Research and Public Health*, **7**, 3704–3729
- Westgarth, C., Pinchbeck, G.I., Bradshaw, J.W.S., Dawson, S., Gaskell, R.M. *et al.* (2010b) Factors associated with cat ownership in a community in the UK. *Veterinary Record*, **166**, 354–357
- Westreich, D. and Greenland, S. (2013) The Table 2 fallacy: presenting and interpreting confounder and modifier coefficients. *American Journal of Epidemiology*, **177**, 292–298
- Westwood, C.T., Bramley, E. and Lean, I.J. (2003) Review of the relationship between nutrition and lameness in pasture-fed dairy cattle. *New Zealand Veterinary Journal*, **51**, 208–218
- Wetherill, G.B. (1969) *Sampling Inspection and Quality Control*. Methuen, London
- Whalley, B. and Hyland, M.E. (2013) Placebo by proxy: the effects of parents' beliefs on therapy for children's temper tantrums. *Journal of Behavioral Medicine*, **36**, 341–346
- Wheeler, D.J. and Chambers D.S. (2010) *Understanding Statistical Process Control*, 3rd edn. SPC Press, Knoxville
- Wheeler, K. (2011) *Impact of Grazing Management on Cattle and Sheep Parasites*. ADAS, Wolverhampton
- Whipple, D.L., Bolin, C.A., Davis, A.J., Jarnagin, J.L., Johnson, D.C. *et al.* (1995) Comparison of the sensitivity of the caudal fold skin test and a commercial γ -interferon assay for diagnosis of bovine tuberculosis. *American Journal of Veterinary Research*, **56**, 415–419
- Whipps, J.M., Lewis, K. and Cooke, R.C. (1988) Mycoparasitism and plant disease control. In: *Fungi in Biological Control Systems*. Ed. Burge, N.M., pp. 161–187. Manchester University Press, Manchester
- Whitaker, D.A. (2000) Use and interpretation of metabolic profiles. In: *The Health of Dairy Cattle*. Ed. Andrews, A.H., pp. 89–107. Blackwell Science, Oxford
- Whitaker, D.A. and Kelly, J.M. (1982) Incidence of clinical and subclinical hypomagnesaemia in dairy cows in England and Wales. *Veterinary Record*, **110**, 450–451
- Whitaker, D.A., Kelly, J.M. and Smith, E.J. (1983) Incidence of lameness in dairy cows. *Veterinary Record*, **113**, 60–62
- Whitaker D.A., Kelly, J.M. and Smith, S. (2000) Disposal and disease rates in 340 British dairy herds. *Veterinary Record*, **146**, 363–367
- Whitaker, D.A., Macrae, A.I. and Burrough, P. (2004) Disposal and disease rates in British dairy herds between April 1998 and March 2002. *Veterinary Record*, **155**, 43–47
- White, F. (1983) *The Vegetation of Africa – A Descriptive Memoir to Accompany the UNESCO/AETFAT/UNSO Vegetation Map of Africa*. United Nations Educational, Scientific and Cultural Organization, Paris
- White, G.C. and Burnham, K.P. (1999) Program MARK: survival estimation from populations of marked animals. *Bird Study*, **46**, Suppl., 120–138
- White, M.E. and Vellake, E. (1980) A coding system for veterinary clinical signs. *Cornell Veterinarian*, **70**, 160–182
- White, P.C.L., Lewis, A.G. and Harris, S. (1997) Fertility control as a means of controlling tuberculosis in badger (*Meles meles*) populations in the south-west of England: predictions from a spatial stochastic simulation model. *Proceedings of the Royal Society of London, Series B*, **264**, 1737–1747
- Whitehead, A. (2002) *Meta-Analysis of Controlled Clinical Trials*. John Wiley and Sons, Chichester
- Whitehead, J. (1993) Sample size calculations for ordered categorical data. *Statistics in Medicine*, **12**, 2257–2272
- Whitehead, J. (1997) *The Design and Analysis of Sequential Clinical Trials*, revised 2nd edn. John Wiley, Chichester

- Whiteley, L.O., Maheswaran, S.K., Weiss, D.J., Ames, T.R. and Kannan, M.S. (1992) *Pasteurella haemolytica* A1 bovine respiratory disease: pathogenesis. *Journal of Veterinary Internal Medicine*, **6**, 11–22
- Whiteside, A. (Ed.) (1998) *Implications of AIDS for Demography and Policy in South Africa*. University of Natal Press, Pietermaritzburg
- Whiting, P., Harbord, R. and Kleijnen, J. (2005) No role for quality scores in systematic reviews of diagnostic accuracy studies. *BMC Medical Research Methodology*, **5**, 19; doi 10.1186/1471-2288-5-19
- Whiting, P., Rutjes, A.W.S., Reitsma, B., Bossuyt, P.M.M. and Kleijnen, J. (2003) The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Medical Research Methodology*, **3**, 25; doi 10.1186/1471-2288-3-25
- Whiting, P., Rutjes, A.W., Reitsma, J.B., Glas, A., Bossuyt, P.M. *et al.* (2004) Sources of variation and bias in studies of diagnostic accuracy: a systematic review. *Annals of Internal Medicine*, **140**, 189–202
- Whiting, P.F., Rutjes, A.W.S., Westwood, M.E., Mallett, S., Deeks, J.J. *et al.* (2011) QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Annals of Internal Medicine*, **155**, 529–536
- Whiting, T.L. and Marion, C.R. (2011) Perpetration-induced traumatic stress – a risk for veterinarians involved in the destruction of healthy animals. *Canadian Veterinary Journal*, **52**, 794–796
- Whitlock, R.H., Wells, S.J., Sweeney, R.W. and Van Tiem, J. (2000) ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. *Veterinary Microbiology*, **77**, 387–398
- Whitmee, S., Haines, A., Beyrer, C., Boltz, F., Capon, A.G. *et al.* (2015) Safeguarding human health in the Anthropocene epoch: report of The Rockefeller Foundation – Lancet Commission on planetary health. *The Lancet*, **386**, 1973–2028
- Whittaker, M. (1960) The supernatural in the Roman Empire. *Church Quarterly Review*, **161**, 185–199
- Whittaker, R.H. (1953) A consideration of climax theory: the climax as a population and pattern. *Ecological Monographs*, **23**, 41–78
- Whittaker, R.H., Levin, S.A. and Root, R.B. (1973) Niche, habitat, and ecotope. *The American Naturalist*, **107**, 321–338
- Whittington, R. (2003) Emerging diseases in Australia. *Commonwealth Veterinary Association News*, **19** (2), 51–60
- WHO (1959) *Immunological and Haematological Surveys*. Report of a Study Group. World Health Organization Technical Report Series No. 181. World Health Organization, Geneva
- WHO (1970) *Multipurpose Serological Surveys and WHO Serum Reference Banks*. Report of a World Health Organization Scientific Group. World Health Organization Technical Report Series No. 454. World Health Organization, Geneva
- WHO (1973) *Adequacy of Sample Size*. Health Statistical Methodology Unit. World Health Organization Publication HSM/73.1. World Health Organization, Geneva
- WHO (1978) Proposals for the nomenclature of salivarian trypanosomes and for the nomenclature of reference collections. *Bulletin of the World Health Organization*, **56**, 467–480
- WHO (1983) *Basic Documents*, 35th edn. World Health Organization, Geneva
- WHO (1993) *Evaluation of Certain Veterinary Drug Residues in Food*. Fortieth Report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization Technical Report Series No. 832. World Health Organization, Geneva
- WHO (1996) *Emerging Foodborne Diseases*. WHO Fact Sheet No. 124. World Health Organization, Geneva
- WHO (2006) *Communicable Disease Surveillance and Response Systems: Guide to Monitoring and Evaluating*. World Health Organization, Geneva
- WHO (2008) *A Guide to Developing Knowledge, Attitude and Practice Surveys*. World Health Organization, Geneva
- WHO (2014) *Basic Documents*, 48th edn. World Health Organization, Geneva
- WHO/FAO (2006) *Food Safety Risk Analysis: A Guide for National Food Safety Authorities*. FAO Food and Nutrition Paper 87. World Health Organization/Food and Agriculture Organization of the United Nations, Geneva/Rome
- Widén, F., Das Neves, C.G., Ruiz-Fons, F., Reid, H.W., Kuiken, T. *et al.* (2012) Herpesvirus infections. In: *Infectious Diseases of Wild Mammals and Birds in Europe*. Eds Gavner-Widén, D., Duff, J.P. and Meredith, A., pp. 3–36. Wiley-Blackwell, Chichester
- Wiener, J.B. and Rogers, M.D. (2002) Comparing precaution in the United States and Europe. *Journal of Risk Research*, **5**, 317–349
- Wierup, M. (1983) The Swedish canine parvovirus epidemic – an epidemiological study in a dog population of defined size. *Preventive Veterinary Medicine*, **1**, 273–288
- Wierup, M. (2012) Principles and strategies for the prevention and control of infectious diseases in livestock and wildlife. In: *Ecology and Animal Health*. Eds Norrgren, L. and Levensgood, J., pp. 203–211. The Baltic University Press, Uppsala
- Wierup, M., Wahlström, H. and Engström, B. (1992) Experience of 10-year use of competitive exclusion

- treatment as part of the *Salmonella* control programme in Sweden. *International Journal of Food Microbiology*, **15**, 287–291
- Wierup, M., Wold-Troell, M., Nurmi, E. and Hakkinen, M. (1988) Epidemiological evaluation of the *Salmonella*-controlling effect of a nationwide use of a competitive exclusion culture in poultry. *Poultry Science*, **67**, 1026–1033
- Wiethoelter, A.K., Beltrán-Alcrudo, D., Kock, R. and Mor, S.M. (2015) Global trends in infectious diseases at the wildlife-livestock interface. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 9662–9667
- Wigley, P. (2004) Genetic resistance to *Salmonella* infection in domestic animals. *Research in Veterinary Science*, **76**, 165–169
- Wilcove, D.S., McLellan, C.H. and Dobson, A.P. (1986) Habitat fragmentation in the temperate zone. In: *Conservation Biology: Science of Scarcity and Diversity*. Ed. Soulé, M., pp. 237–256. Sinauer Associates, Sunderland
- Wilesmith, J.W. (1982) Johne's disease: a retrospective study of vaccinated herds in Great Britain. *British Veterinary Journal*, **138**, 321–331
- Wilesmith, J.W. (1993) BSE: epidemiological approaches, trials and tribulations. *Preventive Veterinary Medicine*, **18**, 33–42
- Wilesmith, J.W. and Ryan, J.B.M. (1992) Bovine spongiform encephalopathy: recent observations on the age-specific incidences. *Veterinary Record*, **130**, 491–492
- Wilesmith, J.W., Little, T.W.A., Thompson, H.V. and Swan, C. (1982) Bovine tuberculosis in domestic and wild mammals in an area of Dorset. I. Tuberculosis in cattle. *Journal of Hygiene, Cambridge*, **89**, 195–210
- Wilesmith, J.W., Wells, G.A.H., Cranwell, M.P. and Ryan, J.B.M. (1988) Bovine spongiform encephalopathy: epidemiological studies. *Veterinary Record*, **123**, 638–644
- Wiley, J.D.J.F., Gordon, Z.J., Odofin, L.U. and Rabinowitz, P.M. (2005) The canary database: animals as sentinels of human environmental health hazards. *Clinical Toxicology*, **43**, 720
- Wilkinson, D.M. (1999) Bacterial ecology, antibiotics and selection for virulence. *Ecology Letters*, **2**, 207–209
- Wilkinson, L. (2006) Revising the Pareto chart. *The American Statistician*, **60**, 332–334
- Wilkinson, P.J. (1984) The persistence of African swine fever in Africa and the Mediterranean. *Preventive Veterinary Medicine*, **2**, 71–82
- Wilkinson, S., Wiener, P., Archibald, A.L., Law, A., Schnabel, R.D. et al. (2011) Evaluation of approaches for identifying population informative markers from high density SNP Chips. *BMC Genetics*, **12**, 45; doi 10.1186/1471-2156-12-45
- Will, R.G. (1997) A new variant of Creutzfeldt-Jakob disease in the UK. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, Chester, 9–11 April 1997. Eds Goodall, E.A. and Thrusfield, M.V., pp. 85–88
- Willcox, A.W. (2002) Mummies and molecules: molecular biology meets paleopathology. *Clinical Microbiology Newsletter*, **24**, 57–60
- Willeberg, P. (1976) Interaction effects of epidemiologic factors in the feline urological syndrome. *Nordisk Veterinærmedicin*, **28**, 193–200
- Willeberg, P. (1977) Animal disease information processing: epidemiologic analyses of the feline urological syndrome. *Acta Veterinaria Scandinavica*, **18**, Suppl. 64, 1–48
- Willeberg, P. (1979) The Danish swine slaughter inspection data bank and some epidemiological applications. In: Proceedings of an International Symposium on Animal Health and Disease Data Banks, 4–6 December 1978, pp. 133–145. Miscellaneous Publication No. 1381. United States Department of Agriculture, Washington DC
- Willeberg, P. (1980a) Abattoir surveillance in Denmark. *The Pig Veterinary Society Proceedings*, **6**, 43–54
- Willeberg, P. (1980b) The analysis and interpretation of epidemiological data. In: *Veterinary Epidemiology and Economics*. Proceedings of the Second International Symposium, Canberra, 7–11 May 1979. Eds Geering, W.A., Roe, R.T. and Chapman, L.A., pp. 185–198. Australian Government Publishing Service, Canberra
- Willeberg, P. (1981) Epidemiology of the feline urological syndrome. *Advances in Veterinary Science*, **25**, 311–344
- Willeberg, P. (1986) Epidemiologic use of routinely collected veterinary data: risks and benefits. In: Proceedings of the 4th International Symposium on Veterinary Epidemiology and Economics, Singapore, 18–22 November 1985, pp. 40–45. Singapore Veterinary Association, Singapore
- Willeberg, P. (1991) Animal welfare studies: epidemiological considerations. In: Society for Veterinary Epidemiology and Preventive Medicine, Proceedings, London, 17–19 April 1991. Ed. Thrusfield, M.V., pp. 76–82
- Willeberg, P. (1992) Disease monitoring in Denmark. In: Minnesota Swine Conference for Veterinarians. High-Health Strategies, College of Veterinary Medicine, University of Minnesota. *Veterinary Continuing Education and Extension, USA*, **19**, 95–101
- Willeberg, P. (1993) Bovine somatotropin and clinical mastitis: epidemiological analysis of the welfare risk. *Livestock Production Science*, **36**, 55–66
- Willeberg, P. (1997) Epidemiology and Animal welfare. In: Proceedings of the 8th International Symposium on

- Veterinary Epidemiology and Economics, Paris, July 1997. *Epidémiologie et Santé Animale*, **31–32** (1), iii–vii
- Willeberg, P., Gardner, I.A., Mortensen, S. and Mousing, J. (1994) Models of herd size effects in swine diseases. In: *Proceedings of the 7th International Symposium on Veterinary Epidemiology and Economics*, Nairobi, 15–19 August 1994. Eds Rowlands, G.J., Kyule, M.N. and Perry, B.D. *The Kenya Veterinarian*, **18** (2), 189–191
- Willeberg, P., Gerbola, M.-A., Kirkegaard Petersen, B. and Andersen, J.B. (1984) The Danish pig health scheme; nationwide computer-based abattoir surveillance and follow-up at the herd level. *Preventive Veterinary Medicine*, **3**, 79–91
- Willems, R.J.L., Top, J., van den Braak, N., van Belkum, A., Endtz, H. *et al.* (2000) Host specificity of vancomycin-resistant *Enterococcus faecium*. *Journal of Infectious Diseases*, **182**, 816–823
- Willemse, T. (1986) Atopic skin disease: a review and a reconsideration of diagnostic criteria. *Journal of Small Animal Practice*, **27**, 771–778
- Willett, W. (2013) *Nutritional Epidemiology*, 3rd edn. Oxford University Press, New York
- Willette, M.M., Ponder, J.B., McRuer, D.L. and Clark, E.E. (2012) Wildlife health monitoring systems in North America: from sentinel species to public policy. In: *New Directions in Conservation Medicine*. Eds Aguirre, A.A., Ostfield, R.S. and Daszak, P., pp. 552–562. Oxford University Press, New York
- Willets, N. and Cobon, G. (1993) *Antiparasite vaccines*. In: *Vaccines for Veterinary Applications*. Ed. Peters, R., pp. 259–294.
- Williams, E.H. and Bunkley-Williams, L. (1990) The world-wide coral reef bleaching cycle and related sources of coral mortality. *Atoll Research Bulletin*, **335**, 1–71
- Williams, E.S. and Young, S. (1980) Chronic wasting disease of captive Mule deer: a spongiform encephalopathy. *Journal of Wildlife Diseases*, **16**, 89–98
- Williams, E.S., Yuill, T., Artois, M., Fisher, J. and Haigh, S. A. (2002) Emerging infectious diseases. *Revue Scientifique et Technique, Office International des Epizooties*, **21**, 139–157
- Williams, G.W. (1984) Time-space clustering of disease. In: *Statistical Methods for Cancer Studies*. Ed. Cornell, R.G., pp. 167–227. Marcel Dekker, New York and Basel
- Williams, P.C.W. and Ward, W.R. (1989a) Development of a coding system for recording clinical findings in farm animal practice. *Veterinary Record*, **124**, 118–122
- Williams, P.C.W. and Ward, W.R. (1989b) Development of a microcomputer system for recording veterinary visits, preparing accounts, and as an aid to herd fertility and health schemes. *Veterinary Record*, **124**, 265–268
- Williams, R.J. and Martinez, N.D. (2000) Simple rules yield complex food webs. *Nature*, **404**, 180–183
- Williamson, L. (1995) Preventative health care. In: *The Horse: Diseases and Clinical Management*. Eds Kobluk, C.N., Ames, T.R. and Geor, R.J., pp. 83–90. W. B. Saunders, Philadelphia
- Williamson, M.H. and Wilson, R.A. (1978) The use of mathematical models for predicting the incidence of fascioliasis. In: *Weather and Parasitic Animal Disease*. World Meteorological Organization Technical Note No. 159. Ed. Gibson, T.E., pp. 39–48. World Meteorological Organization, Geneva
- Williamson, N.B. (1980) The economic efficiency of a veterinary preventive medicine and management program in Victorian dairy herds. *Australian Veterinary Journal*, **56**, 1–9
- Williamson, N.B. (1987) Evaluating herd reproductive status. *Proceedings of the American Association of Bovine Practitioners*, **19**, 117–121
- Williamson, N.B. (1993) The economics of preventive veterinary medicine schemes in sheep and cattle. In: *The William Dick Bicentenary Proceedings*. The University of Edinburgh, 7–10 July 1993, pp. 13–16. Royal (Dick) School of Veterinary Studies, University of Edinburgh
- Wilson, A., Darpel, K. and Mellor, P.S. (2008) Where does bluetongue virus sleep in the winter? *PLoS Biology*, **6**, 8, e210; doi 10.1371/journal.pbio.0060210
- Wilson, A.J. and Mellor, P.S. (2009) Bluetongue in Europe: past, present and future. *Philosophical Transactions of the Royal Society, Series B*, **364**, 2669–2681
- Wilson, C.D. and Richards, M.S. (1980) A survey of mastitis in the British dairy herd. *Veterinary Record*, **106**, 431–435
- Wilson, C.D., Richards, M.S., Stevenson, F.J. and Davies, G. (1983) *The National Mastitis Survey. A Survey of Udder Infection and Related Factors in the British Dairy Herd 1977*. Ministry of Agriculture, Fisheries and Food Booklet 2433
- Wilson, D.W. and Beers, P.T. (2001) Global trade requirements and compliance with World Trade Organization agreements: the role of tracing animals and animal products. *Revue Scientifique et Technique, Office International des Epizooties*, **20**, 379–384
- Wilson, E.B. (1927) Probable inference, the law of succession, and statistical inference. *Journal of the American Statistical Association*, **22**, 209–212
- Wilson, J.M. (1996) Animal models of human disease for gene therapy. *Journal of Clinical Investigation*, **97**, 1138–1141
- Wilson, J.M.G. and Junger, G. (1968) *The Principles and Practice of Screening for Disease*. Public Health Paper 34. World Health Organization, Geneva

- Wilson, J.W., Schurr, M.J., LeBlanc, C.L., Ramamurthy, R., Buchanan, K.L. *et al.* (2002) Mechanisms of bacterial pathogenicity. *Postgraduate Medical Journal*, **78**, 216–224
- Wilson, S.M. (1993) Application of nucleic acid-based technologies to the diagnosis and detection of disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**, 609–611
- Wilson, T.M. and Howes, B. (1980) An epizootic of bovine tuberculosis in Barbados, West Indies. Proceedings of the Second International Symposium of Veterinary Laboratory Diagnosticians, 24–26 June 1980, Lucerne, Switzerland, Vol. 1, pp. 136–144
- Wilson, V. (2014) Creating compliance in a non-compliant world. *In Practice*, **36**, 47–49
- Wineland, N.E. and Dargatz, D.A. (1998) The National Animal Health Monitoring System: a source of on-farm information. *Veterinary Clinics of North America: Food Animal Practice*, **14**, 127–139
- Winkler, W.G. (1975) Fox rabies. In: *The Natural History of Rabies*, Vol. 2. Ed. Baer, G.M., pp. 3–22. Academic Press, New York
- Winslow, C.-E.A. (1934) The epidemiology of Noah Webster. *Transactions of the Connecticut Academy of Arts and Sciences*, **32**, 21–109
- Winter, A.C. and Clarkson, M.J. (2012) *A Handbook for the Sheep Clinician*, 7th edn. CAB International, Wallingford
- Wintle, B.C. and Cleeland, B. (2012) Interpreting risk in international trade. *Journal of Risk Research*, **15**, 293–312
- Wise, J.K. and Yang, J.-J. (1992) Demographic and employment shifts of US veterinarians, 1980 to 1990. *Journal of the American Veterinary Medical Association*, **201**, 684–687
- Withrow, S.J. (2001) Why worry about cancer in pets? In: *Small Animal Clinical Oncology*, 3rd edn. Eds Withrow, S.J. and MacEwan, E.G., pp. 1–3. W.B. Saunders, Philadelphia
- Witowski, J.A. and Parish, L.C. (2002) The story of anthrax from antiquity to the present: a biological weapon of nature and humans. *Clinics in Dermatology*, **20**, 336–342
- Wittes, J. (2002) Sample size calculations for randomised controlled trials. *Epidemiologic Reviews*, **24**, 39–53
- Wittmann, E.J. and Baylis, M. (2000) Climate change: effects on *Culicoides*-transmitted viruses and implications for the UK. *The Veterinary Journal*, **160**, 107–117
- Witz, J. (1998) One hundred years ago: the discovery of the filterability of viruses. *Biology of the Cell*, **90**, 257–258
- Wlaschitz, S. (2004) Grass sickness: a literature review. *Wiener Tierärztliche Monatsschrift*, **91**, 14–22 (in German; English abstract)
- Wobeser, G.A. (2006) *Essentials of Disease in Wild Animals*. Blackwell Publishing, Oxford
- Wojciechowska, J.I. and Hewson, C.J. (2005) Quality-of-life assessment in pet dogs. *Journal of the American Veterinary Medical Association*, **226**, 722–728
- Wojciechowski, K.J. (1991) Global strategies for rinderpest eradication. In: *South Asia Rinderpest Eradication Campaign*. Proceedings of the Regional Expert Consultation on Rinderpest Eradication in South Asia, Bangkok, Thailand, 5–8 June 1990. RAPA Publication 1991/8, pp. 78–90. Food and Agriculture Organization, Bangkok
- Wolfe, N.D., Dunavan, C.P. and Diamond, J. (2007) Origins of major human infectious diseases. *Nature*, **447**, 279–283
- Wong, W.T. and Lee, M.K.C. (1985) Some observations on the population and natal patterns among purebred dogs in Malaysia. *Journal of Small Animal Practice*, **26**, 111–119
- Wood, B., Washino, R., Beck, L., Hibbard, K., Pitcairn, M. *et al.* (1991) Distinguishing high and low anopheline-producing rice fields using remote sensing and GIS technologies. *Preventive Veterinary Medicine*, **11**, 277–288
- Wood, E.N. (1978) The incidence of stillborn piglets associated with high atmospheric carbon monoxide levels. *The Pig Veterinary Society Proceedings*, **3**, 117–118
- Wood, J.L.N., Kelly, L., Cardwell, J.M. and Park, A.W. (2005) Quantitative assessment of the risks of reducing the routine swabbing requirements for the detection of *Tylorella equigenitalis*. *Veterinary Record*, **157**, 41–46
- Wood, J.L.N., Lakhani, K.H. and Henley, W.E. (2004) An epidemiological approach to prevention and control of three common heritable diseases in canine pedigree breeds in the United Kingdom. *The Veterinary Journal*, **168**, 14–27
- Wood, J.M., Major, D., Heath, A., Newman, R.W., Hoschler, K. *et al.* (2012) Reproducibility of serology assays for pandemic influenza H1N1: collaborative study to evaluate a candidate WHO International Standard. *Vaccine*, **30**, 210–217
- Wood S.N. (2006) *Generalized Additive Models: An Introduction with R*. Chapman and Hall/CRC Press, Boca Raton
- Woodley, C.M., Taylor, S.M., Downs, C.A., Austin, T.J., Bothwell, J. *et al.* (2015) *Guidance for an Epidemiological Strategy and Establishing a Disease Surveillance Network for Caribbean Acropora palmata*. NOAA Technical Memorandum NOS NCCOS 197 and CRCP 21. National Oceanic and Atmospheric Administration, Charleston
- Woodruff, A.W. (1976) Toxocariasis as a public health problem. *Environmental Health*, **84**, 29–31

- Woods, A. (2013a) Is prevention better than cure? The rise and fall of veterinary preventive medicine, c.1950–1980. *Social History of Medicine*, **26**, 113–131
- Woods, A. and Bresalier, M. (2014) One health, many histories. *Veterinary Record*, **174**, 650–654
- Woods, C.M. (2013b) Categorical methods. In: *The Oxford Handbook of Quantitative Methods. Volume 2: Statistical Analysis*. Ed. Little, T.D., pp. 52–73. Oxford University Press, New York
- Woodward, R.H. and Goldsmith, P.L. (1964) *Cumulative Sum Techniques*. Imperial Chemical Industries Mathematical and Statistical Techniques for Industry Monograph No. 3. Oliver and Boyd, Edinburgh
- Woolcock, J.B. (1991) Microbial interactions in health and disease. In: *Microbiology of Animals and Animal Products*. World Animal Science, Vol. A6. Ed. Woolcock, J.B., pp. 61–76. Elsevier, Amsterdam
- Wooldridge, M., Hartnett, E., Cox, A. and Seaman, M. (2006) Quantitative risk assessment case study: smuggled meats as disease vectors. *Revue Scientifique et Technique, Office International des Epizooties*, **25**, 105–117
- Wooldridge, M.J.A., Hoinville, L.J. and Wilesmith, J.W. (1992) A scrapie survey by postal questionnaire: aims, problems and results. In: Society for Veterinary Epidemiology and Preventive Medicine, Denary Proceedings, Edinburgh, 1–3 April 1992. Ed. Thrusfield, M.V., pp. 78–89
- Woolf, B. (1955) On estimating the relation between blood group and disease. *Annals of Human Genetics*, **19**, 251–253
- Woolf, S.H. (2008) The meaning of translational research and why it matters. *Journal of the American Medical Association*, **299**, 211–213
- Woolhouse, M.E.J. and Gowtage-Sequeria, S. (2005) Host range and emerging and reemerging pathogens. *Emerging Infectious Diseases*, **11**, 1842–1847
- Woolhouse, M.E.J., Fèvre, E.M., Handel, I., Heller, J., Tildesley, M.J. et al. (2011) *Guide to Good Practice for Quantitative Veterinary Epidemiology*. <http://www.qve-goodpracticeguide.org.uk/> (accessed 23 March 2015)
- Work, T.H., Roderiguez, F.M. and Bhatt, P.N. (1958) Virological epidemiology of the 1958 epidemic of Kyasanur Forest disease. *American Journal of Public Health*, **49**, 869–874
- Work, T.M., Richardson, L.L., Reynolds, T.L. and Willis, B.L. (2008) Biomedical and veterinary science can increase our understanding of coral disease. *Journal of Experimental Marine Biology & Ecology*, **362**, 63–70
- World Bank Group (2015) *Update on the Economic Impact of the 2014–2015 Ebola Epidemic on Liberia, Sierra Leone, and Guinea*, 95804, April 15 2015. Macroeconomics and Fiscal Management, World Bank Group. <http://documents.worldbank.org/curated/en/2014/04/24377008/update-economic-impact-2014-2015-ebola-epidemic-liberia-sierra-leone-guinea> (accessed 10 March 2016)
- Worth, A.R., Thompson, R.C.A. and Lymbery, A.J. (2014) Revaluating the evidence for *Toxoplasma gondii*-induced behavioural changes in rodents. *Advances in Parasitology*, **85**, 109–142
- Worthington, R.W. (1982) Serology as an aid to diagnosis: uses and abuses. *New Zealand Veterinary Journal*, **30**, 93–97
- Wrathall, A.E. (1975) *Reproductive Disorders in Pigs*. Review Series No. 11 of the Commonwealth Bureau of Animal Health. Commonwealth Agricultural Bureaux, Farnham Royal
- Wrathall, A.E. (1997) Risks of transmitting scrapie and bovine spongiform encephalopathy by semen and embryos. *Revue Scientifique et Technique, Office International des Epizooties*, **16**, 240–264
- Wrathall, A.E. and Hebert, C.N. (1982) Monitoring reproductive performance in the pig herd. *The Pig Veterinary Society Proceedings*, **9**, 136–148
- Wratten, S.D., Mead-Briggs, M., Gettinby, G., Ericsson, G. and Baggott, D.G. (1993) An evaluation of the potential effects of ivermectin on the decomposition of cattle dung pats. *Veterinary Record*, **133**, 365–371
- Wray, C. and Woodward, M.J. (1990) Biotechnology and veterinary science. *Revue Scientifique et Technique, Office International des Epizooties*, **9**, 779–794
- Wright, A. and Thrusfield, M. (2016) Perceptions of zoonotic and animal diseases in the Van Gujjar community of North India. *Preventive Veterinary Medicine*, **123**, 143–153
- Wright, P.F., Neumann, G. and Kawaoka, Y. (2013) Orthomyxoviruses. In: *Fields Virology*, 6th edn. Eds Knipe, D.M. and Howey, P.M., pp. 1186–1243. Lippincott Williams and Wilkins, Philadelphia
- Wright, S. (1959) Genetics and hierarchy of biological sciences. *Science, New York*, **130**, 959–965
- Wright, S.P. (1992) Adjusted *P*-values for simultaneous inference. *Biometrics*, **48**, 1005–1013
- Wright, W.C. (1930) *Fracastorius: Contagion, Contagious Diseases and their Treatment*. G.P. Putnam's Sons, New York
- WTO (1998) *World Trade Organization, Annual Report, 1998*. World Trade Organization, Lausanne
- WTO (2011) *Committee on Sanitary and Phytosanitary Measures: Major Decisions and Documents, September 2011*. World Trade Organization, Lausanne
- Wu, J. and Loucks, O.L. (1995) From balance of nature to hierarchical patch dynamics: a paradigm shift in ecology. *Quarterly Review of Biology*, **70**, 439–466

- Wu, S., Powers, S., Zhu, W. and Hannun, Y.A. (2016) Substantial contribution of extrinsic risk factors to cancer development. *Nature*, **529**, 43–47
- Wylie, K.M., Weinstock, G.M. and Storch, G.A. (2012) Emerging view of the human virome. *Translational Research*, **160**, 283–290
- Wynder, E.L. (1985) Applied epidemiology. *American Journal of Epidemiology*, **121**, 781–782
- Wynne, S.G. (1998) Studies on use of homeopathy in animals. *Journal of the American Veterinary Medical Association*, **212**, 719–724
- Wynne-Edwards, V.C. (1962) *Animal Dispersion in Relation to Social Behaviour*. Oliver and Boyd, Edinburgh
- Xinhua News Agency (2014) Rabies deaths lead to massive dog culls in SW China. *Xinhua News Agency*, 7 September. http://news.xinhuanet.com/english/china/2014-09/07/c_133627000.htm (accessed 13 November 2015)
- Yamada, A., Kahn, L.H., Kaplan, B., Monath, T.P., Woodall, J. *et al.* (Eds) (2014) *Confronting Emerging Zoonoses: The One Health Paradigm*. Springer, Tokyo
- Yamaguchi, S., Imada, T. and Kawamura, H. (1979) Characterization of a picornavirus isolated from broiler chicks. *Avian Diseases*, **23**, 571–581
- Yamane, I., Kamata, A., Sugiura, K., Hamaoka, T., Murakami, Y. *et al.* (1997) An epidemiological analysis of foot and mouth disease outbreak in Taiwan using geographic information system. *Journal of the Japan Veterinary Medical Association*, **50**, 583–586 (in Japanese; English abstract)
- Yan, Y., Panos, J.C., McCormick, D.J., Wan, Q., Giraldo, A.A. *et al.* (2001) Characterization of a novel H2A(-)E+ transgenic model susceptible to heterologous but not self thyroglobulin in autoimmune thyroiditis: thyroiditis transfer with Vbeta8+ T cells. *Cellular Immunology*, **212**, 63–70
- Yassin, H., Velthuis, A.G.J., Boerjan, M. and van Riel, J. (2009) Field study on broilers' first-week mortality. *Poultry Science*, **88**, 798–804
- Yates, F. (1981) *Sampling Methods for Censuses and Surveys*, 4th edn. Charles Griffin, London and High Wycombe
- Yates, W.D.G. (1982) Interaction between viruses in bovine respiratory diseases. A review of infectious bovine rhinotracheitis, shipping fever pneumonia and viral bacterial synergism in respiratory diseases in cattle. *Canadian Journal of Comparative Medicine*, **46**, 341–349
- Yekutieli, P. (1980) *Eradication of Infectious Disease – A Critical Study. Contributions to Epidemiology and Biostatistics*, Vol. 2. S. Karger, Basel
- Yerushalmy, J. and Palmer, C.E. (1959) On the methodology of investigations of etiologic factors in chronic diseases. *Journal of Chronic Diseases*, **10**, 27–40
- Yilma, J.M. and Malone, J.B. (1998) A geographic information system forecast model for strategic control of fasciolosis in Ethiopia. *Veterinary Parasitology*, **78**, 103–127
- Yorke, J.A. and London, W.P. (1973) Recurrent outbreaks of measles, chickenpox and mumps. II. Systematic differences in contact rates and stochastic effects. *American Journal of Epidemiology*, **98**, 469–482
- Yoshida, N., Miyamoto, T., Fukusho, N.S. and Tajima, M. (1977) Properties of paramyxovirus isolated from budgerigars with an acute fatal disease. *Journal of the Japanese Veterinary Medical Association*, **30**, 599–603
- Youden, W.J. (1950) Index for rating diagnostic tests. *Cancer*, **3**, 32–35
- Young, G.B., Lee, G.J., Waddington, D., Sales, D.I., Bradley, J.S. *et al.* (1983) Cutting wastage in dairy cows in East Anglia. *Veterinary Record*, **113**, 107–111
- Yuasa, N., Taniguchi, T. and Yashida, I. (1979) Isolation and some characteristics of an agent inducing anaemia in chicks. *Avian Diseases*, **23**, 366–385
- Yuill, T.M. (1986) The ecology of tropical arthropod-borne viruses. *Annual Review of Ecology and Systematics*, **17**, 189–219
- Yusuf, S., Held, P. and Furberg, C. (1991) Update of effects of calcium antagonists in myocardial infarction or angina in light of the second Danish verapamil infarction trials (DAVIT-II) and other recent studies. *American Journal of Cardiology*, **67**, 1295–1297
- Zadoks, R.N. and Schukken, Y.H. (2006) Use of molecular epidemiology in veterinary practice. *Veterinary Clinics of North America: Food Animal Practice*, **22**, 229–261
- Zak, O. and Sande, M.A. (Eds) (1999) *Handbook of Animal Models of Infection*. Academic Press, San Diego
- Zambon, M. (1998) Laboratory diagnosis of influenza. In: *Textbook of Influenza*. Eds Nicholson, K.G., Webster, R.G. and Hay, A.K., pp. 291–313. Blackwell Science, Oxford
- Zambon, M. and Joseph, C. (2001) *The PHLS Plan for Pandemic Influenza*. Public Health Laboratory Service, London
- Zanluca, C., de Melo, V.C.A., Mosimann, A.L.P., dos Santos, G.I.V., dos Santos, C.N.D. *et al.* (2015) First report of autochthonous transmission of Zika virus in Brazil. *Memórias do Instituto Oswaldo Cruz*, **110**, 569–572
- Zappulli, V., De Zan, G., Cardazzo, B., Bargelloni, L. and Castagnaro, M. (2005) Feline mammary tumours in comparative oncology. *Journal of Dairy Research*, **72**, S98–S106
- Zar, J.H. (1996) *Biostatistical Analysis*, 3rd edn. Prentice Hall, Upper Saddle River

- Zar, J.H. (2010) *Biostatistical Analysis*, 5th edn. Pearson Education International, Upper Saddle River
- Zarkovich, S.S. (1966) *Quality of Statistical Data*. Food and Agriculture Organization of the United Nations, Rome
- Zeimes, C.B., Olsson, G.E., Hjertqvist, M. and Vanwambeke, S.O. (2014) Shaping zoonosis risk: landscape ecology vs. landscape attractiveness for people, the case of tick-borne encephalitis in Sweden. *Parasites & Vectors*, **7**, 370; doi 10.1186/1756-3305-7-370
- Zelen, M. (1969) Play the winner rule and the controlled clinical trial. *Journal of the American Statistical Association*, **64**, 131–146
- Zetterquist, P. (1972) *A Clinical and Genetic Study of Congenital Heart Defects*. Institute for Medical Genetics, University of Uppsala, Sweden
- Zhou, X.H. (1998) Correcting for verification bias in studies of a diagnostic test's accuracy. *Statistical Methods in Medical Research*, **7**, 337–353
- ZIMS (2015) *ZIMS for Medical*. <http://www2.isis.org/products/Pages/medical.aspx> (accessed 12 July 2015)
- Zinkernagel, R.M. (2003) On natural and artificial vaccinations. *Annual Review of Immunology*, **21**, 515–546
- Zinsstag, J., Schelling, E., Bonfoh, B., Fooks, A.R., Kasymbekov, J. *et al.* (2009) Towards a 'One Health' research and application tool box. *Veterinaria Italiana*, **45**, 121–133
- Zinsstag, J., Schelling, E.M., Waltner-Toews, D. and Tanner, M. (2011) From 'One Medicine' to 'One Health' and systematic approaches to health and well-being. *Preventive Veterinary Medicine*, **101**, 148–156
- Zinsstag, J., Schelling, E., Waltner-Towes, D., Whittaker, M. and Tanner, M. (Eds) (2015) *One Health: The Theory and Practice of Integrated Health Approaches*. CABI, Wallingford
- Zinsstag, J., Schelling, E., Wyss, K. and Mahamet, M.B. (2005) Potential of cooperation between human and animal health to strengthen health systems. *The Lancet*, **366**, 2142–2145
- Zintl, A., Gray, J.S., Skerrett, H.E. and Mulcahy, G. (2005) Possible mechanisms underlying age-related resistance to bovine babesiosis. *Parasite Immunology*, **27**, 115–120
- Zipperer, A., Konnerth, M.C., Laux, C., Berscheid, A., Janek, D. *et al.* (2016) Human commensals producing a novel antibiotic impair pathogen colonization. *Nature*, **535**, 511–516
- Zlotogora, J. (1998) Recessive or dominant? Reclassification in the molecular age. *Clinical Genetics*, **53**, 423–425
- Zou, G.Y. (2010) Confidence interval estimation under inverse sampling. *Computational Statistics and Data Analysis*, **54**, 55–64
- Zou, K.H., O'Malley, A.J. and Mauri, L. (2007) Receiver-operating characteristic analysis for evaluating diagnostic tests and predictive models. *Circulation*, **115**, 654–657
- Zou, X.-H., Li, S.M. and Gatsonis, C.A. (2008) Wilcoxon-based group sequential designs for comparison of areas under two correlated ROC curves. *Statistics in Medicine*, **27**, 213–223
- Zucker, J.R. (1999) Changing patterns of autochthonous malaria transmission in the United States: a review of recent outbreaks. *Emerging Infectious Diseases*, **2**, 37–43
- Zuckerman, Lord (1980) *Badgers, Cattle and Tuberculosis*. Report to the Right Honourable Peter Walker. Her Majesty's Stationery Office, London
- Zukowski, S.H., Hill, J.M., Jones, F.W. and Malone, J.B. (1991) Development and validation of a soil-based geographic information system model of habitat of *Fossaria bulimoides*, a snail intermediate host of *Fasciola hepatica*. *Preventive Veterinary Medicine*, **11**, 221–227
- zur Hausen, H. (2008) Papillomaviruses – to vaccination and beyond. *Biochemistry (Moscow)*, **73**, 498–503
- Zvuloni, A., Artzy-Randrup, Y., Stone, L., Kramarsky-Winter, E., Barkan, R. *et al.* (2009) Spatio-temporal transmission patterns of black-band disease in a coral community. *PLoS ONE*, **4**, 4, e4993; doi 10.1371/journal.pone.0004993
- Zweig, M.H. and Campbell, G. (1993) Receiver-operating characteristic plots: a fundamental evaluation tool in clinical medicine. *Clinical Chemistry*, **39**, 561–577

Index

a

- Abattoirs, as sources of data 466
- Abduction 43
- Abomasal mucosal hyperplasia 47
- Abortive reaction, in infections 98
- Absolute risk reduction 366
- Abuse (non-accidental injury)
 - animal 23, 222
 - child 23
 - sexual 23
- Accredited/attested herd
 - schemes 597–598
- Accuracy
 - definition 209, 384, 636
 - diagnostic *see* Area under the curve
 - of serological tests 429–430
- Actinobacillosis 29, 52
- Actinobacillus lignieresii* *see* Actinobacillosis
- Action levels
 - in control charts 267
 - in health and productivity
 - schemes 588
- Active data collection 235, 460–461, 619
- Active surveillance *see* Surveillance
- Adjusted (standardized) measures of disease occurrence
 - for age 80
 - calculation 78–80
 - confidence interval 261
 - for lifespan 193–195
- Aedes* spp. 95, 120, 157
- Aetiological fraction *see* Attributable proportion
- African horse sickness 96, 125, 129, 547, 610
- African swine fever 59, 122, 130, 271, 429
- Age
 - comparison of human and animal 193–195
 - as a determinant 90–91
- Age of Enlightenment* *see* 'Enlightenment, The'
- Age-specific rates 78, 195
- Agreement
 - of diagnostic tests 209, 450–454
 - method of 54
- Agresti-Coull confidence interval 259
- Agricultural organizations, as sources of data 468
- Aggregate-level testing 443–444
- AIC *see* Akaike's Information Criterion
- AIDS *see* HIV/AIDS
- Akaike's Information Criterion 505, 509, 513
- ALOHA model 126–127
- Analogy, method of 54–55
- Analysis, errors in 395
- Analytical epidemiology 32
- Animacula 5
- Animal abuse *see* Abuse
- Animal-cemetery records, as sources of demographic data 62
- Animal plagues
 - as current problems 13
 - dates of occurrence 7–8
- Animal-rescue organizations, as sources of demographic data 62
- Animal rights 23
- Animals and plants, relationships between types 152–155
- Animals, domestication of 1–2
- Animal-time at risk 68
- Animal welfare 21–23, 75
- Animism 2
- ANIMO animal movement
 - network 464
- Anthrax, as a plague 5, 7–8
- Anthropology, knowledge of, in disease control 620
- Anthropurgic ecosystems 156
- Antibodies 636 *see also* Serological epidemiology
 - blocking 429
 - comparison of levels 426–427
 - half-life 424
 - methods of expressing
 - amounts of 421–424
 - prevalence 424–425
- Antigenic variation 95, 97
- Antigens 636
- Apsyrus 6
- Aquinas, St Thomas 42
- Arabian medicine 5
- Area incidence ratio 77
- Area under the curve 442–443
- 'Argument from caprice' 50
- Aristotle 42
- Arithmetic mean titre 422
- 'Armchair epidemiology' 34
- Ars Conjectandi* 12
- Arthropods, transmission of infection in 117
- Asbestos, as a carcinogen 193
- Anscombe's Quartet 495
- Association 46–50, 636 *see also*
 - Observational studies;
 - Sampling; Statistics
 demonstrating 296–318
 - non-statistical 46
 - statistical 46–50
 - types of 46–50

- Asymptotic (large sample)
 methods 257
- Atomistic fallacy 355
- Atrophic rhinitis 107, 450–451
- Attack rate 70
- Attested herd schemes *see* Accredited
 herd schemes
- Attributable proportion 327–328
- Attributable rate *see* Attributable risk
- Attributable risk 325–327
- AUC *see* Area under the curve
- Aujeszký's disease, airborne
 transmission 128
- Aurochs 2
- Australia
 control of screw-worm
 fly in 581
 fascioliasis in 83
- Authority, role of, in accepting a
 hypothesis 44
- Autochthonous ecosystems 156
- Ayurvedic medicine 4
- b**
- Babesia* spp. 92, 207
- Backward elimination 508
- Badgers
 density of setts 81
 tuberculosis in 17, 20, 31, 58, 65,
 93, 245, 523, 569, 611
- Balance of nature 142
 disease control and 621
- Bar chart 263
- Basic reproductive number (basic
 reproduction ratio, basic
 reproductive rate)
 169–172, 520, 608
 coefficient of variation 170
 contact rate 169, 524
 effective reproductive number 171
 transmission rate 169
 transmission risk 169
- Bates, Thomas 6
- Bats, rabies in 47
- Bayes, Thomas *see* Bayesian inference
- Bayesian inference 43, 297
- Bayesian Information Criterion 509
- Bean plots 264
- Beef cattle health and productivity
 schemes 594–597
- 'Begging the question' *see* *Petitio
 principii*
- Behaviour
 as a determinant 93, 121
 group 147
- Beijerinck, Martinus 5
- Benefit:cost ratio 580, 588
- Bernoulli
 Daniel 11, 520
 Jakob 12
- Berkson's bias *see* Admission
 rate bias
- Beta-pert distribution 433
- Bhutan, Kingdom of,
 sampling in 273
- Bias *see also* Observational studies
 admission rate 389
 all's well literature 396
 analysis strategy 395
 apprehension 391
 attention 391
 auxiliary hypothesis 386
 Berkson's *see* admission rate bias
 case ascertainment 388
 in case-control studies 352
 cognitive dissonance 386
 in cohort studies 344
 communication 395–396
 competing risks 387
 confirmation 385
 confounding 49–50, 210, 331–
 334, 345, 392–395, 637
 in cross-sectional studies 353
 data capture 391
 data-dredging 396
 data entry 391
 definition of 210
 diagnostic *see* diagnostic suspicion
 diagnostic access 388
 diagnostic suspicion 391
 diplomatic 482
 dry-season 482
 end aversion 391
 exclusion 388
 expectation 386, 391
 exposure identification 391
 exposure suspicion 391
 faking bad 391
 faking good 391
 gender 482
 healthcare access 387
 hot-topic 396
 incidence-prevalence 388
 inclusion 389
 information (observational) 210,
 344, 390–392
 instrument bias 391
 interpretation 385–386
 interview setting 391
 interviewer 210, 226, 354, 391
- language 396
 loss to follow-up 389
 magnitude 396
 matching 389
 measurement 210, 390
 mechanism bias 386
 membership bias 388
 misclassification 210,
 330–331, 390
 missing data 395
 Neyman *see* incidence-prevalence
 non-response 344, 352, 353, 389
 obsequiousness 391
 orientation 386
 outcome identification 391
 outcome reporting 396
 person 482
 popularity 387
 positive results *see* publication
 post-hoc analysis 395
 procedure selection 389
 project 482
 publication 377, 395, 411
 recall 391
 referral filter 387
 rescue 385
 responder 391
 response fatigue 391
 reporting 411
 selection 210, 330, 344, 386–390
 significance 396
 social undesirability *see*
 unacceptability
 spatial 481–482
 spectrum 434
 surveillance 388
 survival *see* incidence-prevalence
 telephone random sampling 388
 tidying-up 395
 time-will-tell 386
 truth-will-out 386
 unacceptability 391
 unacceptable disease 391
 unacceptable exposure 391
 verification 432
 volunteer 388
- BIC *see* Bayesian Information
 Criterion
- 'Big data' 471, 629, 636
- 'hubris' 471
- Bills of Mortality *see* Graunt, John
- Binary outcomes,
 modelling 509–511
- Binomial distribution 255
- Biocenosis 156

- Biological markers 35
 Biomes 139–142
Biomphalaria glabrata 149
 Biosecurity 172, 609, 611, 623, 636
 Biosocial disease model 88
 Biotic community *see* Biocenosis
 Biotopes 156
 Birds, psittacine, preventive
 medicine in 601
 Birth rates 77
 'Black box' approach/methods 48
 Black Death 4, 6, 59, 102, 125, 138, 145
 Bladder cancer
 in dogs 192
 in humans 192
 Bland-Altman plot 452–454
 Blanket slaughter (culling) 606
 Blinding, in clinical trials 369
 Blocking antibodies 429
 Blood flukes *see* Schistosomiasis
 'Blood splashing' 30
 Bluetongue
 climate change and 103
 in Portugal 82
 recombination in 96
 transmission 116, 126, 129
Boophilus microplus 93
 Bootstrap estimates 261–262, 323
Bordetella spp., mutation in 95
 Bovine ephemeral fever virus 116
 Bovine hypomagnesaemia 52
 Bovine petechial fever 55
 Bovine spongiform
 encephalopathy 14, 20,
 30, 39, 53, 315
 Bovine tuberculosis 20
 role of badgers 17, 58, 523
 Bovine virus diarrhoea
 control 598, 622
 latent infection 99
 Box-and-whisker plots *see* Box plots
 Box plots 263
Brachyspira hyodysenteriae 445
 latent infection in 99–100
 Breast cancer *see* Tumours,
 mammary
 Breed societies, as sources of
 data 469
 Breslow-Day test for
 homogeneity 333
 Broad Street pump 49–50
Brucella abortus,
 transmission 116, 121
Brucella suis 469
 Brucellosis
 control and eradication 598, 622
 genetic resistance 614
 screening tests 436
 BSE *see* Bovine spongiform
 encephalopathy
 Buddhist medicine 4
 Budgerigars, goitre in 77
 Burkitt's lymphoma
 and Epstein-Barr virus 110
 and *Plasmodium*
 falciparum 110
- C**
 Calculus, differential, models
 using 521–526
 California Animal Neoplasm
 Registry 191
 Calorific energy flow 155
Campylobacter spp. 21, 39, 121,
 149, 352, 357, 552, 611
 Canary Database 198, 648
 Cancer
 animal models of 191–196
 cause of 110–112
 induction of 110–112
 by bacteria 110
 by chemicals 110
 by helminths 110
 by viruses 110
 investigation of 112
 random model of
 development 110
 stem cell model of
 development 110
 specific cancers *see* Tumours
 Cancer eye 30
 Candidate genes 612
 Canine distemper 7, 43, 86,
 92, 102, 115, 126, 207, 620
 Canine parvovirus infection,
 sentinels for 459
 Capture-release-recapture
 sampling, population
 enumeration by 65
 Carbon monoxide poisoning, in
 pigs 53
 Carcinogenesis 110
 Carcinogens 110
 monitoring 191
 Carriers
 convalescent 99
 of foot-and-mouth disease 99
 incubatory 99
 Case-case-control studies *see*
 Observational studies
 Case-case studies *see* Observational
 studies
 Case-case-time control studies *see*
 Observational studies
 Case-chaos studies *see* Observational
 studies
 Case-cohort studies *see*
 Observational studies
 Case-control studies *see*
 Observational studies
 Case-crossover studies *see*
 Observational studies
 Case fatality 72
 Case-only studies *see* Observational
 studies
 Case-other disease studies *see*
 Observational studies
 Case series 339
 Case studies 339
 Case-time-control studies *see*
 Observational studies
 Casuistry 42
 Cats *see also specific diseases of cats*
 coat colour, skin cancer and 94
 conjunctivitis 46
 health schemes 601
 panleucopenia 129, 178
 urological syndrome/
 urolithiasis 109,
 328–330
 Cattle cultures 2
 Cattle Health Certification
 Standards (UK) 598
 Cattle
 anthrax 7–8
 beef health and productivity
 schemes 594–597
 bluetongue 101, 116, 157,
 461, 605
 bovine virus diarrhoea virus 99,
 107, 282, 426, 455,
 598, 622
 copper deficiency 80
 copper poisoning 625
 dairy health and productivity
 schemes 588–591
 enzootic bovine leucosis 68,
 282, 331
 ephemeral fever virus 116
 foot-and-mouth disease 43,
 59–61, 116, 132, 246,
 606, 615
 Haemonchus contortus
 infection 47
 hypomagnesaemia 52, 103, 179

Cattle (*cont'd*)

Johne's disease
 (paratuberculosis) 290,
 429, 455 *see also specific
 diseases of cattle*
 keratoconjunctivitis 101, 123
 Kikuyu grass poisoning 55
 malignant catarrhal fever 30, 477
 mannosidosis 54, 613
 mastitis control 577, 590, 611
 metabolic profiles 591
 optimum feeding 591
 paratuberculosis *see* Johne's disease
 petechial fever 55
 protoporphyria 613
 rhinotracheitis virus 102
 size of herds 66
 squamous cell carcinoma of the
 eye 30, 53, 77
 Texas fever 5, 604
 ticks 130, 162
 trypanosomiasis
 eradication 605
 tolerance 614
 world productivity 17
 Cattle Tracing System 242
 Causal hypotheses, formulation
 of 53–56
 Causal diagrams 394
 Causal inference 43–44
 Causal models 50–52
 Causal reasoning
 in comparative studies 199
 method of agreement 54
 method of analogy 54–55
 method of concomitant
 variations 54, 101
 method of difference 53–54
 Causal theories 42–43
 Causality 42–56
 Causation, reverse 395
 Cause of disease 2–5, 45–46
 Cause(s)
 direct 47
 efficient 42
 final 42
 formal 42
 indirect 47
 material 42
 necessary 50
 sufficient 50
 Censoring 73
 Censuses 37, 66, 270
 Census study 352
 Centiles 262

Central limit theorem 293
 Centring 494
 Certification schemes, as sources of
 data 469
 Chagas' disease 118, 141
 Chain-binomial models 174
 Character displacement 150
 Check digits 217
 Chemotherapy, therapeutic and
 prophylactic 609
 CHESS *see* Computerized Herd
 Evaluation System for Sows
 Chile, rabies in 185
 Chiron 2
 Chi-squared (χ^2)
 distribution 508, 660
 Chi-squared (χ^2) test 310–311
 Chi-squared (χ^2) test for
 trend 314–315
 Chlamydiosis 464
 Chlorinated hydrocarbon poisoning,
 in foxes 59
 Cholera 5, 49, 55, 59, 125, 199,
 521, 538
 Choroplethic maps 82–83
 Chromosomal disorders 89
 Chromosomal shattering 111
 Chronic disease epidemiology 35
 Chronic pulmonary disease 198
 Chronotones 158
 Chrysoloras, Manuel 42
Chrysomya bezziana
 control, cost-benefit analysis
 of 581–582
 spread 125
 Classification, of disease 205–207
 Classificatory scale of measurement
 see Nominal scale of
 measurement
 Climate
 change 102–103
 classification 101
 as a determinant 101–104
 ecological effects 139–142
 population dispersal and 144
 Clinical epidemiology 33
 Clinical infection 98
 Clinical trials 361–382
 categories 361–362
 community 363
 definition 361
 efficacy 361, 364–367
 vs. effectiveness 361
 interpretation of results 374–375
 meta-analysis 375–380

open 362
 PICO (PICOTT) framework 364
 protocol 364
 randomized controlled 362–363
 admission criteria 368–369
 blinding 369
 compliance 373–374
 exclusion criteria 368–369
 experimental population 368
 experimental unit 367–368
 follow-up 373
 intention-to-treat analysis 374
 minimization 370
 per protocol analysis 374
 placebo/placebo effect 363
 by proxy 363
 primary hypothesis 364
 protocol 364–365
 randomization 369–370
 block 370
 simple 369
 sample size
 determination 372–373
 stratification 370
 termination 374
 types
 adaptive-design 371
 bioequivalence 364
 cluster randomized 363
 confirmatory 363
 cross-over 370–371
 exploratory 363
 equivalence 364, 372–373
 factorial design 372
 multicentre 363
 non-inferiority 363–364,
 372–373
 open label 369
 parallel group (standard) 370
 sequential 371
 superiority 372, 374
 uncontrolled 362
 Clustered data
 modelling of 514–519
 in surveys 274, 277–284, 293
 Coat colour, disease and 94
 Co-carcinogens 111
 Coccidioidomycosis 142, 159
Code of Hammurabi 463
 Codes *see* Data, codes and coding;
 Questionnaires
 Coefficient of variation 170
 Cohen's *d* 378
 Cohort studies *see* Observational
 studies

- Coitus, infection via 124–125
 Coli septicaemia 107
 Colorado tick fever 120
 Commensals 637
 Commercial livestock enterprises, as
 sources of data 468–469
 Companion-animal medicine,
 future of 629
 Companion animals
 demographic studies 63–64
 health schemes 599–602
 cats 601
 dogs 600
 ferrets 601
 horses 602
 psittacines 601
 rabbits 601
 surveillance in 483, 485
 Comparative epidemiology 35,
 189–200
 Comparison of groups 296 *see also*
 Association; Observational
 studies
 three or more samples 299, 301
 two samples
 means 302–304
 medians 304–309
 proportions 309–315
 Competition, ecological 142–144
 Competitive exclusion, principle of
 see Interference,
 epidemiological
 Complementary log-log
 transformation 510
 Complete-subject analysis 228
 Compliance, in clinical trials 373
 Compliance gap analysis 601
 Composite measurement
 scales 204–205
 Computational epidemiology 33
 Computerized Herd Evaluation
 System for Sows 242, 587
 Concomitant variations,
 method of 54
 Concordance 450
 Conditional logistic
 regression 512
 Confidence interval
 definition 257, 637
 of specific parameter *see*
 appropriate parameter
 Confidence limits *see* Confidence
 interval
 Conformation of hosts, as a
 determinant 93
 Confounding 49, 331–335,
 392–395
 Conjugation, bacterial 96
 Connectance (connectivity), of
 networks 153
 Conservation medicine 31, 40
 CONSORT 396, 410
 Constants 641
 Construct validity 205, 364, 450
 Contact-network models
 533–534
 Contact rate 169
 Contagion 5
 Contagious bovine
 pleuropneumonia 7–8,
 10, 13, 30
 Contagious spatial pattern 186
 Contagium animatum 5
 Contagium vivum fluidum 5
 Content validity 205
 Contiguous populations 62–65
 Contiguous slaughter (culling) 606
 Contingent valuation 568
 Control of disease 604–632
 definition 604
 economic assessment 575–582
 strategies 605–616
 Convenience sampling 272
 Convergent evolution 139
 Cook's distance 499
 Copper deficiency 80
 Copper poisoning,
 chronic 625–626
 Coral reefs, diseases of 48
 climate change and 103
 Correlation 316–317
 coefficient 691
 Cost:benefit ratio *see* Benefit:
 cost ratio
 Cost-benefit analysis *see* Economics
 and veterinary
 epidemiology
 Cowpox 5, 30
Coxiella burnetii 121, 151, 423
 Credible interval 257
 Critical community size 168
 Critical knowledge 43
 Crossed data structures 516
 Cross-over trials 370–371
 Cross-product ratio
 see Odds ratio
 Cross-reactions 429
 Cross-sectional studies *see*
 Observational studies
 Cross-sectional surveys 37
 Crude measures of disease
 occurrence 76
 Cryptorchidism, canine 78
Cryptosporidium spp. 21
 CTS *see* Cattle Tracing System
Culicoides spp. 101, 103, 126,
 129, 605
 Cullen, William *see* *Synopsis*
 Nosologiae Methodicae
 Cumulative incidence *see* Incidence
 Cumulative mortality *see* Mortality
 Cusum charts 267–269
 Cut-off points, in diagnostic
 tests 430
 Cyclic neutropenia *see* Gray collie
 syndrome
 Cyclical trends in disease
 178–179
Cysticercus bovis 610
Cysticercus pisiformis 116
Cysticercus tenuicollis 466
Cytoecetes ondiri *see* Bovine
 petechial fever
- d**
 d'Azyr, Felix Vicq 10
 Dairy health
 national schemes 597–599
 private schemes 588–591
 Dairy herds, metabolic profiles 591
 DairyCHAMP 238
 DairyCOMP 305, 238
 Dampening-down (suppressive)
 vaccination 607
Dance of Death 59
 Dangerous contacts, in infectious
 disease outbreaks 623
 Darwin, Charles 138, 149
 Data
 accuracy 209, 429–430
 diagnostic *see* Area under
 the curve
 classification 201–205
 case-specific 229
 categorical 201
 codes and coding 210–217
 alpha 213–214
 alphanumeric 214
 check digits 217
 choice 215
 components 212
 consecutive 212–213
 consistency 217
 error detection 216–217
 'finger trouble' 217

- Data (*cont'd*)
- hierarchical 212–213
 - numeric 212–213
 - of questionnaires 220
 - structure 211–212
 - symbols 215
- collection
- cooperation 462
 - cost 462–463
 - methods 219
 - traceability 463–464
- continuous 201
- definition of 201
- interpretative 207–208
- measurements 201–204
- bias 210
 - composite 204
 - continuous 201
 - discrete 201
 - interval 202
 - Likert 220
 - nominal 202
 - ordinal 202
 - ratio 202
 - visual analogue 203
- numerical presentation 251–269
- observational 207–208
- record-specific 229
- sources 464–471
- quality 228–229
- Databases
- definition of 229
 - examples 237–244
 - macroscale systems 232
 - mesoscale systems 232
 - microscale systems 232
 - models of
 - flat model 229
 - hierarchical model 230
 - network model 230–231
 - record model 229
 - relational model 231
- Day books, as sources of data 231
- Day of Atonement* 3
- DBMS *see* Databases, database management systems
- de Candolle, Alphonse Pyrame 139
- de Lacrois, Francois Bossier *see* *Nosologia Methodica*
- Death rate 72
- Decision boundaries, in control charts 267
- Decision support systems *see* Recording schemes
- Deconfounding principle 348
- Deduction 43
- Deer
- Health Scheme (UK) 598
 - population-size determination 65
- Degrees of freedom 257
- Delphi conference 536
- Delta-beta values 514
- Demographic base maps 80
- Demographic studies, in dogs and cats 63–64
- Demography 62
- De Moivre, Abraham 520
- Demonic theory of disease 2
- Density curve 254
- Density-dependent competition 144
- Density function 254
- Density models 521
- Density strips 264
- Dermacentor andersoni* 120
- Dermatitis interdigitalis *see* Interdigital dermatitis
- Dermatophilosis 93
- Descartes, René, 43
- Descriptive epidemiology 32
- Design effect *see* Sampling, design effect
- Determinants of disease 86–114
- age 90–91
 - agent 94–101
 - associated with host, agent and environment 86–89
 - behaviour 93
 - biological interaction 108–109
 - breed 92–93
 - classification of 86–89
 - climate
 - macroclimate 101–103
 - climate change 102–103
 - global warming 103
 - microclimate 103–104
 - coat colour 94
 - diet 104–105
 - endogenous *see* Intrinsic environment 101–106
 - improvement of, in disease control 611
 - exogenous *see* extrinsic
 - extrinsic 86
 - genetic
 - in agents 94–97
 - in hosts 92
 - hormonal 91
- host 89–94
- housing 104
- husbandry 104–105
- interaction between 106–112
- intrinsic 86
- location 101
- management 105
- microbial colonization of hosts 100–101
- 'occupational', 105
- primary and secondary 86
- sex 91–92
- size and conformation 93
- species 92–93
- statistical interaction 109–110, 328–330, 505–506
- stress 105–106
- Deterministic models 521, 556
- Diagnosis
- bias 210
 - criteria 207–208
 - diagnostic tests
 - accuracy 429, 442
 - aggregate-(herd-) level 443–444
 - agreement between 450–454
 - area under the curve 442–443
 - Bland-Altman plot 452–454
 - diagnostic odds ratio 434
 - gold standard 431
 - absence of 431
 - in import risk assessment 446–447
 - kappa* statistic 450–452
 - latent class analysis 450
 - likelihood ratios 436–441
 - multiple testing
 - negative-herd retesting 446
 - parallel testing 444–445
 - serial testing 445–446
 - positive and negative results 429–430
 - predictive value 434–436
 - reliability 209, 454
 - repeatability 209, 454
 - reproducibility 209, 454
 - robustness 209
 - ruggedness 209
 - ROC curves 441
 - comparing 443
 - sensitivity and specificity 208, 430
 - confidence intervals for 433
 - relationship between 431

- validity of technique 209, 428
 guidelines for
 establishing 447–448
 serological 427–430
 Youden's index 433–436
 performance related 20
 Diagnostic laboratories, as sources of
 data 464
Dictyocaulus viviparus, vaccine
 against 629
 Diet, as a determinant 104–105
 Difference, method of 53–54
 Digitizing tablet 245
 Dinka 2
 Diphtheria, clinical trial of antiserum
 against 362
Diphyllbothrium spp. 116
 Direct adjustment *see also* Adjusted
 (standardized) measures
 of disease occurrence
Dirofilaria immitis 118, 141, 143
 Discounting 580
 Discourse on Airs, Waters and
 Places 5
 Disease
 adjusted (standardized)
 measures 78–80
 animals' niches relating to 149
 assessment of cost 565
 cause(s)
 causal associations of 47–48
 see also Association
 causal postulates 45
 changing concepts of 2–5
 direct 47
 enabling factors 52
 Evans' rules 45–46
 formulating hypotheses 53–56
 indirect 47
 Koch's postulates 45
 models 50–52
 necessary 51
 non-statistical association 46
 place and 53
 population and 53
 precipitating factors 52
 predisposing factors 52
 reinforcing factors 52
 risk factors 47
 risk indicators 47
 risk markers 47
 statistical association 46–49
 dangers of 49
 utility of 48–49
 sufficient 50
 time and 53
 classification 205–207
 control 31, 604–632
 balance of nature and 621
 biological vectors 610
 biosecurity 611
 cause, maintenance and
 transmission
 and 616–617
 chemotherapy 609
 compensation and 621
 cost-benefit analysis 579–582
 definitions of 604
 diagnostic feasibility 619
 ecological consequences 621
 environment, husbandry and
 feeding 611
 factors in 616–623
 financing of 621–622
 fomites, disinfection of 611
 genetic improvement 612–614
 grazing protocols 610
 host/parasite
 relationship in 617
 host range in 617
 legislation 621
 minimal disease methods 614
 movement of hosts 610
 niche filling 611
 planning and monitoring 31
 producers' opinions 619
 public health
 considerations 620–621
 public opinion 620
 quarantine 605–606
 replacement stock in 619
 selecting campaigns
 economic criteria 575–582
 technical criteria 622–623
 slaughtering 606, 616
 strategies 605–616
 surveillance 459
 uncertainty in 580
 vaccination 606–609, 615–616
 veterinary infrastructure
 and 617
 determinants of *see* Determinants
 of disease
 diagnosis 207–208, 421–456
 ecology *see* Ecology
 economics of *see* Economics and
 veterinary epidemiology
 eradication 604–605 *see also*
 Disease, control of
 multifactorial 19, 50, 52, 89, 108
 natural history 30–31, 139
 nomenclature 205–207
 non-infectious 19
 occurrence, describing 58–85
 measures of 67–80
 patterns of 168–188
 population regulation
 by 145–147
 reporting *see* Databases, examples
 of; Monitoring,
 Surveillance
 seasonal trends 178–179
 serological diagnosis 421
 spatial distribution 80–84, 186
 temporal distribution 58–61
 temporal trends
 detection 180–186, 255
 regression analysis 181–186
 rolling (moving) averages 181
 space-time clustering 186
 of unknown cause 18–19
 Disinfection 132, 611
 Dispersal 144
 Dissemination rate 172
 Distance sampling *see* Sampling
 Distemper *see* Canine distemper
 Distribution maps 82
 Divergence of character 150
 Divination 4
 Divine cause of disease 3
 'Doctrine of Signatures' 4
 Dogs *see also specific diseases*
 of dogs
 age conversion to human age
 equivalent 193–195
 cryptorchidism 92
 cyclic neutropenia 612
 deafness 94
 domestication 1, 93
 epilepsy and epileptiform
 seizures 199
 haemophilia 89, 199
 health schemes 600
 heart disease 93
 heartworm *see* *Dirofilaria*
 immitis
 lung disease 198
 mammary tumours 195
 mast cell tumours 93
 parvovirus infection 59, 169,
 392, 459
 progressive retinal atrophy 612
 pyometra 30, 337
 testicular neoplasia 78
 urinary incontinence 310, 312

- Domesday survey, livestock census in 66
- Domestic animals
 demography 62
 domestication 1–2
- Dot maps *see* Point (dot or location) maps
- Drift, antigenic
 in influenza viruses 96
 in trypanosomes 96
- Droplet
 expiratory 123
 nuclei 126
- Drought, as a determinant 102
- Ductus diverticulum 196
- Dummy variables 500
- Dysautonomia, feline 18, 205
- Dystokia, in cows 68
- e**
- Ear tags, in animal
 identification 464
- Ebola virus
 and human cultural practices 93
 and One Health 25
 and quarantine 605
- Echinococcosis, eradication from
 Iceland 620
- Echinococcus granulosus* 153
- Eclipse phase 118
- Ecohealth 31
- Ecological climax 157
- Ecological fallacy 354
- Ecological health 31
- Ecological interfaces *see* Ecotones
- Ecological mosaics 158
- Ecological studies 354–355
- Ecology
 consequences of disease
 control on 621
 definition 138
 of disease 30–31, 138–167
 social 138
- Econometrics 565, 584
- Economics and veterinary
 epidemiology 565–585
 assessment of disease
 cost 576–577
 contingent valuation 568
 normative economics 569
 opportunity cost 568
 partial farm budgets 579
 positive economics 569
 production functions 565–567
- social cost-benefit
 analysis 579–582
 benefit:cost ratio 580
 criteria for selecting a control
 campaign 580–581
 in dairy herd health
 schemes 588
 discounting 580
 example 581
 internal rate of return 581
 net present value 580
 principles 579–580
 problems 582
 shadow prices 580
 uncertainty 580
 technical and economic
 efficiency 568–569
 trade bans 571–575
 willingness-to-pay 567
- Ecosystem health 31, 40
- Ecosystems 31, 155–156
 anthropurgic 156
 autochthonous 156
 definition of 155
 synanthropic 156–157
- Ecotones 157–158
- Ecotope 141
- Ectromelia virus 32
- Edge effect 157
- Effect-measure
 modification 392
- Effect size
 as a general term 378
 in meta-analysis 378–379
- Effective contact 120–121,
 169, 172, 174
- Effectiveness, in clinical
 trials 361
- Efficacy, in clinical trials 361,
 364–367
- Efficiency frontier 576
- Efficiency, technical vs.
 economic 568–569
- EID2 see ENHanCED Infectious
 Disease Database*
- Einstein, Albert 535
- Elementary unit 270, 367
- Elimination of disease 605
- El Niño 102
- Elton, Charles *see* Pyramid
 of numbers
- Embryo, transmission of
 infection to 129
- Emergency vaccination 606
- Emergent/emerging diseases
 14–16
 factors responsible for 160–162
- Empedocles 4
- Empirical models 526–527
- Employment of veterinarians 21
- Enabling factors 52
- Endemic disease occurrence 58–84
- Endemic pulsations 178
- Endogenous determinants *see*
 Intrinsic determinants
- Endogenous pathogens 100
- ENHanCED Infectious Disease
 Database* 243–244
- ‘Enlightenment, The’* 10–12, 43
- Enteric disease, mixed
 infections in 107
- Environment
 as a determinant 101–106
 importance of 24, 45
 social 31
- Environmental epidemiology 35
- Environmental mastitis 88
- Environmental resistance 142
- Enzootic bovine leucosis 68, 125,
 282, 331, 605
- Enzootic pneumonia 325
- Epidemics
 basic reproductive
 number 169–172
 common source 172–173
 definition 28, 58–59
 dissemination rate 172
 epidemic curves 168–177
 epidemic disease
 occurrence 58–59
 epidemic waves 175
 estimated dissemination rate 172
 point source 172
 propagating 172–173
 recurrent 178
 Reed-Frost model 173–175
 R_0 *see* Basic reproductive
 number
 synchrony in 145
 ‘Epidemiologic’ vs.
 ‘epidemiological’ 29
- Epidemiological error 383–384
- Epidemiological interference *see*
 Interference,
 epidemiological
- Epidemiological investigation, types
 of 32–35
- Epidemiological rates 76

- Epidemiological ratios 76, 192
 Epidemiological unit 271
 Epidemiology
 analytical 32, 340–359
 applied 34
 ‘armchair’ 34
 chronic disease 35
 clinical 33
 comparative 189–200
 components 35–39
 computational 33
 definition 1, 28–29
 descriptive 32
 environmental 35
 experimental 32–33
 field 33–34
 forensic 35
 genetic 33
 horizontal 158
 intervention 33
 macro- 35
 micro- 35
 molecular 34–35
 nutritional 35
 participatory 34, 475–483
 psychosocial 35
 qualitative 35–36
 quantitative 36–39
 role 1, 40
 scope 28–40
 ‘shoe-leather’, 33
 social 35
 socio-psychological 35
 subclinical 35
 theoretical 33
 types 32–35
 uses 29–32
 veterinary 1
 Epileptiform seizures 199
 EpiMAN 242–243
 Epiphytotic 29
 Epistemology 42
 Epizootic 28
 Epizootiology 28
 Epornitic 28
 Equine influenza
 antigenic types 428
 as a plague 7–8
 Equine motor neuron
 disease 186, 187
 Equine parasitic mange, eradication
 of, in the UK 10
 Eradication of disease *see also*
 Disease, control of
 definition 604–605
 early examples 10
 strategies 605–616
 Error detection, in coding 216–217
 Error grid analysis 452
Escherichia coli 19, 21, 88, 92, 100,
 107, 207, 608
 Estimated dissemination
 rate 172, 173
 Ethnoveterinary medicine 34
 European Union, rabies
 control in 605
 Evans, Alfred *see* Evans’ rules
 Evans’ rules 45–46
 Evasion, of demons 2
 Evidence-based medicine 33
 levels of evidence in 376
 Evolution, convergent 139
 Exact confidence interval for a
 proportion 259
 Exogenous determinants *see*
 Extrinsic determinants
 Exogenous pathogens 100
 Exorcism, to remove demons 2
 Exotic diseases *see* Transboundary
 diseases
 Experiential analysis 43
 Experimental models 189
 Experimental epidemiology 32–33
 Experimental science, *vs.*
 observational science 38
 Expert systems 33, 243
 Exponential decay, models of 521,
 525–526
 Exponential function 641
 External validity 368, 384
 Externalities 570
 Extinction
 of infectious agents 604
 of populations 145
 Extrinsic determinants 86
 Extrinsic incubation period 119
 Eyam plague village 520
- f**
F-distribution 427, 692–693
F-test 299, 509, 513
 Face validity 205
 Factorial-design trials 372
 Factorials 642
 Faecal-oral transmission cycle 121
 FAIDS 35
 False-positive paradox 436
 Farming, spread of in Europe 2
- Farms
 hygiene 88
 profiles 587
 records, as sources of data 469
 Farr, William 11, 49, 215, 457
 Farr’s Law 520
Fasciola hepatica see Fascioliasis
 Fascioliasis
 in Australia 84
 control 610
 models 526–527
 survival 30
 Feline dysautonomia 18, 205
 Feline leukaemia
 latent infection 99, 100
 spatial clustering 186
 Feline panleucopenia, seasonal
 variation in 178
 Feline urolithiasis *see* Feline
 urological syndrome
 Feline urological syndrome 30, 109,
 328–329
 Ferret, preventive medicine in 601
 Fertex index 589
 Fertility rates 77
 Foetal death rate 77
 Foetal death ratio 77
 Fiducial interval 257
 Field epidemiology 33–34, 623
 Field trials *see* Clinical trials
 Finagle’s Laws 235
 ‘Finger trouble’ 217
 ‘Fingerprinting’, nucleic acid 34
 ‘Fire-brigade’ treatment 20, 586
 Fisher’s exact test 311–313
 ‘Fitness valley’, in microbial
 colonisation 92
 Fitzherbert, John 6
 Fixed-effect model
 in clinical trials 378–379
 in statistical modelling 515
 Five-point summary 254
 Flat database model 229
 Fleas, host/parasite
 relationships 134
 Fluke, life cycle of 154 *see also*
 Fascioliasis
 Fomites
 definition 117
 disinfection 132, 611
 Food chains
 definition 152
 relationship between predator and
 prey 155

- Food chains (*cont'd*)
 trophic cascades 152
 trophic levels 152
 Food, competition for 142
 Food poisoning 53, 172
 Food quality 21
 Food webs 152–154
 animal size and 153
 connectance and 153
 Foot-and-mouth disease
 airborne spread 126–128
 as a plague 7–8
 carrier state 99
 cyclical trend in 185
 epidemics in the UK 60
 eradication 605
 neighbourhood analysis 246
 nucleotide sequencing 34, 97
 outbreaks
 definition of 61
 in Paraguay 185
 in the UK 7–8, 12, 13, 60
 post-epidemic
 serosurveillance 246, 285
 serotypes 97
 survival 121
 topotypes 97
 vaccination 606, 615
 Footrot 108, 366
 Forecasting systems, for
 fluke 526–527
 Formations, of vegetation 139
 Forward selection 509
 Foxes
 chlorinated hydrocarbon
 poisoning in 59
 movement of 160
 rabies in 148, 155, 169, 523–525
 Fracastorius, Hieronymous
 (Fracastoro, Girolamo) 5
 Fragmentation, of habitats 163
Framingham Heart Study 48
 Free-roaming populations 62
 Frequency polygon 253
 Frequentist statistics 297
 Friedrich William, King of
 Prussia 463
 Frosch, Paul 5
 Fucosidosis 613
 FUS *see* Feline urological syndrome
- g**
 Gaia hypothesis/theory 31, 142
 Galen, Claudius 3, 5
 Galileo (Galilei, Galileo) 43
 'Sin of' 538
 Gastroenteritis, of pigs 178
 Gause, Georgyi
 Frantsevitch 142, 149
 Gauss, Carl Frederick 254
 Gaussian distribution *see* Normal
 distribution
 Gaussian plume model of
 atmospheric
 diffusion 126
 General adaptation syndrome 105
 Generality, of a theory 535
 Generalized additive model 514
 Generalized linear mixed model 516
 General linear model 499
 Generation time 119
 Gene-modified models *see*
 Transgenic models
 Genes, role of in disease
 pathogenesis 34
 Genetic determinants
 in the host 92
 in infectious agents 94–97
 Genetic diseases, animal
 models of 196
 Genetic epidemiology 33
 Genetic markers, in animal
 identification 464
 Genetic improvement, in disease
 control 612–614
 selective breeding 614
 transgenic biology 614
 Genetic reassortment 95, 96
 Genetic screening 612
 candidate genes 612
 linked markers 612
 Genotype *see* Genetic
 determinants
 Geographic base maps
 80–84
 Geographical information
 systems 244–248
 Geometric mean titre 422
 GIS *see* Geographical information
 systems
 Glanders 10, 604
 Glass's delta 378
 Global Health 24, 40
 Global surveillance *see* Surveillance
 Global warming, as a
 determinant 103
 Goats, domestication of 2
 Goitre, in budgerigars 77
 'Gold standard', in diagnostic
 tests 431
 absence of 448–450
 Gompertz's Law 193
 Google™, as a source of surveillance
 data 471
 Gosset, William 257
 Government veterinary
 organizations, as sources of
 data 464–465
 Grass sickness 18, 30
 Graunt, John 10, 520
 Gray collie syndrome 612
 Grazing, and disease control 610
 Grebes, parasites in 151
 Greek medicine 2, 4–6, 629
 'Greenhouse effect' *see* Global
 warming
 Grey literature 401, 478
 Grey-scale figures 266
 Gross margin analysis 579
 Group effects, in clinical trials 368
 Group behaviour, of animals 147
- h**
 Habitat 138
 HACCP (Hazard Analysis Critical
 Control Point) 21,
 549–550
 Haeckel, Ernst 138
Haemaphysalis spinigera 164
Haemobartonella spp. *see*
 Candidatus spp.
Haemonchus contortus 47
 Haemophilia, canine 92
 Half-life, of antibodies 424
 Halley, Edmund 11
 Hares, *Brucella suis* from 469
 'Harking' 379
 Haygarth, John 363
 Hazard ratio 322, 400
 Healers, primitive 1, 2
Healex index 586
 Health
 definition 23
 ecosystem 40
 Health schemes
 companion animals
 birds 601
 cats 601
 dogs 600
 ferrets 601
 horses 602
 rabbits 601

- national schemes
 accredited/attested
 herds 597–598
 health schemes 598–599
 private health and productivity
 schemes
 action level 588
 components 588–591
 beef cattle 594–597
 dairy cattle 588–591
 interference level *see*
 action level
 pigs 591–592
 sheep 592–593
 justification 588
 objectives 586–587
 structure 586–588
 targets 587–588
 uptake 588, 601
 Healthy-worker effect 344, 388
 Heart disease, in dogs 93
 Heartwater, risk of introduction
 into the US 126
 Heartworm *see* *Dirofilaria immitis*
 Hedges' *g* 378
 Hefts, as epidemiological units 271
 Hekistotherms 139
Helicobacter pylori, and gastric
 cancer 110, 385
 Helminthiasis
 climate change and 103
 in dogs 620
 environment and 30
 Henle-Koch postulates *see* Koch's
 postulates
 Herd effects *see* Group effects
 Herd-level testing *see* Aggregate-level
 testing
 Herd management software 242
 Herpesvirus, causing malignant
 catarrhal fever 30
 Herschel, John Frederick 38, 43
 Heuristics 386
 Hierarchical statistical model *see*
 Mixed effects model
 Hierarchic codes 212–213
 Hierarchic database model 230
 Higgin's & Thompson's *H*
 heterogeneity index 334
 Hill, Austin Bradford *see* Hill's
 criteria
 Hill's criteria 55–56
 Hippocrates of Cos 5, 629
 Histogram 252, 264–265
 HIV/AIDS 35, 124, 138
 as a population regulator 146
 Home range 147
 Homeopathic remedies,
 assessment of 361
 Hookworm 122
 Horizontal transmission 115–129
 Hormonal determinants 91
 Horse cultures 2
 Horses *see also specific diseases of*
 horses
 grass sickness 18, 30
 health programmes 602
 motor neuron disease 187
 Hosmer-Lemeshow goodness-of-fit
 test 514
 Host(s)
 aberrant *see* secondary
 accidental *see* incidental
 amplifier 116
 dead-end *see* incidental
 definitive 115–116
 determinants 89–94
 environment within 131
 final 116
 hibernating 116
 incidental 116
 infectiousness 118
 link 116
 intermediate 116
 maintenance *see* primary
 microbial colonization
 100–101
 mobility, and transmission of
 infection 125–126
 natural *see* primary
 paratenic 116
 primary 116
 reservoir 116
 secondary 116
 susceptibility 118
 Host/parasite relationship,
 in disease control 617
 Housing, as a determinant 104
 Household surveys, to estimate
 population sizes 62
 Humanosis 29, 637
 Humboldt, Alexander von 139
 Hume, David 43, 56
 Humoral pathology 4
 Humours, of the body 4
 Husbandry, as a determinant 86
 Huxley, Thomas Henry 45
 line 139
 Hybridization, nucleic acid 34
 Hyperendemic disease 58
 Hyperkeratosis 55
 Hypocupraemia 80
 Hypomagnesaemia 52, 54, 98,
 103, 179
 Hypospadias 197
 Hypotheses
 characteristics 536
 definition 44
 formulation 53–56
 statistical testing of
 vs. estimation 300
 significance tests 297
i
 Identification of animals 62, 463
 Immunity, sterile 98
 Immunization
 history of 9
 natural 607–608
 feedback 608
 Immunological status, vertical
 transmission
 and 129–130
 Immunosuppression, as a
 microbial maintenance
 strategy 133
 Inapparent infection 98
 Incantation, in disease control 2
 Incidence
 cumulative incidence 67–68
 confidence interval 259
 incidence density 70
 incidence rate 68–70
 confidence interval 261
 relationship to
 prevalence 70–71
 Incidence density *see* Incidence
 Incubation period 45, 118
 Index case 173
 Indian Ocean Dipole 102
 Indicator variables *see* Dummy
 variables
 Indirect adjustment *see* Adjusted
 measures of disease
 occurrence
 Individual-value plots 264
 Induced models *see* Experimental
 models
 Induction
 of cancer 110–112
 in causal inference 43
 'Inductivist turkey' 44

- Infection(s)
- agents 94–101
 - antigens on 429
 - biomes affecting 139–142
 - classification 100, 146, 521
 - genotypic changes in 94–97
 - hazards to 131–132
 - resistant forms 133
 - basic reproductive
 - number 169–172
 - characteristics of pathogens 120
 - clinical 98
 - effective contact 120–121, 169, 172
 - emergent/emerging 14, 161
 - extrinsic incubation period 119
 - generation time 119
 - gradient of 97–98
 - group types 89
 - host and vector types 115–118
 - host/parasite
 - relationships 134, 617
 - inapparent (silent) 98
 - incubation period 118
 - latent 99–100
 - latent period 118
 - maintenance of
 - environment in host 131
 - extension of host
 - range 134–135
 - external
 - environment 131–132
 - persistence in host 133–134
 - 'rapidly in, rapidly out'
 - strategy 133
 - resistance and 133
 - strategies 132–135
 - mixed 17, 106–108
 - novel 15–16
 - outcome of 98–100
 - subclinical 98
 - transmission 115–137, 147
 - aerial 123–124
 - aerosol *see* aerial
 - ascending 129
 - biological 117–118
 - characteristics of
 - hosts 118–120
 - characteristics of
 - pathogens 120
 - coitus 124–125
 - congenital 129
 - contact 124
 - cyclopropagative 118
 - developmental 118
 - direct 115
 - droplet nuclei 126
 - epidemiological interference
 - in 151–152
 - factors in 118–121
 - food webs and 153–155
 - germinative 129
 - hereditary 129
 - horizontal 115–129
 - host mobility 125–126
 - iatrogenic 124–125
 - indirect 115
 - ingestion 123
 - inhalation *see* aerial transmission
 - inoculation 124
 - lateral *see* horizontal
 - long-distance 125–129
 - oral route of 121
 - at parturition 129
 - percutaneous 122
 - propagative 118
 - pseudovertical 115
 - respiratory route of 122
 - salivarian 118
 - stercorarian 118
 - transovarial 130–131
 - trans-stadial 130–131
 - vectors 117–118
 - vertical 129–131
 - via embryos 129
 - via skin, cornea and mucous
 - membranes 122–123
 - vectors 117–118
 - virulence 94
- Infectious disease *see also* Infection
- as a regulator of
 - populations 145–147
 - complex 17, 106–108
 - diagnosis 457
 - emergent/emerging 14, 161
 - factors in control 616–623
 - novel 15–16
- Infectious kerato-
- conjunctivitis 101, 123
- Infectiousness, of hosts 118
- Infectivity, of pathogens 120
- Influential data 498–499
- Influenza viruses
- antigenic drift in 96
 - antigenic shift in 96
 - classification 428
 - serological tests 209 *see also* Equine influenza
- 'Infodemiology' 471
- Informatics 638
- Information, definition of 234
- Information systems *see* Recording schemes
- Inhalation, infection by *see* aerial transmission
- Inheritance
- Mendelian 89, 90, 92
 - multifactorial 89–90
 - polygenic 89
- Inhibitors, in serological tests 429
- 'Inner perfection', of a theory 535–536
- Inoculation, infection by 124
- Instrumental knowledge 43
- Insurance companies, pet, as sources of demographic data 62
- as sources of surveillance data 468
- Intangibles, in economics 565, 567, 581
- Interaction 106–112
- biological 108, 638
 - mechanical 108
 - statistical 109–110, 328–330, 392, 638
- Interactive knowledge 43
- Interactive site segregation 150
- Interference, epidemiological 151–152, 611
- Interference levels *see* Action levels
- Interherd 242
- Internal parasitism, niches 150
- Internal rate of return 581
- Internal time component 68
- Internal validity 368, 384
- Internet, The
- as a source of surveillance data 471
 - sites relevant to epidemiology 648–649
- Interval estimation 257–262 *see also specific parameters*
- Interval scale of measurement 202
- Interviews 226–227
- computer-assisted 227
 - semi-structured 479
 - telephone 226–227

- Intracellular parasitism, as a
microbial maintenance
strategy 150–151
- Intra-class correlation
coefficient 518
see also Sampling, rate of
homogeneity
- Intrinsic determinants 86
- Intuition, role of, in accepting a
hypothesis 44
- Investigation, epidemiological, types
of 32–35
- Irish Potato Famine 102
- Isomorbs 83
- Isomorts 83
- Isoplethic maps 83–84
- Iwanowsky (Ivanovsky), Dmitri 5
- j**
- Jaagsiekte 597, 614
- Jackknife residuals 496
- Jaw tumours, in sheep 80, 101
- Jenner, Edward 5, 30
- Johne's disease 123, 224, 290, 355
- JP15 campaign 16
- k**
- K88 antigen 92, 108
- KAP survey *see* Knowledge, Attitude
and Practice survey
- Kaplan-Meier analysis 74
- Kappa statistic 450–452
- Kendall's Threshold
Theorem 168–169
- Kendall's waves 175–177
- Keratoconjunctivitis,
bovine 101, 123
in hens 104
superficial, in dogs (Uberreiter's
syndrome) 102
- Kermack-McKendrick model 521
- Ketosis 18, 47
- Key-Gaskell syndrome *see* Feline
dysautonomia
- Kikuyu grass poisoning 55
- Kilbourne, Fred 5
- Knacker yards, as sources of
data 467
- Knowledge
Attitude and Practice survey 479
critical 43
interactive 43
instrumental 43
- Koch, Robert 5 *see also* Koch's
postulates
- Koch's postulates 45
- Koppen, Waldimir 139
- Kuhn, Thomas 5, 44
- Kyasanur Forest disease, landscape
epidemiology of 162–164
- l**
- Lameness
in cattle, survey of 223
in pigs, assessment of 203
- Lancisi, Giovanni 5, 30, 606, 614
- Landscape epidemiology 158–165
- Landscapes 156
- Laplace, Pierre-Simon 12
- Lassa fever 117, 178
- Latent infection 99–100
- Latent period 118
- Lateral transmission *see* Horizontal
transmission
- Law (s)
of diminishing returns 566
scientific 536
- Lebeau's method of lifespan
adjustment 194
- Lee waves 126
- Leibnitz, Gottfried 6
- Leptospira* spp. *see* Leptospirosis
- Leptospirosis
epidemic 173
genetic resistance 614
hosts 30
landscape epidemiology of 161
seasonal trends 178–179
- Leukaemia, in cats, spatial
clustering of 186
- Leverage 496
- Liber de Wintonia (Book of
Winchester)* *see* Domesday
survey
- Lice, niches of 150
- Life cycles
evolution of 153
of infectious agents 115
- Life-table analysis 73–74
- Lifespan adjustment 194
- Life zones 140
- Likelihood 638
ratios 436–441
ratio test 508, 510, 512, 513
- Likert scale 220
- Lind, James 361
- Line of best fit *see* Regression line
- Linear mixed model 515
- Linear regression 183, 492
- Linked markers 612
- Link function 511
- Listeria* spp. *see* Listeriosis
- Listeriosis 89, 342
- Live birth rate 77
- Livestock
health schemes
accredited/attested herds
597–598
national 598–599
private 586–597
beef cattle 594–597
dairy cattle 588–591
pigs 591–592
sheep 592–593
medicine, future of 628
population of Great Britain 18
production, as an economic
process 566
world populations of 17
- Lobb, Thomas 5
- Location maps *see* Point maps
- Loeffler, Friedrich 5
- Logarithms 641
- Logistic model, in ecology 144
- Logistic regression 510
diagnostic checking 513–514
model selection 512
- Logistic transformation 510
- Logit transformation 510
- Lognormal distribution 256
- Longevity, studies of,
in animals 75
- Longitudinal studies 320
- Longitudinal surveys 37
- Lorenz, Konrad 40
- Lotka, Alfred *see* Lotka-Volterra
equations
- Lotka-Volterra equations 148
- Louis, Pierre-Charles-Alexander 12
- Louping-ill 70
- Louse infestations 150
- Lucké's frog virus 148
- Lucretius Carus, Titus 5
- Lymphocytic choriomeningitis 130
- Lysogeny 95
- Lysosomal storage disease
196, 613
- m**
- Macroclimate *see* Climate
- Macro-epidemiology 35
- Macroparasites
definition 146
mathematical models 521
- Maedi-visna, control of 618

- Magnesium
 deficiency 52, 86, 179
 levels 54, 86, 179
 Malignant catarrhal fever
 30, 477
 Malignant hyperthermia *see* Porcine
 stress syndrome
 Malthus, Thomas Robert 146
 Management, as a determinant 105
 'Manhattan Principles' 24
Mannheimia spp. *see* *Pasteurella* spp.
 Mannosidosis 613
 Manpower, veterinary,
 in disease control 617, 618
 distribution of 21
 Mantel-Haenszel
 procedure 332–333
 Maps
 choroplethic 82–83
 demographic base 80
 distribution 82
 geographic base 80
 grid-based 245
 isoplethic 83–84
 participatory 480
 point (dot or location) 81
 proportional circle 82
 proportional sphere 82
 raster-based *see* grid-based
 vector-based 245
 Margin of clinical equivalence 374
 Marginal cost 572
 Marginal analysis 577
Marisa cornuarietis 149
 Masking, in clinical trials *see* Blinding
 Mass screening 37
 Mastitis
 control 590, 597
 economic study of 577–579
 environmental 88
 summer 107
Mastomys natalensis 116
 Matching 334, 351, 395
 Maternal mortality ratio 77
 Mathematical models 520–539
 Mathematics, notation and
 terms 641–642
 Matrices, models using 530–532
 Matrix scoring, in participatory
 epidemiology 479–480
 Maximum likelihood 494, 510
 McNemar's change test 313
 Mean 253
 arithmetic 422
 confidence interval for
 difference between two
 means 303–304
 single sample 257–258
 geometric 422
 Measurement error 390
 Measurements *see* Data,
 measurements
 Mechanistic causal approach 48
 Median 253
 confidence interval for
 difference between two
 medians 308–309
 single sample 258
 Medical records, as sources of
 data 231–232
 Meerkats, tuberculosis in 93
 Megatherms 139
 Mendelian disorders 92
 Merriam, Clinton Hart 140
 Mesothelioma 193
 Mesotherms 139
 Meta-analysis 205, 375–380,
 412–416
 Metabolic profiles 591,
 593, 594
 Metadata 243
 Metaphysical medicine 4
 Method
 of agreement 54
 of analogy 54–55
 of concomitant variations 54
 of difference 53–54
 Miasmata (miasmas) 4, 5, 6,
 49, 123
 Microbiome 141
 Microbiota 141
 Microchips, in animal
 identification 62, 464
 Microclimate *see* Climate
 Micro-epidemiology 35
 Microparasites
 definition 146
 mathematical models 521
 Microtherms 139
 Military campaigns, spread of
 disease by 9
 Milk yields, in productivity
 schemes 588
 Mill, John Stuart 43, 53–55
 Minimal disease methods 614
 Misclassification 330–331, 390
 Missing values, in data
 analysis 228–229, 387
 Mixed effects logistic
 regression 519
 Mixed effects model 516
 Mixed infections 106–108
 Models and modelling 38
 biological 189–199
 types of 189–190
 causal 50–52
 mathematical 520–539
 types of 521
 statistical 492–519
 additive 109, 502
 Moduli 311, 642
 Molecular epidemiology 34–35
 Monitoring
 definition 37, 457, 638
 disease 20 *see also* Surveillance
 Monte Carlo models 242, 323,
 528–529
 Moran effect 144–145
Moraxella bovis *see*
 Keratoconjunctivitis,
 bovine
 Morbidity
 definition 62
 measures of 67–80
 true and false changes in 180
 Mortality
 cumulative mortality 72
 confidence interval 260
 definition 62
 human 11
 caused by the Black Death 59
 mortality rate 72
 Mosquito, in yellow fever,
 transmission 158
 Mouse, Lassa virus infection
 in 117, 178
 Movement, of animals
 in control of disease 609–610
 as a determinant 125–126
 Moving averages *see*
 Rolling averages
 Multifactorial causes 50–52
 Multifactorial diseases 19–20, 88
 Multifactorial inheritance 89–90
 Multi-level model *see* Mixed
 effects model
 Multinomial distribution 260
 Multiple linear regression *see*
 Multivariable linear
 regression
 Multiple logistic
 regression 511–512

- Multivariable analysis,
 definition 317, 499, 638
- Multivariable linear
 regression 499
- Multivariate analysis,
 definition 317, 499, 638
- Muscle, haemorrhages in 30
- Mutation, in infectious
 agents 94–95
- Muzzle patterns, in animal
 identification 464
- Mycobacterium paratuberculosis*
 (previously *M. johnei*) *see*
 Johne's disease
- Mycobacterium tuberculosis* *see*
 Badgers, tuberculosis in;
 Opposums, tuberculosis in;
 Tuberculosis
- Mycoplasma* spp. 30, 46
- Myxomatosis 179
- n**
- Naegleria fowleri* 94
- NADIS *see* National Animal Disease
 Information System
- NAHMS *see* National Animal Health
 Monitoring System
- National Animal Disease Information
 System 237–239
- National Animal Health Monitoring
 System (US) 239–241
- National Companion Animal
 Surveillance Program 244
- National Scrapie Plan (GB) 612
- Natural experiment 32–33
- Natural history of disease 17, 30–
 31, 35, 139, 237, 534, 616
- Natural law, universe of 4–5
- Natural models *see* Spontaneous
 models
- Nature, balance of 142
- NCASP *see* National Companion
 Animal Surveillance
 Program
- Nebuchadnezzar 361
- Negative-herd retesting 446
- Negative models 189
- Neisseria gonorrhoeae* 96
- Nematodiriasis, control by alternate
 grazing 610
- Nematodirus* spp. *see* Nematodiriasis
- Neorickettsia*
 helminthoeca 153, 154
- Nested data structures 516
- Net Present Value 579, 580
- Network(s)
 database model 230–231
 general 153
 mathematical models
 using 532–534
 surveillance 475
- New Zealand
 EpiMAN decision support system,
 development in 242–243
 leptospirosis in 54
 tuberculosis in opossums in 615
- Newcastle disease
 outbreaks 61, 94, 176
 virulence 94
- Niches, ecological 150–152
 filling, disease control and 611
 fundamental 150
 realized 150
- Nidality 159–161
- NNT *see* Number needed to treat
- Noise, as a determinant 101
- Nomenclature of disease
 205–207
- Nominal scale of measurement 202
- Non-compensating error 271
- 'Non-naturals' 5
- Non-parametric tests 300
- Nonreactive models *see* Negative
 models
- Non-sampling error 271
- Normal distribution
 approximations 257
 definition 254–255
- Normative economics 569
- North Atlantic Oscillation 102
- Nosoareas 159
- Nosocomial infections 96
- Nosogenic territories 159
- Nosologia Methodica* 211
- Novel infectious diseases 15–16
- Nuer 3, 479
- Null hypothesis 297–298
- Number needed to harm 367
- Number needed to treat 366–367
- Numerical rating scale *see* Ordinal
 scale of measurement
- Nurmi phenomenon *see* Interference,
 epidemiological
- Nuthatches, character
 displacement in 150
- Nutrition, as a determinant *see* Diet,
 as a determinant
- Nutritional epidemiology 35
- o**
- Observational studies 319–360
 bias 330–335, 344–346, 352
 case-case 357
 case-case-time 359
 case-chaos 359
 case-cohort 357
 case-control 319
 design 346–352
 hospital-based 350
 nested 356–357
 case-crossover 358
 case-only 358
 case-other disease 358
 case-time-control 358–359
 cohort 319
 design 340–346
 comparison of types 321
 confounding 331, 332–335
 cross-sectional 320
 design 352–354
 ecological 354–355
 interaction 328–330, 505–506
 longitudinal 320
 Mantel-Haenszel
 procedure 332–333
 matching 334–335, 351
 frequency 334, 351
 individual 334, 351
 misclassification 330–331
 multivariable
 techniques 499–500
 nomenclature 320
 panel 355
 parameters estimated in *see also*
 specific parameters
 aetiological fraction *see*
 attributable proportion
 attributable
 proportion 327–328
 attributable risk 325–327
 cross-product ratio *see* odds ratio
 odds ratio 323–325
 prevalence ratio 324
 rate ratio *see* relative risk
 relative odds *see* odds ratio
 relative risk 321–323
 risk ratio *see* Relative risk
 power calculation 336–337
 prospective 320
 repeated surveys 355
 retrospective 320
 sample size
 determination 335–36

- Observational studies (*cont'd*)
 spatial correlation 354
 time series studies 355
 types 319–321
 upper confidence limits 337–338
- Occam (Ockham), William of *see*
 ‘Occam’s razor’
 ‘Occam’s (Ockham’s) razor’, 45
 ‘Occupation’, animal, as a
 determinant 105
- Odds ratio 323–325
 calculation of confidence
 intervals for 324–325
 logarithmic-based
 method 324
 test-based method 325
 calculation of upper confidence
 limits 337–338
 definition 323
 diagnostic 434
- Office International des Epizooties*
 (World Organisation for
 Animal Health) 23, 60, 237
- OIE *see* *Office International des*
Epizooties
- Oncogenes 111
- Ondiri disease *see* Bovine
 petechial fever
- One Health 24–25, 40
- One-tailed tests 298–299
- Opossums, tuberculosis in 615
- Opportunistic pathogens 19,
 100–101
- Opportunity cost 568
- Orbiviruses, antigenic change in 96
- Ordinal scale of measurement 202,
 364, 430–431
- Ornithodoros moubata* 130
- Orphan models 189–190
- Origin of Species* *see* Darwin, Charles
- Osteosarcoma
 in children 93
 in dogs 93
- Ostertagia* spp. *see* *Ostertagiasis*
- Ostertagiasis*
 control of, by mixed grazing 610
 models of 533
- Ostler, William 24
- Outbreak of disease
 definition 60–61
 investigation of 623–628
 epidemiological approach
 to 626–628
- Outliers 498–499
- Overmatching 334, 350–351
- Oxytrema silicula* 154
- Ozone, depletion of, as a
 determinant 102
- p**
- Pain, assessment of 203, 204
- Palliative care 604
- Pandemics 59
- Panel studies 355
- Panel surveys 352
- Papyrus of Kahun* 2
- Parallel testing 444
- Paramecium* spp., population growth
 curve of 142
- Parameter 252, 641
- Parametric tests 299
- Parasitic mange, eradication of 10
- Parasitism, intracellular 150–151
- Parasitocenosis 159
- Paratuberculosis *see* Johne’s disease
- Parelaphostrongylus tenuis* 149
- Partial farm budgets 579
- Pareto charts 263
- Participatory appraisal *see*
 Participatory epidemiology
- Participatory epidemiology 34,
 475–483
- Participatory mapping 480
- Particle:infectivity ratio 120
- Parturition, infection at 129
- Parvovirus, canine 59, 92
- Passive data collection 235,
 460, 475
- Passive surveillance *see* Surveillance
- ‘Passports’, horse 64
- Pasteur, Louis 5, 362
- Pasteurella haemolytica* 18
- Pasturella* spp., in mixed
 infections 107
- Pasteurellosis 51
- Patch
 ecological types of 156
 parameters and disease
 dynamics 165
- Patent ductus arteriosus 196
- Pathogenicity 94
- ‘Pathogenicity islands’ 96
- Pathogens
 characteristics of 120
 endogenous 100
 exogenous 100
 opportunistic 100–101
- Pavlovsky, Evgeny Nikanorovich 159
- PCR *see* Polymerase chain reaction
- Peirce, Charles Sanders 43
- Pellagra 361
- Pentateuch, dietary laws of 540
- Peptide mapping 34
- Percentiles *see* Centiles
- Performance-related diagnosis 20
- Perkins’ tractors 363
- Personalized medicine 629
- Petitio principii* 538
- Pet-food manufacturers, as sources of
 data 469
- Pet shops, as sources of data 469
- ‘PETS’ travel scheme 605
- Pharmaceutical sales, as sources of
 data 468
- Phase variation 95
- Pie charts 263
- Piece-wise regression 502
- Pig Health Scheme (UK) 599
- PigCHAMP* 242, 594
- PigORACLE* 242
- Pigs *see also* specific diseases in pigs
 adenomatosis 17
 atrophic rhinitis 450–451
 disease eradication 615
 enzootic pneumonia 324–325
 foot lesions 104
 gastric torsion 105
 gastroenteritis 102, 179
 health and productivity
 schemes 591–592
 herd structure 19
 leptospirosis 59
 lymphosarcoma 112
 parvovirus 20, 89
 porcine stress syndrome 106
 reproductive failure 88
 respiratory disease 324–325
 stillbirths 53
- Placation, to remove demons 2
- Placebo/placebo effect 363
 by proxy 363
- Plague(s)
 biblical 5
 definition 6
 occurrence 49, 520
- Planetary health 31, 138
- Plants and animals, relationships
 between types
 152–155
- Plasmids 96
- Plasmodium* spp. 116
- ‘Play the winner’ rule 370

- Pleurisy, in sheep, temporal occurrence 181
- Pleuropneumonia 9, 30, 136
eradication 9, 604
- Pneumonia
acute bacterial 71
causes 52
enzootic 324–325
in sheep, temporal occurrence 181
- Point (dot or location) maps 81
- Point estimation 257
- Poisson distribution 255–256
confidence interval for 259–260
- Poisson, Siméon-Denis 12, 255
- Policy briefs, evidence-based
advocacy briefs 604
objective briefs 604
- Poliovirus immunization,
natural 152
- Pollution, environmental 101, 198
- Polygenic inheritance 89
- Polymerase chain reaction 34, 430
- Popper, Karl 44, 536
- Population(s)
of cats and dogs 63
competitive exclusion 149
contiguous 62–65
control of, by disease 145
control of, implications for disease 148
density-dependent competition 144
dispersal 144
distribution 139–142
biomes 139–142
vegetational zones 139
experimental 368–369
extinction 145
group behaviour 147
home range 147
of horses 64
interspecific competition 149
intraspecific competition 148
life zones 140, 141
of livestock 66
Moran effect 144–145
niches 148–150
predator/prey relationship 145, 155
pyramids 90, 153
regulation of size 142–148
at risk 61
separated 65–67
serological estimations and comparisons in 424–427
size
competition for food and 142
control by competition 142–144
estimation of 62–65, 66–67
logistic equation 144
predation and 145
regulation 142–148
social dominance 147
Wynne-Edwards hypothesis 147–148
structure of 62–67
study population 270
surveys of prevalence in 270–295
sympatric species 149
synchrony 145
target population 270, 386
of wild animals 64–65
- Porcine stress syndrome 106
PorkCHOP 242
- Positive economics 569
- Positivism 43
- Postcapture myopathy syndrome 106
- Posterior probability 43
- Post-test probability *see* Posterior probability
- Potato Famine, Irish *see* Irish Potato Famine
- Pott, Percivall 110
- Poultry, disease eradication in 615
see also specific diseases of poultry
- Poultry Health Scheme (UK) 599
- Poultry slaughterhouses, as sources of data 467
- Power, statistical
calculation, in observational studies 336–337
definition 298
- PPS sampling *see* Sampling, using probability proportional to size
- Precautionary principle 543
- Precipitating factors 52
- Precision 209, 257, 384
of a theory 535
- Precision medicine 629
- Predation 145, 155
- Predictive value 434–436
- Predisposing factors 52
- Pre-emptive slaughter (culling) 606
- Pregnancy rate 77
- Premium Sheep and Goat Health Scheme (UK) 599
- Prepatent period 118
- Prescriptive screening 37
- Pre-test probability *see* Prior probability
- Prevalence 67
annual 67
calculation of incidence rate from 71
confidence intervals for difference between two samples 313
one sample
aggregate-level 292
cluster sampling 293–294
simple random sampling 291–292
imperfect tests 292
low prevalence 292–293
small samples 292
stratified sampling 293
systematic, sampling 293
definition of 67
detectable 285
determination of sample size to estimate difference between two samples 313–314
one sample
aggregate-level 289–290
cluster sampling 277–284
simple random sampling 275–277
stratified sampling 277
systematic sampling 277
presence of disease 284–289
lifetime 67
period 67, 352
point 67
relationship to incidence rate 70–71
- Prevalence models 521
- Prevalence ratio 324
- Prevalence studies 339
- Prevention of disease 586
primary 604
secondary 604
tertiary 604
- Preventive medicine programmes 586
- Primary case, in epidemics 173
- Primary data collection 219
- Primary determinants 86

- Principle of parsimony *see* 'Occam's razor'
- Prior probability 43
- Probability
 conditional 251
 definition 251
 interval 257, 639
- Probing, in participatory epidemiology 479
- Probit transformation 510
- Production
 as an economic process 565
 shortfalls 588
- Production functions 565–567
- Productivity, of cattle 18
 historical improvements in 17
- Professions, characteristics of 40
- Pro-formas, as sources of data 232
- Progressive retinal atrophy 612
- Proportional circle maps 82
- Proportional morbidity rate 77
- Proportional morbidity ratio 192
- Proportional mortality rate 77
- Proportional piling, in participatory epidemiology 479
- Proportions
 confidence interval for
 difference between two proportions 313–314
 multinomial, proportions 260–261
 single sample 258–259, 433
 Agresti-Coull method 259
 exact method 259
 Wald's method 259
 Wilson's method 259
 sample-size determination
 difference between two proportions 313–314
 single sample 275–276
- Prospective studies 320
- Prospective surveys 37
- Protection zones, in disease control 610
- Proteus* spp., conjugation in 96
- Protoporphyrin 613
- Pruritus, in dogs 87, 364
- Pseudomonas* spp.
 iatrogenic transmission of 124
 transduction in 96
- Psittacines, preventive medicine in 601
- Psychometric approach to measurement 205
- Psychosocial epidemiology 35
- Public health, veterinary 21
- Pulex irritans*, survival of infectious agents in 134
- Pulmonary disease, chronic canine 198
- Purposive sampling 272
- Pyometra, canine 337
- Pyramid of numbers 153
- q**
 Q fever 53, 78, 121
 QQ (quantile-quantile) plot 498
 Quality of life, in pets 75
 Quantal assay 423–424
 Quantification
 dangers of 12
 evolution of, in medicine 12
 Quantiles 253
 Quarantine 6, 125, 543, 605–606
 Quartiles 253
 Questionnaires *see* Surveys
 Quetelet, Adolphe 12
- r**
 R_0 *see* Basic reproductive number
 Rabbit haemorrhagic disease 485
 Rabbit, preventive medicine in 601
 Rabies
 in bats 48
 in Chile 185
 endemic occurrence 160
 in foxes 148, 160, 169, 523–525
 iatrogenic transmission 124
 legal aspects 621
 long-term (secular) trends 180
 in skunks 83
 sporadic cases 59
 in the US 83, 180
 Radiation, solar 101–102
 Ramazzini, Bernardino 9
 Random-digit dialling 271, 350
 Random-effects model 516
 in clinical trials 378–379
 in statistical modelling 515
 Random intercept model 517
 Random numbers 661–662
 Random slope 517
 Randomization
 in clinical trials 369–370
 in controlling confounding 395
 Ranks *see* Ordinal scale of measurement
 Rapid rural appraisal *see* Participatory epidemiology
- Rate ratio *see* Relative risk
- Rate of homogeneity *see* Sampling, rate of homogeneity
- Rates 76–77
- Ratio scale of measurement 202
- Ratio(s) 76–77
 particle:infectivity 120
- Rats
 leptospirosis and 157
 plague and 5
 scrub typhus and 147
 in synanthropic ecosystems 156–157
- Receiver-operating characteristic curve *see* ROC curve
- Recombination 95–96
- Record cards, as sources of data 231
- Record database model 229
- Recording schemes
 examples 237–244
 implementation 236–237
 objectives 236
 scales of 232
- Reductionism 48
- Reed-Frost model 173–175
- Reed-Muench titration 423
- Refinement, definition 209
 of serological tests 427–428
- 'Refutedness', of a theory 535
- Registries, as sources of data 467
- Regression analysis 180–185, 492–505
- Regression line 494
- Reinforcing factors 52
- Related samples 299
- Relational database model 231
- Relative odds *see* Odds ratio
- Relative risk 321–323
 calculation of confidence intervals for
 logarithmic-based method 323
 test-based method 323
 definition 321
- Relative risk increase 367
- Relative risk reduction 365–366
- Reliability 209–211, 454
 of questionnaires 227
- Renatus, Publius Flavius Vegetius 6
- Renaissance, The* 59
- Repeatability 209, 454
- Reporting systems *see* Recording schemes
- Reproducibility 209, 454

- Reproductive failure, in pig herds 88
- Research laboratories, as sources of data 469
- Residuals 493
 empirical 496
 jackknife 496
 ordinary 496
 standardized 496
 studentised 496
 theoretical 496
- Residues, antibiotic 23
- Response rate, in interviews and questionnaires 221
- Restriction enzyme analysis 34
- Restriction, in controlling confounding 394
- Retinal scans, in animal identification 464
- Retrospective studies 320
- Retrospective surveys 37
- Reverse causation 395
- Rhinopneumonitis virus 118
- Rhinotracheitis virus 102
- Rhipicephalus* spp., nidus of 159
- Rift Valley fever 102, 118, 141, 152, 160, 618
- Rimpuf* model 127
- Rinderpest
 control 5, 10, 16, 284, 287, 457, 463, 614, 616
 economic aspects 579
 eradication of 16
 sample size determination for 287
 excretion 119
 JP15 campaign 16
 military campaigns disseminating 9
Pan-African Rinderpest Campaign 284
 pandemic 59
 as a plague 7–8
 stimulus for founding veterinary schools 10
- Ring vaccination 606
- Risk analysis 39, 540–564
 acceptable risk 560–563
 application of diagnostic tests to 446–447
 hazard identification 546
 microbial 39
 precautional quarantine 543
 precautionary principle 543
 risk assessment 546–548
 consequence assessment 546
 entry assessment 546
 exposure assessment 546
 hazard characterization 546
 risk characterization 546
 risk estimation 546
 uncertainty 547
 aleatory 547
 epistemic 547
 stochastic 547
 variability 547
 risk communication 551
 risk management 548–550
 HACCP (Hazard Analysis Critical Control Point) 549–550
 qualitative 551–556
 quantitative 556–563
 probability distributions 557–558
 anchoring 557
 semi-quantitative 551–552
- Risk-based surveillance *see* Surveillance
- Risk, definition 540
- Risk-difference modification 392
- Risk factors 47
- Risk indicators 47
- Risk markers 47
- Risk ratio *see* Relative risk
- Risk-ratio modification 392
- Robustness, of a diagnostic test 209
- ROC curves 441–443
- Rocky Mountain spotted fever 159
- Rolling (moving) averages 181, 423
- Ross River virus 120
- Ross, Ronald 48
- Rotaviruses 121
- Rous, Francis Peyton 5
- Routes of transmission, of infectious agents *see* Infection, transmission
- Ruggedness, of a diagnostic test 209
- Ruini, Carlo 6
- Ruminal boluses, in animal identification 464
- Russell, Bertrand 44, 535–536
- S**
- Sacrifice, to remove demons 2
- Salihotriya* 6
- Salivarian transmission of infection 118
- Salmon poisoning 153–154
- Salmonella* spp. *see* Salmonellosis
- Salmonellosis 53, 100, 159, 614
- Sampling *see also* Prevalence; Statistics; Surveys
 capture-release-recapture 65
 in clinical trials 362–364
 cluster 274–275, 277–285, 293
 convenience 272
 design effect 279
 distance 65
 error 271, 639
 fraction 271
 frame 271, 386, 387
 imperfect diagnostic tests in 276–277, 288–289
 intra-class correlation coefficient *see* rate of homogeneity
 multistage 274
 non-probability 272, 388
 in observational studies 319–320
 optimization 285–287
 probability 272–275
 proportional allocation 273–274
 purposive sampling 272
 random-digit dialling 271
 random numbers 661–662
 rate of homogeneity 279
 sample size
 in clinical trials 372–373
 comparing two samples 302–314
 disease detection 284–290
 in observational studies 335–336
 prevalence 275–284 *see also* appropriate parameter
 simple random 275–277, 291–292
 stratified 273–274, 277, 293
 systematic 272–273, 277, 293
 types of 272–275
 unit 271
 using probability proportional to size 283–284
 variation 639
 without replacement 288, 661
 with replacement 288, 661
- SARS 126, 620
- SAVSNET *see* Small Animal Veterinary Surveillance Network
- Scanning surveillance *see* Surveillance
- Scapegoats 3
- Scatter plots 265

- Schistosoma* spp. *see* Schistosomiasis
 Schistosomiasis 149, 154, 159, 462
 and bladder cancer 110
 Scholasticism *see* The Scholastics
 Scholastics, The 42
 Schwarz's Bayesian Information Criterion *see* Bayesian Information Criterion
 Scientific inquiry, role of, in accepting a hypothesis 45
 Scientific revolutions 10
 Scoring schemes/systems 203–204
 Scrapie 133, 440, 464, 612
 Screening 37, 431, 434, 435
 genetic 612
 mass 37
 prescriptive 37
 strategic 37
 Screw-worm fly 125
 cost-benefit analysis of control of 581
 Scrub typhus 147, 158
 Scurvy, early clinical trial 361
 Seasonal calendars, in participatory epidemiology 480
 Seasonal trends in disease 178–179
 Secondary attack rate 70
 Secondary cases, in epidemics 173
 Secondary data collection 219
 Secondary determinants 86
 Sectioned density plots 264
 Secular trends in disease 179–180
 Security interval 257
 Segmented regression *see* Piece-wise regression
 Selenium, plant uptake by 138
 Semantic differential questions 220
 Semi-interquartile range 254
 Semmelweis, Ignaz 9, 53, 362
 Semmelweis effect (reflex) 9
 Sensitivity
 analysis 537
 analytical 423, 430
 diagnostic 208–209, 430
 aggregate-(herd-) level 443
 calculation of confidence intervals for 433
 parallel 444
 serial 445–446
 Sentinels 459–460
 environmental 191, 198
 Sentinel health events 460
 Sentinel surveillance *see* Surveillance
 Separated populations 65–67
 Sequential-design trials 371–372
 Serial interval 119
 Serial measurements 302
 Serial testing 445–446
 Serological epidemiology
 antibody assay
 titres 423–424
 arithmetic mean 367, 422
 geometric mean 367, 422
 multiple serial dilution 423
 Spearman-Kärber
 titration 423–424
 relative analytical
 sensitivity of 430
 single serial dilution 423
 antigenic tolerance 429
 definition of 421
 interpreting tests 427–430
 seroconversion 422
 calculation of rate of 426
 tests *see* Diagnosis, diagnostic tests
Serpulina hyodysenteriae *see* *Brachyspira hyodysenteriae*
 Serum banks
 applications 470
 collection of serum 470
 definition 470
 selection bias in 470
 as sources of data 470
 Severe acute respiratory syndrome *see* SARS
 Sex, as a determinant 91–92
 Sex chromosomes 92
 Sex-influenced inheritance 92
 Sex-limited inheritance 92
 Sex-linked inheritance 92
 Shadow prices 580
 Sheep *see also specific diseases in sheep*
 disease eradication in 615
 domestication 2
 footrot 107
 efficacy of vaccine against 365–367
 health schemes 592–593
 jaw tumours 80
 pleurisy 181–185
 pneumonia 181–185
 Sheep pox, introduction of into the UK 9
 Sheep ticks, populations of 528–530
 Shewhart charts 267
 Shift, antigenic, in influenza viruses 96
SHIFT veterinary import network 464
Shigella spp. 96
 Shipping fever 18, 106
 Significance
 clinical (biological) 300
 statistical 297
 tests 296–297
 Silent infection *see* Inapparent infection
 Simplicity
 of a hypothesis 45
 of a theory 535
 Simulation models 526–530
 'Sin of Galileo' 538
 SIR models 521
Sitta spp. 150
 Size of hosts, as a determinant 93
 Skin cancer, coat colour and 94
 Skin, infection via 122
 Skunks, map of rabies in 83
 Slaughter (culling),
 of badgers, tuberculosis and 17
 as a control measure 615
Small Animal Veterinary Surveillance Network 244
 Smallpox 5, 9, 11, 30, 123, 126, 170, 520, 604, 608, 617
 SMEDI viruses 89
 Smith, Adam *see* 'Wealth of Nations'
 Smith, Theobald 5
 Smog, cause of respiratory-disease epidemic 59
 Smoking, cause of lung cancer 54
 Snails, control of 149
 SNOMED 214
 SNOP 214
 SNOVET 214
 Snow, John 49, 50, 55, 520, 538
 SNVDO 212
 Social cost-benefit analysis *see* Economics and veterinary epidemiology
 Social dominance 147
 Social ecology 138
 Social epidemiology 35
 Software, computer, veterinary epidemiological packages 643–647
 Spatial correlation study 354
 Southern Oscillation 102
 Spatial distribution of disease 186–187

- Spearman-Kärber titration 423–424
- Species, as a determinant 92–93
- Specific measures of disease occurrence 76–78
- Specificity
- analytical 430
 - diagnostic 208–209, 430
 - aggregate-(herd-) level 443
 - calculation of confidence intervals for 433
 - parallel 444–445
 - serial 445–446
- Spiroceria lupi*, and canine oesophageal carcinoma 112
- Spontaneous models 190
- Sporadic disease occurrence 59–60
- Squamous cell carcinoma
- in cats 47
 - in cattle 30
- Stability, of pathogens 120
- 'Stamping-out policy' 606, 609, 640
- modified 'stamping-out policy' 640
- Standard deviation 254
- Standard error
- definition of 257
 - of specific parameter *see appropriate parameter*
- Standard Nomenclature of Veterinary Diseases and Operations see SNVDO*
- Standardized measures of disease occurrence *see* Adjusted measures of disease occurrence
- Standardized morbidity ratio *see* Adjusted measures of disease occurrence
- Standardized mortality ratio *see* Adjusted measures of disease occurrence
- Standardized Normal deviate 254
- Staphylococcus* spp., conjugation in 96
- State-transition models 523
- Statistical packages 318, 643–647
- Statistics *see also* Association; Observational studies; Sampling; Surveys
- asymptotic methods 257
 - basic definitions 251–252
 - binomial distribution 255
 - Normal approximation 257
- Bland–Altman plot 452–454
- centiles 253
- Chi-squared (χ^2) distribution 660
- confidence intervals and limits *see specific parameters*
- constants 641
- correlation 316–317
- correlation coefficient 691
- degrees of freedom 257
- descriptive 252–254
- distributions 254–257
- errors of inference 297–298
- generalized linear models 511–519
- independent and related samples 299
- interaction 109–110, 328–330, 505–506
- interval estimation 257–262 *see also specific parameter*
- kappa* statistic 450–452
- logistic regression 510
- Mantel-Haenszel procedure 332–333
- mean 253
- measures of position 253–254
- measures of spread 254
- median 253
- multiple significance testing 298
- Bonferroni correction 298
- multivariable analysis 317
- multivariate analysis 317
- Normal distribution 254–255
- null hypothesis 297
- one-and two-tailed tests 298–299
- parameter 252
- parametric vs. non-parametric techniques 299–300
- Poisson distribution 255–256
 - Normal approximation 257
- power 298, 336–338
- probability 251
- quantiles 253
- quartiles 253
- random numbers 661–662
- regression 183, 492–499
- sample sizes 300 *see also* Sampling, and specific parameters
- standard deviation 254
- standard error 257
- tests
- χ^2 -test 310–313
 - χ^2 -test for trend 314–315
 - Fisher's exact test 311–313
 - McNemar's change test 313, 452
 - Wilcoxon–Mann–Whitney test 304–306
 - Wilcoxon signed ranks test 306–308
 - Student's *t*-test 302–303
- triangular distribution 557
- variables 46, 251–252
- variance-ratio (*F*) distribution 692–693
- Stercorarian transmission of infection 118
- Sterile immunity 98
- St Louis encephalitis virus 459
- Stochastic models 521, 525, 529, 556, 559
- Stochastic variation 493, 547
- Strategic screening 37
- Strategic vaccination 606–607
- Stratification
- in clinical trials 370
 - in observational studies 334
 - in surveys 273
- Streptobacillus* spp. 123
- Streptococcus uberis*, and environmental mastitis 88
- Stepwise regression 509
- Stress 105–106
- cold 101
 - in veterinarians 105
- Stressors *see* Stress
- STROBE 396
- Student's *t*-distribution 257, 262, 650
- Student's *t*-test 302–303
- Studies, observational *see* Observational studies
- Subclinical disease 97
- Subclinical epidemiology 35
- Subclinical infection 97
- Subgroup analyses 395
- Suk 2
- Summa Theologica* 42
- Summary plots 264
- Summer mastitis 107
- Suppressive (dampening-down) vaccination 607
- Surveillance 457–491
- aquatic-animal 485–486

- Surveillance (*cont'd*)
 assessing the performance
 of 486–488
 companion-animal 483, 485
 definition 457
 goals 458–459
 mechanisms 471–475
 networks 475
 sources of data for 464–471
 systems 475
 types of
 active 460–461
 disease 20, 232, 459
 early-warning 461
 epidemiological 459
 passive 460–461
 post-authorization drug 362
 risk-based 461
 scanning (global) 461
 sentinel 459
 serological 460
 syndromic 461
 targeted 461
 wildlife 485
 'Surveillance committees', in the
 Reign of Terror 457
 Surveillance zones, in disease
 control 246, 285, 610
 Survey(s) *see also* Prevalence,
 Sampling, Statistics 37,
 270–295
 cost of 290
 cross-sectional 37
 Domesday 66
 detecting presence of
 disease 284–290
 household, to estimate population
 sizes 62
 longitudinal 37
 prevalence 275–284
 questionnaires 219–228
 closed questions 219
 coding 220–221
 computer-assisted 227
 criteria for success 227–228
 design 219–226
 interviews 226–227
 mailed and self-completed 226
 missing values 228–229
 open-ended questions 219
 presentation and wording 221
 question structure 221
 response rate 221
 structure 219–220
 telephone 226–227
 testing 227
 types of 272–275
 Survival 73–75
 confidence interval 260
 Susceptibility, of hosts 118
 Swine dysentery, eradication of 100
 Swine fever, eradication 615
 Sympatric species 149
 'Symptom iceberg', 465
 Synanthropic ecosystems 156–157
 Syndemics 108–109
 Syndrome, definition of 461
 Synergism
 biological 108
 statistical 109
Synopsis Nosologiae Methodicae, 211
Syphacia obvelata 58
 Systematic reviews 376, 397–420
 clinical heterogeneity 403, 408
 Cochran's Q statistic 412
 collecting data 407–409
 comparison with narrative
 reviews 398
 contextual heterogeneity 408
 DerSimonian and Laird weighting
 method 416
 effect modification 408
 electronic databases 401
 evidence synthesis 397
 extraction of outcomes 407–408
 extraction of study
 characteristics 408–409
 forest plot 415
 funnel plot 414
 GRADE working group 417
 heterogeneity 412–415
 interpretation 419
 inverse variance method *see*
 DerSimonian and Laird
 weighting method
 literature searching 399–400
 Mantel-Haenszel weighting
 method 416
 meta-analysis 412
 meta-regression 416
 methodological heterogeneity 408
 narrative review 398
 overall effect estimate 412
 PECO(S) systematic review
 question 403
 Peto weighting method 416
 PICO(S) systematic review
 question 364, 399
 PICOTT systematic review
 question 364, 403
 PIT(S) systematic review
 question 403
 PO(S) systematic review
 question 403
 pooled effect estimate 412
 presentation of results 416–419
 PRISMA 415
 Protocols 398
 publication bias 411
 QUADAS 411
 quantitative synthesis 412
 reporting bias 411
 risk of bias 400, 409–412
 assessment tool 411
 screening 406
 selection of relevant
 studies 406–407
 STARD 411
 statistical heterogeneity 408
 steps in 398
 summary effect measure 412
 summary effect size 412
 synthesis of results 412–416
 Tau-squared 414
 Trichomonas foetus 411
 types of review question 399
 vote counting 407
*Systematized Nomenclature of
 Veterinary Medicine see
 SNOVET*
*Systematized Nomenclature of
 Human and Veterinary
 Medicine see SNOMED*
 Systems models 534
t
 Tables 262–263
 Tail docking
 in dogs 23
 in horses 23
 Talmud, The 5
 Targets, in health
 schemes 587–588
 Targeted surveillance *see*
 Surveillance
 Tarone's test for homogeneity 333
 Tattoos, in animal
 identification 62
 Taxonomy, microbial 97
 Temporal distribution of
 disease 177–186, 255
 Tenacity, role of, in accepting a
 hypothesis 44
 Territoriality 147
 Tertiary cases 70
 'Test and removal' control
 strategy 606
 'Testedness', of a theory 535

- Testicular neoplasia 78
 Texas fever, eradication of 5,
 604–605
 Theileriosis 130
 Theoretical epidemiology 33
 Theories 535
 ‘Theory of Elevation’ 49
 ‘Theory of Everything’ 536
 Threshold levels
 in diagnostic tests 422
 in epidemics 168
 of infectious agents 120
 of multifactorial traits 90
 in vaccination 608
 Ticks
 climate change and 103
 disease transmission by 5,
 130, 164
 in sheep 529–530
 Time-invariant data 212
 Time lines 266
 Time-series analysis 180–186
 Time-series studies 355
 Time series plots *see* Time trend plots
 Time trend plots 265
 Titre *see also* Serological
 epidemiology
 definition of 421
 estimation 423–424
 logarithmic
 transformation 256, 422
 Tobacco rattle virus 96
 Tolerance, immune 129, 130,
 133, 429
 ‘Tombstone data’ *see* Time-
 invariant data
 Topley, William 32
 Topotypes, of foot-and-mouth
 disease 97
 TOST procedure *see* Two one-sided
 tests procedure
Totalvet 242
Toxoplasma gondii 133
 Traceability 463–464
 Tracing
 in infectious disease
 outbreaks 623–624
 systems 463
TRACES 464
 Trade bans, economic effects
 of 571–575
 Transboundary diseases 135–136
 Transduction 96
 Transference, to remove demons 3
 Transformation
 in bacteria 96
 mathematical 256
 in regression 502
 Transect walks, in participatory
 epidemiology 480
 Transgenic biology 614
 Transgenic models 190
 Translational medicine
 (research) 25
 Transmission of disease *see* Infection,
 transmission
 Transmission rate 169
 Transmission risk 169
 Transposition/transposons,
 bacterial 95
 Treatment of disease, ancient
 methods of 2–4
 Trends in disease occurrence
 spatial 186–187
 temporal 177–186
 cyclical 178
 long-term (secular) 179–180
 seasonal 178–179
 short-term 177
 Trends in veterinary
 medicine 20–25
 Trepanation, to remove demons 3
Treponema hyodysenteriae *see*
 Brachyspira hyodysenteriae
 Triad, of determinants 88
 Triangular distribution 557
 Triangulation, in participatory
 epidemiology 478
Trichinella spiralis 98
 Trophic cascades 152
 Trophic levels of food chains 152
Trypanosoma spp.
 antigenic drift in 96
 transmission 118
 Trypanosomiasis 16
 eradication 605
 tolerance to 614
 translocation by cattle 126
 Tsetse fly 31, 124, 480, 605
 Tuberculosis *see also* Badgers
 bovine, badgers and 17,
 31, 615
 in cattle 247
 eradication 615
 negative-herd retesting 446
 in opossums 31, 615
 in white-tailed deer 615
 Tularaemia, landscape epidemiology
 of 161–162
 Tumour registries, as sources of data
 see Registries
 Tumour-inducing viruses 110
 Tumour-suppressing genes 111
 Tumours
 bladder 110, 191–192
 cervix 33, 110
 fibrosarcoma 112
 gastric 110
 horn 112
 leucosis 110
 leukaemia 110
 mammary 193
 mesothelioma 192
 oesophageal 112
 osteosarcoma 93
 scrotal 110
 Turkeys, importation to Europe 2
 Two one-sided tests procedure 375
 Two-tailed tests 298–299
 Type I and Type II error 297–298
 ‘Typhoid Mary’ 99
- U**
 Uberreiter’s syndrome *see*
 Keratoconjunctivitis,
 superficial, in dogs
 Ultraviolet radiation, as a
 determinant *see* Solar
 radiation
 Unapparent infection *see* Inapparent
 infection
 Uncertainty
 in economics 580
 in risk analysis 547
 United Kingdom
 disease eradication in 615
 health schemes in
 national 597–599
 private 586–588
 holdings by size of herd 66
 pig herd structure 19
 United States
 heartworm in *see* *Dirofilaria*
 immitis
 leptospirosis in 179
 rabies in 83, 180
 Universal constant 641
 USSR, leptospirosis,
 epidemic in 173
Utricularia spp. 154
- V**
 Vaccines and vaccination
 barrier 606
 critical vaccination threshold 608
 early 5, 9
 clinical trial of 362
 efficacy 366

- Vaccines and vaccination (*cont'd*)
 emergency 606, 609, 615
 frontier *see* barrier
 immune-belt *see* barrier
 inactivated (killed) 607
 live 607
 new technology 608
 preventive 606
 ring 606
 routine 606
 vs. slaughter 615
 strategic 606
 suppressive (dampening down) 607, 615
 use in disease control 606, 609, 615
 'vaccination to live' 616
- Validity 209–210
 construct 205, 364, 450
 content 205, 364
 in epidemiological studies 383–396
 external 368, 384
 face 205
 internal 368, 384
 of mathematical models 537
 of questionnaires 227–228
- Variables 46, 251–252, 641
 dependent *see* response
 explanatory 252
 independent *see* explanatory
 input *see* explanatory
 outcome *see* response
 response 252
 study 252
- Variance 254
- Variance-ratio (*F*)
 distribution 692–693
- Variant surface glycoprotein (VSG), in
 trypanosomes 96
- Variates 251
- Variolation 9, 520
- Vase plots 264
- Vectors, types of 117–118
- Vegetational physiognomy 141
- Vegetational zones 139
- Velvet removal 23
- VeNom* 214
- Venezuelan equine encephalitis virus 459
- Vertical transmission 129–131
- VetCompass* *see* *Veterinary Companion Animal Surveillance System*
- Veterinarians, employment trends 21
- Veterinary Companion Animal Surveillance System* 244
- Veterinary Investigation Diagnosis Analysis* 242
- Veterinary Medical Databases* 191, 241–242
- Veterinary medicine
 contemporary 12–25, 628–629
 development of 1–25
- Veterinary practices
 computerized records in 465–466, 485
 as sources of data 465
- Veterinary schools
 founding of 9–10
 as sources of data 241, 469
- VETSTAT* 468
- VIDA* *see* *Veterinary Investigation Diagnosis Analysis*
- Violin plots 264
- Virchow, Rudolf 24
- Virome 141
- Virulence 32, 94, 120, 146, 153, 640
 evolution of, in parasites 94, 153
- Viruses
 initiation of cancer by 110
 particle:infectivity ratio 120
- Visual analogue scale 203–204, 299, 364
- VMDB* *see* *Veterinary Medical Databases*
- Volterra, Vito *see* Lotka-Volterra equations
- von Willebrand's disease *see* Haemophilia, canine
- VSG *see* Variant surface glycoprotein
- W**
- WAHID* *see* *World Animal Health Information Database*
- WAHIS* *see* *World Animal Health Information System*
- Wald confidence interval 259
- Wald test 512
- Wales, pig herd structure in 19
- Wallace, Alfred Russel *see* Wallace Line
- Wallace Line 139
- Warble fly infestation 609
- 'Wealth of Nations' 543
- 'Web of causation' 52
- Webster, Noah 5
- Whewell, William 43
- Wilcoxon–Mann–Whitney test 304–306
- Wilcoxon signed ranks test 306–308
- Wild-animal organizations, as sources of data 469
- William of Occam (Ockham) *see* 'Occam's razor'
- Willingness-to-pay 567
- Wilson confidence interval 259
- Wind-chill index 102
- Witches and witchcraft, as causes of disease 2
- Wolf, as ancestor of dogs 1, 93
- 'Wooden tongue' *see* Actinobacillosis
- Woolf's test for homogeneity 333
- World Animal Health Information Database* 237
- World Animal Health Information System* 237
- World Organisation for Animal Health* *see* Office International des Epizooties
- World Trade Organization 23, 237, 543, 574
- WTO *see* World Trade Organization
- 'Wynne-Edwards' hypothesis 147–148
- Wynne-Edwards, Vero Copner *see* 'Wynne-Edwards' hypothesis
- X**
- 'X disease' *see* Hyperkeratosis
- Xerophiles 139
- Y**
- Yearbooks, as sources of demographic data 66
- Yellow fever, transmission of 55, 152, 157, 158, 199
- Yersinia enterocolitica* 429
- Youatt, William 4
- Youden's index 433
- Z**
- Zika virus 126
- Zoography 62
- Zoological gardens, as sources of data 468
- Zoonosis incidence ratio 77
- Zoonoses 5, 29, 101, 620, 640
 economic aspects 570
 as opportunists 101