# The role of Australian native wildlife in Q fever

Karen Olivia Mathews

BSc, BAppSc (Hons I)

A thesis submitted to fulfil the requirement for the degree of

Doctor of Philosophy

Sydney School of Veterinary Science

Faculty of Science

University of Sydney

2022

# Declaration

This thesis is submitted to the University of Sydney in fulfilment of the requirements for the Degree of Doctor of Philosophy.

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text.

I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

Parts of this thesis have been published in the candidate's name.

Karen Olivia Mathews

BSc, BAppSc (Hons l)

30th June 2022

# Acknowledgements

Firstly I would like to acknowledge and express my gratitude to my primary supervisor Associate Professor Katrina Bosward, for encouraging me to embark on this PhD and for her guidance and support throughout this journey. I would also like to thank the other members of my supervisory team, Professor David Phalen, Professor Jacqui Norris, Professor Paul Sheehy and Associate Professor Jenny-Ann Toribio for your help and support during this time.

To Nick Malikides who provided support for the epidemiological analysis in Chapters 3 and 4, thank you for all your help and enthusiasm with this component of the PhD. I would like to acknowledge Catherine Savage whose MVPH thesis proved a useful study guide throughout this PhD, and formed the basis for the questionnaires used in this thesis.

I would like to thank Maryanne Large and the AWRC 2018 organising committee for allowing the serological survey to proceed at the conference, and also to thank the Australian wildlife rehabilitators attending the AWRC who enthusiastically participated in the surveys.

I would also like to acknowledge and thank the Australian wildlife rehabilitators who participated in the online KAP survey, as well as Tiggy Grillo, Keren Cox-Witton and others who piloted the survey, and the wildlife organisations and groups who assisted with survey dissemination.

To Cathie Herbert and Maquel Brandimarti, thank you for collaborating and for providing the kangaroo tissue samples for DNA extraction and PCR.

I would like to acknowledge David Gaffney for enabling me to collect the tissue samples from the kangaroos at Dubbo, and the numerous others who helped with the tissue collection at Canberra.

I would like to extend my gratitude to Queensland Parks and Wildlife Service for providing the opportunity to investigate Red-necked wallabies on St Helena Island.

I would like to thank Damien Higgins and other collaborators from the Koala Health Hub for providing the koala DNA samples. with special mention to Andrea Casteriano for looking through spreadsheets, digging around in freezers to find the samples and patiently responding to my emails.

I would like to thank Nicholas Wood for his help with the serological survey, and collaborators at the Australian Rickettsial Reference Laboratory, Stephen Graves and John Stenos for your contribution, help and guidance with various components of my thesis. I would also like to thank Chelsea Nguyen and Mythili Tadepalli for processing serological samples, and 'showing me the ropes' while I was visiting the lab in Geelong.

I would like to offer my sincere thanks and appreciation to the Marie Bashir Institute, Dr Ramune "Rami" Cobb and Pfizer International Inc and the NHMRC for providing funding to support this research into understanding Q fever.

To my fellow students at Camden Campus thanks for the chats they were good while they lasted. Our PhDs were undertaken during unprecedented times – drought, fires, floods, two years of COVID and more floods.

To my babies Amber-Jayne, Charlotte-Emily and Ryan-James, thank you for your love, support and encouragement throughout my PhD…I promise no more studying! I hope that I have shown you that anything is possible if you put your mind to it.

To my mum and dad, I am glad that I have made you proud, and now you will not have to ask me "Are you nearly there?"

Finally, I would like to thank my husband James for his ongoing love, support and patience throughout this journey, which was somewhat challenging for us at times. Thank you for listening to me while I talked about my PhD, but consider it payback for me having to listen about your running expeditions and more recently about motorbikes. You can have me back now and I promise……no more degrees - my cup is now full 😊

## Abstract

Q fever is a zoonosis caused by the bacterium, *Coxiella burnetii,* which mainly affects people in close contact with domestic ruminants (cattle, sheep, goats). Traditional at-risk groups have been identified and include meat and livestock industry workers, shearers and veterinarians. Australia is the only country to have a licenced Q fever vaccine for use in humans, which is recommended for groups who are at increased risk of occupationally acquired Q fever.

Although domestic ruminants are regarded as the main source of human disease, over the past decade, Australian wildlife (in particular kangaroos) have been implicated as a source of Q fever for humans, though, the evidence to support this widely held belief has remained largely circumstantial. Nevertheless, owing to speculation that Australian wildlife rehabilitators (AWR) may be potentially at risk of acquiring Q fever by handling sick, injured and orphaned wildlife, in 2015, wildlife and zoo workers were added to the Australian government's list of occupational groups for whom Q fever vaccination (QFV) is recommended. Despite this recommendation, it is unknown if AWR share the lack of awareness of the Q fever vaccine and shortfalls in QFV that have been identified in other at-risk groups who are advised to be vaccinated. There have been no studies that have investigated whether AWR are aware of the Q fever vaccine, and the level of QFV uptake in AWR is unknown.

The overall aim of this project was, therefore, to further understand the role of Australian native wildlife (and macropods in particular) as a source of Q fever for humans. The project consisted of three arms including:

1) a seroprevalence study to assess *C. burnetii* exposure levels in AWR;

2) a knowledge, attitudes and practices online survey in AWR which was intended to

i) identify associations between self-reported Q fever in AWR and risk factors for exposure to *C. burnetii*;

ii) determine factors associated with QFV; and

iii) describe AWR attitudes and potential barriers towards QFV;

3) a molecular investigation of tissue samples collected opportunistically from wildlife species was undertaken to determine the prevalence of *C. burnetii* DNA and identify potential shedding routes.

The findings from these studies demonstrated that AWR are almost twice as likely to be exposed to *C. burnetii* and self-report a higher level of medically diagnosed Q fever than the general Australian population. *Coxiella burnetii* seropositivity was not associated with demographic or animal-related risk factors including exposure to wildlife species, domestic ruminants, and other domestic animals. Similarly, no association between self-reported medically diagnosed Q fever and contact with Australian native wildlife was identified in unvaccinated AWR. Rather the findings indicated that unvaccinated AWR are likely to become infected with *C. burnetii* and develop Q fever through exposure to traditional sources such as domestic ruminants, or associations with veterinary clinics whilst rehabilitating wildlife. In addition, substantial shortfalls in Q fever vaccine uptake by AWR were observed. Barriers to QFV included: lack of knowledge of Q fever and the Q fever vaccine, complacency, uncertainty surrounding the importance, safety and efficacy of the Q fever vaccine, cost and vaccine access. Moreover, shortfalls in the general biosecurity practices employed by those working closely with wildlife species were also identified in this study.

Overall, these findings reinforce the importance and need for QFV in this less recognised at-risk group and highlight the need to convey to both the AWR themselves and the medical personnel that they are at risk of contracting Q fever and to educate the AWR about the health consequences of contracting the disease. The findings also underscore the need for veterinary clinics to educate all employees, including support staff and AWR associated with veterinary clinics, about the risks and potential health consequences of Q fever and recommend QFV to ensure the health and safety of all employees.

The low levels of vaccination in AWR highlight the need for implementing interventions that will address some of the key barriers to QFV and improve vaccine uptake. This may include educating AWR about Q fever and the importance of QFV, and the introduction of a subsidised vaccination program to facilitate vaccination by making it more accessible.

A low *C. burnetii* DNA prevalence was observed in the wildlife examined in this study, with only the samples from the two positive animals (a cloacal swab from a kangaroo and a urogenital swab from a koala) being amplified at a relatively low concentration of approximately 11 genome equivalents per reaction. These findings suggest that macropods and other Australian native wildlife species may not be a major source of *C. burnetii* for humans in comparison to other species such as livestock, and aligns with the above-mentioned results of the investigations into AWR themselves, where no association between exposure to wildlife species and *C. burnetii* seropositivity or Q fever was demonstrated. However, people in close contact with Australian native wildlife and their habitats remain at risk of contracting Q fever due to the low infectious dose, the aerosol transmission route and the prolonged survival of *C. burnetii* in the environment, all of which are purported in the *C. burnetii* literature. This, combined with the severe consequences for some people who contract Q fever, means that QFV should be recommended for people in close contact with Australian native wildlife and their habitats.

Although not the primary focus of this research, the sera provided by participants in the *C. burnetii* serosurvey was utilised in a second opportunistic serosurvey to investigate exposure to rickettsia. among AWR. This study demonstrated an elevated seroprevalence to *Rickettsia* spp. in AWR compared to control groups in other Australian studies, with most infections attributable to tick-transmitted *Rickettsia* spp. from the Spotted Fever Group. These findings suggest that AWR are at increased risk of contracting rickettsia-related illnesses and future studies should be directed at providing clarity around tick exposure in this cohort. Furthermore, the increased risk of tick exposure

in AWR suggests that AWR would also be more likely to be exposed to other pathogenic tick-borne zoonoses.

This research demonstrated that AWR are at risk of exposure to two zoonotic pathogens, *Coxiella burnetii* and *Rickettsia* spp. Given that the majority of zoonotic diseases emerge from wildlife, and that AWR are on the 'front line' working closely with Australian native wildlife, it is likely that AWR have been exposed to other zoonoses. The impact and risks of diseases that may emerge from the humans-wildlife interface have been highlighted by the COVID-19 global pandemic. This underscores the need for increased awareness of zoonoses, and the adoption of appropriate biosecurity practices by wildlife rehabilitators world-wide, to protect themselves and the greater community from known and unknown zoonotic pathogens.

# Table of contents

Declaration .......... 2

Acknowledgements .......... 3

Abstract .......... 5

Table of contents .......... 9

List of Figures .......... 13

List of Tables .......... 15

List of Abbreviations .......... 19

List of Peer Reviewed Publications .......... 22

Contribution to Conference Proceedings .......... 23

List of Awards and Grants .......... 25

Disclosure and Author Contribution Statement .......... 26

Chapter 1 Literature Review .......... 30

1.1 Discovery of the Q fever agent .......... 30

1.2 Bacteriology of *Coxiella burnetii* .......... 31

1.3 Epidemiology of Q fever .......... 40

1.4 Disease manifestation and symptoms .......... 49

1.5 Detection of *Coxiella burnetii* and Q fever diagnosis .......... 54

1.6 Prevention and control .......... 57

1.7 Q fever in Australia .......... 60

1.8 Study scope and objectives .......... 71

Chapter 2 *Coxiella burnetii* seroprevalence and Q fever in Australian wildlife rehabilitators ............... 73

2.1 Abstract ........ 74

2.2 Introduction ........ 75

2.3 Materials and Methods ........ 77

2.4 Statistical Analysis ........ 79

2.5 Results ........ 82

2.6 Discussion ........ 91

2.7 Conclusion ........ 97

Chapter 3 Risk factors associated with self-reported Q fever in Australian wildlife rehabilitators: findings from an online survey ........ 99

3.1 Abstract ........ 100

3.2 Introduction ........ 100

3.3 Materials and Methods ........ 103

3.4 Results ........ 106

3.5 Discussion ........ 111

3.6 Conclusion ........ 117

Chapter 4 Factors associated with Q fever vaccination in Australian wildlife rehabilitators ........ 118

4.1 Abstract ........ 119

4.2 Introduction ........ 120

4.3 Materials and Methods ........ 122

4.4 Results ........ 126

4.5 Discussion ........ 132

4.6 Conclusion ... 139

Chapter 5 Detection of *Coxiella burnetii* DNA in Australian native wildlife ... 141

5.1 Abstract ... 141

5.2 Background ... 142

5.3 Materials and Methods ... 143

5.4 Results ... 159

5.5 Discussion ... 165

5.6 Conclusion ... 173

Chapter 6 Serological evidence of exposure to Spotted Fever Group and Typhus Group rickettsiae in Australian wildlife rehabilitators ... 174

6.1 Preface ... 174

6.2 Abstract ... 175

6.3 Introduction ... 176

6.4 Methods ... 179

6.5 Results ... 185

6.6 Discussion ... 193

6.7 Conclusion ... 200

Chapter 7 General Discussion ... 201

7.1 Key findings, conclusions, and future directions ... 201

7.2 Australian wildlife rehabilitators - an 'at-risk' group ... 205

7.3 Factors contributing to elevated *C. burnetii* seroprevalence and higher rates of Q fever ... 206

7.4 *Coxiella burnetii* in Australian native wildlife ... 209

7.5 Gene targets and qPCR assay specificity ........................................ 212

7.6 Closing comments ........................................ 213

References........................................ 215

Appendix A  Questionnaire for Serological Survey........................................ 244

Appendix B Knowledge, Attitudes and Practices online questionnaire........................................ 253

Appendix C Univariable analysis for risk factors associated with self-reported Q fever ........................................ 274

Appendix D Factors associated with Q fever vaccination........................................ 275

Appendix E CoxMP assay development and wildlife PCR results........................................ 278

# List of Figures

**Figure 1.1** Phylogenetic network using concatenated 16S rRNA, 23S rRNA, *GroEL*, *rpoB* and *dnaK* sequences constructed using 71 *Coxiella*-like strains of ticks, 15 *Coxiella burnetii* reference strains, and bacterial outgroups. The *Coxiella* clades are labelled A to D ................................................................. 33

**Figure 1.2** Murine fibroblast cell (L929) infected with *Coxiella burnetii* ................................................ 37

**Figure 1.3** Electron micrograph of *Coxiella burnetii* large and small cell variants in the phagolysosome ........................................................................................................................................... 38

**Figure 1.4** Disease progression pathways following human *Coxiella burnetii* infection ........................ 50

**Figure 1.5** Typical human serological and molecular response to *Coxiella burnetii* infection............... 55

**Figure 1.6** Q fever notification rate by geographic area (cases per 100 000) in Australia for 2015 ...... 61

**Figure 1.7** Q fever notification rates for Australia (National), Queensland and New South Wales by year, 1991 2014 ........................................................................................................................ 63

**Figure 3.1** Breakdown of responses from Australian wildlife rehabilitators participating in a nationwide online survey regarding Q fever conducted in 2018. ............................................................................ 107

**Figure 4.1** Flow diagram of responses to questions exploring Q fever vaccine awareness and vaccination status of Australian wildlife rehabilitators participating in a nationwide online survey on Q fever knowledge, attitudes and practices conducted in 2018. ............................................................ 126

**Figure 5.1** The geographical locations in New South Wales and Queensland, Australia from which Australian native wildlife tissues were obtained and analysed for the presence of *Coxiella burnetii* DNA Inset shows the location of these states within Australia. .................................................................... 144

**Figure 5.2** The geographical locations in the Australian Capital Territory and New South Wales, Australia from which Eastern Grey kangaroo (*Macropus giganteus*) tissues were obtained and analysed for the presence of *Coxiella burnetii* DNA in Case study 1.................................................... 145

**Figure 5.3** Geographical location in New South Wales, Australia from which Australian native wildlife samples were obtained to investigate for the presence of *Coxiella burnetii* DNA in Case study 2...... 147

**Figure 5.4** Geographical location, in New South Wales, Australia, from which urogenital swabs of koalas (*Phascolarctos cinereus*) were obtained to investigate for the presence of *Coxiella burnetii* DNA in Case study 3. .......... 148

**Figure 5.5** Geographical location, in Queensland, Australia, from which Red-necked wallaby (*Macropus rufogriseus*) environmental faecal (scat) samples were obtained to investigate for the presence of *Coxiella burnetii* DNA in Case study 4. .......... 149

**Figure 5.6** Australian geographical location of wildlife samples tested for the presence of *Coxiella burnetii* DNA, mapped to New South Wales, Q fever incidence rate (2018) by local government area .......... 171

**Figure 6.1** Location of residence of 122 Australian wildlife rehabilitators participating in rickettsia seroprevalence survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. .......... 187

# List of Tables

**Table 0.1** List of presentations and conferences attended during the PhD canditature of Karen Mathews (2018-2022) ........ 23

**Table 1.1** Summary of notable Q fever outbreaks in Australia 1979-2018 ........ 42

**Table 1.2** Counts, percentages and rates of Q fever notification by Australian jurisdiction, 1991-2014. ........ 61

**Table 1.3** Studies reporting serological evidence of *Coxiella burnetii* exposure in Australian native wildlife. ........ 68

**Table 1.4** Published literature reporting the detection of *Coxiella* spp. or *Coxiella burnetii* DNA in Australian native wildlife by PCR. ........ 70

**Table 1.5** Knowledge gaps, research aims and rationale of the PhD of Ms Karen Mathews at The University of Sydney School of Veterinary Science entitled 'The role of Australian native wildlife in Q fever' ........ 71

**Table 2.1** Biosecurity practices reported by 158 Australian wildlife rehabilitators when handling animals and cleaning animal enclosures. Results obtained from a survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. ........ 85

**Table 2.2** Serological results, and self-reported Q fever vaccination of, and Q fever disease in, wildlife rehabilitators participating in a *Coxiella burnetii* seroprevalence survey at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. ........ 87

**Table 2.3** Univariable logistic regression analysis of positive serological result for *C. burnetii* exposure among Australian wildlife rehabilitators participating in a survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018 ........ 88

**Table 2.4** Univariable logistic regression analysis of Q fever vaccination among Australian wildlife rehabilitators participating in a survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018 ........ 89

**Table 2.5** Final multivariable logistic regression analysis of Q fever vaccination among Australian wildlife rehabilitators participating in a survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. .......... 90

**Table 3.1** Characteristics of Australian wildlife rehabilitators participating in a nationwide online survey conducted in 2018 .......... 109

**Table 3.2** Results of final multivariable analysis for risk factors associated with self-reported Q fever among 287 unvaccinated Australian wildlife rehabilitators participating in a nationwide online survey conducted in 2018. .......... 111

**Table 4.1** Results of multivariable logistic regression analysis for variables associated with Q fever vaccination status among Australian wildlife rehabilitators participating in a nationwide online survey conducted in 2018 (n=338). .......... 128

**Table 4.2** Descriptive (n, %, totals) and odds ratio (and Chi-square *p*-value) results for variables significantly associated with awareness of Q fever and Q fever vaccination among Australian wildlife rehabilitators participating in a nationwide online survey conducted in 2018 (n=338). .......... 128

**Table 4.3** Descriptive (n, %, totals) and odds ratio (and Chi-square *p*-value) results for variables significantly associated with Q fever vaccination status among Australian wildlife rehabilitators who were aware of Q fever and the availability of Q fever vaccine (n=200). .......... 130

**Table 4.4** Reasons cited for not being vaccinated against Q fever by 148 unvaccinated Australian wildlife rehabilitators participating in a nationwide online survey conducted in 2018. .......... 132

**Table 5.1** Details of four case studies that were undertaken to investigate for the presence of *Coxiella burnetii* DNA in tissues obtained from Australian native wildlife species residing in New South Wales, Australian Capital Territory and Queensland, Australia. .......... 143

**Table 5.2** Summary of DNA extraction and PCR methodologies utilised for the four case studies that were undertaken to investigate Australian native wildlife species for the presence of *Coxiella burnetii* DNA. .......... 151

**Table 5.3** Primer and probe sequences for endogenous control and *Coxiella burnetii* PCR assays. .......... 158

**Table 5.4** Breakdown of the number of samples and sample types collected from Australian native wildlife species in various regions in New South Wales and Australian Capital Territory, Australia and on St Helena Island, Queensland for Case studies 1-4 which underwent investigation for the presence of *Coxiella burnetii* DNA. ........ 160

**Table 5.5** Summary PCR results for Australian native wildlife species classified as positive or suspect for the presence of *Coxiella burnetii* as determined using a multiplex qPCR assay. ........ 161

**Table 5.6** *Coxiella burnetii* DNA prevalence in Australian native wildlife species from different geographical locations in New South Wales, Australian Capital Territory and St Helena's Island in Queensland, Australia ........ 162

**Table 5.7** Number and sex distribution of native wildlife species from the Camden region of New South Wales, Australia investigated for the presence of *Coxiella burnetii* DNA via qPCR in Case study 2. ........ 163

**Table 5.8** Details of koala (*Phascolarctos cinereus*), urogenital swabs in Case study 3 collected from individual animals and tested for the presence of *Coxiella burnetii* DNA using qPCR in New South Wales, Australia. ........ 164

**Table 6.1** Sequence and product lengths of target gene primers used to detect SFG and TG DNA (citrate synthase) and human β-actin DNA (internal reference gene to verify DNA quality) in the whole blood and serum DNA extracts of Australian wildlife rehabilitators participating in a survey at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. ........ 183

**Table 6.2** Biosecurity practices reported by 120 Australian wildlife rehabilitators when handling animals and cleaning enclosures. Results obtained from a survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. ........ 188

**Table 6.3** Serological results (reciprocal titres) and antigenic classification of seropositive wildlife rehabilitators participating in a rickettsia seroprevalence survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. ........ 189

Table 6.4 Univariable logistic regression analysis of a positive serological result to *Rickettsia* spp. exposure among Australian wildlife rehabilitators participating in a survey at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. ................................................................................ 191

Table 6.5 Final multivariable logistic regression results for exposure to *Rickettsia* spp. among Australian wildlife rehabilitators participating in a survey at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. ........................................................................................................ 192

Table 7.1 Summary of key findings, limitations, and suggestions for future research arising from the PhD thesis undertaken by Ms Karen Mathews at The University of Sydney School of Veterinary Science entitled 'The role of Australian native wildlife in Q fever'..................................................................... 202

## List of Abbreviations

| | |
|---|---|
| ABS | Australian Bureau of Statistics |
| ACT | Australian Capital Territory |
| AEFI | Adverse events following immunisation |
| ARRL | Australian Rickettsial Reference Laboratory |
| ASGS | Australian Statistical Geography Standard |
| AWR/AWRs | Australian wildlife rehabilitators |
| AWRC | Australian Wildlife Rehabilitation Conference |
| AUD | Australian dollars |
| AVA | Australian Veterinary Association |
| BHQ | Black Hole Quencher |
| ºC | degrees celsius |
| CDC | USA Centers for Disease Control and Prevention |
| CFT | complement fixation test |
| CI | confidence interval |
| CLE | *Coxiella*-like endosymbionts |
| *com1* | Outer membrane protein |
| Cq | cycle quantification |
| CoxMP | *Coxiella burnetii* multiplex PCR |
| CR3 | complement receptor 3 |
| DNA | deoxyribonucleic acid |
| dNTPs | deoxynucleotide triphosphates |
| EC | extraction control |
| EDTA | ethylenediaminetetraacetic acid |
| EGK | Eastern Grey Kangaroo |
| ELISA | enzyme-linked immunosorbent assay |
| EMA | ethidium monoazide |
| GE | genome equivalents |
| GIT | gastrointestinal tract |

| | |
|---|---|
| *gltA* | citrate synthase gene *gltA* |
| *groEL* | *htpAB* heat shock operon |
| IAP | integrin associated protein |
| IFA | indirect immunofluorescence assay |
| Ig | immunoglobulin |
| IHC | immunohistochemistry |
| IS | insertion sequence |
| Ig | immunoglobulin |
| KangCytb | Kangaroo cytochrome b PCR |
| Koalaβ-actin | Koala β-actin PCR |
| LCV | large cell variant |
| LPS | lipopolysaccharide |
| $MgCl_2$ | magnesium chloride |
| mg | milligrams |
| mL | millilitre |
| MIQE | Minimum Information for Publication of Quantitative Real-Time PCR Experiments |
| MLVA | multiple locus variable number tandem repeat analysis |
| mM | millimolar |
| NHMRC | National Health and Medical Research Council |
| NSW | New South Wales |
| NT | Northern Territory |
| NTC | no template control |
| OR | odds ratio |
| PBS | phosphate buffered saline |
| PCR | polymerase chain reaction |
| PIS | participant information statement |
| PPE | personal protective equipment |
| PV | parasitophorous vacuole |
| QFD | Q fever disease |
| QFDS | Q fever disease status |

| | |
|---|---|
| QFSS | Q fever serostatus |
| QFS | Q fever syndrome |
| QFV | Q fever vaccination |
| QFVS | Q fever vaccination status |
| QLD | Queensland |
| qPCR | quantitative PCR |
| RNW | Red-necked wallaby |
| SA | South Australia |
| SE | standard error |
| SCV | small cell variant |
| SFG | Spotted Fever Group |
| STG | Scrub Typhus Group |
| spp. | species |
| subsp | subspecies |
| TG | Typhus Group |
| U.S.A | United States of America |
| Mg | micrograms |
| µL | microliter |
| µM | micromolar |
| UGT | urogenital |
| USYD | University of Sydney |
| UV | ultraviolet |
| VIC | Victoria |
| WA | Western Australia |
| WGK | Western Grey kangaroo |
| WH&S | Workplace Health and Safety |

# List of Peer Reviewed Publications

1. **Mathews, K. O**., Toribio, J. A., Norris, J. M., Phalen, D., Wood, N., Graves, S. R., Sheehy, P. A., & Bosward, K. L. (2020). *Coxiella burnetii* seroprevalence and Q fever in Australian wildlife rehabilitators. *One health*, *12*, 100197.https://doi.org/10.1016/j.onehlt.2020.100197
2. **Mathews, K. O.,** Phalen, D., Norris, J. M., Stenos, J., Toribio, J.-A., Wood, N., Graves, S., Sheehy, P. A., Nguyen, C. & Bosward, K. L. (2021). Serological Evidence of Exposure to Spotted Fever Group and Typhus Group Rickettsiae in Australian Wildlife Rehabilitators. *Pathogens*, 10(6), 745. https://doi:10.3390/pathogens10060745
3. **Mathews, K. O**., Savage, C., Norris, J. M., Phalen, D., Malikides, N., Sheehy, P. A., & Bosward, K. L. (2022). Risk factors associated with self-reported Q fever in Australian wildlife rehabilitators: findings from an online survey. Submitted to *Zoonoses and Public Health*, (currently under review).
4. **Mathews, K. O**., Norris, J. M., Phalen, D., Malikides, N., Savage, C., Sheehy, P. A., & Bosward, K. L. (2022). Factors associated with Q fever vaccination in Australian wildlife rehabilitators. Submitted to *Vaccine*, (currently under review).

## Contribution to Conference Proceedings

Table 0.1 List of presentations and conferences attended during the PhD candidature of Karen Mathews (2018-2022)

| Author | Title | Contribution | Conference details |
|---|---|---|---|
| **Karen Mathews,** David Phalen, Jacqueline Norris, John Stenos Jenny-Ann Toribio, Nicholas Wood, Stephen Graves, Paul A Sheehy, Chelsea Nguyen, Katrina Bosward | Q fever in Australian wildlife rehabilitators | Invited speaker | Australian Veterinary Association Conference (2022) |
| **Karen Mathews,** David Phalen, Jacqueline Norris, John Stenos Jenny-Ann Toribio, Nicholas Wood, Stephen Graves, Paul A Sheehy, Chelsea Nguyen, Katrina Bosward | Serological evidence of exposure to Spotted Fever Group and Typhus Group rickettsiae in Australian wildlife rehabilitators | Invited speaker, conference abstract and poster | Australian Society for Infectious Diseases Zoonoses Conference (2021) |
| **Karen Mathews**, Jenny-Ann Toribio, Jacqueline Norris, David Phalen, Nicholas Wood, Stephen Graves, Paul Sheehy, Katrina Bosward | Are Australian wildlife rehabilitators at risk of contracting Q fever by caring for macropods? | Conference abstract and poster | Australian Society for Infectious Diseases Zoonoses Conference (2021) |
| **Karen Mathews,** David Phalen, Jacqueline Norris, John Stenos Jenny-Ann Toribio, Nicholas Wood, Stephen Graves, Paul A Sheehy, Chelsea Nguyen, Katrina Bosward | Exposure to *Coxiella burnetii* (Q fever) and *Rickettsia* spp. in Australian wildlife rehabilitators | Invited speaker, conference abstract | Australian Wildlife Rehabilitation Conference (2021) |
| **Karen Mathews,** David Phalen, Jacqueline Norris, John Stenos Jenny-Ann Toribio, Nicholas Wood, Stephen Graves, Paul A Sheehy, Chelsea Nguyen, Katrina Bosward | Risk factors associated with seropositivity to *Coxiella burnetii* and *Rickettsia* spp. in Australian wildlife rehabilitators caring for Australian mammals | Oral presentation and conference abstract | Science Week-Australian and New Zealand College of Veterinary Science (2021) |

| Author | Title | Contribution | Conference details |
| --- | --- | --- | --- |
| **Karen Mathews**, Jacqueline Norris, David Phalen, Paul Sheehy, Nicholas Malikides , Katrina Bosward | Risk factors for Q fever disease in Australian wildlife rehabilitators | Oral presentation | Marie Bashir Institute Early Career Researcher “Muster up” (2020) |
| **Karen Mathews**, Jenny-Ann Toribio, Jacqueline Norris, David Phalen, Nicholas Wood, Stephen Graves, Paul Sheehy, Katrina Bosward | Are Australian wildlife rehabilitators at risk of contracting Q fever by caring for macropods? | Conference abstract and poster | Marie Bashir Institute Colloquium (2019) |

# List of Awards and Grants

Paulette Isabel Jones Completion Scholarship (2021)

Zoonoses 2021 Conference (Australasian Society for Infectious Diseases) Poster Prize (2021)

First place in Faculty of Science, USYD heat of 3 Minute Thesis (2021)

University of Sydney Post Graduate Research Support Scheme Scholarship (2021)

MBI Early Career Researcher “Muster up” - ‘Gold’ prize for the best presentation (2020)

University of Sydney Post Graduate Research Support Scheme Scholarship (2020)

Sydney University School of Veterinary Science Post Graduate Conference-best mid-candidature poster and accompanying presentation (2019)

RTP Stipend Scholarship (2018)

# Disclosure and Author Contribution Statement

Chapters 2 and 6 are published in peer-reviewed journals. Chapter 3 and 4 are currently under review.

# Chapter 2

**Mathews, K. O**., Toribio, J. A., Norris, J. M., Phalen, D., Wood, N., Graves, S. R., ... & Bosward, K. L. (2020). *Coxiella burnetii* seroprevalence and Q fever in Australian wildlife rehabilitators. *One Health*, 12, 100197. doi:10.1016/j.onehlt.2020.100197

I, Karen Mathews was responsible for the conceptualisation, methodology, investigation, formal analysis and the original preparation and editing of this manuscript.

**Contributions to this manuscript made by all authors were as follows:**

**Karen O. Mathews** Conceptualisation, Writing - original draft, Formal analysis, Investigation, Methodology

**Jenny-Ann Toribio:** Writing-review & editing, Supervision, Methodology, Analysis

**Jacqueline M. Norris** Conceptualisation, Writing - review & editing, Methodology, Investigation, Funding acquisition

**David Phalen** Conceptualisation, Writing - review & editing, Methodology, Funding acquisition

**Stephen Graves** Funding acquisition. Writing - review & editing, Methodology, Funding acquisition

**Nicholas Wood** Funding acquisition, Writing - review & editing, Methodology, investigation

**Paul A. Sheehy** Conceptualisation Writing - review & editing, Methodology

**Katrina L. Bosward** Conceptualisation, Funding acquisition, Investigation, Writing - review & editing, Supervision, Methodology, Project administration

As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Signature: Katrina Bosward Date: 30th June 2022

# Chapter 3

**Mathews, K. O**., Savage, C., Norris, J. M., Phalen, D., Malikides, N., Sheehy, P. A., & Bosward, K. L. (2022). Risk factors associated with self-reported Q fever in Australian wildlife rehabilitators: findings from an online survey. Submitted to *Zoonoses and Public Health*, (currently under review).

I, Karen Mathews was responsible for the conceptualisation, methodology, investigation, formal analysis and the original preparation and editing of this manuscript.

**Contributions to this manuscript made by all authors were as follows:**

**Karen O. Mathews** Conceptualisation, Writing - original draft, Formal analysis, Investigation, Methodology

**Cathie Savage** Conceptualisation, Writing - review & editing, Methodology

**Jacqueline M. Norris** Conceptualisation, Writing - review & editing, Investigation, Supervision, Methodology

**David Phalen** Conceptualisation, Writing - review & editing, Methodology, Investigation, Supervision,

**Nicholas Malikides** Writing - review & editing, Formal analysis

**Paul A. Sheehy** Conceptualisation Writing - review & editing, Methodology, Supervision

**Katrina L. Bosward** Conceptualisation, Funding acquisition, Investigation, Writing - review & editing, Supervision, Methodology, Project administration

As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Signature: Katrina Bosward Date: 30th June 2022

# Chapter 4

**Mathews, K. O**., Norris, J. M., Phalen, D., Malikides, N., Savage, C., Sheehy, P. A., & Bosward, K. L. (2022). Factors associated with Q fever vaccination in Australian wildlife rehabilitators. Submitted to *Vaccine*, (currently under review).

I, Karen Mathews was responsible for the conceptualisation, methodology, investigation, formal analysis and the original preparation and editing of this manuscript.

**Contributions to this manuscript made by all authors were as follows:**

**Karen O. Mathews** Conceptualisation, Writing - original draft, Formal analysis, Investigation, Methodology

**Jacqueline M. Norris** Conceptualisation, Writing - review & editing, Investigation, Supervision, Methodology

**David Phalen** Conceptualisation, Writing - review & editing, Methodology, Investigation, Supervision.

**Nicholas Malikides** Writing - review & editing, Formal analysis

**Cathie Savage** Conceptualisation, Writing - review & editing, Methodology

**Paul A. Sheehy** Conceptualisation, Writing - review & editing, Methodology, Supervision

**Katrina L. Bosward** Conceptualisation, Funding acquisition, Investigation, Writing - review & editing, Supervision, Methodology, Project administration

As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Signature: Katrina Bosward Date: 30th June 2022

# Chapter 7

**Mathews, K. O.,** Phalen, D., Norris, J. M., Stenos, J., Toribio, J.-A., Wood, N., Graves, S., Sheehy, P. A., Nguyen, C. & Bosward, K. L. (2021). Serological Evidence of Exposure to Spotted Fever Group and Typhus Group Rickettsiae in Australian Wildlife Rehabilitators. *Pathogens*, 10(6), 745. https://doi:10.3390/pathogens10060745

I, Karen Mathews was responsible for the conceptualisation, methodology, investigation, formal analysis and the original preparation and editing of this manuscript.

**Contributions to this manuscript made by all authors were as follows:**

**Karen O. Mathews** Conceptualisation, Writing - original draft, Formal analysis, Investigation, Methodology.

**Jenny-Ann Toribio** Writing-review & editing, Supervision, Formal Analysis

**Jacqueline M. Norris** Conceptualisation, Funding acquisition, Investigation, Writing - review & editing, Supervision, Methodology

**David Phalen** Conceptualisation, Funding acquisition, Writing - review & editing, Supervision, Methodology

**John Stenos** Writing - review & editing, Methodology, Formal analysis, Validation

**Stephen Graves** Funding acquisition. Writing - review & editing, Methodology, Funding acquisition

**Nicholas Wood** Funding acquisition, Investigation, Writing - review & editing, Methodology

**Paul A. Sheehy** Conceptualisation, Writing - review & editing, Methodology

**Chelsea Nguyen** Writing - review & editing, Methodology

**Katrina L. Bosward** Conceptualisation, Funding acquisition, Investigation, Writing - review & editing, Supervision, Methodology, Project administration

As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Signature: Katrina Bosward Date: 30th June 2022

# Chapter 1 Literature Review

## 1.1 Discovery of the Q fever agent

Q fever was first described in 1935, following an outbreak of an acute febrile illness amongst abattoir workers in Brisbane, Queensland, Australia (Derrick, 1937). After ruling out other diseases known at that time, Derrick suspected that the illness had a novel cause and named it Q (for query) fever "until fuller knowledge should allow a better name". Although Derrick successfully replicated the illness in guinea pigs following their inoculation with blood or urine from infected patients, he was unable to visualise or isolate the causative agent, and therefore he suspected that the infectious agent was a virus. Derrick forwarded liver emulsions from experimentally infected guinea pigs to his colleague, Frank M Burnet, in Melbourne, Victoria, Australia who observed large numbers of "rickettsia-like" organisms in splenic and liver samples of mice infected with these preparations (Burnet & Freeman, 1937). However Burnet concluded that the Q fever agent was different to other rickettsial organisms he had previously observed (Burnet & Freeman, 1937).

Around the same time, in the Rocky Mountain Laboratories in Montana, USA, Gordon Davis and Herald Rea Cox were characterising the 'Nine Mile Agent'; a rickettsia-like organism isolated from a *Dermacentor andersoni* tick, which induced a febrile illness in guinea pigs (Davis et al., 1938).

The link between the findings of the American and Australian groups was established in 1938 when Rolla Eugene Dyer became infected with the 'Nine Mile agent' whilst visiting the Rocky Mountain Laboratories. Guinea pigs inoculated with Dyer's blood became febrile and 'rickettsia-like' organisms were identified in the spleens of these infected animals. Collaborative studies between the American and Australian groups demonstrated that guinea pigs previously infected with Burnet's mouse spleen samples (containing the Q fever agent from Australia) were protected when inoculated with Dyer's blood (containing the Nine Nile agent from America). It was therefore postulated that the 'Nine Mile Agent' and the 'Q fever agent' were the same or closely related organisms (Dyer, 1938).

Originally, Derrick suggested that the new bacterium be named *Rickettsia burneti* (with this spelling) in honour of Frank M Burnet (Derrick, 1939), while the American group named it *Rickettsia diaporica* due to its ability to pass through filters (Cox & Bell, 1939). In 1948, due to notable differences in biochemical and cultural characteristics compared to other rickettsia, *R. burneti* was classified into a new genus and renamed *Coxiella burnetii* (Philip, 1948).

## 1.2 Bacteriology of *Coxiella burnetii*

### 1.2.1 Classification

*Coxiella burnetii* is an obligate intracellular bacterium (Maurin & Raoult, 1999) that propagates in the phagolysosomes of eukaryotic cells (Woldehiwet, 2004). Based on its cell wall structure and composition, *C. burnetii* is classified as a Gram negative bacterium, however it stains poorly with the Gram stain technique, and the Gimenez's method (Gimenez, 1964) is preferred for its visualisation in laboratory culture or clinical specimens (Maurin & Raoult, 1999).

*Coxiella burnetii* was originally classified in the $\alpha$ subdivision of proteobacteria in the *Rickettsiaceae* family of the order Rickettsiales, however following phylogenic analysis of the 16S rRNA gene sequences in the early 1990s, it was reclassified, into the $\gamma$ subdivision of Proteobacteria in the *Coxiellaceae* family in the order Legionellales due to its genetic similarities with *Legionella pneumophila* (a facultative intracellular human pathogen) the causative agent of Legionnaires' disease (Weisburg et al., 1989) and the intracellular arthropod pathogen *Rickettsia grylli*. Currently, there are 43 strains of *C. burnetii* listed in the National Centre for Biotechnology taxonomy database (Schoch et al., 2020) and a comparison of 16S rRNA gene sequences between strains demonstrated high levels (>99%) of sequence homology (Duron et al., 2015; Stein et al., 1997). The only other member within the *Coxiella* genus that has been formally identified is *Candidatus Coxiella* cheraxi, which has been associated with high mortality rates in Australian freshwater crayfish (*Cherax quadricarinatus*), however this organism remains to be isolated from culture (Elliman & Owens, 2020).

### 1.2.2 *Coxiella*-like endosymbionts

Many strains of *Coxiella*-like endosymbionts (CLE) have been described in ticks and, over the past 25 years, the origin of CLE has been a point in question. An early study by Noda et al. (1997) speculated that CLE originated from bacterial pathogens of vertebrates that were acquired by ticks while feeding on bacteraemic hosts. Some years later Zhong et al. (2007) suggested that *C. burnetii* was the progenitor of CLE. Following whole genome sequencing of the CLE obtained from *Ambylomma americanum,* Smith et al. (2015) hypothesised that CLE and *C. burnetii* evolved from a common ancestor. These theories were recently tested in a study by Duron et al. (2015) who utilised a multi-locus typing methodology to characterise diversity within the *Coxiella* genus. The analysis of concatenated sequences of five genes (16S rRNA, 23S rRNA, *GroEL*, *rpoB* and *dnaK*), from 71 *Coxiella*-like strains, isolated from various tick species, and 15 *C. burnetii* reference strains, split the Coxiella genus into four distinct and genetically diverse clades (**Figure 1.1**). A high level of genetic diversity was present between the 43 genotypes of CLE, with none being genetically identical to *C. burnetii.* The clustering of all *C. burnetii* strains within the A clade, and the low genetic diversity amongst *C. burnetii* strains suggests that *C. burnetii* is a recently emerging pathogen, whose ancestor was a soft tick symbiont, which, following the acquisition of virulence factors, was able to infect vertebrates (Duron et al., 2015).

*Coxiella*-like endosymbionts appear to be important for the health of ticks. Although CLE have a reduced genome size compared to *C. burnetii,* the retention of genes associated with biosynthetic pathways suggests that CLE may synthesise metabolites for their haematophagous arthropod hosts, that are not available in mammalian blood. This phenomenon has been observed in other haematophagous species that are dependent on bacterial endosymbionts to supply essential vitamins and cofactors such as tsetse flies (*Glossina morsitans*) (Akman et al., 2002) and bedbugs (*Cimex lectularius*) (Hosokawa et al., 2010). The reduced fecundity of *A. americanum* following the elimination of CLE via antibiotic treatment (Zhong et al., 2007), and the presence of CLE in Malpighian

tubules also suggests a potential role for CLE in nutrition, osmoregulation and excretion (Angelakis et al., 2016) providing further evidence that these organisms are important for tick fitness.

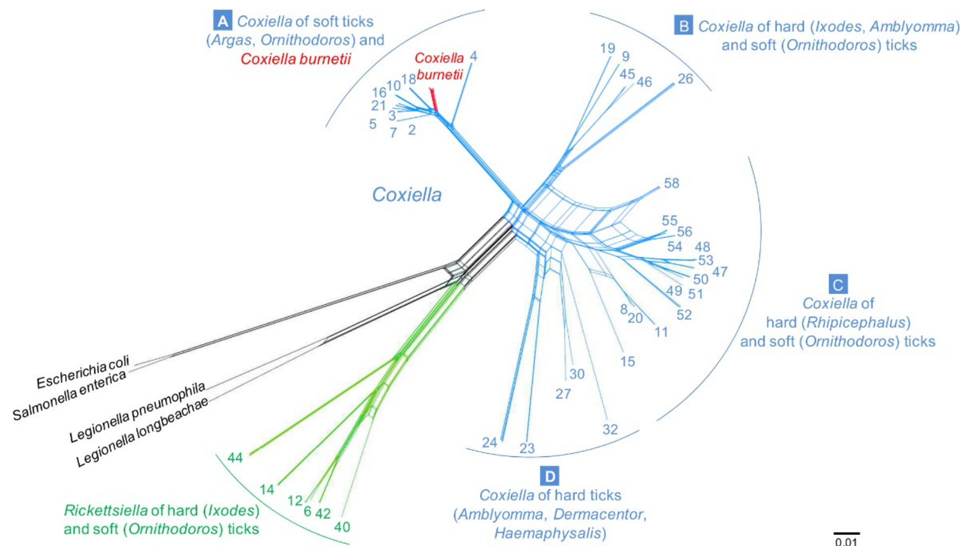


**Figure 1.1** Phylogenetic network using concatenated 16S rRNA, 23S rRNA, *GroEL*, *rpoB* and *dnaK* sequences constructed using 71 *Coxiella*-like strains of ticks, 15 *Coxiella burnetii* reference strains, and bacterial outgroups. The *Coxiella* clades are labelled A to D (Duron et al., 2015).

Due to their symbiotic role in maintaining tick health, CLE are not considered to be pathogenic to vertebrates (Smith et al., 2015), however lethal infections in domestic birds due to CLE have been described (Shivaprasad et al., 2008; Vapniarsky et al., 2012; Woc-Colburn et al., 2008). *Coxiella*-like endosymbionts have also been detected in horses from South Korea via PCR, however the clinical significance of this finding remains unclear (Seo et al., 2016). The concept that CLE are non-pathogenic has also been challenged with two candidate species *Candidatus* C. massiliensis and *Ca.* C. mudrowiae being recently proposed as potential agents of human disease in France following their identification via PCR in skin biopsies blood and ticks obtained from patients presenting with scalp eschars (Angelakis et al., 2016; Guimard et al., 2017). A recent Australian study by Oskam et al. (2017) identified *Ca.* C. massiliensis in *Rhipicephalus sanguineus* ticks collected from dogs from four Australian jurisdictions using a *Coxiella*-genus specific PCR. To date, efforts to culture CLE have been unsuccessful.

### 1.2.3 Genome organisation

The complete genome sequence of *C. burnetii* was published in 2003 (Seshadri et al., 2003). Sequencing of the Nine Mile Phase 1 RSA 493 strain originally isolated from ticks revealed a 1,995, 275bp circular chromosome, with a G+C content of 42.6% and an extrachromosomal plasmid QpH1 of 37,393bp (Roest, Bossers, et al., 2013). Although *C. burnetii* exhibits similarities to other obligate intracellular pathogens such as *Chylamidia* spp. and *Rickettsia* spp., its genome differs from these species considerably. For example, the number of pseudogenes within the *C. burnetii* genome is higher in comparison to other intracellular pathogens. From an evolutionary perspective, this indicates that *C. burnetii* genome reduction has commenced relatively recently (Brenner et al., 2021). In contrast to genomes of other intracellular pathogens which rarely contain insertion sequence (IS) elements, 29 IS elements were identified in the *C. burnetii* Nine Mile Phase 1 RSA493 strain (Seshadri et al., 2003). Furthermore, the *C. burnetii* genome encodes for genes that provide the organism with greater metabolic and transport capabilities reflecting adaptations that allow *C. burnetii* to thrive in its intracellular niche (Seshadri et al., 2003).

In addition to the QpH1 plasmid harboured by the Nine Mile strain, three plasmids harboured by other strains of *C. burnetii* have also been described: QpRS (Lautenschläger et al., 2000), QpDG (Hendrix et al., 1991) and QpDV (Valková & Kazár, 1995). Although plasmidless strains of *C. burnetii* also exist, such strains contain an integrated plasmid-like sequence (IPS) within their genome (Savinelli & Mallavia, 1990). Initial findings led to the hypothesis that plasmid type was associated with strain pathogenicity and disease state with a study by Samuel et al. (1985) reporting that QpH1 was only identified in *C. burnetii* isolates obtained from patients with acute Q fever, as well as ticks and animals, whereas isolates from patients with Q fever endocarditis (a clinical manifestation associated with chronic or persistent forms of Q fever) were found to contain QpRS or IPS. However, this hypothesis was rejected following the findings of a later study that detected QpH1 in *C. burnetii* isolates obtained from patients with Q fever endocarditis (Stein & Raoult, 1993).

### 1.2.4 Phase Variation

*Coxiella burnetii* exists as two distinct phase variants termed Phase 1 and Phase 2. Phase 1 organisms correspond to the wild-type virulent form of *C. burnetii* which is only found in nature and may be isolated from infected hosts (Hotta et al., 2002). Conversely, Phase 2 organisms are an artificial avirulent form of *C. burnetii*, that are not found in nature, and only emerge following the serial passage of Phase 1 organisms in laboratory environments such as embryonated eggs, cell culture systems or synthetic media (Hotta et al., 2002; Kersh et al., 2011; Stoker & Fiset, 1956).

The differences between the Phase 1 and Phase 2 *C. burnetii* cells are due to variations in the structure of the lipopolysaccharide (LPS), which is an important virulence determinant of *C. burnetii* (Hackstadt, 1990; Porter et al., 2011). The virulent Phase 1 cells express full length LPS which contains the O-antigen and two unusual sugars, virenose and dihydrohydroxystreptose, both of which appear to be unique to *C. burnetii* (Schramek et al., 1985), whereas the LPS of avirulent Phase 2 cells, lacks the O-antigen and these unique sugars moieties (Toman et al., 2009). The switch from Phase 1 to Phase 2 is poorly understood, but is thought to occur due to the removal of immune pressure (normally supplied by the animal host) to produce full length LPS and thus serve as an energy conservation strategy when growing in a non-animal environment (Lukácová et al., 2008). In the Nine Mile strain, the transition from Phase 1 to Phase 2 is accompanied by a large 25,992bp deletion (Hoover et al., 2002) encoding multiple genes involved in the synthesis of the O-polysaccharide chain, although this deletion is not consistently observed across all Phase 2 forms of all *C. burnetii* strains (Thompson et al., 2003). Due to the gene deletion, Nine Mile Phase 2 is avirulent and may be grown in Biosafety Level 2 facilities (Howe et al., 2010).

The different antigenic presentation of Phase 1 and Phase 2 cells forms the basis of Q fever serodiagnosis. Following human infection with virulent Phase 1 *C. burnetii*, anti-Phase 2 antibodies appear first and predominate during the acute stage of infection, whereas anti-Phase 1 antibodies appear later and are associated with a longer duration of infection and are seen in persistent

infections (Maurin & Raoult, 1999). The lag in the immune response to Phase 1 LPS antigens may be understood based on the knowledge that both surface protein antigens, and LPS antigens present on the virulent Phase 1 cells are recognised by the immune system; however surface protein antigens, which are common to both Phase 1 and Phase 2 cells, evoke an earlier and stronger immune response in comparison to the LPS antigen (Hackstadt, 1988). This results in an initial anti-Phase 2 response against surface protein antigens, followed by an anti-Phase 1 response to LPS antigen.

An early investigation of the protective potency of Q fever vaccine in guinea pigs demonstrated that vaccines produced using Phase 1 organisms were 100-300 times more protective than their Phase 2 equivalents (Ormsbee et al., 1964). The currently available Q fever vaccine in Australia is a whole cell vaccine produced using purified Phase 1 *C. burnetii* Henzerling strain (Seqirus, 2019).

### 1.2.5 Life cycle in vertebrate hosts

#### *1.2.5.1 Phagocytosis of Coxiella burnetii*

Mononuclear phagocytic cells (monocytes and macrophages) are the preferred target cells for *C. burnetii* in vertebrates, however the attachment and internalisation pathway differs between avirulent (Phase 2) and virulent (Phase 1) forms (Honstettre et al., 2004). Following adherence to monocyte cell surface receptors, the internalisation of avirulent Phase 2 cells is mediated by αvβ3 integrin and complement receptor 3 (CR3), with the phagocytic activity of CR3 being dependent upon its activation via αvβ3 and integrin associated protein (IAP). In contrast, Phase 1 organisms are poorly internalised, and phagocytosis is mediated by the αvβ3 integrin alone. Phase 1 organisms induce the formation of 'ruffles' on the surface of monocytes consequent to actin cytoskeleton rearrangement. This interferes with the coupling of αvβ3 integrin and IAP, resulting in the impairment CR3 activity which reduces internalisation efficiency. Due to greater internalisation efficiency, Phase 2 cells grow at a faster rate than Phase 1 (Capo et al., 1999).

*1.2.5.2 Parasitophorous vacuole formation*

The phagolysosomal pathway is an important component of the host's defence mechanism against pathogen invasion (Lee et al., 2020). Following internalisation, invading pathogens are contained within the early phagosome, which 'matures' and becomes increasingly acidified via a series of endosome-associated fusion events, before finally fusing with secondary lysosomes, forming a phagolysosome which functions to destroy the invading pathogen (Berón et al., 2002). Following internalisation, Phase 2 *C. burnetii* organisms progress down the phagolysosomal pathway and are rapidly destroyed, however Phase 1 *C. burnetii* hijacks this process before lysosomal fusion occurs, resulting in the formation of a large acidified parasitophorous vacuole (PV). This hostile acidic environment does not kill *C. burnetii*, but instead provides an intracellular compartment (**Figure 1.2**) for the bacterium to live and replicate (Hackstadt & Williams, 1981).

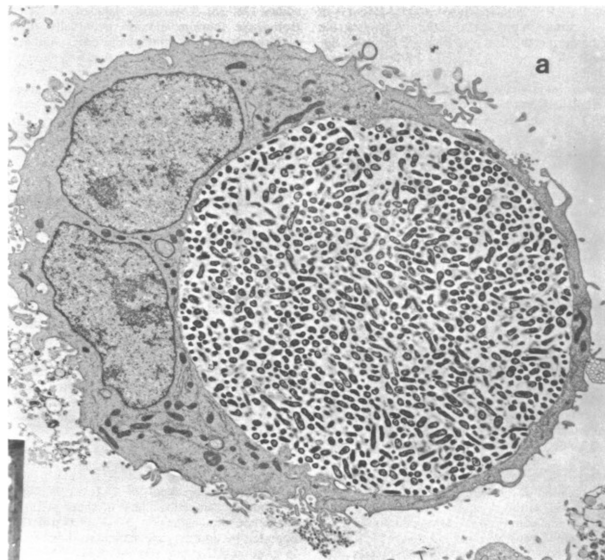


**Figure 1.2** Murine fibroblast cell (L929) infected with *Coxiella burnetii* (Baca & Paretsky, 1983).

*1.2.5.3 Morphological variants*

The pleomorphic phenotype of *C. burnetii* was first noted in early experiments by Davis and Cox, who, using light microscopy, noted granular and bacillary forms of the bacterium in the spleens of infected guinea pigs (Cox, 1938). Almost 50 years later, McCaul and Williams (1981) proposed a model of *C.*

*burnetii* differentiation, describing two distinct morphotypes termed large cell variants (LCV) and small-cell variants (SCV) (**Figure 1.3**). When viewed using electron microscopy, SCV are typically rod shaped, 0.2-0.5µm in length, and contain highly condensed chromatin, an extensive array of intracytoplasmic membranes and a dense peptidoglycan layer between their cytoplasmic and outer membranes. The SCV represents the dormant extracellular spore-like form, with their small size accounting for the property of filterability observed by Cox in 1938 (Cox, 1938). In contrast, LCV resemble Gram negative bacteria, and represent the metabolically active intracellular form of *C. burnetii* (McCaul & Williams, 1981). They can exceed 1µm in length and contain dispersed chromatin, lack intracytoplasmic membranes and exhibit a distinct periplasmic space between their cytoplasmic and outer membranes (McCaul & Williams, 1981).

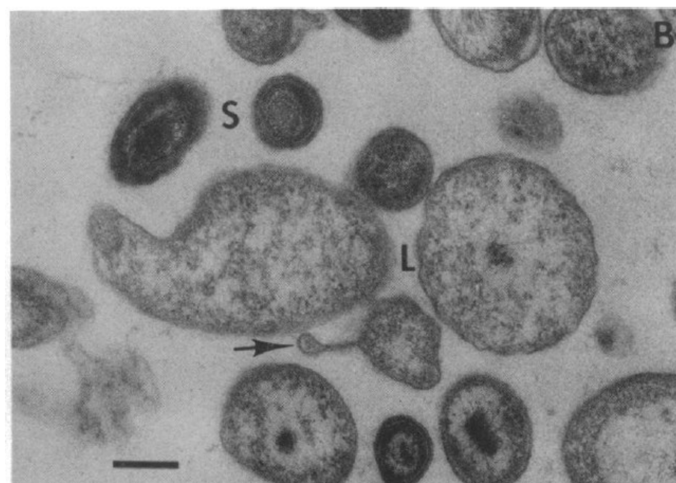


**Figure 1.3** Electron micrograph of *Coxiella burnetii* large and small cell variants in the phagolysosome L - large cell variants, S - small cell variants, Marker = 0.2µM , arrow indicates the bleb formation on large cell variants (McCaul & Williams, 1981).

The SCV is the infectious form of *C. burnetii* and it has been estimated that inhalation of as little as one organism is required to initiate infection in humans (Jones et al., 2006). The SCV is highly resistant to stressors including elevated temperatures, osmotic pressure, UV radiation, desiccation and heat, enabling it to persist in the environment for extended periods whilst retaining its viability (McCaul & Williams, 1981). Due to the low infectious dose, environmental stability and the aerosol transmission

route, *C. burnetii* is classified as a Category B Bioterrorism agent by the Centres for Disease Control and Prevention (Centres of Disease Control and Prevention, 2021), and within Australia and New Zealand the pathogen is listed as a Risk Group 3 microorganism due its ability to cause serious disease in humans (Standards Australia, 2010). The ability of the SCV to resist high temperatures also gave rise to the implementation of high-temperature pasteurisation (71.7°C) protocols in the 1950s (Enright et al., 1957).

*In vivo,* the SCV is associated with persistent infections including Q fever endocarditis, and it has been suggested that its resistive properties may contribute to the refractory nature of such infections to antimicrobial therapy (Maurin & Raoult, 1999). The LCV is thought to be important for the cell-to-cell spread of *C. burnetii* during the acute stages of infection and the formation of the PV via the secretion of proteins that promote its fusion with other cellular vesicles (Coleman et al., 2004).

#### *1.2.5.4 Growth and development*

The growth and developmental cycles of *C. burnetii* were investigated by Coleman et al., (2004) in Vero cells infected with purified suspensions of the SCV. The generated growth curve exhibited features of a typical closed bacterial replication system with distinct lag, exponential and stationary phases. Less than an hour post-infection, the SCV is contained within the membrane of the PV signifying the commencement of a two-day lag phase, during which the non-replicative SCV morphs into the replicative LCV. Exponential replication of the LCV ensues over the next four days, with the concomitant appearance of the large PV harbouring the replicating LCV morphotype almost exclusively. The stationary phase is reached around six days post infection, and coincides with LCV to SCV morphogenesis, and by day eight the PV is comprised of approximately 50% SCV. Finally, the SCV is released into the extracellular environment however the mechanism by which this occurs is unclear (Coleman et al., 2004).

## 1.3 Epidemiology of Q fever

### 1.3.1 Host range and sources of infection

*Coxiella burnetii* can infect an extensive range of vertebrate and invertebrate hosts including wild and domestic mammals, birds, and arthropods (Maurin & Raoult, 1999; Parker et al., 2006; Tissot-Dupont & Raoult, 2008).

#### *1.3.1.1 Ruminants*

Domestic ruminants (cattle, sheep and goats) are considered to be the primary source of *C. burnetii* infection for humans with the majority of Q fever outbreaks globally attributed to these three species (Angelakis & Raoult, 2010; Eldin et al., 2017). Serological evidence of *C. burnetii* infection in domestic ruminants has been reported in most parts of the world except New Zealand, with the estimated apparent prevalence in cattle being slightly higher than in sheep and goats (20% and 15% respectively) (Guatteo et al., 2011). Infected animals shed the organism in high concentrations during parturition via birth products and fluids (up to $10^9$ organisms per gram have been reported for placental tissue of sheep) (Hartwell et al., 1951), resulting in massive environmental contamination. Consequently, exposure to birth products of *C. burnetii* infected animals is regarded as an important risk factor for Q fever (Sloan-Gardner et al., 2017, Wade et al., 2006, Welsh et al., 1958). Infected animals also shed the pathogen in their milk, urine and faeces, although the shedding route and duration differ between species (Rodolakis, 2009). Infected cattle and goats shed *C. burnetii* mainly in milk, while infected sheep shed *C. burnetii* primarily via faeces (Rodolakis, 2009).

The vast majority of Q fever outbreaks worldwide are attributed to domestic ruminants or their products. The largest outbreak to date occurred in the Netherlands from 2007-2010 which involved over 4,000 human Q fever cases (van der Hoek et al., 2012). Infected dairy goats were identified as the primary source of the outbreak, and many human cases occurred in the vicinity of dairy goat farms with a recent history of Q fever induced abortion waves (Karagiannis et al., 2009). To control the outbreak more than 50,000 pregnant sheep and goats from infected farms were culled from

December 2009 to 2010 (van der Hoek et al., 2012). Other notable Q fever outbreaks in Europe have been associated with small ruminants including an outbreak resulting in over 500 Q fever cases which were associated with kidding goats in the Panagyurische region of Bulgaria in the early 1990s (Serbezov et al., 1999). Another outbreak in Switzerland in 1983, involving 415 serologically confirmed cases residing in several villages in a Swiss alpine valley, was linked to the movement of flocks of sheep (~500 animals) from alpine regions to pastures in the valley near the affected villages (Dupuis et al., 1987).

Since the first outbreak of Q fever in a Brisbane abattoir in 1935 (Derrick, 1937), several Q fever outbreaks have been described in Australia. A summary of, and references for, these outbreaks is/are provided in Table 1.1, with the majority of Australian outbreaks also associated with domestic ruminants or their products.

*1.3.1.2 Cats and dogs*

Kosatsky (1984) described the first cat-associated case of Q fever which occurred in Nova Scotia, Canada. The outbreak was linked to a parturient cat and her kittens and affected 13 adults, nine of whom were hospitalised. Exposure to *C. burnetii* infected cats is considered a major risk factor for the acquisition of Q fever in this region (Kosatsky, 1984; Langley et al., 1988; Marrie et al., 1988). Q fever outbreaks associated with parturient cats have also been documented in the USA (Pinsky et al., 1991) and Australia (Table 1.1) (Kopecny et al., 2013; Malo et al., 2018). *Coxiella burnetii* DNA has been detected in the birth fluids of parturient cats in Egypt (Abdel-Moein & Zaher, 2021), and in uterine biopsies of healthy (Cairns et al., 2007) and reproductively abnormal (Fujishiro et al., 2016) pet cats in the USA. Cats are considered to be an important reservoir species in Japan (Komiya, Sadamasu, Kang, et al., 2003), where the pathogen has been isolated from feline vaginal swabs (Nagaoka et al., 1998) and the sera of cats raised by Q fever patients (Nagaoka et al., 1996).

**Table 1.1** Summary of notable Q fever outbreaks in Australia 1979-2018 Adapted from (New South Wales Health, 2019).

| Year | State | Outbreak setting | Outbreak details | Reference |
|---|---|---|---|---|
| 1979 | VIC | Abattoir | 110 abattoir workers | (Buckley, 1980) |
| 1998 | NSW | Abattoir | 29 confirmed and 8 suspected cases | (National Centre for Disease Control, 1998) |
| 2003 | QLD | Goat farm | 5 cases | (Miller et al., 2005) |
| 2004 | SA | Animal sale yard | 25 cases exposed to infected sheep and dust | (O'Connor et al., 2015) |
| 2006 | VIC | Cosmetics factory | 4 cases linked to processing sheep placentas and foetal tissue | (Wade et al., 2006) |
| 2007 | SA | Abattoir | 5 confirmed cases and 1 possible fatal case | (International Society for Infectious Diseases, 2007) |
| 2010 | NSW | Veterinary hospital | 9 veterinary personnel and 1 cat owner linked to an infected parturient cat undergoing a caesarean section | (Kopecny et al., 2013) |
| 2012 | NSW | Veterinary hospital | 3 veterinary nurses attending to an infected parturient dog undergoing a caesarean section | (Gibbons & White, 2014) |
| 2012-2014 | VIC | Goat dairy farm | 18 confirmed cases | (Bond et al., 2016) |
| 2014-2015 | NSW | Remote rural town in NSW | 14 confirmed cases with 3 in high-risk occupations and 11 in non-animal related occupations | (Archer et al., 2017) |
| 2015 | NSW | Abattoir | 7 confirmed cases including 1 fatal case and 1 suspected case | (Lord et al., 2016) |
| 2018 | QLD | Animal refuge/ veterinary hospital | 6 confirmed cases and 1 probable case | (Malo et al., 2018) |

VIC- Victoria, NSW – New South Wales, SA- South Australia, QLD- Queensland

Similar to cats, the first dog-associated Q fever outbreak also occurred in Nova Scotia (Buhariwalla et al., 1996). This outbreak affected three members of one family who developed *C. burnetii* pneumonia following exposure to a *C. burnetii*-infected parturient dog. A Q fever outbreak amongst veterinary personnel in Sydney, Australia (Table 1.1) following exposure to an infected dog undergoing a caesarean section has also been described (Gibbons & White, 2014). *Coxiella burnetii* has been isolated from, and detected in the sera of dogs associated with Q fever cases at a Japanese veterinary hospital (Komiya, Sadamasu, Toriniwa, et al., 2003), in the blood of stray dogs in Taiwan (Chou et al., 2014) and the urine of domestic dogs in Australia (Tozer et al., 2014).

Seroprevalence studies provide further evidence that cats and dogs can be reservoirs for *C. burnetii*. Antibodies against *C. burnetii* have been detected in cats from many countries including Iran (Mousapour et al., 2020), Japan (Komiya, Sadamasu, Kang, et al., 2003) Canada (Cyr et al., 2021) and the United Kingdom (Meredith et al., 2015). An Australian study by Shapiro et al. (2015) reported *C. burnetii* seroprevalence of 5.1% in pet cats and 9.3% in breeding cats in NSW and a study by Ma et al., (2020) reported 13.1% seropositivity in cats in communities across remote NSW. Seropositivity has been documented in dogs in North America (Campagna et al., 2011), Europe (Boni et al., 1998; Ebani, 2020), Asia (Saengsawang et al., 2022), the Middle East (Havas & Burkman, 2011) and Africa (Boni et al., 1998). There is also a growing body of evidence of *C. burnetii* exposure in dogs in Australia (Cooper, Goullet, et al., 2012; Cooper et al., 2011; Ma et al., 2020; Orr et al., 2022; Shapiro, Brown, et al., 2017; Shapiro et al., 2016; Tozer et al., 2014).

*1.3.1.3 Horses*

Although horses are susceptible to *C. burnetii* infection, their role in the epidemiology of Q fever is unclear. Equine seroprevalence studies have demonstrated evidence of *C. burnetii* infection in horses with seroprevalence ranging from 0% to 52.5% reported for various countries (Marenzoni et al., 2013). In France, *C. burnetii* has been suggested as an abortogenic agent in horses, and *C. burnetii*

DNA has been detected in aborted foetuses and equine placentas via qPCR (Leon et al., 2012). A pooled *C. burnetii* DNA detection rate of 11.9% has been reported for equine urine and blood sampled in Australia (Tozer et al., 2014). The pathogen has been recently recognised as a potential source of equine abortion in Australia, with *C. burnetii* DNA detected in 4.0% of aborted equine foetal tissues obtained from animals in NSW and VIC (Akter et al., 2020). Although cases of Q fever have been reported in horseback riders and people visiting horse facilities, the source of *C. burnetii* infection in these cases was likely due to ruminants, and to date, no cases of human Q fever have been definitively attributable to horses (Desjardins et al., 2018; Porter et al., 2011).

#### *1.3.1.4 Wildlife*

The frequency of sporadic Q fever cases attributed to exposure to wildlife is increasing (Flint et al., 2016; Stevenson et al., 2015). A systematic review of the literature on wildlife *C. burnetii* reservoirs worldwide, reported that infection and/or exposure to *C. burnetii* had been identified in 109 species of wild mammals (González-Barrio & Ruiz-Fons, 2019).

In Europe, *C. burnetii* infection has been described in wild ungulates, carnivores, lagomorphs and wild birds (Yon et al., 2019). Studies in wild European rabbit (*Oryctolagus cuniculus*) populations in Portugal and Spain demonstrated high seroprevalence, systemic infection and shedding via vaginal secretions, implicating these species as potential reservoirs of *C. burnetii* infection for humans and livestock in Europe (González-Barrio et al., 2015). Molecular evidence of *C. burnetii* infection has also been demonstrated in genital swabs of wild rodents in Canada (Thompson et al., 2012), and, in the United Kingdom, serological evidence of *C. burnetii* infection has been reported in wild rodents and foxes (Meredith et al., 2015). Recently an epidemiological investigation into a Q fever outbreak in a military training camp in French Guiana identified the three-toed sloth (*Bradypus tridactylus*) as a reservoir for a particularly virulent strain of *C. burnetii* (Pommier de Santi et al., 2018). Infection with this strain produced high antibody titres, symptoms in 100% of those identified as infected and

increased symptom severity. Severe Q fever pneumonia requiring hospitalisation occurred in around 40% of cases (Mahamat et al., 2013). Interestingly *C. burnetii* infection is yet to be described in domestic ruminants in French Guiana.

Australian studies have reported evidence of *C. burnetii* infection in Australian marsupials including kangaroos (Banazis et al., 2010; Bennett et al., 2011; Pope et al., 1960; Potter et al., 2011), bandicoots (Bennett et al., 2011; Derrick et al., 1939) and koalas (Tozer et al., 2014) as well as introduced feral species such as foxes, cats, pigs and goats (Cooper, 2011; Cooper, Goullet, et al., 2012). A detailed discussion on *C. burnetii* in Australian mammals is provided in section **1.7.3**.

#### *1.3.1.5 Ticks*

*Coxiella burnetii* was originally isolated from a *Dermacentor andersoni* tick in the 1930s by Davis et al. (1938) and it has since been reported that over 40 tick species are naturally infected with *C. burnetii* (Maurin & Raoult, 1999). However the role of ticks in the epidemiology of Q fever is poorly understood and is further complicated by the recent discovery that CLE are widespread in ticks (Duron et al., 2015).

Although ticks are not considered essential for the maintenance of *C. burnetii* infections in livestock or humans (Maurin & Raoult, 1999), they may play an important role in the circulation of *C. burnetii* in wildlife populations (Eldin et al., 2017). *Coxiella burnetii* has been detected in ticks collected from Australian wildlife including; the ornate kangaroo tick (*Amblyomma triguttatum*) (Cooper et al., 2013; Pope et al., 1960), the paralysis tick *Ixodes holocyclus* (Cooper et al., 2013; Graves et al., 2016) and bandicoot ticks (*Haemaphysalis humerosa)* (Bennett et al., 2011; Cooper et al., 2013), suggesting that *C. burnetii* is being maintained in nature via a tick-wildlife sylvatic cycle. Of these tick species, *A. triguttatum* and *I. holocyclus* are also known to bite humans.

It is also worth noting that *C. burnetii* has also been described in other arthropod vectors including bed bugs (*Cimex lectularius*), fleas (Psaroulaki et al., 2014) and a variety of species of mites

(Mamatkulov et al., 2019), however a recent Australian molecular study did not detect *C. burnetii* DNA in cat fleas (*Ctenocephalides felis)* opportunistically obtained from dogs and cats (Huang et al., 2021).

## 1.3.2 Transmission

### *1.3.2.1 Aerosol transmission*

Inhalation of contaminated aerosols or dust is the most common route through which *C. burnetii* is transmitted to humans (Angelakis & Raoult, 2010; Eldin et al., 2017; Gidding et al., 2009). People may become infected via direct contact with tissue, excreta, and fluids from infected animals, particularly during slaughtering or parturition (Kersh, Fitzpatrick, Self, Priestley, et al., 2013). Infection may also occur indirectly via inhalation of contaminated dust released from fomites such as wool (Abinanti et al., 1955), clothing (Oliphant et al., 1949) and manure used as fertiliser (Berri et al., 2003). A Q fever outbreak in an art college in England was linked to contaminated straw used for packing (Harvey et al., 1951) and four employees in a cosmetic processing factory in Victoria Australia developed Q fever following exposure to sheep foetal products (Wade et al., 2006). *Coxiella burnetii* is highly infectious to humans (Jones et al., 2006) and therefore people who are directly or indirectly exposed to low numbers of Phase 1 organisms may become infected and develop Q fever.

In pregnant mammals, *C. burnetii* demonstrates a predilection for placental tissue (Kazar, 2005) where it replicates extensively within placental trophoblasts (Sánchez et al., 2006). Birth products are recognised as a major infection source (Marrie, 1990; Welsh et al., 1958), owing to large numbers of the bacterium excreted via foetal membranes, birth fluids and placenta, which is known to occur during parturition in both abortion and normal deliveries (Roest et al., 2012). Up to $10^9$ *C. burnetii* organisms per gram of placenta have been found in sheep (World Organisation for Animal Health, 2018) and shedding of *C. burnetii* via milk, faeces and vaginal discharges may continue for several months post parturition (Arricau Bouvery et al., 2003).

Owing to the ability of *C. burnetii* to persist for extended periods in soil (Kersh, Fitzpatrick, Self, Priestley, et al., 2013) and withstand harsh environmental conditions, infectious aerosols may remain long after environmental shedding by infected animals. Environmental *C. burnetii* also has the potential to be spread over large distances in contaminated dust in dry and windy conditions. Windborne spread of *C. burnetii* has been attributed to Q fever outbreaks in several countries including France (Tissot-Dupont et al., 2004) the United Kingdom (Hawker et al., 1998), the Netherlands (Hackert et al., 2012) and Australia (O'Connor et al., 2015).

Since *C. burnetii* may be shed in the milk of infected ruminants, the ingestion of contaminated unpasteurised dairy products represents a potential route of infection. However the importance of dairy products as a source of food-borne Q fever in humans is controversial, because the results of different studies and investigations vary (Cerf & Condron, 2006). In an early epidemiological investigation conducted by Marmion and Stoker (1958), several sporadic Q fever cases were attributed to the consumption of unpasteurised milk. However another study in a penitentiary demonstrated that, while many of the inmates consuming only raw milk developed antibodies to *C. burnetii,* none developed clinical Q fever (Benson et al., 1963), while another involving volunteers who consumed raw milk, resulted in no clinical cases of Q fever or seroconversion in the study group (Krumbiegel & Wisniewski, 1970). More recently higher *C. burnetii* seroprevalence and Q fever incidence were reported in people consuming raw milk products, however these findings were subject to confounding bias, whereby people consuming raw milk may be more likely to be exposed to *C. burnetii* from ruminants due to residing in rural locations, or visiting farms to obtain their raw milk, therefore, infection via the aerosol route could not be discounted (Eldin et al., 2013). Currently, the commonly held view is that, while the risk of *C. burnetii* infection and the development of Q fever following ingestion of unpasteurised dairy products is not negligible, it is considered much lower in comparison to transmission via inhalation of aerosols from livestock contact and parturient products (Gale et al., 2015; World Organisation for Animal Health, 2018).

*1.3.2.2 The role of ticks in transmission*

There is little evidence to suggest that ticks are a major source of infection for humans. Cases of Q fever following a tick bite have been described, although rarely (Beaman & Hung, 1989; Graves et al., 2020). Tick-associated Q fever has also been reported in the absence of tick bite in a patient who crushed ticks between their fingers after removing them from clothing (Eklund et al., 1947). Because the patient did not report being bitten by a tick, the mechanism of infection proposed was via entry through a skin abrasion or wound. Given that *C. burnetii* infected ticks have been demonstrated to shed the organism in large numbers (up to $10^{10}$ organisms per gram) in their faeces (Philip, 1948), this mechanism of infection is feasible. Indeed, *Rickettsia prowazekii*, the agent of louse borne epidemic typhus is transmitted similarly (Körner et al., 2020). However, given that *C. burnetii* is an airborne pathogen, and that the aerosol route of infection is proposed to be the most efficient route of infection (Million & Raoult, 2015), transmission could have also occurred via inhalation of aerosolised *C. burnetii*-contaminated tick excreta (Körner et al., 2020).

*1.3.2.3 Person-to-person transmission*

Cases of person-to-person transmission of *C. burnetii* are documented, though rarely, and include cases in mortuary workers and doctors attending post-mortems of an infected cadaver (Harman, 1949), and Q fever in a patient following a bone marrow transplant (Kanfer et al., 1988). Sexual transmission in humans (Milazzo et al., 2001) has also been reported, which is supported by similar findings in mice (Kruszewska & Tylewska-Wierzbanowska, 1993). Pregnant women may shed *C. burnetii* in their breastmilk, placenta and vaginal secretions, and therefore potentially pose a transmission risk to other patients (Amit et al., 2014), medical staff (Deutsch & Peterson, 1950) and their unborn child (Fiset et al., 1975).

## 1.4 Disease manifestation and symptoms

### 1.4.1 Q fever in humans

The majority of *C. burnetii* infections in humans are asymptomatic. However, in some people, *C. burnetii* infection can manifest as a serious illness and produce long term health consequences regardless of the initial clinical presentation (**Figure 1.4**) (Eldin et al., 2017). Due to its non-specific clinical presentation, which renders Q fever indistinguishable from other febrile illnesses, Q fever is frequently misdiagnosed (Eastwood et al., 2018).

Confirmation of disease requires a high index of suspicion by medical practitioners and the use of specialised serological and molecular diagnostic assays, usually performed by reference laboratories. However, early and accurate diagnosis of Q fever is vital given the better outcomes achieved with the current recommended treatment for patients with acute symptomatic Q fever (doxycycline for 14 days) (Eastwood et al., 2018). This regime is effective at preventing severe long-term complications, providing treatment commences within the first three days of symptom onset (Anderson et al., 2013). As such, it is recommended that if a patient is suspected of having Q fever, appropriate empirical antimicrobial treatment should be administered immediately, rather than withholding treatment until diagnostic test results are received (Anderson et al., 2013).

#### *1.4.1.1 Acute Q fever*

Acute symptomatic Q fever is estimated to occur in approximately 50% of people infected with *C. burnetii*, with symptoms appearing after an incubation period of approximately 20 days, however the incubation period may vary depending on the inoculum dose (Anderson et al., 2013). The symptoms of acute Q fever manifest as a self-limiting influenza-like illness with an abrupt onset, characterised by high-grade fevers, chills, severe headache, fatigue and myalgia (Eastwood et al., 2018). Manifestations and severity of acute disease vary according to host factors including age, gender and immune status, as well as pathogen factors such as inoculum dose and infecting strain (Raoult et al., 2018). Fevers

may persist for between one and three weeks and may occur in conjunction with complications including hepatitis and pneumonia (Eldin et al., 2017; P. E. Fournier et al., 1998). In Australian Q fever patients, hepatitis appears to manifest more frequently than pneumonia (Graves & Islam, 2016), whereas pneumonia is a more common sequela among patients in Maritime Canada. Similarly, in French Guiana, a unique *C. burnetii* strain is responsible for high hospitalisation rates with Q fever pneumonia (Eldin et al., 2014; Million & Raoult, 2015).

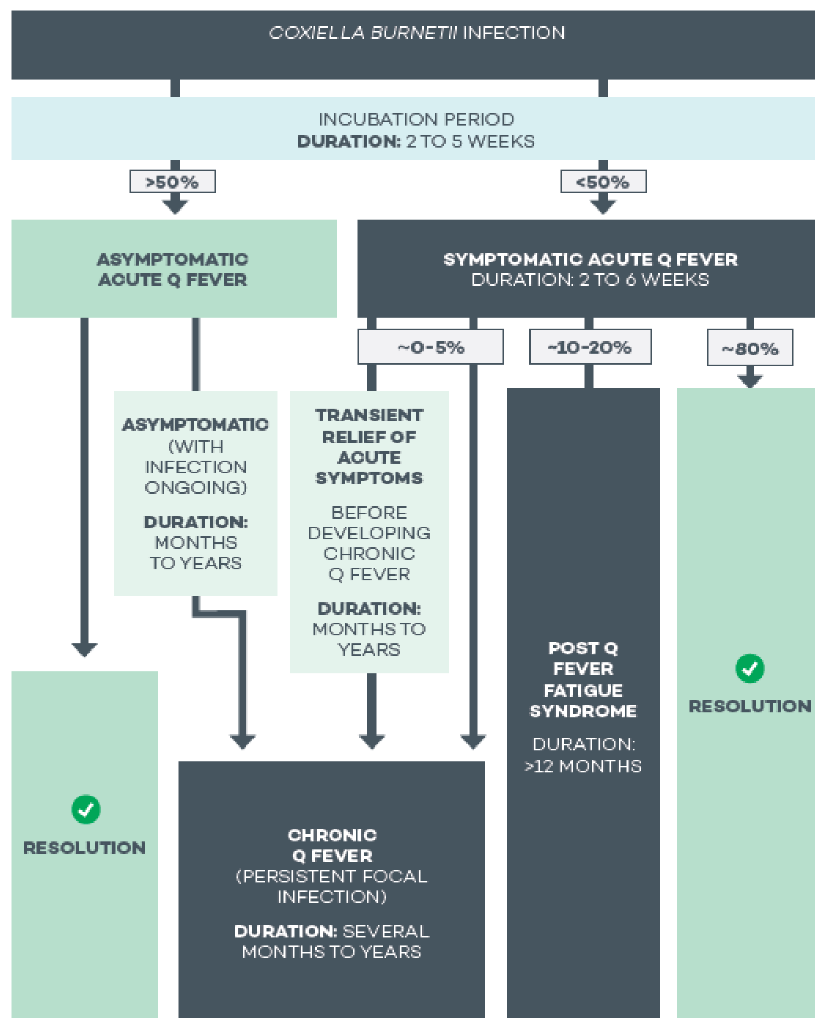


**Figure 1.4** Disease progression pathways following human *Coxiella burnetii* infection (Seqirus, 2021).

*1.4.1.2 Persistent focal infection*

Persistent focal infections (PFI) of *C. burnetii*, which may develop in Q fever patients following symptomatic or asymptomatic infection, are attributable to the persistent focus of *C. burnetii* bacteria at one or more anatomical locations (Eldin et al., 2017). Previously patients who developed PFI were classified as having chronic Q fever, however, in some instances, the diagnosis of 'chronic Q fever' occurred in the absence of a clear focus of infection, therefore the term PFI is now preferred, as it more accurately describes the clinical manifestation of the disease in the patient (Eldin et al., 2017). It is estimated that up to 5% of medically diagnosed Q fever cases will develop a PFI which is more likely in people who are immunocompromised or have underlying valvular or vascular disease (Wielders et al., 2013). The most common and serious manifestation described among patients with PFI is Q fever endocarditis (Angelakis & Raoult, 2010), which may develop insidiously, years after acute disease (P. E. Fournier et al., 1998). People at greatest risk of developing endocarditis are males, >40 years of age and those with pre-existing valvular disease or who are immunocompromised (Brouqui et al., 1993; Raoult et al., 1992). Diagnosis of Q fever endocarditis is often delayed due to non-specific clinical symptoms, and the fact that *C. burnetii* does not grow on media utilised routinely; with the diagnostic delay resulting in an increased mortality rate (Fenollar et al., 2001). Other less common clinical manifestations of PFI include vascular infection (Wegdam-Blans et al., 2011), osteomyelitis (Merhej et al., 2012) and non-Hodgkin's lymphoma (Melenotte et al., 2016). Persistent infection with *C. burnetii* is associated with high mortality and morbidity, therefore early detection and treatment is essential for the best prognostic outcomes. Serological follow up following acute *C. burnetii* infection is recommended to monitor for progression to a persistently infected form (Wielders et al., 2013).

*1.4.1.3 Q fever fatigue syndrome*

Post Q fever fatigue syndrome (QFS) is a clinical manifestation of Q fever characterised by a state of prolonged and debilitating fatigue, that may occur following asymptomatic or symptomatic infection. Symptoms of QFS include persistent fatigue, alcohol intolerance, breathlessness and increased sweating (Marmion et al., 1996). Although QFS was first documented in Australian abattoir workers,

and was originally thought to be specific to Australia, it was later described in Q fever patients from Canada (Hatchette et al., 2003), the United Kingdom (Wildman et al., 2002) and the Netherlands (Morroy et al., 2011), and is now an internationally recognised sequela of *C. burnetii* infection. It is estimated that up to 20% of acute Q fever cases, will develop QFS (Centres for Disease Control and Prevention, 2013), however the pathogenesis of QFS is poorly understood (Raijmakers et al., 2019). One hypothesis is that it is due to the chronic stimulation of the immune system by persistent *C. burnetii* antigens or non-viable *C. burnetii* cells which results in cytokine dysregulation (Marmion et al., 2009; Penttila et al., 1998). The only risk factor so far identified as being associated with the development of QFS, is the severity of the initial infection (Hickie et al., 2006) and, to date, there is no effective and proven treatment strategy for QFS (Raijmakers et al., 2019). Studies assessing the efficacy of antimicrobial treatment for QFS have shown conflicting results (Arashima et al., 2004; Iwakami et al., 2005; Ledina et al., 2007), however ongoing cognitive behaviour therapy has recently been recommended as a promising treatment modality (Raijmakers et al., 2019).

*1.4.1.4 Q fever during pregnancy*

The outcomes resulting from *C. burnetii* infection in pregnant women vary and may be dependent on geographical region (Angelakis et al., 2013). Pregnant women infected with *C. burnetii* are significantly more likely to present as asymptomatic in up to 90% of cases in some regions despite serology suggestive of recent infection (Tissot-Dupont et al., 2007). However, both symptomatic and asymptomatic infection during pregnancy has also been shown to result in severe adverse outcomes including miscarriage, intrauterine growth retardation, intrauterine foetal death and preterm delivery (Carcopino et al., 2007; Million et al., 2014; Nielsen et al., 2014; Raoult et al., 2002). In those women with adverse outcomes, the manifestation appears to depend on the trimester in which *C. burnetii* infection occurs. Women who become infected during their first trimester are more likely to experience miscarriage (Million et al., 2014), while pre-term delivery or intrauterine foetal demise are more likely outcomes in women who become infected during the later stages of their pregnancy (Carcopino et al., 2007; Ghanem-Zoubi & Paul, 2020). In some studies, women who become infected

with *C. burnetii* during pregnancy are reported to be at increased risk of developing persistent infections (Carcopino et al., 2009) and recrudescent infections during subsequent pregnancies. Angelakis et al. (2013) found that the placentas of infected women who experienced abortion, more frequently contained *C. burnetii* strains harbouring the QpDV plasmid. This suggests that obstetrical morbidity may be influenced by the genotype of the infecting strain, which in turn may be related to geographical region given that many genotypes have apparent limited geographical distribution (Glazunova et al., 2005; Million & Raoult, 2015).

### 1.4.2 Coxiellosis in animals

*Coxiella burnetii* infection in animals is known as coxiellosis and, in contrast to humans, infected animals remain mostly asymptomatic (Maurin & Raoult, 1999). However *C*. *burnetii* can be associated with reproductive disorders in animals with manifestations including abortion, still birth, weak offspring and placentitis demonstrated in ruminants (cattle, sheep, goats, deer) (Maurin & Raoult, 1999) (Agerholm, 2013) (Z. Kreizinger et al., 2015) and other mammals such as cats (Kopecny et al., 2013), dogs (Buhariwalla et al., 1996), fur seals (Kersh et al., 2012) and sea lions (Duncan et al., 2012).

Coxiellosis has been well described in experimentally infected laboratory animals. Guinea pigs intraperitoneally or intranasally infected with *C. burnetii* become febrile and develop mononuclear cell granulomas in their bone marrow, liver, spleen, kidney and lungs (Lillie, 1942). Guinea pigs typically recover from infection without sequelae however, as in humans, they may remain latently infected (Maurin & Raoult, 1999). Similar to guinea pigs, mice infected intraperitoneally or intranasally with *C. burnetii* develop granulomas in their organs (Perrin & Bengtson, 1942). However in contrast to guinea pigs, mice do not become febrile, remain mostly asymptomatic and infection in these species may persist for months with prolonged shedding of *C. burnetii* in faeces and urine (Oyston & Davies, 2011). The presence of *C. burnetii* has also been demonstrated in the liver, kidney, lung, heart, spleen and testes of cynomolgus monkeys infected via the aerosol route (Gonder et al., 1979).

## 1.5 Detection of *Coxiella burnetii* and Q fever diagnosis

### 1.5.1 Culture

*Coxiella burnetii* is designated a "Category B pathogen" requiring biosafety level 3 facilities for its isolation due to its extreme infectivity, therefore isolation of *C. burnetii* is not routinely used for the diagnosis of Q fever. If required, isolation of *C. burnetii* from clinical specimens such as heart valves is mostly undertaken by authorised reference laboratories (P.-E. Fournier et al., 1998). *Coxiella burnetii* is also listed as a Category A infectious substance due to its ability to cause permanent disability, or life-threatening or fatal disease in otherwise healthy humans or animals following exposure (World Health Organisation, 2021).

*Coxiella burnetii* is unable to be cultured using conventional microbiological methods due to its intracellular lifestyle. Isolation of *C. burnetii* can be achieved for a variety of sample types via inoculation of specimens onto Vero cells (grown using conventional cell culture) or into embryonated eggs (Ormsbee, 1952) or laboratory animals such as guinea pigs and mice (Williams et al., 1986). Historically, isolation of *C. burnetii* was performed in guinea pigs, and although not widely used in modern times, guinea pig or mouse passage represents an effective way of isolating *C. burnetii* from samples contaminated with other bacteria, with the spleen being the most useful target organ for the recovery of *C. burnetii* (Maurin & Raoult, 1999). A major achievement for *C. burnetii* research was the development of the acidified citrate cysteine medium enabling cell-free growth of *C. burnetii*. With its low pH (4.75) and oxygen tension (2.5%) this axenic growth medium mimics the conditions in the parasitophorous vacuole and supports vigorous growth of *C. burnetii* (Omsland, 2012).

### 1.5.2 Serology

Serological diagnosis of acute Q fever in humans relies on the detection of *C. burnetii* antibodies which typically begin to appear 7-15 days after symptom onset (Maurin & Raoult, 1999). As discussed in section 1.2.4, Phase 1 and Phase 2 *C. burnetii* antigens appear in a characteristic temporal pattern (Figure 1.5) enabling the differentiation between acute and chronic forms of Q fever in humans.

Antibodies to Phase 2 *C. burnetii* appear first and are associated with acute infection whereas Phase 1 antibodies appear later and are associated with chronic disease (Dupont et al., 1994).

The serological pattern of antibody production in animals is less well understood. The evolution of *C. burnetii* infection in guinea pigs however is similar to that of humans. Bacteraemia is evident for 5-7 days post *C. burnetii* challenge, and serum antibodies to Phase 2 and Phase 1 *C. burnetii* antigens appear 15 and 21 days post infection respectively (Kishimoto & Burger, 1977). In a study in goats, a strong Phase 2 antibody response appeared around 14 days post infection while the Phase 1 antibody appeared later and was less pronounced (Roest, Post, et al., 2013). While little is known about the kinetics of antibody response following *C. burnetii* infection in animals these findings suggest they may be similar between species.

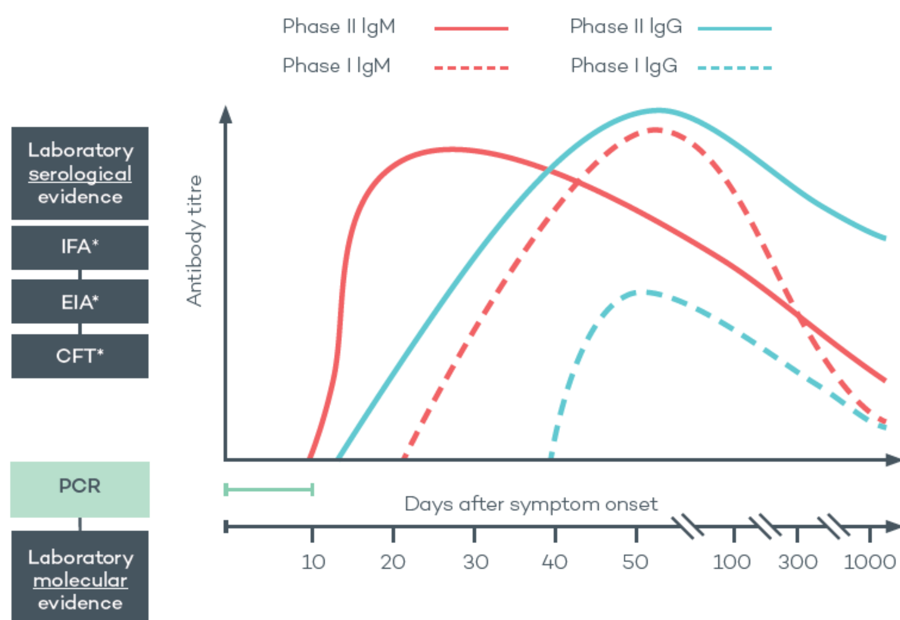


**Figure 1.5** Typical human serological and molecular response to *Coxiella burnetii* infection (Seqirus, 2021).

The current reference method for the serological diagnosis of Q fever in humans is the immunofluorescence assay IFA (Schneeberger et al., 2010). The IFA is highly sensitive and can

differentiate between different antibody classes and Phase variants. Diagnosis is performed by testing acute and convalescent serum (taken around 3-4 weeks apart) and is confirmed by seroconversion, or a four-fold increase in antibody titre using paired serum samples (Raoult et al., 2000). Although IFA is considered highly specific, samples collected during early infection may produce negative results due to the lag in antibody response. Although IFA is the 'gold standard' for Q fever serodiagnosis, enzyme-linked immunosorbent assay (ELISA) is a useful alternative for screening large numbers of samples such as in an outbreak setting (Jager et al., 2011).

Confirmation of exposure to *C. burnetii* in animals can also be achieved using serological methods. Although the complement fixation test (CFT) was the previous reference test as per the World Organisation for Animal Health, it is no longer recommended due to its poor sensitivity compared to ELISA and IFA (World Organisation for Animal Health, 2018). The ELISA is the preferred method for ruminants (Kittelberger et al., 2009) as it has comparatively high sensitivity and good specificity, and is convenient for screening large numbers of animals (World Organisation for Animal Health, 2018). The IFA may also be used in animals and has been demonstrated to have superior sensitivity to ELISA in some species including goats (Muleme et al., 2016), cats (Shapiro et al., 2015) and alpacas (Tellis et al., 2022). However, IFA is labour intensive and requires technical expertise, and is therefore unsuitable for screening large numbers of samples (Sahu et al., 2020).

### 1.5.3 Molecular detection via polymerase chain reaction

Several PCR-based methodologies (conventional, nested and quantitative PCR [qPCR]) have been utilised to detect *C. burnetii* DNA in a variety of sample types for research and diagnostic purposes. These samples include clinical specimens (e.g. necropsy tissues, vaginal swabs, faeces, blood, serum and milk), processed foods (e.g. cheese and pasteurised milk) and environmental samples (e.g. air, dust and soil) (Van den Brom et al., 2015). These PCR assays target a variety of single copy genes including those encoding the heat shock protein *GroEL/htp*AB (Bond et al., 2016), the metabolic enzyme, isocitrate dehydrogenase (*icd*) (Klee et al., 2006), and *com1*, the gene encoding a 27-kDa

outer membrane protein (de Bruin et al., 2011). Assays targeting the multicopy transposase gene of the insertion element, IS*1111*, are widely used as they offer the advantage of increased sensitivity compared to single copy gene targets. However, this gene's demonstrated variation of 7-110 copies across different *C. burnetii* strains, means that the IS*1111* gene target is not recommended for quantitative PCR (qPCR) (Klee et al., 2006). Furthermore, the presence of IS*1111* has recently been confirmed in CLE of ticks, representing a potential lack of specificity for this gene target (Duron, 2015). Therefore, findings from studies that have relied solely on IS*1111* for the detection of *C. burnetii* DNA should be interpreted with this limitation in mind. While well-designed qPCR assays can confirm the presence of *C. burnetii* in samples, they are unable to determine the infectious potential of the sample, due to their inability to differentiate between viable and non-viable bacteria. A study by Mori et al. (2013) reported a qPCR methodology incorporating the live/dead stain ethidium monoazide (EMA) which circumvents this issue. The EMA stain only penetrates dead cells with compromised cell walls and DNA which is bound to EMA cannot be amplified via PCR, therefore only DNA from live cells will be detected (Rudi et al., 2005).

## 1.6 Prevention and control

### 1.6.1 Q fever Vaccination

#### *1.6.1.1 Q-Vax® and Q fever vaccination*

Currently, Australia is the only country with a licenced human Q fever vaccine (Q-Vax®; Seqirus, Parkville, VIC). Q-Vax® is a whole-cell formalin-inactivated vaccine produced using purified Phase 1 *C. burnetii* Henzerling strain (Seqirus, 2019). Clinical trials for Q-Vax® were conducted between 1981 and 1988, whereby the vaccine was trialled in over 4,000 South Australian abattoir workers and was licenced for use in Australia in 1989 (Marmion et al., 1990).

Due to the risk of hypersensitivity reactions in people who are already sensitised to *C. burnetii* antigen, the administration of Q-Vax® is contraindicated in individuals who have a history of medically diagnosed Q fever; or have experienced a Q fever-like illness following potential exposure to *C.*

*burnetii;* or who have previously received the Q fever vaccine (Marmion et al., 1990; Seqirus, 2021). Potential vaccinees must undergo stringent pre-vaccination screening to detect pre-existing immunity to *C. burnetii*. This involves serological testing (measurement of IgG antibodies to Phase 2 *C. burnetii* antigen), an intradermal Q-Vax® Skin Test (Seqirus, 2019) and the collection of the patient's history by the vaccine provider to identify potential exposure events (Seqirus, 2021). For the interpretation of the pre-vaccination screening results, patients must attend a follow-up appointment seven days after receiving the intradermal skin test, and any patient returning a positive skin test and/or identified as being serologically positive for *C. burnetii* should not be vaccinated (Seqirus, 2021). Currently, Q fever vaccination (QFV) is not recommended for persons who are immunocompromised, pregnant or under 15 years of age, as its safety and efficacy are yet to be established in these populations (Seqirus, 2021).

#### *1.6.1.2 Efficacy of Q-Vax®*

Q-Vax® is highly effective in preventing Q fever with a review based on several Australian studies of vaccinated abattoir workers reporting a protective efficacy ranging from 83 to 100% (Bond et al., 2017; Chiu & Durrheim, 2007; Woldeyohannes et al., 2020). The high efficacy of this vaccine is attributed to the ability of Q-Vax® to stimulate a long-term T-cell memory response (Kersh, Fitzpatrick, Self, Biggerstaff, et al., 2013; Marmion et al., 1990). While the duration of protective immunity afforded by Q-Vax® is not known, it is estimated to be at least five years in vaccinated abattoir workers, however, it remains unclear whether this immunity was due solely to the vaccine *per se*, or a combined effect of the vaccine, and boosted immunity due to environmental exposure to *C. burnetii* infected animals (Ackland et al., 1994; Kersh, Fitzpatrick, Self, Biggerstaff, et al., 2013).

#### *1.6.1.3 Safety of Q-Vax®*

Local and systemic adverse events have been reported following Q-Vax® immunisation in people who were negative in pre-vaccination screening (AEFI) (Gidding et al., 2009; Marmion et al., 1990; Schoffelen et al., 2014; Sellens, Bosward, et al., 2018). An injection site reaction, characterised by pain

or swelling is the most frequently reported local AEFI, whilst systemic reactions to QFV may include fever, headache and arthralgia. However AEFI are mostly non-severe, few require medical attention and no deaths have been reported as a result of vaccine administration (Therapeutic Goods Administration Database of Adverse Event Notifications—Medicines, 2022).

*1.6.1.4 Groups for whom Q-Vax® is recommended*

Due to the airborne transmission of *C. burnetii* and its prolonged survival in the environment, vaccination is regarded as the highest order risk control measure for the prevention of Q fever (Australian Technical Advisory Group on Immunisation, 2021; Safe Work NSW, 2022; WorkSafe QLD, 2022). The Australian Technical Advisory Group on Immunisation (AGATI) currently recommend QFV for high-risk occupational groups including meat and livestock industry workers, professional dog and cat breeders and veterinary personnel (Australian Technical Advisory Group on Immunisation, 2021). In 2018, these recommendations were extended to wildlife and zoo workers who have contact with kangaroos and bandicoots, and people who cull or process kangaroos (Australian Technical Advisory Group on Immunisation, 2021). Despite these recommendations, and the availability of Q-Vax®, low levels of vaccine uptake have been reported for at-risk groups including veterinary nurses (29%) (Sellens et al., 2016), farmers (18-43%) (Gidding et al., 2009), goat producers (17%) (Gunther et al., 2019) and cat breeders (2%) (Shapiro, Norris, et al., 2017).

### 1.6.2 Biosecurity and Q fever prevention

Biosecurity guidelines are available for specific cohorts including the Guidelines for Veterinary Personal Biosecurity (Australian Veterinary Association, 2017) issued by the Australian Veterinary Association, and The National Biosecurity Guidelines issued by Wildlife Health Australia. The latter recommend that when interacting with wildlife, basic biosecurity practices should be adopted at all times regardless of the perceived risks (Wildlife Health Australia, 2018). Basic biosecurity practices include, but are not limited to, regular hand washing, the appropriate management of laundry and use of personal protective equipment (PPE) such as disposable gloves and coveralls. The main

biosecurity practices listed specifically for Q fever in the guidelines are ventilation controls, dust management, P2/N95 face mask and QFV. Although wearing a P2/N95 face mask may reduce the risk of airborne *C. burnetii* transmission, vaccination remains the most effective means of preventing Q fever in humans given the long survival time of *Coxiella* in the air and environment (New South Wales Health, 2019).

## 1.7 Q fever in Australia

### 1.7.1 Q fever in humans

#### *1.7.1.1 Notifications*

Q fever has been a notifiable disease in all Australian states and territories since 1977 (Garner et al., 1997), and with around 400-500 cases notified annually, it is the most commonly notified non-foodborne zoonosis in Australia (National Notifiable Diseases Surveillance System, 2021). Q fever notification rates in Australia are also one of the highest reported globally. For example, in 2019 the national notification rate of 2.2 cases per 100,000 population (National Notifiable Diseases Surveillance System, 2021) was approximately seven and ten times higher than notification rates in the United States of America (0.23 cases per 100,000 population) (Centres for Disease Control and Prevention, 2021) and European countries (0.18 cases per 100,000 population) (European Centre for Disease Prevention and Control, 2021). However the Q fever notification data are likely to underestimate the true disease prevalence, due to many *C. burnetii* infections being asymptomatic and the non-specific nature of disease symptoms resulting in under and misdiagnosis (Garner et al., 1997; Gidding et al., 2019; Tozer et al., 2011).

Cases of Q fever are notifiable in all Australian states and territories, however the eastern states of New South Wales (NSW) and Queensland (QLD) account for the majority of annual Q fever notifications, reflecting, in part, the intensity of cattle, sheep and goat farming and associated industries in these states (Table 1.2 and Figure 1.7) (Seqirus, 2021). Analysis of the 1991-2014 national Q fever notification data by Sloan-Gardner et al. (2017) revealed that there were 12,164

notified cases of Q fever in Australia during that period. The states of QLD and NSW collectively accounted for 87% of notifications with the rate in QLD (6.26 per 100,000 population) being almost twice as high as NSW (3.07 per 100,000 population). In comparison, notification rates in other Australian jurisdictions were much lower over the same period (**Table 1.2**).

**Table 1.2** Counts, percentages and rates of Q fever notification by Australian jurisdiction, 1991-2014. Adapted from (Sloan-Gardner et al., 2017).

| Jurisdiction | n | % | Rate/100 000 |
|---|---|---|---|
| Queensland | 5730 | 47 | 6·26 |
| New South Wales | 4893 | 40 | 3·07 |
| Victoria | 837 | 7 | 0·70 |
| South Australia | 391 | 3 | 1·06 |
| Western Australia | 269 | 2 | 0·56 |
| Northern Territory | 27 | <1 | 0·56 |
| Australian Capital Territory | 14 | <1 | 0·13 |
| Tasmania | 3 | <1 | 0·05 |

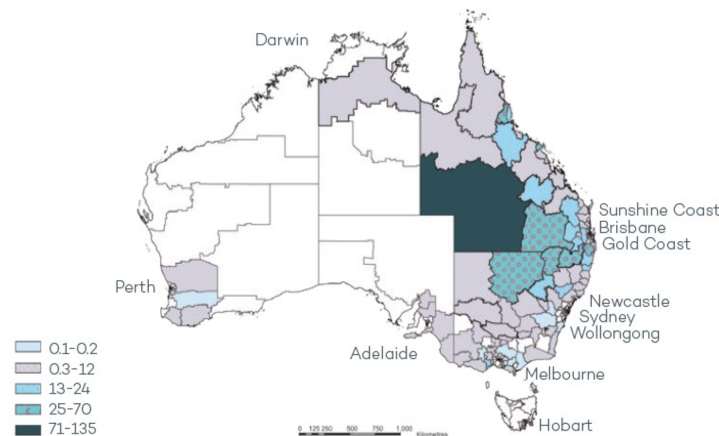


**Figure 1.6** Q fever notification rate by geographic area (cases per 100 000) in Australia for 2015 (Seqirus, 2021).

The majority of Q fever notifications in Australia are in adult males working in traditional high risk industries such as abattoir and meat processing, farming, shearing, and livestock transportation (Communicable Diseases Network Australia, 2018; Eastwood et al., 2018; Garner et al., 1997; Lowbridge et al., 2012; Sloan-Gardner et al., 2017), with slaughtering and assisting with animals births considered particularly high risk activities (Beech et al., 1962; Sloan-Gardner et al., 2017).

To increase Q fever vaccine uptake in high-risk occupational groups, the federal government funded National Q fever Management Program (NQFMP) was implemented between 2001 -2006. The NQFMP provided subsidised vaccinations for persons in high-risk occupations; initially targeting those working in, or associated with, abattoirs, and shearers; but was later expanded to include dairy, sheep, and beef cattle farmers as well as their family members and employees (Gidding et al., 2009). During the NQFMP, Australia's national Q fever notification rate declined by over 50% from 4.0 cases per 100,000 population in 2001 to 1.7 cases per 100,000 in 2006 (**Figure 1.6**). The number of notifications continued to decline following the cessation of the NQFMP to the lowest rate on record of 1.4 cases per 100,000 population in 2009. However since then, Australia's national Q fever notification rate has been steadily increasing and was recorded as 2.3 per 100,000 population in 2021 (National Notifiable Diseases Surveillance System, 2021).

Following the introduction of the NQFMP, the proportion of Q fever cases in traditional at-risk groups including abattoir workers has declined (Lowbridge et al., 2012; Massey et al., 2009). In a review of Q fever notifications in NSW and QLD for the period 2007-2013, where data on occupation was recorded, 64% of cases were associated with traditional high-risk occupations, however 36% of cases worked in occupations that posed no known risk of exposure to *C. burnetii* (Sloan-Gardner et al., 2017). Similarly of the Q fever notifications in QLD in the period 2013-2017, 51% of cases we not considered to be occupationally at-risk (Tozer et al., 2020). Another study identified that 22% of interviewees with medically diagnosed Q fever did not live on farms or in rural areas, and 26% of patients reported exposure to kangaroos and wallabies although many of these cases reported multiple co-exposures to a variety of animal species including cattle (81%) and sheep (38%) (Massey et al., 2009). While occupation remains an important risk factor for Q fever this finding highlights the changing epidemiology of Q fever and suggests that occupation alone is not a reliable predictor for *C. burnetii* infection.

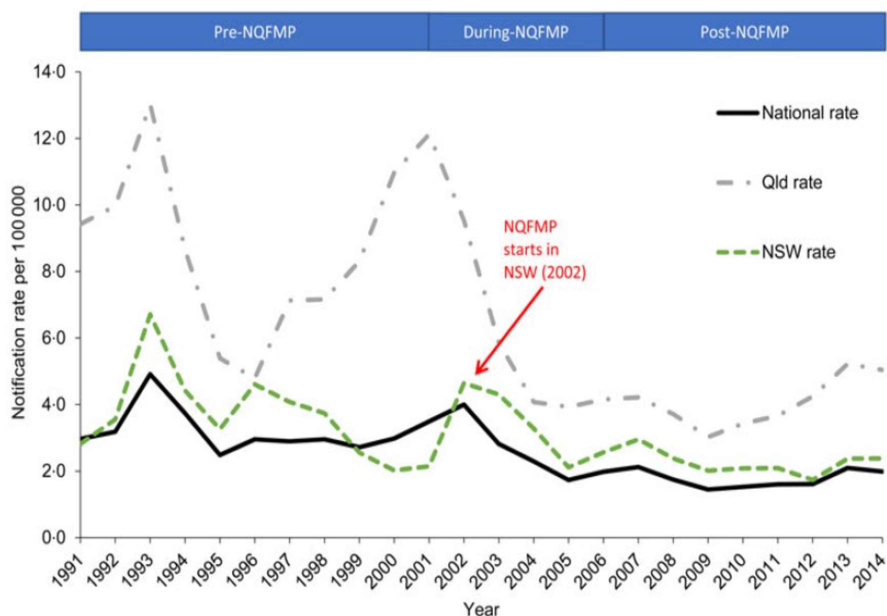


Figure 1.7 Q fever national notification rates for Australia, Queensland and New South Wales by year, 1991 2014 (Sloan-Gardner et al., 2017).

*1.7.1.2 Seroprevalence studies*

Several serological studies (which utilised samples opportunistically obtained from serum banks), have reported *C. burnetii* seroprevalence in the general Australian population ranging from 5.0-7.4%, (Gidding et al., 2020; Islam et al., 2011; Parker et al., 2010; Tozer et al., 2011). Islam et al. (2011), estimated an overall seroprevalence of 7.4% in the Hunter New England region of NSW, and Gidding et al. (2020), reported a nationwide *C. burnetii* seroprevalence of 5.6%. A seroprevalence study in QLD demonstrated a similar *C. burnetii* seroprevalence in metropolitan Brisbane compared to the non-metropolitan remote/rural area (5.0% and 5.3% respectively) where exposure to traditional sources of *C. burnetii* infection would have been expected to be more common (Tozer et al., 2011). However the abovementioned studies used sera from pathology laboratories that was surplus to requirement for diagnostic workup and so may have represented people who were more likely to be unwell. Therefore, the 3.6% *C. burnetii* seroprevalence determined in Red Cross blood donors from NSW and QLD (Gidding et al., 2019) may offer a closer approximation of the *C. burnetii* seroprevalence in the general Australian population than seroprevalence based on serum banks, because blood donors

must be of good general health and meet specific eligibility criteria to donate blood (Australian Red Cross Blood Service, 2019).

Seroprevalence studies on at-risk populations in Australia are scarce. Studies conducted in the early 2000s using pre-vaccination skin test results as a proxy for exposure, estimated *C. burnetii* seroprevalence between 4.2% and 21.2% in agricultural and livestock industry workers (Parker et al., 2010). More recently a seroprevalence of 19% was reported for a cohort of unvaccinated Australian veterinary workers, where increased exposure to ruminants was identified as a significant risk factor for seropositivity (Sellens et al., 2020). The seroprevalence in other at-risk occupational groups including professional dog and cat breeders, animal refuge workers, and wildlife and zoo workers remains unstudied (Australian Technical Advisory Group on Immunisation, 2021). However, evidence of *C. burnetii* exposure has been documented in a survey of Australian cat breeders, whereby medically diagnosed Q fever was self-reported by 6% of participants (Shapiro, Norris, et al., 2017).

### 1.7.2 Q fever and contact with Australian wildlife

Over the past decade contact with Australian wildlife has been suggested as a risk factor for contracting Q fever and, in 2017, wildlife and zoo workers were added to The Australian Immunisation Handbook as an at-risk population for whom QFV is recommended, with kangaroos particularly mentioned as high-risk animals (Australian Technical Advisory Group on Immunisation, 2021). This addition was, in part, based on studies on Australian wildlife themselves, demonstrating serological exposure to, and molecular evidence of, *C. burnetii* in kangaroos and other wildlife species discussed in section 1.8.4. Furthermore, there are also several case reports where prior macropod exposure has been mentioned or identified in investigations of cases of clinical disease, and considered a possible source of infection. One such case is the report of life-threatening Q fever resulting in multiorgan failure described in a park ranger in Central Queensland who had ongoing exposure to kangaroo and wallaby carcases as part of their occupation. In this case the source of *C. burnetii* infection could not be determined, and, although macropod exposure was suspected as the potential infection source,

given that the case occurred in an area of high Q fever notifications, and that the patient also lived approximately 2km from a paddock harbouring cattle, transmission via other traditional sources of infection could not be ruled out (Stevenson et al., 2015). Another two Q fever cases with atypical risk exposures were reported in northern NSW (Flint et al., 2016). Traditional exposure sources were not identified for these cases, and both patients (from different workplace locations) reported working outdoors in areas inhabited by kangaroos, mowing lawns contaminated with kangaroo faeces and, during their presumed incubation period, both had handled joeys. However, serological testing and intradermal skin tests of co-workers were negative and tick specimens and kangaroo tissue samples tested negative for *C. burnetii* DNA via PCR.

In a surveillance report on Q fever notifications in NSW during the period 2011-2015, the most common exposures noted for indirect exposure to wildlife were mowing areas contaminated with faeces from native animals, and contact with macropods and their faeces (Clutterbuck et al., 2018). Another surveillance report on Q fever notifications in QLD identified that, of the 1,170 cases of Q fever recorded during the period 2013-2017, just over half (50.2%; 587/1170) reported contact with Australian wildlife in the month prior to disease onset, the majority (71.2%; 418/587) of whom had contact with kangaroos (Tozer et al., 2020).

A Q fever outbreak investigation in Lightning Ridge in rural northern NSW identified that very few cases worked in high-risk occupations; most reported no high-risk exposure activities during their incubation period, however a significant proportion had been exposed to ticks and had sighted kangaroos on their property (Archer et al., 2017). Similarly, exposure to macropods was identified as a risk factor in Q fever patients in an endemic region of North QLD with 42.9% of patients reporting contact with macropods, and 69.8% observing macropods near, or on their property (Sivabalan et al., 2017).

### 1.7.3 Potential wildlife reservoirs for *Coxiella burnetii*

The search for possible wildlife reservoirs for *C. burnetii* began shortly after the discovery of the pathogen, with bandicoots (*Isoodon torosus*) being the first Australian mammals to come under investigation. Derrick et al. (1939) successfully recovered *C. burnetii* from the organs of experimentally infected bandicoots demonstrating that bandicoots are susceptible to infection. Derrick also demonstrated that sera from captured bandicoots were seroreactive to *C. burnetii* antigen via CFT, providing evidence of a natural infection cycle, however his attempts to isolate *C. burnetii* from naturally infected bandicoots were unsuccessful.

More recent serosurveys involving Western barred bandicoots (*Perameles bougainville*) from Bernier and Dorre Islands in WA, and the Northern bandicoot (*Isoodon torosus*) from QLD, have reported 8.6% (Bennett et al., 2011) and 23.9% (Cooper, Goullet, et al., 2012) seropositivity respectively to *C. burnetii* using ELISA. In addition, the former study also detected *C. burnetii* DNA via qPCR targeting the IS*1111* gene in bandicoot faeces and a *Haemaphysalis humerosa* tick parasitising a bandicoot at 3.7 and 2.7 genome equivalents/µL respectively. Sequence comparison of *com1* PCR products generated from the bandicoot faecal sample showed high similarity (>99%) to the Dugway strain of *C. burnetii.* Phylogenetically the Dugway strain of *C. burnetii* represents an evolutionarily distinct genomic group which has not undergone genome reduction - a process that is associated with pathogenicity in other strains of *C. burnetii* (Beare et al., 2009). Dugway-like *C. burnetii* strains are yet to be isolated from animals or human Q fever cases (Arricau-Bouvery & Rodolakis, 2005), suggesting that they are less likely to cause pathology in animals and human hosts.

The possibility that kangaroos are potential reservoirs for *C. burnetii* was first suggested by Pope et al. (1960) who demonstrated evidence of *C. burnetii* infection in kangaroos via CFT and isolated the organism from the blood of an infected kangaroo. The discovery of *C. burnetii* in kangaroo ticks (*Amblyomma triguttatum*) lead to speculation that kangaroos are reservoirs of *C. burnetii* and that a sylvatic kangaroo-tick cycle existed.

More recent seroprevalence studies lent support to the belief that kangaroos, and other wildlife species are reservoirs for *C. burnetii*. Seroprevalence studies in Western grey kangaroos (WGK) (*Macropus fuliginosus*) have reported *C. burnetii* seroprevalence ranging from 24-33% using ELISA (Banazis et al., 2010; Potter et al., 2011). Seroprevalence of 20.8% was documented in macropod species from WA and QLD (Cooper, Barnes, et al., 2012). Multispecies serological studies have found evidence of *C. burnetii* exposure in a range of wildlife species from QLD including Eastern grey kangaroos (EGK) (Cooper, Goullet, et al., 2012; Cooper et al., 2013). The first two abovementioned studies (Banazis et al., 2010; Potter et al., 2011), used pooled low and high reacting serum in the same ELISA for negative and positive controls respectively, while the ELISA utilised in the multispecies studies was optimised using sera obtained from naive and *C. burnetii* infected dogs and mice (Cooper, Barnes, et al., 2012; Cooper, Goullet, et al., 2012). In these instances, the use of species specific positive and negative controls validated in other serological assays or via other serological methodologies would have added greater weight to the validity of the results by further ruling out the possibility of cross-reactivity.

Molecular evidence of *C. burnetii* DNA has also been demonstrated in several species of Australian wildlife from QLD and Western Australia (WA) (**Table 1.4**). Banazis et al. (2010) and Potter et al. (2011) detected *C. burnetii* DNA via qPCR targeting the IS*1111* gene in 12.3% and 4.1% of faecal samples respectively from Western Grey Kangaroos (WGK). Although the presence of *C. burnetii* DNA with high homology to the Dugway strain (mentioned above) in kangaroo faeces was confirmed by DNA sequencing, attempts to isolate *C. burnetii* from faecal samples were unsuccessful. The authors of these studies acknowledge that it was unclear whether the detection of *C. burnetii* DNA in kangaroo faeces reflected true bacterial shedding from the gastrointestinal tract (GIT), and therefore infection in the kangaroo, or passage of the organism through the GIT following ingestion of pasture contaminated by either other kangaroos or co-grazing ruminants. *Coxiella burnetii* DNA has also been detected in a range of other Australian wildlife species including koalas, where it was detected in

blood, urine and faeces, (Tozer et al., 2014) and possums where it was identified in blood (Cooper et al., 2013).

Table 1.3 Studies reporting serological evidence of *Coxiella burnetii* exposure in Australian native wildlife. Scientific names of species are listed below table.

| Australian State | Species | Sample number (n) | Seroprevalence (%) | Comments | Reference |
|---|---|---|---|---|---|
| Western Australia | Western grey kangaroo | 343 | 33.5 | Utilised pooled low and high reacting serum for negative and positive controls respectively | (Banazis et al., 2010) |
| | | 1017 | 24.0 | | (Potter et al., 2011) |
| | Western barred bandicoot | 35 | 8.6 | | (Bennett et al., 2011) |
| Queensland and Western Australia | Various macropod species | 500 | 20.8 | ELISA was optimised using sera obtained from naive and *C. burnetii* infected dogs and mice | (Cooper, Barnes, et al., 2012) |
| Queensland | Brushtail possum | 56 | 10.7 | | (Cooper, Goullet, et al., 2012) |
| | Northern bandicoot | 46 | 23.9 | | |
| | Northern bandicoot | 35 | 31.4 | | (Cooper et al., 2013) |
| | Eastern grey kangaroo | 17 | 41.1 | | |
| | Agile Wallaby | 5 | 60.0 | | |
| | Red kangaroo | 4 | - | | |
| | Common wallaroo | 3 | 66.2 | | |
| | Brushtail possum | 2 | - | | |
| | Rufous bettong | 1 | - | | |
| | Blacked-striped wallaby | 1 | - | | |

Western grey kangaroo (*Macropus fuliginosus*), Western barred bandicoot (*Perameles bougainville*), macropod species (*Macropus* spp.), Brushtail possum (*Trichosurus vulpecula*), Northern bandicoot (*Isoodon macrourus*), Eastern grey kangaroo (*Macropus giganteus),* Agile Wallaby (*Macropus agilis*), Red kangaroo (*Macropus rufus*), Common wallaroo (*Macropus robustus*), Rufous bettong (*Aepyprymnus rufescens*), Blacked-striped wallaby (*Macropus dorsalis*). ELISA- enzyme-linked immunosorbent assay

Additional evidence of *C. burnetii* infection in kangaroos was recently reported by Shapiro et al. (2020) in a study investigating for the presence of *C. burnetii* DNA in raw meat containing kangaroo sold for pet consumption. In this study, 29% of the packets tested positive for *C. burnetii* via qPCR (using IS*1111*, *com1* and *htp*AB gene targets) with DNA most commonly detected in offal samples (Shapiro et al., 2020). Genotypes recovered from the samples aligned to genotypes recovered from human Q fever patients. While this finding suggests the existence of a sylvatic cycle in kangaroos and that

kangaroos may be capable of amplifying *C. burnetii* in their tissues, the possibility of *C. burnetii* contamination occurring during the processing of the samples could not be ruled out.

Although molecular evidence of *C. burnetii* infection has been reported in WA and QLD wildlife species as outlined in Table 1.4, no such studies have yet been undertaken on wildlife residing in NSW. Moreover, there is considerable variability between these studies regarding the qPCR methodology used to detect and classify samples as positive or negative for *C. burnetii* DNA rendering comparisons between studies difficult. The Minimum Information for Publication of Quantitative Real-time PCR Experiments (MIQE) guidelines promote robust experimental design and transparency in reporting of qPCR results among researchers (Bustin et al., 2009). Among the recommendations in these guidelines are the reporting of assay validation and limit of detection, however, these were not reported by any of the studies in Table 1.4 rendering between-study comparisons difficult, and may be considered a limitation of these studies. Moreover, all studies considered a sample to be positive for *C. burnetii* DNA based solely on the amplification of the IS*1111* gene. Although IS*1111* offers increased sensitivity compared to single copy gene targets, due to its identification in CLE of ticks, IS*1111* is no longer considered specific to *C. burnetii* (Duron, 2015). Therefore, studies employing only this gene in their detection strategy, could have potentially misidentified CLE as *C. burnetii*.

.

**Table 1.4** Published literature reporting the detection of *Coxiella* spp. or *Coxiella burnetii* DNA in Australian native wildlife by PCR. Scientific names of species are found below table.

| Australian State | Species | Sample type | Target gene | Sample classification criteria | Sample number (n) | *Coxiella burnetii* DNA prevalence (%) | Sequenced | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Western Australia | Western grey kangaroo | faeces | *IS*1111* and ***JB*153-3* | positive for *IS*1111* | 42/343 | 12.3 | Yes *com1*- 99% Dugway strain | Utilised two gene targets but based prevalence off IS*1111* only | (Banazis et al., 2010) |
| | | | | | 6/343 | | | | |
| | | faeces | | | 41/990 | 4.1 | No | | (Potter et al., 2011) |
| | Western Barred bandicoot | faeces | | | 1/12 | 8.3 | Yes *com1*- 99% Dugway strain | | (Bennett et al., 2011) |
| Queensland | Common northern bandicoot | whole blood | *IS*1111* | positive for any gene target | 6/35 | 25.0 | Yes using *com1* with 98% homology to Nine Mile Clone 4 but it is unclear if the PCR products were amplified from ticks or animals | In the discussion (rather than the methods section of the paper) it was mentioned that the study utilised three gene targets, but criteria used to classify samples as positive and negative is unclear, and it appears that classification as positive was based on the amplification of only one gene target. | (Cooper et al., 2013) |
| | Eastern grey kangaroo | | | | 6/17 | | | | |
| | Agile wallaby | | | | 1/5 | | | | |
| | Red kangaroo | | | | 1/4 | | | | |
| | Common wallaroo | | | | 1/3 | | | | |
| | Brushtail possum | | | | 1/2 | | | | |
| | Blacked-striped wallaby | | | | 1/1 | | | | |
| Queensland | Eastern grey kangaroo | blood | *IS*1111* and ***com1* | amplification observed for either gene target | 0/3 | 5.8 | Not attempted | The paper lacked a lot of essential information regarding how samples were classified, and a breakdown of which samples were positive for each animal was not provided | (Tozer et al., 2014) |
| | Koala | blood, faeces, urine | | | 5/99 | | | | |
| | Flying foxes | urine | | | 7/90 | | | | |
| | Wallaby | faeces, urine | | | 0/5 | | | | |
| | Wombat | blood, faeces, urine | | | 0/10 | | | | |

Western grey kangaroo (*Macropus fuliginosus*), Western barred bandicoot (*Perameles bougainville*), Northern bandicoot (*Isoodon macrourus*), Eastern grey kangaroo (*Macropus giganteus*), Agile Wallaby (*Macropus agilis*), Red kangaroo (*Macropus rufus*), Common wallaroo (*Macropus robustus*), Brushtail possum (*Trichosurus vulpecula*), Blacked-striped wallaby (*Macropus dorsalis*), Koala (*Phascolarctos cinereus*), Flying fox (*Pteropus* spp.), Wallaby (*Macropus* spp.), *Insertion sequence *1111* (IS*1111*), **Outer membrane protein (*com1*), *** gene encoding 2-Oxoacid dehydrogenase (*JB153-3*).

## 1.8 Study scope and objectives

This research was designed to enhance the understanding of the connection between Q fever in people and *C. burnetii* infection and shedding in Australian native wildlife. The specific aims of this thesis are presented in **Table 1.5**.

**Table 1.5** Knowledge gaps, research aims and rationale of the PhD of Ms Karen Mathews at The University of Sydney School of Veterinary Science entitled 'The role of Australian native wildlife in Q fever'.

| Knowledge Gap | Aims | Rationale |
|---|---|---|
| *There are no studies that have investigated the levels of C. burnetii exposure in AWR.* | Investigate *C. burnetii* seroprevalence in AWR and identify factors associated with seropositivity. | There is a growing body of evidence from human epidemiological data and animal studies implicating Australian wildlife as sources of Q fever for humans, therefore AWR may be at risk of *C. burnetii* infection and developing Q fever by caring for Australian wildlife. The correlation of seropositivity and Q fever prevalence in AWR with demographic and animal exposure history will enable the identification of potential risk factors associated with *C. burnetii* exposure and Q fever and help to inform intervention strategies for disease prevention and risk factor management in this group. |
| *There are no studies that have investigated Q fever prevalence in AWR.* | Investigate *Q fever* prevalence in AWR and identify factors associated with Q fever disease status. | |
| *Despite national guidelines recommending Q fever for AWR, vaccination uptake in this population is not known, nor is it known whether AWR have sufficient knowledge and awareness of Q fever and Q fever vaccination.* | Investigate the knowledge, attitudes and awareness of Q fever and QFV in AWR and determine the vaccination status in this population. | Q fever is a vaccine preventable disease and AWR are considered an at-risk group for whom vaccination is recommended. Studies on other at-risk groups in Australia have identified insufficiencies in vaccine uptake and knowledge gaps regarding Q fever and QFV. Determining the proportion of vaccinated AWR and Identifying knowledge gaps will inform intervention strategies to improve vaccine uptake in AWR. |
| *Australian native wildlife, particularly kangaroos, have been suggested as reservoirs for C. burnetii, thereby representing a potential infection source for humans. However, there is no direct evidence that they amplify and shed the organism from their tissues, as occurs in ruminants. Therefore, the role of wildlife as reservoirs for C. burnetii remains unclear.* | Determine the prevalence of *C. burnetii* in Australian native wildlife and identify potential shedding routes. | Domestic ruminants have long been regarded as the primary source of *C. burnetii* infection in humans. Serological and molecular evidence of *C. burnetii* infection in Australian native wildlife species has also been reported, however the cycle of *C. burnetii* infection in wildlife and their potential role in the transmission of *C. burnetii* to humans remains poorly understood. Determining the presence and location of *C. burnetii* in wildlife tissues and excretions will enable a better understanding of the infection cycle in wildlife and the risk they pose for human transmission. |

*C. burnetii – Coxiella burnetii*, AWR-Australian wildlife rehabilitator, QFV-Q fever vaccination

This research is intended to provide insight into the role of Australian wildlife (with particular emphasis on kangaroos) in the transmission of *C. burnetii* to humans. An understanding of the risk that wildlife poses to humans is of relevance to those who have regular wildlife contact including Australian wildlife rehabilitators (AWR), veterinary personnel, livestock workers, farmers, kangaroo harvesters as well as the general public who visit farms, wildlife parks, golf courses and camping grounds where wildlife reside and roam free. This will, in turn, provide evidence to inform QFV policy and other biosecurity guidelines and recommendations for these cohorts of the Australian population.

# Chapter 2 *Coxiella burnetii* seroprevalence and Q fever in Australian wildlife rehabilitators

This chapter appears as the following published paper in the international peer reviewed scientific journal, *One health* (citation below). Only the format has been changed for consistency of style in this thesis.

**Mathews, K. O**., Toribio, J. A., Norris, J. M., Phalen, D., Wood, N., Graves, S. R., Sheehy, P. A., & Bosward, K. L. (2020). *Coxiella burnetii* seroprevalence and Q fever in Australian wildlife rehabilitators. *One health*, *12*, 100197.https://doi.org/10.1016/j.onehlt.2020.100197

## 2.1 Abstract

*Coxiella burnetii* is the causative bacterium of the zoonotic disease Q fever, which is recognised as a public health concern globally. Macropods have been suggested as a potential source of *C. burnetii* infection for humans. The aim of this cross-sectional study was to determine the prevalence of *C. burnetii* exposure in a cohort of Australian wildlife rehabilitators (AWRs) and assess Q fever disease and vaccination status within this population. Blood samples were collected from adult participants attending the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. Participants completed a questionnaire at the time of blood collection. Antibody titres (IgG, IgA and IgM) against Phase 1 and Phase 2 *C. burnetii* antigens as determined by immunofluorescence assay, revealed that of the unvaccinated participants, 6.1% (9/147) had evidence of exposure to *C. burnetii*. Of the total participants, 8.1% (13/160) had received Q fever vaccination, four of whom remained seropositive at the time of blood collection. Participants reporting occupational contact with ruminants, were eight times more likely to have been vaccinated against Q fever, than those reporting no occupational animal contact (OR 8.1; 95% CI 1.85 – 45.08). Three AWRs (2%) reported having had medically diagnosed Q fever, two of whom remained seropositive at the time of blood collection. Despite the lack of association between macropod contacts and *C. burnetii* seropositivity in this cohort, these findings suggest that AWRs are approximately twice as likely to be exposed to *C. burnetii*, compared with the general Australian population. This provides support for the recommendation of Q fever vaccination for this potentially 'at-risk' population. The role of macropods in human Q fever disease remains unclear, and further research into *C. burnetii* infection in macropods including: infection rate and transmission cycles between vectors, macropods as reservoirs, other animals and humans is required.

**Keywords:** *Coxiella burnetii*; Q fever; seroprevalence; Australia, wildlife rehabilitators; macropods, kangaroos

## 2.2 Introduction

*Coxiella burnetii* is the causative agent of the zoonotic disease Q fever, which is recognised as a public health concern globally (Maurin and Raoult, 1999). Infection is typically acquired via the inhalation of aerosols contaminated with the bacterium. Although domestic ruminants are the main reservoirs of human disease (Marrie, 1990), direct evidence of *C. burnetii* infection has also been identified in a variety of wild and domestic animal species including: dogs (Shapiro et al., 2016), cats (Kopecny et al., 2013), horses (Marenzoni et al., 2013) birds (Agerholm, 2013) and macropods (Banazis et al., 2010; Pope et al., 1960; Shapiro et al., 2020). Following human infection, clinical outcomes vary in severity, ranging from asymptomatic infection with seroconversion, to a flu-like illness. In some instances, Q fever may progress to chronic forms including endocarditis that may result in death (Raoult et al., 2005). Additionally, post Q fever fatigue syndrome is a relatively common clinical sequela to Q fever disease (Marmion et al., 2009). The economic impact of Q fever disease in Australia is considerable with the cost of compensation alone estimated to exceed $AU1.3 million ($US960 000) annually (M. Kermode et al., 2003).

In Australia, Q fever has been a notifiable human disease in all states and territories since 1977 (Garner et al., 1997). It is the most frequently reported directly transmitted zoonosis (National Notifiable Diseases Surveillance System, 2016) with the highest Q fever notification rates typically associated with livestock/meat industry workers in New South Wales (NSW) and Queensland (QLD) (National Notifiable Diseases Surveillance System, 2016). A safe and highly effective human Q fever vaccine (Q-Vax®; Seqirus, Parkville, Vic.) has been available in Australia since 1989, and vaccination is recommended for high-risk occupational groups such as veterinary personnel, and abattoir and livestock workers (Australian Technical Advisory Group on Immunisation, 2021). Recently, the recommendation for Q fever vaccination (QFV), has been extended to wildlife and zoo workers, with kangaroos particularly mentioned amongst the list of 'high risk' animals (Australian Technical Advisory Group on Immunisation, 2021).

Over the past decade in Australia, there has been an increased incidence in Q fever notifications with minimal known exposure to well-documented risk factors (Chong et al., 2003; Clark et al., 2020; Clutterbuck et al., 2018; Gale et al., 2007; Islam et al., 2011; Parker et al., 2010; Tozer et al., 2011), and there is a growing body of evidence suggesting macropods, in particular kangaroos, represent a potential source of *C. burnetii* infection for humans. *Coxiella burnetii* has been isolated from the ticks of infected kangaroos (Pope et al., 1960), and *C. burnetii* DNA has been identified in kangaroos (Banazis et al., 2010; Cooper et al., 2013; Potter et al., 2011; Shapiro et al., 2020) and other wildlife including bandicoots (Bennett et al., 2011) and their associated ticks (Bennett et al., 2011; Cooper et al., 2013). A Western Australian study found *C. burnetii* DNA in the faeces of kangaroos co-grazing with livestock, along with a *C. burnetii* seroprevalence of 33% in these same animals (Banazis et al., 2010). Furthermore, *C. burnetii* DNA was recently detected in samples of raw meat containing kangaroo sold for pet consumption (Shapiro et al., 2020). Ongoing occupational exposure to kangaroo and wallaby carcasses was postulated as a possible source of *C. burnetii* infection for a Queensland park ranger who contracted Q fever in 2015 (Stevenson et al., 2015). Q fever has also been reported in individuals working in outdoor environments inhabited by kangaroos, or on grounds heavily contaminated with kangaroo faeces, and in those handling juvenile joeys (Flint et al., 2016). Although molecular evidence of *C. burnetii* was not found in any of the kangaroo samples tested, the association with macropods in these cases was still considered a plausible risk factor for *C. burnetii* transmission. Combined, these studies suggest that wildlife rehabilitators can potentially acquire Q fever by handling sick, injured and orphaned wildlife.

This study aimed to measure the seroprevalence of *C. burnetii* (Q fever) antibodies in Australian wildlife rehabilitators attending a wildlife rehabilitator conference, and investigate the association of seropositivity with risk factors for *C. burnetii* exposure to determine: 1) the level of exposure to *C. burnetii* in rehabilitators of Australian mammalian wildlife (AWRs), and 2) the potential sources of exposure.

## 2.3 Materials and Methods

### 2.3.1 Study Design and Recruitment

This cross-sectional study targeted AWRs over 18 years of age attending the Australian Wildlife Rehabilitation Conference (AWRC), held on the Camperdown campus of the University of Sydney, Sydney Australia, in July 2018. Participants were recruited from the conference delegation over the three days of the conference to complete a self-administered questionnaire and have a blood sample collected. Participation was voluntary. This research was approved by the Human Research Ethics Committee of the University of Sydney (project number 2018/457).

### 2.3.2 Sample size calculation

Since the population size of wildlife rehabilitators across the whole of Australia was not available, an estimation based on the known number of rehabilitators in NSW was made. The population of NSW was 7.7 million of which approximately 4600 (0.06%) (Savage, 2017) people engaged in wildlife rehabilitation. The relative proportion of wildlife rehabilitators residing in other Australian states and territories was presumed to be similar to NSW and were subsequently calculated using the Australian Bureau of Statistics 2016 population figures (Australian Bureau of Statistics, 2016). The population estimates from each jurisdiction were summated to give an estimated national wildlife rehabilitator population size of 14,358. The sample size for this study was calculated using Statulator software (Dhand & Khatkar, 2014). Assuming a nationwide average of 3% seroprevalence of Immunoglobulin G (IgG) antibodies to *C. burnetii* (Gidding et al., 2019), an expected response rate of 15% [based on a serosurvey of veterinary workers (Sellens et al., 2020)] and a national wildlife rehabilitator population size of 14,358, this study would require a sample size of 117 AWRs for estimating *C. burnetii* seroprevalence with 8% absolute precision and 95% confidence.

### 2.3.3 Questionnaire

Participants completed a questionnaire to accompany their blood sample (**Appendix A**). A unique identification number assigned to each participant was used to label their questionnaire and

corresponding blood collection tube. The questionnaire consisted of 32 questions (24 closed and eight Likert scale) and was divided across four sections containing questions on (i) demographics of the rehabilitator and where they rehabilitated wildlife, (ii) the type of wildlife they rehabilitated and other animals located on or nearby to the caring residence (iii) their rehabilitation and husbandry practices, (iv) a history of Q fever disease (QFD) and vaccination status. Each participant was provided with an information statement explaining the purpose and expected outcomes of the research, and written consent was obtained prior to study participation. At the end of the questionnaire, participants could opt to be notified of their individual serological results, as well as a receive a summary of the study outcomes.

## 2.3.4 Laboratory Methods

*2.3.4.1 Blood Sample Collection*

Approximately 8mL of blood was drawn from the median cubital vein of each participant into serum separator tubes (Interpath, Victoria, Australia) by a certified venepuncturist or registered doctor. The blood was centrifuged at 4,000xg for 5 minutes, after which the serum was removed and stored at 20°C until transportation to the laboratory.

*2.3.4.2 Indirect immunofluorescence antibody testing*

The serum samples were analysed at the Australian Rickettsial Reference Laboratory (ARRL), Geelong, Australia using an in-house indirect immunofluorescence assay (IFA) accredited by the *National Association of Testing Authorities* (accreditation No. 14342). Initial screening of serum samples was conducted using a 1/25 and a 1/400 (to detect prozone phenomenon) dilution of sera in 2% casein. Approximately 2μL of diluted serum was spotted in duplicate onto a glass slide coated with *C. burnetii* Phase 1 or Phase 2 antigen (Virion/Serion, Germany). After incubation at 35°C for 40 minutes, the slides were washed with PBS (diluted 1/10) and air-dried before adding a combined conjugate containing fluorescein-labelled goat anti-human IgA+IgG+IgM (H+L). The incubation and wash steps were repeated, the slides were dried, mounted with a cover slip and read using a fluorescent

microscope (400×; Axioskop 40; Zeiss). Positive sera underwent a doubling dilution series (1/25 to 1/3200 in 2% casein) with and without rheumatoid factor removal reagent (Virion/Serion, Germany) to reduce non-specific binding. Each serum dilution was tested against three fluorescein labelled goat anti-human conjugates, anti-IgM, anti-IgG anti-IgA, and total conjugate containing anti- IgA+IgG+IgM (H+L) using the methodology described above. For both screening and titration, positive and negative human serum samples were included on each slide as controls and serum was considered positive if fluorescence was observed at a dilution of 1/25 or greater. All antibodies were manufactured by KPL/ SeraCare (USA). Criteria adapted from Healy et al., (2011) was used to classify exposure with relatively recent exposure considered if Phase 1 and/or Phase 2 IgG ≥1/50 and Phase 2 IgM ≥1/50 and past exposure if Phase 1 and/or Phase 2 IgG ≥1/50 and Phase 2 IgM <1/50.

## 2.4 Statistical Analysis

### 2.4.1 Data Management

Participants completed paper questionnaires, and the data was manually entered into the secure online platform REDCap (Research Electronic Data Capture) (Harris et al., 2019; Harris et al., 2009), hosted at The University of Sydney, Australia. A subset (10%) of randomly selected questionnaires were checked for transcription errors, after which the data was exported into Microsoft® Excel® (Microsoft Corporation, Washington, USA) for cleaning and processing. Data analysis was performed using R statistical program® (R Core Team).

### 2.4.2 Outcome variables and risk factors

The primary outcome variable was whether the rehabilitator was *C. burnetii* seropositive or seronegative [Q fever serostatus (QFSS)]. Secondary outcome variables included whether the participant had or had not been vaccinated against Q fever [Q fever vaccination status (QFVS)] and whether or not the participant had been medically diagnosed with Q fever disease [(Q fever disease status (QFDS)]. All outcome variables were dichotomous. Descriptive statistics (mean, median and range for continuous variables, bar charts for categorical variables) were generated to obtain

information regarding the distribution of each variable. The continuous variables age and number of animals cared for per year were categorised and then collapsed into condensed categories for further analyses. Questions regarding animal exposure were collapsed into four groups as follows: ruminants (cattle, goats, sheep), macropods (kangaroos and wallabies), domestic species (dogs, cats, pigs, horses, poultry) and other wildlife species (bandicoots, possums, flying foxes, koalas, wombats and other wildlife). Responses to questions that utilised a Likert scale (frequently, occasionally, rarely, never) were collapsed and categorised as 'yes' if the response was frequently or occasionally, and 'no', if the response was rarely or never. Variables with 10% missing data were not included in the statistical analysis. Participants unsure of their QFVS (5/165) were excluded from the final data set.

Two additional variables were generated from postcode of residence: (1) Australian state of residence and (2) Australian Statistical Geography Standard (ASGS) Remoteness Area. Participant's postcodes were matched to the corresponding remoteness area, according to the Australian Bureau of Statistics (ABS) remoteness structure, which divides Australia into five geographic regions of relative remoteness (Major cities of Australia, Inner Regional Australia, Outer Regional Australia, Remote Australia and Very Remote Australia) (Australian Bureau of Statistics, 2016). Postcodes spanning more than one remoteness category, were allocated to the category that contained the majority of the geographic area of the postcode.

Biosecurity practices were based on two questions in which participants indicated how frequently ('always', 'frequently', 'occasionally', 'rarely' or 'never') they utilised the following infection control practices while handling animals and cleaning enclosures: overalls/protective outerwear, disposable gloves, safety glasses, P2 face mask, and prompt hand washing. Biosecurity practices were deemed inadequate if participants 'never' used any form of PPE when handling animals or cleaning enclosures. The use of each type of infection control was considered adequate if 'always' or 'frequently' was selected. A participant's biosecurity practice was considered adequate if they always or frequently used overalls/protective outerwear and practiced prompt hand washing when handling animals, and

additionally wore disposable gloves when cleaning enclosures. Respondents were considered to practice enhanced biosecurity when handling animals if they 'always' or 'frequently' used overalls/protective outerwear, practiced prompt hand washing and wore disposable gloves, or if all five methods of infection control were practiced when cleaning enclosures. This assessment and classification of adequate and enhanced biosecurity were established by the authors, using recommendations from the Australian Veterinary Association Guidelines for Veterinary Personal Biosecurity (Australian Veterinary Association, 2017), in combination with National Wildlife Biosecurity Guidelines (Wildlife Health Australia, 2018).

The fourteen potential risk factors that underwent univariable analysis for the outcome variables QFSS and QFVS were: age, state of residence, remoteness area, total years rehabilitating wildlife, total weeks per year rehabilitating wildlife, animal species residing on their property or within a 2km radius, rehabilitating wildlife on own property, number of people in the household rehabilitating wildlife, wildlife species rehabilitated during rehabilitation career, total number of animals rehabilitated per year, occupational animal contact, present or assisting with the birth of non-human mammalian species, and biosecurity practices when handling animals and cleaning enclosures. An additional four risk factors were considered for the outcome variable QFSS: frequency of rehabilitating macropods over the rehabilitation career and during the past year, handling orphan joeys and whether the rehabilitator reported having been bitten by a tick.

### 2.4.3 Modelling

Univariable logistic regression was conducted to identify associations between potential risk factors and the outcome variables QFSS and QFVS. Risk factors with $p < 0.3$ in the univariable analysis were progressed to multivariable analysis after evaluation of the strength of association between these risk factors using the Cramer's V statistic. When the correlation coefficient for a pair of risk factors was $> 0.7$ only the variable deemed more biologically plausible was included in subsequent multivariable analysis. Multivariable modelling was performed using backward selection where the variable with

the least significance (Wald test) was removed sequentially. Variables with p-values < 0.1 were retained in the final model. Occupational animal contact was considered a potential confounder and included in the multivariable model for the outcome variable QFSS *a priori* due to its association with positive serology.

## 2.5 Results

### 2.5.1 Responses

Out of 350 AWRC delegates, 165 AWRs volunteered to donate a blood sample and complete the questionnaire, corresponding to a response rate of 47.1%. Five participants were removed from the study due to their inability to recall their QFVS, leaving 160 participants in the final data set.

### 2.5.2 Demographics of Australian wildlife rehabilitators

Of the 160 AWRs, 93.8% (150/160) were female and the median age of the cohort was 54 years (158/160; 21-79; IQR 45-62). All respondents had been actively rehabilitating wildlife for the past five years, and 50.6% (81/160) had been rehabilitating wildlife for more than 10 years. Most participants (96.9%; 155/160) identified their association with wildlife was as a rehabilitator, and of these, 29.7% (46/155) performed other wildlife-associated roles. Amongst the cohort were: 26 (16.3%) veterinary nurses, six wildlife researchers (3.8%) and one veterinarian (1%), most of whom (apart from two veterinary nurses) also classified themselves as a wildlife rehabilitator.

Participants were predominantly from NSW (53.8%; 86/160) followed by Western Australia (WA; 13.1%, 21/160), Victoria (VIC; 12.5%, 20/160), QLD (9.4%; 15/160), South Australia (SA; 6.3%, 10/160), Tasmania (TAS; 1.9%, 3/160), Northern Territory (NT; 1.3%, 2/160) and the Australian Capital Territory (ACT;1.9%, 3/160). Although all Australian states and territories were represented, the proportion of AWRs residing in NSW was higher, and the proportions in VIC and QLD were lower (53.8%, 12.5% and 9.4% respectively), compared to the available total national population estimates for these states (32%, 26 % and 20% respectively) (Australian Bureau of Statistics, 2018). The proportions within the remaining jurisdictions of WA, SA, TAS, ACT and NT (combined 24.4%) were comparable to the

Australian population distribution. According to the available data on population distribution via state and remoteness area (National Rural Health Alliance, 2011), the proportion of the cohort living in major cities was lower (48 % vs 70%), while the proportion living in inner regional Australia was higher (39% vs 18%) than the distribution of the general Australian population. Thirteen percent (20/160) of AWRs resided in outer regional/remote areas, which was similar to the population distribution for these remoteness categories (11%).

### 2.5.3 Wildlife rehabilitating practices

Of the 160 AWRs, 98.1% (157/160) who rehabilitated animals in the same state as their residence, 83.7% (134/160) resided in the same geographical postcode in which their wildlife rehabilitation was undertaken, and 78.6% (125/159) of rehabilitators spent more than 30 weeks per year caring for wildlife. The number of animals cared for per year ranged from 2 to 1500, with three rehabilitators reporting having cared for over 1,000 animals per year. Of the 93.1% (149/160) of AWRs who rehabilitated animals on their own property, 20.1% (30/149) housed animals exclusively within their home, 8.7% (13/149) used outdoor enclosures and 71.1% (106/149) practiced both housing arrangements. Regarding the primary location at which rehabilitation was undertaken, 89.4% (143/160) of respondents rehabilitated wildlife primarily in their home or someone else's home, 25.0% (40/160) in a wildlife rescue centre/dedicated wildlife hospital, 13.8% (22/160) in a veterinary clinic that also treats wildlife and 3.8% (6/160) primarily rehabilitated wildlife in a zoo. Of the 58.8% (94/160) of AWRs who reported occupational contact with animals, 37.2% (35/94) had been exposed to ruminants, 78.7% (74/94) to domestic animals, 53.2% (50/94) to macropods and 71.3% (67/94) to other animals including wildlife. Over half of the participants had frequently rehabilitated macropods throughout their wildlife rehabilitation career and in the year prior (61.9%; 99/160 and 52.9%; 83/157 respectively). Overall, the most commonly and frequently rehabilitated species, over the duration of their rehabilitation career and in the year prior (data not shown) were possums and gliders followed by macropods (kangaroos and wallabies). Almost all (96.2%, 152/158) had handled orphaned joeys,

43.8% (70/160) had been bitten by a tick, and 27.5% (44/160) had been present or assisted with a non-human birth.

Biosecurity practices adopted when handling animals and cleaning enclosures are presented in Table 2.1. While the majority of AWRs practiced prompt hand washing after handling animals and cleaning enclosures, 3.2% (5/160) of AWRs indicated that they did not practice any form of biosecurity during or after either activity. Disposable gloves were worn more frequently when cleaning enclosures than when handling animals (p=0.002), however the vast majority of AWRs did not meet 'adequate' biosecurity requirements in either situation, and only 1.9% (3/160) practiced 'enhanced' biosecurity' when cleaning enclosures.

### 2.5.4 Q fever serostatus and investigated potential risk factors

Serological results of vaccinated and unvaccinated rehabilitators are presented in Table 2.2. Nine (6.1%; 95% CI 2.8% – 11.3%) of the 147 unvaccinated participants were *C. burnetii* seropositive, and all except one rehabilitator resided in either NSW or QLD. The two participants whose serological response was classified as 'recent exposure' also resided in NSW and QLD, were unvaccinated and one described themselves as a wildlife rehabilitator, and the other a wildlife researcher/student. Seven of the nine (77.7%) seropositive participants had rehabilitated macropods, 5/9 (55.6%) had been present at, or assisted with non-human births, and 7/9 (77.7%) had been exposed to animals through their occupation.

Of the 18 potential risk factors investigated for association with positive *C. burnetii* serostatus amongst the 147 unvaccinated AWRs, six had a p<0.3 in the univariable analyses (Table 2.3). Participants were more likely (OR 3.7 95%; CI 0.92 – 15.60) to be seropositive if they had been present at, or assisted with non-human births, and participants residing in QLD were twice as likely (OR 2.3; 95% CI 0.38 – 14.54) to be seropositive than those living in NSW. All measures of association returned a Cramer's V p value <0.7; therefore all six variables were included in the multivariable model. Although not significant (p=0.535) in the univariable analysis, 'occupational animal contact'

was considered a confounder, and therefore included in the model. Multivariable analysis was unsuccessful in producing a final model of risk factors associated with a positive QFSS.

Table 2.1 Biosecurity practices reported by 158 Australian wildlife rehabilitators when handling animals and cleaning animal enclosures. Results obtained from a survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018.

| Biosecurity Practices | Number (%) of participants when handling animals | Number (%) of participants when cleaning enclosures |
|---|---|---|
| No PPE | 5 (3.2) | 5 (3.2) |
| Prompt hand washing | 153 (96.8) | 153 (96.8) |
| Overalls/protective outerwear | 20 (12.7) | 29 (18.4) |
| Disposable gloves | 36 (22.8) | 61 (38.6) |
| Safety glasses | 5 (3.2) | 10 (6.3) |
| Face mask | 4 (2.5) | 8(5.1) |
| | | |
| Level of biosecurity practice* | | |
| Inadequate | 138 (87.3) | 136 (86.1) |
| Adequate | 20 (12.7) | 22 (13.9) |
| Enhanced | 0 (0) | 3 (1.9) |

*Level of biosecurity practice was based on reported personal protection equipment (PPE) use and recommendations from the Australian Veterinary Association Guidelines for Veterinary Personal Biosecurity (Australian Veterinary Association, 2017) and National Wildlife Biosecurity Guidelines (Wildlife Health Australia, 2018.

## 2.5.5 Q fever diagnosis

Three (2%; 95% CI 0.4% – 5.8%) of the 147 unvaccinated participants self-reported having been medically diagnosed with QFD. Two of these participants (one seropositive and one was seronegative at the time of blood collection) indicated that their QFD diagnosis occurred ≥20 years ago, and both reported animal-associated occupations (beef cattle breeder and veterinary nurse) and having been present at the birth of mammals other than humans. The third participant (who was seropositive at the time of blood collection) reported a more recent diagnosis of QFD (2017). This participant was an engineer whose employment was non-animal-associated and who had not attended non-human births. All three QFD confirmed rehabilitators indicated that they had rehabilitated macropods. Out of the 147 unvaccinated participants, two reported having had self-diagnosed QFD (without laboratory testing), one of whom indicated they were ineligible for the Q fever vaccine due to a positive pre-vaccination screening result in 2012. Although this participant was seronegative at the time of blood

collection, the reported positive pre-vaccination test was evidence that this participant had been exposed to *C. burnetii.* Due to the small number of participants reporting having had medically diagnosed QFD, logistic regression analysis was not performed for this outcome variable.

### 2.5.6 Q fever vaccination and investigated potential risk factors

Thirteen (8.1%; 95% CI 4.4% – 13.5%) of the 160 participants self-reported having been vaccinated against Q fever and all reported never being diagnosed with QFD. The majority (84.6%; 11/13) of the 13 vaccinated participants resided in NSW (7/13) or QLD (4/13) and reported having received the Q fever vaccine through their General Practitioner (GP) (6/13) or a workplace/university vaccination program (7/13). All rehabilitators who had been vaccinated through a vaccination program, reported occupational contact with ruminants. Of the 26 (26/160; 15.5%) veterinary nurses participating in this study, 93.3% (24/26) were not vaccinated two (8.3%) of whom were seropositive.

Univariate logistic regression identified six risk factors (out of 14) that were associated with having received QFV ($p<0.3$) (**Table 2.4**). Of these, 'occupational animal contact' was highly significant; AWRs reporting occupational contact with ruminants were six times more likely to have received QFV (OR 6.2; 95% CI 1.66 – 30.09) than those reporting no occupational contact with animals. The risk factor 'state of residence' was also significant; AWRs residing in QLD were four times more likely to have been vaccinated (OR 4.26; 95% CI 0.99 – 16.68) than those residing in NSW or other Australian jurisdictions. Of the six risk factors considered in the multivariable analysis, three were retained in the final model (**Table 2.5**). After accounting for the state of residence, and the total number of animals rehabilitated per year, 'occupational animal contact' was the only significant risk factor for QFV; AWRs reporting occupational contact with ruminants were eight times more likely to have received QFV (OR 8.1; 95% CI 1.85 – 45.09) than those who had no occupational contact with animals.

.

**Table 2.2** Serological results, and self-reported Q fever vaccination of, and Q fever disease in, wildlife rehabilitators participating in a *Coxiella burnetii* seroprevalence survey at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018.

| | | | | Phase 2 *C. burnetii* antigens | | | Phase 1 *C. burnetii* antigens | | |
|---|---|---|---|---|---|---|---|---|---|
| **Occupation** | **State of Residence** | **Year vaccinated** | **Year diagnosed** | **IgA** | **IgM** | **IgG** | **IgA** | **IgM** | **IgG** |
| **Persons with self-reported Q Fever disease** | | | | | | | | | |
| Veterinary nurse/teacher | VIC | - | 1992 | - | - | - | - | - | - |
| Beef cattle breeder | NSW | - | 2000 | - | - | 1600 | - | - | 1600 |
| Engineer | NSW | - | 2017 | 100 | - | 400 | - | - | 200 |
| **Persons seropositive and therefore assumed to have been exposed to *Coxiella burnetii* (clinical or subclinical infection)** | | | | | | | | | |
| Wildlife rehabilitator | QLD | - | - | 200 | 50 | 400 | 50 | 25 | 100 |
| Retired teacher | NSW | - | - | - | - | 25 | - | - | - |
| Veterinary nurse | NSW | - | - | - | - | 50 | - | - | 25 |
| Wildlife researcher/student | NSW | - | - | - | 200 | ≥3200 | - | - | 100 |
| Writer/editor | NSW | - | - | - | - | 50 | - | - | 25 |
| Veterinary nurse | QLD | - | - | 50 | - | 50 | - | - | - |
| Company director/farmer | WA | - | - | - | - | 25 | - | - | 50 |
| **Persons reporting having received Q fever vaccination** | | | | | | | | | |
| Retired/ farmer /journalist | NSW | 2013 | - | - | - | 50 | - | - | 50 |
| Wildlife catcher/spotter | QLD | 1998 | - | - | - | - | - | - | - |
| Wildlife rehabilitator | QLD | 1999 | - | - | - | - | - | - | - |
| Service | QLD | 2005 | - | - | - | - | - | - | - |
| Home duties | NSW | 2010 | - | - | - | 800 | 200 | 50 | 100 |
| Wildlife catcher/spotter | QLD | 2010 | - | - | - | - | - | - | - |
| Rescue officer | SA | 2014 | - | - | - | - | - | - | - |
| Midwife | NSW | 2015 | - | - | - | - | - | - | - |
| Veterinarian | NSW | 2015 | - | - | - | - | - | - | - |
| Veterinary nurse/zookeeper | NSW | 2015 | - | - | - | - | - | - | - |
| Veterinary nurse/admin Assistant | NSW | 2016 | - | - | 25 | - | - | 50 | 50 |
| Veterinary student | SA | 2017 | - | - | - | - | - | - | - |
| Retired librarian | NSW | 2017 | - | - | - | 50 | - | - | 100 |

Numbers correspond to reciprocal antibody titres; Dash (-) = reciprocal antibody titre <25, VIC - Victoria, NSW - New South Wales, QLD - Queensland, WA - Western Australia, SA - South Australia.

**Table 2.3** Univariable logistic regression analysis of positive serological result for *C. burnetii* exposure among Australian wildlife rehabilitators participating in a survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018 (p <0.3).

| | | Q fever Serostatus | | | | | |
|---|---|---|---|---|---|---|---|
| Variable name and description | Total number | Seropositive | Seronegative | Odds ratio | 95% LCL | 95% UCL | P-value |
| **State of residence** | 147 | | | | | | 0.111 |
| NSW/ACT | | 6 | 76 | 1 | | | |
| Queensland | | 2 | 9 | 2.814 | 0.375 | 14.544 | 0.245 |
| Other | | 1 | 53 | 0.240 | 0.012 | 1.454 | 0.191 |
| **Total years rehabilitating wildlife** | 147 | | | | | | 0.288 |
| 1-10 | | 3 | 71 | 1 | | | |
| more than 10 | | 6 | 67 | 2.119 | 0.537 | 10.359 | |
| **Total number of animals rehabilitated per year** | 143 | | | | | | 0.291 |
| 0-30 | | 3 | 64 | 1 | | | |
| 31-100 | | 5 | 40 | 2.667 | 0.620 | 13.575 | 0.195 |
| >100 | | 1 | 30 | 0.771 | 0.034 | 5.810 | 0.772 |
| **Frequency of caring for macropods over rehabilitation career** | 147 | | | | | | 0.275 |
| Infrequently | | 2 | 55 | 1 | | | |
| Frequently | | 7 | 83 | 2.318 | 15.954 | 2.769 | |
| **Present at or assisting with the birth of non-human mammalian species** | 147 | | | | | | 0.063 |
| No | | 4 | 103 | 1 | | | |
| Yes | | 5 | 35 | 3.667 | 0.924 | 15.596 | |
| **Biosecurity practices when cleaning enclosures** | 145 | | | | | | 0.265 |
| None/hand wash only | | 6 | 76 | 1 | | | |
| Handwash + other | | 2 | 61 | 0.415 | 0.059 | 1.875 | |
| **Occupational animal contact** | 147 | | | | | | 0.535* |
| No animal contact | | 3 | 60 | 1 | | | |
| Contact with ruminants | | 3 | 24 | 2.5 | 0.436 | 14.349 | 0.281 |
| Contact with other animals | | 3 | 54 | 1.11 | 0.198 | 6.220 | 0.900 |

*p>0.3 but considered a confounder *a priori* and therefore included in the multivariable analysis, LCL -lower confidence interval, UCL upper confidence interval.

**Table 2.4** Univariable logistic regression analysis of Q fever vaccination among Australian wildlife rehabilitators participating in a survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018 (p<0.3).

| | | Q fever Vaccination Status | | | | | |
|---|---|---|---|---|---|---|---|
| Variable Name and Description | Total number | Vaccinated | Unvaccinated | Odds ratio | 95% LCL | 95% UCL | P-value |
| **State of residence** | 160 | | | | | | 0.038 |
| NSW/ACT | | 7 | 82 | 1 | | | |
| Queensland | | 4 | 11 | 4.260 | 0.989 | 16.681 | 0.039 |
| Other | | 2 | 54 | 0.433 | 0.063 | 1.870 | 0.308 |
| **Rehabilitating wildlife on own property** | 160 | | | | | | 0.264 |
| No | | 2 | 9 | 1 | | | |
| Yes | | 11 | 138 | 0.359 | 0.079 | 2.54 | 0.223 |
| **Number of people in house caring for wildlife** | 157 | | | | | | 0.186 |
| 1 | | 5 | 89 | 1 | | | |
| >1 | | 7 | 56 | 2.225 | 0.674 | 7.340 | 0.189 |
| **Number of animals per year cared for per year** | 156 | | | | | | 0.089 |
| 0-30 | | 4 | 67 | 1 | | | |
| 31-100 | | 8 | 45 | 2.978 | 0.883 | 11.704 | 0.089 |
| >100 | | 1 | 31 | 0.541 | 0.027 | 3.840 | 0.582 |
| **Occupational animal contact** | 160 | | | | | | 0.005 |
| No animal contact | | 3 | 63 | 1 | | | |
| Contact with ruminants | | 8 | 27 | 6.222 | 1.660 | 30.090 | 0.01 |
| Contact with other animals | | 2 | 57 | 0.737 | 0.094 | 4.579 | 0.743 |
| **Biosecurity handling animals** | 158 | | | | | | 0.065 |
| None/hand wash only | | 6 | 104 | 1.000 | | | |
| Handwash + other | | 7 | 41 | 2.959 | 0.930 | 9.700 | 0.065 |

*p>0.3 but considered a confounder *a priori* and therefore included in the multivariable analysis, NSW-New South Wales, ACT-Australian Capital Territory, , LCL -lower confidence interval, UCL upper confidence interval.

**Table 2.5** Final multivariable logistic regression analysis of Q fever vaccination among Australian wildlife rehabilitators participating in a survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. (p<0.1).

| | Q fever Vaccination Status | | | | | |
|---|---|---|---|---|---|---|
| Variable Name and Description | Vaccinated | Not Vaccinated | Odds ratio | 95% LCL | 95% UCL | P-value Wald |
| State of residence | | | | | | 0.061 |
| NSW/ACT | 7 | 82 | 1 | | | |
| Queensland | 4 | 11 | 2.041 | 0.346 | 10.427 | 0.404 |
| Other | 2 | 54 | 0.231 | 0.030 | 1.156 | 0.103 |
| Number of animals per year cared for per year | | | | | | 0.063 |
| 0-30 | 4 | 67 | 1 | | | |
| 31-100 | 8 | 45 | 2.795 | 0.722 | 12.353 | 0.145 |
| >100 | 1 | 31 | 0.314 | 0.015 | 2.544 | 0.336 |
| Occupational animal contact | | | | | | 0.008 |
| No animal contact | 3 | 63 | 1 | | | |
| Contact with ruminants | 8 | 27 | 8.111 | 1.852 | 45.087 | 0.008 |
| Contact with other animals | 2 | 57 | 0.974 | 0.117 | 6.187 | 0.955 |

NSW-New South Wales, ACT-Australian Capital Territory, , LCL -lower confidence interval, UCL upper confidence interval.

## 2.6 Discussion

This is the first study to investigate *C. burnetii* exposure among Australian wildlife rehabilitators. We report an overall seroprevalence for this cohort of 6.1%, which is 70% greater than the recently reported 3.6% in an Australian study of Red Cross blood donors from non-metropolitan NSW and QLD (using the same laboratory techniques as the current study) (Gidding et al., 2019). Two other Australian studies (also employing the same laboratory methods as the current study), have reported higher general population *C. burnetii* seroprevalence: (Islam et al., 2011) estimated an overall seroprevalence of 7% in the Hunter New England region of NSW, and more recently Gidding et al. (2020) reported a 5.6% nationwide *C. burnetii* seroprevalence, however these two studies used archived sera that were opportunistically obtained from pathology laboratories. We believe that the Red Cross blood donor study is a closer approximation to the assumed healthy participants in the current study, as blood donors must be of good general health and meet specific eligibility criteria to donate blood (Australian Red Cross Blood Service, 2019). Furthermore, participants in the current study, and the blood donor study, completed a questionnaire which accompanied their blood sample that contained specific questions regarding their demographic details, QFDS, QFVS and potential exposure history. This enabled a detailed analysis of the respective data sets to identify potential risk factors associated with seropositivity and having received Q fever vaccination. The 6.1% seroprevalence observed in the current study is lower than the 19% *C. burnetii* seroprevalence reported for a cohort of unvaccinated Australian veterinary workers, where increasing exposure to ruminants was identified as a significant risk factor for seropositivity (Sellens et al., 2020). International studies of livestock veterinarians have reported seroprevalence for *C. burnetii* as high as 65.1% (Van den Brom et al., 2013). Our findings suggest that rehabilitators of Australian wildlife are almost twice as likely to be exposed to *C. burnetii* compared to the general population, but only a third as likely to be exposed as Australian veterinarians associating with ruminants.

The current study utilised IFA to confirm *C. burnetii* exposure by measuring levels of circulating *C. burnetii* antibody at a 'point in time'. Although IFA is considered the 'gold standard' for human Q fever

diagnosis (Maurin & Raoult, 1999), due to temporal decline in antibody levels and the variability in immune responses between individuals (Sellens et al., 2020), the 6.1% seroprevalence observed in this study likely represents the minimum level of *C. burnetii* exposure amongst this cohort. This heterogeneity in antibody titres is demonstrated by the finding that only 30.7% (4/13) of vaccinated participants and two of the three participants with medically diagnosed QFD were seropositive at the time of blood collection (Table 2.2). Similarly, in the study by Gidding et al. (2019), only 10% of vaccinated blood donors and 39% of donors with a history of QFD were seropositive. Additional AWRs with previous *C. burnetii* exposure may have been identified via intradermal skin testing or the measurement of interferon gamma production in response to *C. burnetii* antigenic stimulation (Schoffelen et al., 2013), however such tests were beyond the scope of this study.

Currently there is limited information available on the demographics of AWRs, however one recent study on NSW rehabilitators reported that 79% were female and over half were >65 years (Haering et al., 2020), which was reflected in the findings of this study. A potential source of bias in this study is the representativeness of the cohort with respect to the proportion of wildlife rehabilitators in Australia and notifications across states. In this study the state of NSW was overrepresented by 22%, which could have resulted in a higher number of seropositive participants given that 43% of the national Q fever notifications in 2018 were from NSW (National Notifiable Diseases Surveillance System, 2021). However, this was offset to some extent by an 11% underrepresentation of participants from QLD which has similarly high notification rates as NSW (National Notifiable Diseases Surveillance System, 2021). The higher number of participants from NSW compared to other states was not unexpected given it was the host state, making travel and attendance easier for these participants. It is recognised that there may be self-selection bias with rehabilitators choosing to participate in the study because of previous experience or association with Q fever, however with half the participants of the conference engaging in the study, the impact of this potential bias is likely limited.

While this study demonstrated that *C. burnetii* exposure was higher in AWRs compared to the general population, and although QFD notification data suggests that macropods are potential sources of infection (Clark et al., 2020; Clutterbuck et al., 2018; Graves & Islam, 2016), we were unable to demonstrate a positive correlation between *C. burnetii* seropositivity and exposure to macropods (adults or juvenile joeys) within this cohort based on the responses to the questionnaire. Nor were we able to identify that exposure to ruminants, other domestic animals or other wildlife, or being present at non-human births were risk factors for *C. burnetii* seropositivity. This was surprising given that the majority of QFD notifications are ruminant associated (Clark et al., 2020; Clutterbuck et al., 2018; Graves & Islam, 2016), and that birth products of infected animals, particularly ruminants, can potentially contain high levels of *C. burnetii* (Maurin & Raoult, 1999; Welsh et al., 1958). Future *C. burnetii* seroprevalence studies in AWR should use a questionnaire specifically designed to differentially explore ruminant-associated and traditional risk factors versus wildlife associated risk factors.

In this study, seropositivity was also not associated with tick bites. Similarly, a study of Q fever in Belgian veterinarians also reported a lack of association between tick bites and *C. burnetii* exposure (Dal Pozzo et al., 2017). Early investigations by Pope et al., (1960) in which *C. burnetii* was isolated from the ticks of infected kangaroos, and more recent Australian studies which detected *C. burnetii* DNA in several wildlife-associated tick species including *A. triguttatum* (ornate kangaroo tick), (Cooper et al., 2013) and *Ixodes holocyclus* (paralysis tick) (Cooper et al., 2013; Graves et al., 2016), suggests that a tick-wildlife transmission cycle exists. It is therefore possible that spillover from infected kangaroos to humans may occur, however whether ticks are a direct source of *C. burnetii* infection for humans has not yet been demonstrated. In reports of cases of Q fever in which tick bites were a part of the clinical history, it was hypothesised that the tick was the source of infection for the affected patients, however, infection from other sources, particularly contaminated aerosols could not be discounted (Beaman & Hung, 1989; Eklund et al., 1947). The link between *C. burnetii* and ticks has long been established (Davis et al., 1938), and given that ticks can excrete large amounts of *C. burnetii*

organisms in their faeces during feeding (Philip, 1948), it is plausible that direct transmissions to humans could potentially occur via inhalation of aerosolised tick excreta, or through direct contamination of the bite site. More research focussing on ticks as a direct source of *C. burnetii* infection for humans is needed. The discovery that many tick species harbour genetically-related *Coxiella*-like endosymbionts (Duron et al., 2015), further complicates the role of ticks in *C. burnetii* transmission, and highlights the need for robust serological (Angelakis et al., 2016) and molecular (Duron, 2015) assays, which are able to definitively differentiate between these two *Coxiella burnetii and non-burnetii* species.

While the source of infection for the seropositive participants in this study remains unknown, the possibility that macropods can occasionally be an infection source for AWRs cannot be ruled out given the serological evidence that macropods can become infected with *C. burnetii* (Banazis et al., 2010; Pope et al., 1960), and potentially shed the bacterium (Banazis et al., 2010; Potter et al., 2011; Shapiro et al., 2020). Further research is required to determine whether macropods are reservoirs for *C. burnetii*, and whether they are capable of shedding viable organism which can subsequently infect humans and cause QFD. This is particularly important, given the growing number of Q fever notifications citing exposure to macropods, without exposure to other well-known infection sources such as ruminants.

Overall, three (2%) out of the 147 unvaccinated AWRs in this cohort self-reported having been medically diagnosed with QFD. This finding is similar to what was found in a study on Australian veterinary workers by Sellens et al. (2020), where 2% (4/192) of the cohort reported having QFD, but lower in comparison to another study of Australian cat breeders in which 6% (7/123) of the study population reported having had medically diagnosed QFD (Shapiro et al., 2017). In all three studies, the level of QFD is substantially higher than the Australian annual notification rate of 0.002% (National Notifiable Diseases Surveillance System, 2021). Due to the non-specific symptoms, many cases of Q fever go undiagnosed (Sloan-Gardner et al., 2017). It has been suggested that occurrence

of QFD could be more than three times higher than that recorded in the notification data (Kaufman et al., 2018). Interestingly, one of the participants in the current study reported being ineligible for the Q fever vaccine due to a positive pre-vaccination screening result. Patients such as these who have been exposed to *C. burnetii* but are not medically diagnosed, contribute to the underestimation of the actual QFD burden. Although age and gender breakdown of Q fever notifications reveal an overrepresentation of males in the 40-69 year age group (Sloan-Gardner et al., 2017), given the elevated seroprevalence in this cohort of AWRs, who were predominantly female, practitioners should not discount the possibility of QFD as a differential diagnosis in female AWRs presenting with an acute febrile illness.

Multivariable modelling for QFVS revealed that the strongest predictor of having been vaccinated against Q fever in this study was occupational animal contact, in particular ruminant contact, (**Table 2.5**) with rehabilitators reporting occupational contact with ruminants eight times more likely to have been vaccinated against Q fever compared to those reporting no contact with animals. Rehabilitators residing in QLD were more likely to have been vaccinated against Q fever than those residing in NSW (OR 2.04) or other Australian jurisdictions (OR:0.231). This was not unexpected given the majority of vaccinated (11/13) participants in this study resided in NSW and QLD, and that the vast majority Q fever notifications originate in these states (National Notifiable Diseases Surveillance System, 2021).

Currently, the Australian Immunisation Handbook recommends QFV for wildlife and zoo workers who have contact with at-risk animals, including kangaroos and bandicoots (Australian Technical Advisory Group on Immunisation, 2021), however only 8.1% (13/160) AWRs in this study had undergone vaccination. This is consistent with other Australian studies which have also reported low levels of vaccine uptake in groups for whom vaccination is recommended (Gidding et al., 2019; Irwin et al., 2009; Lower et al., 2017; Sellens et al., 2016), Similarly, this handbook recommends QFV for veterinary nurses, but alarmingly 93.3% (24/26) of the veterinary nurses participating in this study were not vaccinated. Furthermore, evidence of *C. burnetii* antibody was observed in two of the 24

(8.3%) unvaccinated veterinary nurses (Table 2.2) indicating exposure to *C. burnetii* and reinforcing the need for QFV amongst this group. This low rate of vaccination is consistent with the findings of Sellens et al. (2016) who surveyed Australia's veterinary workforce and found that only 29% veterinary nurses had sought QFV, compared to 74% of veterinarians. Poor knowledge and awareness of QFD and vaccination were cited as notable barriers for not having sought the Q fever vaccine amongst the veterinary nurse cohort. From a workplace health and safety (WH&S) perspective, veterinary employers and veterinarians have a legal and ethical responsibility to reduce or eliminate hazards or threats within the workplace such as those posed by diseases such as Q fever (Sellens, Norris, et al., 2018). Low rates of Q fever vaccine uptake in 'at-risk' groups such as AWRs and veterinary nurses, places them at unnecessary risk of *C. burnetii* infection. Overall these findings reinforce the need for greater Work Health & Safety promotion amongst employers by the delivery of targeted education programs to 'at-risk' groups regarding the risks of *C. burnetii* exposure, and appropriate risk prevention strategies, the most important of which is vaccination. The need for a national Q fever vaccine register was highlighted by five study participants stating that they were 'unsure' of their vaccination status. Given previous QFV is a contraindication for subsequent vaccination due to serious adverse events in those previously exposed to the vaccine, knowledge of vaccination status is vital. None of the vaccinated participants reported having been diagnosed with QFD, which supports the effectiveness of the vaccine (Gefenaite et al., 2011).

Comprehensive National Wildlife Biosecurity Guidelines issued by Wildlife Health Australia (Wildlife Health Australia, 2018) state that wildlife rehabilitators should be aware of, and implement, basic biosecurity practices at all times regardless of the animal species or perceived disease risk, and, in particular for Q fever, recommend that biosecurity practices include ventilation controls, P2/N95 particulate respirator, dust management, and QFV. Although approximately 95% of AWRs in this study reported practicing appropriate hand hygiene, a finding which is consistent with other studies of wildlife health professionals (Bosch et al., 2013; Garland-Lewis et al., 2017), overall we discovered a shortfall in the biosecurity practices within this cohort according to these guidelines. Given that

wildlife can serve as reservoirs of known and potentially novel zoonotic pathogens which can be transmitted to humans and domestic animals through bites, scratches and contact with bodily fluid such as urine and faeces (Garland-Lewis et al., 2017), it is essential for wildlife rehabilitators to adopt appropriate biosecurity practices (including the use of PPE) to help mitigate the risk of contracting Q fever and other zoonotic diseases. The reasons for the deficiency in biosecurity practices amongst this cohort are unclear. Significant knowledge gaps regarding Q fever have been identified in Australian cat breeders (Shapiro, Norris, et al., 2017) and Australian veterinary personnel (Sellens et al., 2016). A study of Australian veterinarians reported that a lack of perceived risk of zoonotic disease exposure and awareness of industry guidelines contributed to poor infection control practices and insufficient PPE usage (Dowd et al., 2013). It is anticipated that wildlife rehabilitators may have similar knowledge gaps regarding the availability of the National Wildlife Biosecurity Guidelines document, the health risks posed by zoonotic diseases, and what constitutes high-risk activities when rehabilitating wildlife. Future studies investigating the knowledge, attitudes and practices regarding zoonotic diseases among AWRs are required for the development and delivery of targeted education programs, aimed at improving biosecurity practices and preventing zoonotic disease transmission to this population. Although ensuring best practice biosecurity will aid in the prevention of many zoonotic diseases, the risk of contracting Q fever from infected animals is still possible due to the transmission mode and environmental persistence of *C. burnetii* (Maurin & Raoult, 1999; Raoult et al., 2005). Therefore, it is recommended that vaccination is a major component of the Q fever prevention strategy for at-risk populations.

## 2.7 Conclusion

This is the first study to investigate the level of *C. burnetii* exposure in rehabilitators of Australian wildlife and correlate seroprevalence with potential risk factors. We observed elevated *C. burnetii* seroprevalence and a higher rate of self-reported QFD in this cohort compared to the general Australian population, however only 8.1% of the cohort had received QFV. Although the source of their increased *C. burnetii* seropositivity requires further clarification, the increased exposure rates,

and the finding that wildlife rehabilitators as a group have a broad range of animal exposures suggest that rehabilitators of Australian wildlife would benefit from QFV. Therefore, as per national guidelines, QFV is recommended for this group (Australian Technical Advisory Group on Immunisation, 2021), and efforts are needed to increase awareness and uptake of vaccine uptake in this group. Shortfalls in the biosecurity practices employed by AWRs identified in this study has important implications, not only for Q fever, but for a range of zoonotic diseases.

# Chapter 3 Risk factors associated with self-reported Q fever in Australian wildlife rehabilitators: findings from an online survey

The content of this chapter is under review for publication in the international peer reviewed scientific journal *Zoonoses and Public Health* (anticipate citation below). Only the format has been changed for consistency of style in this thesis.

**Mathews, K. O**., Savage, C., Norris, J. M., Phalen, D., Malikides, N., Sheehy, P. A., & Bosward, K. L. (2022). Risk factors associated with self-reported Q fever in Australian wildlife rehabilitators: findings from an online survey. Submitted to *Zoonoses and Public Health*, (currently under review).

## 3.1 Abstract

Australian wildlife rehabilitators (AWR) are at increased risk of developing Q fever, a serious zoonotic disease caused by the intracellular bacterium *Coxiella burnetii*. Previous studies have suggested that Australian wildlife may be a potential *C. burnetii* infection source for humans. However, a recent serological survey of AWR found no association between *C. burnetii* exposure and direct contact with any wildlife species. To further explore the potential risk that wildlife may pose, this study aimed to identify associations between self-reported Q fever in AWR and risk factors for exposure to *C. burnetii*. An online cross-sectional survey was implemented in 2018 targeting AWR nationwide. Risk factors for self-reported Q fever were determined using multivariable logistic regression. Medically diagnosed Q fever was self-reported in 4.5% (13/287) of unvaccinated respondents. Rehabilitators who self-reported medically diagnosed Q fever were significantly more likely to: primarily rehabilitate wildlife at a veterinary clinic (OR 17.87, 95% CI: 3.09-110.92), have domestic ruminants residing on the property where they rehabilitate wildlife (OR 11.75, 95% CI: 2.91 – 57.42), have been educated at a High school/Technical and Further Education level (OR 10.29, 95% CI: 2.13-84.03) and be aged >50 years (OR 6.61, 95% CI: 1.60-38.35). No association was found between self-reported Q fever and direct contact with wildlife. These findings support previous work suggesting that AWR are at increased risk of *C. burnetii* infection and may develop Q fever potentially via exposure to traditional infection sources including livestock, other domestic animals or contaminated environments, in association with their rehabilitation practices and lifestyle. Although Q fever vaccination is recommended for AWR, vaccine uptake is low in this population. Future studies are required to identify barriers to Q fever vaccination in this at-risk group. The difficulty in accessing the AWR population also highlights the need for a national centralised AWR database.

## 3.2 Introduction

Q fever is a zoonotic disease initially described in 1935 amongst abattoir workers in Queensland, Australia (Derrick, 1937), but has since been found worldwide, except for New Zealand (Hilbink et al.,

1993). The Q fever agent *C. burnetii*, is an obligate intracellular bacterium that may cause acute and chronic human infections (Angelakis & Raoult, 2010; Marrie, 1990). *Coxiella burnetii* also exists as a highly infectious extracellular spore-like form, which can persist in the environment for at least 12 months (Kersh, Fitzpatrick, Self, Priestley, et al., 2013) and can be easily dispersed by the wind over long distances (Hawker et al., 1998). Domestic ruminants are regarded as the major reservoirs of human infection (Marrie et al., 1996). Infected ruminants contaminate the environment by shedding *C. burnetii* in their milk, urine, faeces and, to a greater extent, products of parturition (Marrie, 1990). Infection is mostly acquired following inhalation of contaminated aerosols.

In humans, the clinical manifestations of *C. burnetii* infection are broad, ranging from asymptomatic seroconversion in approximately in 20-80% of cases, to acute disease, which typically presents as a self-limiting 'influenza-like' illness, characterised by high fevers, headaches, chills and fatigue, with hepatitis and pneumonia as potential complications (Million & Raoult, 2015). Post-Q fever fatigue syndrome and persistent focal infection (previously 'chronic Q fever') are well recognised sequelae of *C. burnetii* infection, which may manifest years after primary infection, regardless of the initial clinical presentation (Eldin et al., 2017; Maurin & Raoult, 1999). Due to non-specific clinical symptoms, Q fever cases may go undiagnosed or result in delayed diagnosis (Million & Raoult, 2015). In Australia, Q fever has been nationally notifiable since 1977 (Garner et al., 1997), with approximately 500 human cases notified annually (National Notifiable Diseases Surveillance System, 2021). Australia is the only country where an effective licensed human Q fever vaccine (Q-Vax®; Seqirus, Parkville, Vic.) is available. Q fever vaccination is recommended for those engaged in high-risk occupations including abattoir workers, veterinarians and zoo and wildlife workers (Australian Technical Advisory Group on Immunisation, 2021).

In addition to traditional domestic animal sources, Australian wildlife have been suggested as potential sources of *C. burnetii* infection for humans. Evidence of *C. burnetii* exposure or infection has been observed in many wildlife species including bandicoots, possums, koalas, flying foxes (Bennett et

al., 2011; Tozer et al., 2014) and macropods (Banazis et al., 2010; Pope et al., 1960; Potter et al., 2011; Shapiro et al., 2020). Seroprevalence rates of between 21-33% have been reported in kangaroos in Western Australia (WA) and Queensland (QLD) (Banazis et al., 2010; Cooper, Barnes, et al., 2012; Potter et al., 2011). The detection of *C. burnetii* DNA in macropod faeces (Banazis et al., 2010; Cooper, Barnes, et al., 2012; Potter et al., 2011) and in raw meat containing kangaroo intended for pet consumption (Shapiro et al., 2020), suggests that macropods exposed to *C. burnetii* may become infected and subsequently amplify and shed the bacterium. Studies examining Q fever notification data have identified macropod exposure as a possible risk factor for *C. burnetii* infection in people with limited or no known exposure to the traditional high risk animals (Chong et al., 2003; Clutterbuck et al., 2018; Gale et al., 2007; Islam et al., 2011; Parker et al., 2010). Additionally, human Q fever cases in which patients were exposed to kangaroo and wallaby carcasses (Stevenson et al., 2015), kangaroo faeces and joeys, and worked in outdoor environments inhabited by kangaroos (Flint et al., 2016; Pickard, 2016), in the absence of exposure to traditional reservoir species such as livestock, have been reported. However, the link between Q fever and macropods remains circumstantial, and the role of macropods in *C. burnetii* transmission to humans remains poorly understood.

A recent serological survey investigating the link between wildlife exposure and Q fever identified Australian wildlife rehabilitators (AWR) as an at-risk population for *C. burnetii* infection (Mathews, Toribio, et al., 2021) with the 6.1% *C. burnetii* seropositivity among the cohort being 70% greater than that reported in a study of healthy Australian blood donors (3.6%) (Gidding et al., 2019). Furthermore, 2% of the unvaccinated AWR participants self-reported having had medically diagnosed Q fever. However, an association between direct wildlife exposure and *C. burnetii* seropositivity was not identified in the study, and risk factors for self-reported Q fever were unable to be evaluated due to the limited number of medically diagnosed Q fever cases (Mathews, Toribio, et al., 2021).

This study aims to build on the findings of Mathews, Toribio, et al. (2021) by using an online survey directed at AWR to (1) identify the association between self-reported medically diagnosed Q fever and risk factors for exposure to *C. burnetii* and (2) determine the proportion of AWR who had undergone Q fever vaccination.

## 3.3 Materials and Methods

### 3.3.1 Study design and recruitment

This cross-sectional online survey targeted AWR over 18 years of age from all Australian states and territories. Study data were collected and managed using REDCap electronic data capture tools hosted at The University of Sydney (Harris et al., 2019; Harris et al., 2009). REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources. Wildlife rehabilitators were recruited from June through to August 2019, with survey distribution aided by the following organisations: Wildlife Health Australia, For Australian Wildlife Needing Aid (FAWNA), Western Australian Wildlife Rehabilitation Council Inc., Tasmanian Wildlife Rehabilitation Council, Wildlife Victoria, Australian Wildlife Carer's Network Inc., and New South Wales (NSW) Wildlife Council, who advertised the survey to their members via email, newsletters and postings to social media groups and on websites. Reminders were sent after approximately 12 weeks. To maximise the response rate, the opportunity to win an electronic tablet was used as an incentive to motivate participation in the survey.

### 3.3.2 Sample size calculation

An estimated prevalence of 8% was used to calculate the minimum required sample size for this study. This was based on the prevalence of self-reported medically diagnosed Q fever in other Australian cohorts identified as being at high risk of Q fever (Australian Technical Advisory Group on

Immunisation, 2021) including: AWR (2%) (Mathews, Toribio, et al., 2021), veterinary personnel (2%) (Sellens et al., 2016) and cat breeders (6%) (Shapiro, Norris, et al., 2017). Using this assumption and a <1% prevalence of medically diagnosed Q fever in the general Australian population (National Notifiable Diseases Surveillance System, 2021), this study would require a sample size of 324, to achieve a power of 80% for detecting a difference in proportions of 7% between exposed and unexposed groups with a two sided p-value of 0.05 (Dhand & Khatkar, 2014).

### 3.3.3 Questionnaire design and implementation

The questionnaire (**Appendix B**) was developed with reference to previous studies (Guy & Banks, 2012; Sánchez & Baker, 2016; Sellens et al., 2016; Shapiro, Norris, et al., 2017), and in consultation with key stakeholders, including wildlife public health researchers, wildlife veterinarians and wildlife rehabilitators. Pretesting of the questionnaire via a pilot testing group allowed questions to be modified for clarity. The questionnaire consisted of 12 open, 23 closed, 16 checklist, nine Likert scale and four multiple choice questions which were divided across six sections. Questions focused on (i) the rehabilitator and the geographical and physical location used to rehabilitate wildlife, (ii) the type of wildlife rehabilitated and other animals residing nearby, (iii) rehabilitation and husbandry practices, iv) knowledge and attitudes regarding Q fever and its causative agent *C. burnetii*, (v) Q fever vaccination status, and (vi) Q fever disease and exposure to the agent. Participants were required to answer all questions and branching logic was employed to direct them through the questionnaire. Participants accessed the questionnaire via a hyperlink distributed via email, web page or social media. A participant information statement was provided explaining the purpose and expected outcomes of the research, and consent was obtained before questionnaire commencement. The study was approved by the Human Research Ethics Committee of the University of Sydney (project number 2018/270).

### 3.3.4 Data management and analysis

Upon survey closure, the data was exported from REDCap (Harris et al., 2019; Harris et al., 2009) into Microsoft® Excel® (Microsoft Corporation, Washington, USA) for preliminary exploration and processing and statistical analysis was performed using R statistical program® (R Core Team).

#### *3.3.4.1 Explanatory variables*

Descriptive statistics including mean (± standard error; SE), median (interquartile range; IQR) and range for continuous variables, and contingency tables for categorical variables, were generated to obtain information regarding their distribution. Continuous variables were transformed into practically plausible categorical variables. Where necessary, categorical variables were recategorised based on their distribution, biological plausibility and previous studies (Mathews, Toribio, et al., 2021).

#### *3.3.4.2 Outcome variable Q fever*

The primary outcome variable was Q fever status. Participants were classified as having had Q fever if they had self-reported, medically diagnosed Q fever. Participants who had not heard of Q fever before survey participation were also classified as not having had Q fever, based on the assumption that they would have remembered being medically diagnosed given the uncommon diagnosis. Similarly, participants who were unvaccinated for Q fever or were unsure of their vaccination status were classified as non-vaccinates, given that Q fever vaccination is a multi-step process and therefore more likely to be 'memorable' (Sellens, Norris, et al., 2018). Vaccinated participants were excluded from the logistic regression analysis as no vaccinated respondents self-reported having had Q fever.

#### *3.3.4.3 Univariable analysis*

Univariable logistic regression was conducted to evaluate the associations between potential risk factors and the outcome variable Q fever. All potential risk factors were screened and unadjusted odds ratios were calculated. Variables significantly associated with Q fever ($P < 0.2$) were included in multivariable analyses. Highly correlated variables were identified if Cramer's V statistic was > 0.7.

Only the variable deemed more biologically plausible was included in subsequent multivariable analysis.

*3.3.4.4 Multivariable analysis*

Multivariable logistic regression was undertaken to examine relationships between screened risk factors and the outcome variable Q fever. Biologically or practically relevant two-way interactions between explanatory variables were evaluated. Each interaction term was added to the base model and removed if the likelihood ratio statistic was insignificant ($P > 0.01$). A backward elimination approach was used to build the final model. All relevant risk factor variables were placed in the multivariable model and evaluated for confounding. Each variable was removed sequentially (starting with the variable with the highest *P*-value) and was considered to be a confounder, and therefore retained in the model, if it was significant ($P < 0.05$) or if its removal resulted in > 10% change in parameter estimates of explanatory variables, irrespective of its significance level. Multicollinearity between variables in the final multivariable model was identified when the variance inflation factor (VIF) was > 5.

## 3.4 Results

### 3.4.1 Response rate and descriptive analysis

In total, 405 participants accessed the questionnaire via the hyperlink and the final data set consisted of 338 (338/398; 84.9%) questionnaire responses (**Figure 3.1**). The survey response rate could not be determined because the survey was administered via electronic means (through email lists, websites, social media and newsletters), therefore the total number of people who received the survey is unknown.

Characteristics of the survey respondents are presented in **Table 3.1**. Participants were primarily female (282/338; 83.4%) and the median age of respondents was 52 years (19-80; IQR 42-62) with 54.7% (185/338) >50 years of age. Although all Australian states and territories were represented, compared to the available total national population estimates, the proportion of respondents residing

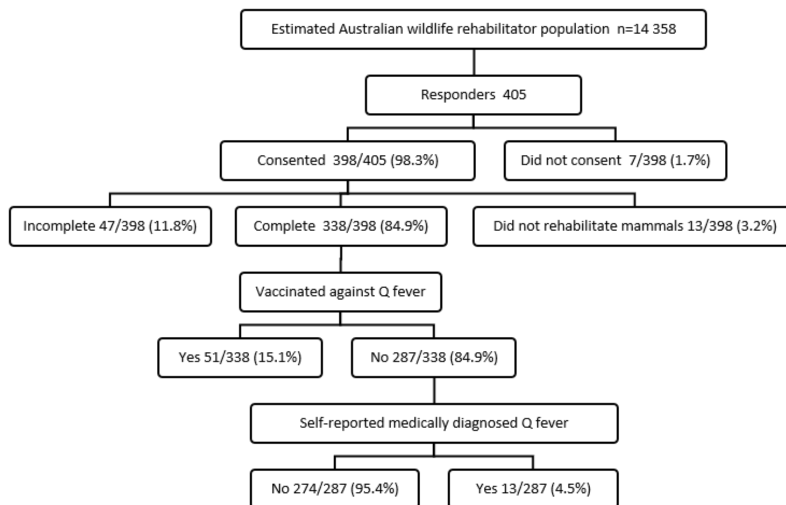


Figure 3.1 Breakdown of responses from Australian wildlife rehabilitators participating in a nationwide online survey regarding Q fever conducted in 2018. Estimation of the nationwide Australian wildlife rehabilitator population (n=14,358) is described in Mathews, Toribio, et al. (2021).

in NSW and Tasmania (TAS) was higher (24% and 8% respectively), the proportions in Victoria (VIC), South Australia (SA) and WA were lower (20%, 6% and 5% respectively), and the proportions within QLD, Northern Territory (NT) and Australian Capital Territory (ACT) (combined 23%) were comparable to the Australian population distribution (Australian Bureau of Statistics, 2018). The proportion of the cohort living in major cities (99/338; 29.3%) was lower, and the proportion living in inner regional Australia (167/338; 49.4%) was higher compared to the distribution of the general Australian population (70% and 18% respectively). Twenty one percent (72/338) of respondents resided in outer regional/remote areas, which is approximately double the population distribution (11%) for these remoteness categories. (National Rural Health Alliance, 2011).

Most participants (91.7%; 310/338) were associated with a wildlife group. Approximately 102 individual wildlife groups associations were reported across the cohort with the greatest number of representatives associated with NSW Wildlife Information, Rescue and Education Inc. (WIRES; 76/310; 24.5%), followed by Wildcare Australia (50/310; 16.1%), FAWNA (30/310; 9.7%), Sydney Metropolitan Wildlife Services (SMWS; 27/310; 8.7%), Wildlife Victoria (13/310; 4.2%) and Northern Rivers Wildlife

Carers (NRWLC; 12/310; 3.9%). Most participants (260/336; 77.4%) rehabilitated <50 animals per year. Possums and gliders were the most commonly rehabilitated animals being cared for by 80.5% (272/338) of respondents, followed by macropods (255/338; 75.4%), monotremes (272/338; 35.5%), flying foxes and microbats/bats (111/338; 32.8%), bandicoots (93/338; 27.5%), wombats (83/338; 24.6%), koalas (69/338; 20.4%), dasyurids (e.g. quolls and antechinus) and small marsupials (41/338;12.1%), and birds and reptiles (21/338; 6.2%). Just over half (53.3%; 180/338) reported having been present at, or assisting with, a non-human birth. Of these 46.7% (84/180) of births attended were ruminant, 48.9% (88/180) cat and dog, 16.1% (29/180) horses and 32.8% (59/180) other species including alpacas, cheetahs, giraffes and rodents.

### 3.4.2 Self-reported Q fever diagnosis

Overall, 51 (51/338; 15.1%) participants reported having been vaccinated against Q fever and were excluded from modelling for the outcome variable Q fever, leaving 287 unvaccinated participants in this dataset. Of the 287 (287/338; 84.9%) unvaccinated participants, 13 (13/287) self-reported having been medically diagnosed with Q fever (using laboratory testing), corresponding to a Q fever prevalence of 4.5% (95% CI 2.4% – 7.6%). A further seven (7/287; 2.4%) reported being ineligible to receive the vaccine due to a positive pre-vaccination screening test. Self-reported Q fever diagnosis occurred over 18 years (from 2000 to 2018), and the age at which the patients were diagnosed ranged from 20-64 years (median 52 years; IQR 12 years). Over half (8/13; 61.5%) were from NSW and most had been educated to a High School/ TAFE level (11/13; 84.6%). Just under half (6/13; 46.2%) of the medically diagnosed respondents were hospitalised due to their illness, with the duration of hospitalisation ranging from 2-21 days (mean 6.2 ± 3.0 days). The most frequently reported symptoms were chills (13/13; 100%), joint pain (13/13; 100%), fatigue (13/13; 100%), and sweat (12/13; 92.3%). Five participants (5/13; 38.5%) developed pneumonia, two (2/13; 23.1%) hepatitis and one (1/13; 15.4%) endocarditis. No pregnancy associated complications were reported.

**Table 3.1** Characteristics of Australian wildlife rehabilitators participating in a nationwide online survey conducted in 2018

| Variable | Category | Number | Proportion (%) |
|---|---|---|---|
| Gender | Female | 282 | 83.4 |
| | Male | 51 | 15.1 |
| | Prefer not to say | 5 | 1.5 |
| Age | >50 | 185 | 54.7 |
| | ≤50 | 153 | 45.3 |
| Level of education | University/Postgraduate | 153 | 45.3 |
| | High School Level/TAFE or private college | 185 | 54.7 |
| State of residence | New South Wales | 189 | 55.9 |
| | Queensland | 71 | 21.0 |
| | Tasmania | 31 | 9.2 |
| | Victoria | 21 | 6.2 |
| | Western Australia | 17 | 5.0 |
| | South Australia | 3 | 0.9 |
| | Northern Territory | 5 | 1.5 |
| | Australian Capital Territory | 1 | 0.3 |
| Remoteness classification | Major Cities of Australia | 99 | 29.3 |
| | Inner regional Australia | 167 | 49.4 |
| | Outer regional Australia/remote Australia/very remote Australia | 72 | 21.3 |
| Active Rehabilitator | No | 15 | 4.4 |
| | Yes | 323 | 95.6 |
| Years rehabilitating Australian mammals | 1-10 | 182 | 53.8 |
| | >10 | 156 | 46.2 |
| Number of animals cared for per year† | 1-50 | 260 | 77.4 |
| | more than 50 | 76 | 22.6 |
| Associated with wildlife groups | No | 28 | 8.3 |
| | Yes | 310 | 91.7 |
| Primary location of rehabilitating wildlife | Wildlife rescue/rehabilitation facility closed to the public | 44 | 13.0 |
| | Animal facility open to public | 14 | 4.1 |
| | Veterinary clinic | 30 | 8.9 |
| | Private residence | 296 | 87.6 |
| Care for wildlife on own property | No | 18 | 5.3 |
| | Yes | 320 | 94.7 |
| Number of people in household | <3 | 123 | 36.4 |
| | ≥3 | 95 | 28.3 |
| Occupational animal contact | No occupational contact | 213 | 63.0 |
| | Cattle sheep goats (ruminants) | 50 | 14.8 |
| | Non- ruminant occupational contact | 75 | 35.2 |
| Present at non-human birth | No | 158 | 46.7 |
| | Yes | 180 | 53.3 |
| Hand reared joeys | No | 50 | 14.8 |
| | Yes | 288 | 85.2 |
| Tick bite | No | 185 | 55.1 |
| | Yes | 151 | 44.9 |

† missing data n=2

Eight (8/13; 61.5%) participants self-reporting a Q fever diagnosis reported being present at, or assisting with, a non-human birth, of which six (6/8; 75%) were ruminant births. Of the respondents that reported handling joeys (10/13; 76.9%), nine (9/10; 90%) had handled macropod joeys and one (1/13; 7.7%) had handled possum and koala joeys. Eleven participants (11/13; 84.6%) had rehabilitated kangaroos or wallabies, and of the six (6/13; 46.2%) participants reporting occupational contact with animals, five (5/6; 83.3%) had ruminant contact and two (2/6 33.3%) had contact with kangaroos or wallabies.

### 3.4.3 Univariable analysis

Of the 27 risk factors investigated for association with Q fever amongst the 287 unvaccinated AWR, eight (**Appendix C**) progressed to multivariable analysis and no collinearity between any variable was identified (Cramers V < 0.7).

### 3.4.4 Multivariable analysis

None of the interaction terms were significant at the 1% level and therefore were not considered in the final model. Multivariable modelling identified four variables significantly associated with Q fever (**Table 3.2**). After controlling for the other variables in the model, AWR with medically diagnosed Q fever were more likely to: primarily rehabilitate wildlife at a veterinary clinic ($P < 0.002$), rehabilitate wildlife on a property in which domestic ruminants also resided ($P< 0.001$), have secondary or Technical and Further Education (TAFE) level education rather than tertiary level education ($P = 0.010$) and be aged > 50 years ($P = 0.017$). Occupational contact with ruminants was non-significant ($P = 0.074$) but was included in the final model as it confounded the relationship between other variables. Multicollinearity was not observed between the variables in the final model.

**Table 3.2** Results of final multivariable analysis for risk factors associated with self-reported Q fever among 287 unvaccinated Australian wildlife rehabilitators participating in a nationwide online survey conducted in 2018.

| Description | β | SE (β) | Adjusted odds ratio | 95% CI | *P*-value |
|---|---|---|---|---|---|
| Intercept | -7.81 | 1.41 | | | < 0.001 |
| Domestic ruminants living on the same property | | | | | 0.001 |
| No | | | 1 | | |
| Yes | 2.46 | 0.74 | 11.75 | 2.91-57.42 | |
| Primary rehabilitated Australian wildlife at a veterinary clinic | | | | | 0.002 |
| No | | | 1 | | |
| Yes | 2.88 | 0.89 | 17.87 | 3.09-110.92 | |
| Education level | | | | | 0.010 |
| University/Postgraduate | | | 1 | | |
| High School Level/TAFE or private college | 2.33 | 0.91 | 10.29 | 2.13-84.03 | |
| Age | | | | | 0.017 |
| ≤50 | | | 1 | | |
| >50 | 1.88 | 0.79 | 6.61 | 1.60-38.35 | |
| Occupational exposure to ruminants | | | | | 0.074 |
| No | | | 1 | | |
| Yes | 1.30 | 0.73 | 3.67 | 0.85-15.53 | |

TAFE - Technical and Further Education, CI – confidence intervals

## 3.5 Discussion

The 4.5% prevalence of medically diagnosed Q fever observed in this study is higher than, but similar to the 2.1% (n=3/147) self-reported Q fever prevalence found in a cohort of AWR attending a nationwide conference (Mathews, Toribio, et al., 2021). This 4.5% prevalence also extrapolates to approximately 4,530 cases of Q fever per 100,000 in AWRs over the 18 years (2000 – 2018) in which AWR in this study reported having been medically diagnosed with Q fever. This number is approximately 100 fold greater than the cumulative Australian Q fever notifications over the same 18 year period (2000-2018; 43 notifications per 100,000 of population) (National Notifiable Diseases Surveillance System, 2021). Together these studies provide further evidence that AWR are at increased risk of *C. burnetii* infection and developing Q fever than the Australian general population. In addition, the 4.5% self-reported Q fever prevalence in this cohort of AWR is comparable to that reported in other high-risk groups in Australia, including unvaccinated veterinary personnel (2%) (Sellens et al., 2016), cat breeders (6%) (Shapiro, Norris, et al., 2017) and goat producers (6%) (Gunther et al., 2019). This indicates that AWR have a similar risk of infection to these at-risk groups.

Given that many cases of Q fever are undiagnosed or misdiagnosed due to non-specific symptoms (Michelle Kermode et al., 2003), the 4.5% Q fever prevalence observed in this study may be an underestimation of the true Q fever prevalence within this cohort. This is supported by the finding that seven study participants returned a positive pre-vaccination screening test and were ineligible for vaccination.

However, it is possible that respondents who were aware of, had experience with, or were interested in Q fever may have been more likely to respond to the survey (Tripepi et al., 2010), resulting in a potential overrepresentation of people who had experienced Q fever. Given that this low magnitude of sampling bias may be offset by the possible effect of underdiagnosis and that around 95% of respondents in this study did not report Q fever, the Q fever prevalence determined in the current study probably still indicates that AWR have a higher risk of contracting Q fever. In addition, while participants were asked to self-identify as having been medically diagnosed with Q fever, the questionnaire did not ask them to specify the diagnostic test used for diagnosis. Therefore, there is potential for some degree of measurement bias, given the variation in sensitivity and specificity of the various serological assays (P.-E. Fournier et al., 1998). It is reasonable to assume, however, that those with significant clinical disease were accurately represented and diagnosed in this study.

The results of this study demonstrated that AWR who self-reported medically diagnosed Q fever were 18 times more likely to have primarily rehabilitated wildlife at a veterinary clinic. This finding is probably due to factors associated with veterinary clinics which may increase the likelihood of AWR being directly or indirectly exposed to *C. burnetii.* Small, large, and mixed animal veterinary clinics treat a variety of animal species known to be potential reservoirs of *C. burnetii,* including livestock species (Marrie, 1990) and companion animals such as cats and dogs (Kopecny et al., 2013; Shapiro et al., 2016) In addition, animals visiting veterinary clinics for reproductive and obstetric procedures, particularly those who are periparturient, may present a greater risk due to the organism's predilection for the products of conception (Welsh et al., 1958). Q fever outbreaks among veterinary

personnel have been associated with indirect or direct contact with birth products following dog and cat caesareans in small animal veterinary clinics (Gibbons & White, 2014; Kopecny et al., 2013). Furthermore, *C. burnetii* can survive in the air for up to two weeks (Welsh et al., 1958), and therefore infection is possible in people without direct exposure to infected animals or their products.

Another possible explanation for why rehabilitating wildlife in a veterinary clinic setting may result in an increased risk for *C. burnetii* infection is the low levels of QFV in AWR. Although excluded from analysis, only 15.1% of the study cohort reported having been vaccinated, which is slightly higher, but similar to the 8% vaccination rate reported by Mathews, Toribio, et al. (2021). These low vaccination rates are a significant concern for a population for whom vaccination is recommended by the Australian government (Australian Technical Advisory Group on Immunisation, 2021). Other Australian studies have observed similarly low vaccination rates in high-risk groups for whom QFV is recommended, such as veterinary personnel and livestock industry workers (Gidding et al., 2019; Lower et al., 2017; Massey et al., 2009; Sellens et al., 2016). The occupation of participants in the current study was not reported, however, it is possible that some of those rehabilitating in a veterinary clinic setting were ancillary veterinary workers (e.g., veterinary nurses or reception staff). Future work should aim to determine the level of Q fever awareness, identify barriers to QFV in AWR, and help formulate strategies for enhancing vaccine uptake in this group, which may help to enhance uptake in other at-risk groups.

In this study, rehabilitators reporting having been diagnosed with Q fever were more than 10 times as likely to have reported achieving a lower level of education (high school, TAFE or private college). Although, as mentioned above, occupation was unmeasured in this study, a potential explanation for this association could be that many AWR are employed as para-veterinary staff who, unlike those who have been enrolled in veterinary and animal science degrees (Sellens et al., 2016), are not required to be vaccinated as part of their training. These findings are supported by those of Sellens et al. (2016) and emphasise the need to better educate all veterinary clinic employees, and AWR associated with

veterinary clinics, about the potential risk of exposure to *C. burnetii* and the importance of Q fever vaccination.

The finding that AWR self-reporting Q fever were almost 12 times more likely to rehabilitate wildlife on a property that housed domestic ruminants was not surprising, given that contact with domestic ruminants is an important and well-known risk factor for human *C. burnetii* infection (Angelakis & Raoult, 2010). Infected ruminants contaminate the environment by shedding *C. burnetii* in high numbers in their birth products and to a lesser extent in their milk, urine and faeces (Maurin & Raoult, 1999). *Coxiella burnetii* transmission to AWR potentially occurred via inhalation of aerosolised organisms through direct contact with ruminants, and/or indirectly through contact with environments contaminated by livestock species.

Finally, the results of this analysis showed that AWR who self-reported Q fever were more likely to be aged >50 years at the time of the survey. This is commonly reported in Q fever notification data (Clutterbuck et al., 2018; Sloan-Gardner et al., 2017), and is thought to be due to the cumulative increased risk of exposure over time and/or the concomitant decline in cellular immunity during the aging process (Weiskopf et al., 2009). While eleven of thirteen AWR self-reporting Q fever were female, gender was also not a risk factor for Q fever. A consistent observation across AWR study cohorts (including the current study cohort) is that most AWR are female (Englefield, Candy, et al., 2019; Guy & Banks, 2012; Mathews, Toribio, et al., 2021; Tribe & Brown, 2000). Q fever has traditionally been associated with males, most likely as a consequence of the occupations (e.g., abattoir workers, farmers, etc) in which men predominate and where the risk of exposure is high (Chiu et al., 2010; Sloan-Gardner et al., 2017). However, given the results of this study, and the elevated *C. burnetii* seroprevalence observed in female AWR (Mathews, Toribio, et al., 2021), medical practitioners should not discount Q fever in their differential diagnosis in female AWR presenting with an acute flu-like illness.

Consistent with a recent serosurvey in AWR where direct contact with wildlife species was not identified as a risk factor for *C. burnetii* seropositivity (Mathews, Toribio, et al., 2021), this study did not identify contact with kangaroos or other wildlife species as a risk factor for *C. burnetii* infection. While there is a body of evidence implicating macropods as a source of *C. burnetii* infection for humans (Banazis et al., 2010; Clutterbuck et al., 2018; Cooper, Barnes, et al., 2012; Flint et al., 2016; Pope et al., 1960; Potter et al., 2011; Shapiro et al., 2020; Stevenson et al., 2015), the evidence is still largely circumstantial. Additionally, the mechanism by which *C. burnetii* is amplified and shed into the environment by macropods or other wildlife species remains poorly understood and is an area at which future research should be directed.

Accessing the entire AWR population for this study proved difficult because, currently, members of the Australian wildlife and rehabilitation sector are registered with different state or territory authorities governed by different licensing arrangements (Englefield, Blackman, et al., 2019; Englefield et al., 2018; Haering et al., 2020) with no unifying national governing body through which AWR can be contacted. In addition to difficulties contacting rehabilitators, calculation of a response rate, which is reliant on a known numerator (number of AWR the survey reached) and denominator (number of AWR nationwide), was not possible because neither of these values could be determined with accuracy. Regarding the numerator, the questionnaire was disseminated electronically via social media, which is currently a common, cost effective, and convenient way of managing surveys (Wright, 2017). However, the 'snowball effect' of social media sharing rendered estimating the number of people the survey reached impossible. Regarding the denominator, the number of people involved in rehabilitating wildlife nationwide is unknown, although it has been estimated at 14 358 by Mathews, Toribio, et al. (2021) and 17 000 by Englefield, Candy, et al. (2019).

Despite the inability to determine an accurate response rate, it is important to note that the participants of this study reported being associated with approximately 102 different individual wildlife rehabilitator groups/associations, and the number of survey responses received (n= 338) was

higher than, though in a similar order of magnitude to, that reported in another national online survey of AWR (n=270) investigating the mental, physical and financial challenges faced by this population (Englefield, Candy, et al., 2019). Furthermore, the age and sex distribution amongst the current cohort aligns with other Australian studies on AWR, with most being female and a high proportion aged >50 years (Englefield, Candy, et al., 2019; Guy & Banks, 2012; Mathews, Toribio, et al., 2021; Tribe & Brown, 2000), suggesting this is likely a representation of the broader population. All Australian states and territories were represented amongst the study participants, although the distribution of survey respondents according to state and territory differed from the national general population distribution, with a larger proportion of respondents residing in NSW and TAS (24% and 8% respectively) and the proportions residing in VIC, SA and WA were lower (20%, 6% and 5% respectively). The proportions within QLD, NT and ACT (combined 23%) were comparable to the Australian population distribution. It remains unclear, however, whether the geographical distribution of the current cohort is representative of the broader AWR population, due to the paucity of studies on AWR and the lack of a centralised database from which to access such information. Despite these limitations, the current AWR cohort were probably a reasonable representation of the AWR population in Australia, and the sample size was sufficient to achieve the 80% power required to detect the significant and high magnitude risk variable associations found in those self-reporting Q fever.

Given that wildlife are acknowledged as major reservoirs for transmitting emerging and zoonotic agents to humans and domestic animals (Kruse et al., 2004), the difficulty accessing the AWR population in a coordinated way is of concern. Rehabilitators at the forefront of the human-wildlife interface are at increased risk of directly and/or indirectly contracting Q fever and other zoonoses (through vectors and contaminated environments) including rickettsioses (Mathews, Phalen, et al., 2021), Australian bat lyssavirus (Wildlife Health Australia, 2019), salmonellosis (Wildlife Health Australia, 2018), tularaemia (Wildlife Health Australia, 2020) and psittacosis (Wildlife Health Australia, 2017). A centralised national database operating through an organisation such as Wildlife Health

Australia (https://www.wildlifehealthaustralia.com.au/), may facilitate a channel of coordinated contact with the majority of AWR, providing a means to efficiently relay critical information on wildlife biosecurity and about the risks, prevention, and management of zoonoses specific to Australia. A centralised database would also serve as a surveillance tool to help identify new and emerging diseases and assist with the effective management of any new disease outbreaks.

## 3.6 Conclusion

The higher prevalence of self-reported medically diagnosed Q fever observed in this AWR population is consistent with the findings of a recent serosurvey in AWR (Mathews, Toribio, et al., 2021), providing further evidence to support the recommendation of Q fever vaccination for rehabilitators of Australian wildlife. Rehabilitating wildlife on a property that housed domestic ruminants and associations with veterinary clinics were risk factors for Q fever in this study. However, associations between Q fever and direct contact with specific wildlife species including macropods were not identified. These findings suggest that AWR may be exposed to *C. burnetii* and develop Q fever via associations with traditionally recognised animal and environmental sources of infection such as livestock, and potentially through the environment via their wildlife rehabilitation-associated activities (e.g., collecting feed sources such as browse and recovering or releasing animals), but not necessarily through direct contact with the wildlife themselves. However, given the established evidence that wildlife can become infected with *C. burnetii*, further research is needed to understand the pathogenesis of infection in wildlife and potential routes of shedding. This study also highlighted that Q fever vaccination rates in AWR are low despite their recognition as an at-risk population and therefore future studies are needed to identify barriers to vaccination in this group. The difficulty in accessing the AWR population experienced in this study also highlights the need for a national centralised AWR database.

# Chapter 4 Factors associated with Q fever vaccination in Australian wildlife rehabilitators

The content of this chapter is currently under review in the international peer reviewed scientific journal *Vaccine* (anticipated citation below). Only the format has been changed for consistency of style in this thesis.

**Mathews, K. O**., Norris, J. M., Phalen, D., Malikides, N., Savage, C., Sheehy, P. A., & Bosward, K. L. (2022). Factors associated with Q fever vaccination in Australian wildlife rehabilitators. Submitted to *Vaccine*, (currently under review).

## 4.1 Abstract

Australian wildlife rehabilitators (AWR) are at risk of contracting Q fever, a serious zoonotic disease caused by *Coxiella burnetii*. Despite Australian government recommendations for AWR to be vaccinated against Q fever, and the availability of a safe and effective vaccine in Australia, shortfalls in vaccine uptake have been observed in this group. This study aimed to determine factors associated with vaccination and describe AWR attitudes and potential barriers towards Q fever vaccination (QFV). Data used in this analysis was obtained from a nationwide, online, cross-sectional survey of AWR undertaken in 2018. Approximately three quarters (200/265; 75.5%) of those that had heard of Q fever were also aware of the Q fever vaccine and, of those, 25% (51/200) were vaccinated. Barriers to QFV, among unvaccinated (149/200; 74.0%) respondents who had also heard of Q fever and the vaccine, included concerns regarding the safety, efficacy and importance of the Q fever vaccine. Vaccine complacency, convenience of vaccination and a lack of Q fever knowledge were also notable barriers. Only 27.7% (41/148) of respondents reported having had vaccination recommended to them. Multivariable logistic regression identified that vaccinated AWR were more likely to be aged≤ 50 years (OR 4.51, 95% CI: 2.14-10.11), have university level education (OR 2.78, 95% CI: 1.39-5.73), have attended an animal birth (OR 2.14, 95% CI: 1.02-4.73) and were more likely to reside in New South Wales/Australian Capital Territory and Queensland than in other Australian jurisdictions (OR 2.9, 95% CI: 1.10-8.83 and OR 4.82, 95% CI: 1.64-16.00 respectively). The knowledge gaps regarding Q fever and QFV in AWR demonstrate the need for interventions to raise the awareness of the potential health consequences of *C. burnetii* exposure and prevention of Q fever. Education programs to allow AWR to develop an informed perspective of Q fever and QFV, coupled with improvements in vaccine affordability and the implementation of programs to enhance accessibility may also increase vaccine uptake.

## 4.2 Introduction

Q fever is a zoonosis caused by the intracellular bacterium *Coxiella burnetii* (Maurin & Raoult, 1999), cases of which have been documented globally, except for New Zealand (Hilbink et al., 1993). The main mode of C. burnetii transmission to humans is via inhalation of aerosols or dust contaminated with the bacterium, which is shed into the environment in the milk, faeces and birth products of infected animals. Domestic ruminants (cattle, sheep and goats) are regarded as the main reservoirs of *C. burnetii* and the most important sources of human infection (Marrie, 1990). However, evidence of *C. burnetii* infection has also been identified in a range of animal species including cats (Kopecny et al., 2013), dogs (Shapiro et al., 2016), horses (Marenzoni et al., 2013), birds (To et al., 1998), wildlife (Banazis et al., 2010; Bennett et al., 2011; Cooper et al., 2013; Lockhart et al., 2011; Potter et al., 2011) and wildlife ticks (Cooper et al., 2013; Pope et al., 1960).

The clinical manifestations of Q fever vary depending on the age, country of residence, sex of those infected (Million & Raoult, 2015) and *C. burnetii* strain (Long et al., 2019). Acute Q fever is asymptomatic in 20-80% (Million & Raoult, 2015) of cases, and symptomatic infections most commonly present as a self-limiting 'flu-like' illness characterised by headaches, chills, high fevers and night sweats (Raoult et al., 2005). Persistent focal infection (previously chronic Q fever), and post-Q fever fatigue syndrome are also well known sequelae of Q fever; the former most commonly presents as endocarditis with increased susceptibility in people with pre-existing heart valve defects (Eldin et al., 2017). *Coxiella burnetii* infection during pregnancy may lead to adverse pregnancy outcomes including miscarriage and pre-term birth (Mboussou et al., 2019; Raoult et al., 2005). Due to non-specific clinical presentations, Q fever diagnosis is often delayed or missed in the absence of a high index of suspicion, highlighting the importance of prevention in at-risk populations (Healy et al., 2011).

Currently, Australia is the only country with a licenced human Q fever vaccine (Q-Vax®; Seqirus, Parkville, Vic.), which has been available since 1989 (Marmion et al., 1990). Q-Vax® is a whole-cell

formalin inactivated vaccine produced using purified Phase 1 *C. burnetii* Henzerling strain (Seqirus, 2019), and is highly effective in preventing *C. burnetii* infection in humans (Chiu & Durrheim, 2007; Gefenaite et al., 2011; Woldeyohannes et al., 2020). Because of the airborne transmission of *C. burnetii*, vaccination remains the most effective strategy for preventing Q fever and, in Australia, it is recommended for high-risk occupational groups such as farmers, veterinary personnel, abattoir, wildlife and zoo workers (Australian Technical Advisory Group on Immunisation, 2021). However, despite the availability of Q-Vax®, on average approximately 400-500 Q fever notifications are recorded in Australia annually (National Notifiable Diseases Surveillance System, 2021).

Q fever vaccine uptake among high-risk populations in Australia is variable. Uptake prevalences of 50-100% have been reported in abattoir workers and shearers who are routinely vaccinated by their employers before commencing employment (Gidding et al., 2009). Uptake in veterinarians, who are vaccinated as a requirement of their university studies, is estimated at 74% (Sellens et al., 2016). In contrast, lower uptake has been reported for veterinary nurses (29%) (Sellens et al., 2016), who have not historically been offered vaccination as part of their training or through their workplace. Low vaccine uptake has also been reported for other at-risk groups who are largely self-employed including farmers (18-43%) (Gidding et al., 2009), goat producers (17%) (Gunther et al., 2019) and for other at-risk groups such as Australian cat breeders (2%) (Shapiro, Norris, et al., 2017).

Australian wildlife rehabilitators (AWR) have recently been confirmed as a group who are at increased risk of exposure to *C. burnetii* and developing Q fever (Mathews et al., 2022; Mathews, Toribio, et al., 2021). Although national guidelines recommend Q fever vaccination (QFV) for wildlife workers (Australian Technical Advisory Group on Immunisation, 2021), vaccination prevalences of less than 16% have been observed in AWR cohorts (Mathews et al., 2022; Mathews, Toribio, et al., 2021). Reasons for this shortfall in vaccine uptake by AWR are unknown, although lack of awareness of the existence of the Q fever vaccine, lack of perceived susceptibility, vaccine access, and financial expense have been cited as contributing to shortfalls in vaccine uptake among other high-risk groups (Gidding

et al., 2019; Lower et al., 2017; Massey et al., 2009; Sellens et al., 2016; Shapiro, Norris, et al., 2017). An understanding of the factors associated with QFV status, Q fever vaccine awareness, and knowledge of potential barriers associated with becoming vaccinated against Q fever in AWR may help to inform strategies for improving Q fever vaccine uptake in this population.

Therefore, this study aimed to use an online survey targeting AWR to: (1) investigate the association between demographic and other factors and QFV status in AWR; (2); describe attitudes towards vaccines in general and QFV specifically in AWR; and (3) identify potential barriers to QFV in AWR.

## 4.3 Materials and Methods

### 4.3.1 Study design and recruitment

Study data were obtained from a nationwide online cross-sectional survey undertaken in 2018 targeting AWR over 18 years of age, the design, method and results of which have been published elsewhere [companion manuscript (Mathews et al., 2022)]. The questionnaire (**Appendix B**) consisted of open, closed, checklist, Likert scale and multiple choice questions, which were divided across six sections: (i) the rehabilitator and the geographical and physical location used to rehabilitate wildlife, (ii) the type of wildlife rehabilitated and other animals residing nearby, (iii) rehabilitation and husbandry practices, iv) knowledge and attitudes regarding Q fever and its causative agent *C. burnetii*, (v) Q fever vaccination status, and (vi) Q fever disease and exposure to the agent (Supplementary Material 1). Participants were required to answer all questions and branching logic was employed to direct them through the questionnaire. The study was approved by the University of Sydney Human Research Ethics Committee (project number 2018/270).

### 4.3.2 Sample size calculation

An estimated QFV prevalence of 8-15% in exposed (vaccinated) AWR (Mathews et al., 2022; Mathews, Toribio, et al., 2021) was used to calculate the required sample size for this study. Using this assumption and a ≤ 1% prevalence of QFV in unexposed (non-vaccinated AWR; which included those unaware of Q fever and QFV), this study would require a sample size of between 138-324, to

achieve a power of 80% for detecting a difference in proportions of 7-14% between exposed and unexposed groups with a two-sided *p*-value of 0.05 (Dhand & Khatkar, 2014).

### 4.3.3 Data management and analysis

Study data were collected using Research Electronic Data Capture (REDCap) tools hosted at The University of Sydney (Harris et al., 2019; Harris et al., 2009). This platform is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages, and 4) procedures for importing data from external sources. Survey data was explored and processed using Microsoft® Excel® (Microsoft Corporation, Washington, USA) and analysed using R statistical program® (R Core Team).

Four analyses were performed. In the first, data from the entire AWR cohort (n=338) were used to identify associations between QFV status and demographic and other variables using multivariable logistic regression. In the second analysis, two subgroups were formed whereby AWR with prior awareness of Q fever and prior awareness of QFV (n=200; classified as being 'aware' of Q fever) and AWR who were either aware of Q fever but unaware of QFV, or unaware of Q fever and QFV (n=138; classified as being 'unaware' of Q fever). Descriptive statistics regarding demographic and other factors of each subgroup were generated, and, where necessary, to emphasise a statistically significant difference, Chi-squared or Fisher's exact tests were performed. In the third analysis, descriptive statistics regarding attitudes towards QFV and vaccination in general, and associations between QFV status and the source from which rehabilitators had heard of Q fever were explored in the sub-population of 'aware' AWR (n=200). Finally, in the fourth analysis barriers to QFV were explored in the subgroup of 'aware' respondents who had knowledge of the Q fever vaccine but had not been vaccinated (n=148). Statistical significance was set at $p < 0.05$.

*4.3.3.1 Explanatory variables*

Descriptive statistics including median (interquartile range; IQR), mean (± standard error; SE), range for continuous variables, and contingency tables for categorical variables, were generated to obtain information regarding their distribution. Where necessary continuous variables were transformed into practically plausible categorical variables for further analyses, based on biological plausibility, distribution and previous studies (Mathews, Toribio, et al., 2021).

Responses to attitudinal statements regarding vaccination that used a Likert scale (i.e., strongly agree, agree, neither agree nor disagree, disagree, strongly disagree) were collapsed as follows: respondents were categorised as 'agree' if they selected strongly agree, or agree; 'disagree' if they selected strongly disagree, or disagree; and 'unsure' if they selected 'neither agree nor disagree'. Questions regarding where participants had heard of Q fever were collapsed into groups as follows: wildlife associated activities (wildlife rehabilitation group, wildlife conference, another AWR, AWR training session); veterinary personnel (veterinarian/veterinary nurse); media (social media, web-based article, newspaper or magazines, TV, radio) and occupation/education (workplace/TAFE (Technical and Further Education)/government agency). The reasons cited for not being vaccinated against Q fever (barriers) by unvaccinated participants or those unsure of their vaccination status were grouped into themes of 'complacency and lack of knowledge', 'convenience', 'hesitancy', and 'pre-existing immunity'.

*4.3.3.2 Q fever vaccination status*

The outcome variable 'QFV status' was defined as those AWR who self-reported having previously received QFV, had previously heard about Q fever and were aware of the availability of vaccination (Figure 1). Respondents who self-reported being unvaccinated or who were unsure of their vaccination status were classified as non-vaccinates; the latter justified on the basis that QFV is a multi-step process and therefore more likely to be 'memorable' (Sellens, Norris, et al., 2018). Similarly, those participants who had not previously heard about Q fever, or had previously heard

about Q fever but were unaware of the availability of vaccination were also classified as non-vaccinates.

*4.3.3.3 Univariable analysis*

Univariable logistic regression was undertaken to identify associations between explanatory variables and QFV status. Initially, all variables were screened and unadjusted odds ratios calculated. Variables significantly associated with QFV status ($p < 0.1$) were progressed to the multivariable analysis after screening for collinearity. Highly correlated variables were identified if Cramer's V statistic was > 0.7. Only the variable considered more biologically plausible was progressed to multivariable analysis.

*4.3.3.4 Multivariable analysis*

Multivariable logistic regression was undertaken to evaluate relationships between screened explanatory variables and the outcome variable QFV status. Firstly, all biologically plausible two-way interactions between explanatory variables were evaluated. Each interaction term was added to the base model and removed if the likelihood ratio statistic was insignificant ($p > 0.01$). The final model was built using a backward elimination approach, whereby all relevant variables were placed in the multivariable model and evaluated for confounding. Starting with the variable with the highest P-value, each variable was removed sequentially and was considered to be a confounder, and thus retained in the model, if it was significant ($p < 0.05$) or if its removal resulted in > 10% change in parameter estimates of explanatory variables, regardless of its significance level. Multicollinearity between variables in the final multivariable model was identified when the variance inflation factor (VIF) was >5. The model was determined to be a good fit for the data if the Hosmer and Lemeshow Goodness of Fit test returned a $p$ >0.05.

## 4.4 Results

### 4.4.1 Study population

A flow diagram of responses to questions exploring Q fever and Q fever vaccine awareness and QFV status for all 338 AWR respondents is presented in Figure 1. The total number of responses for some questions varies due to some participants not responding to every question.

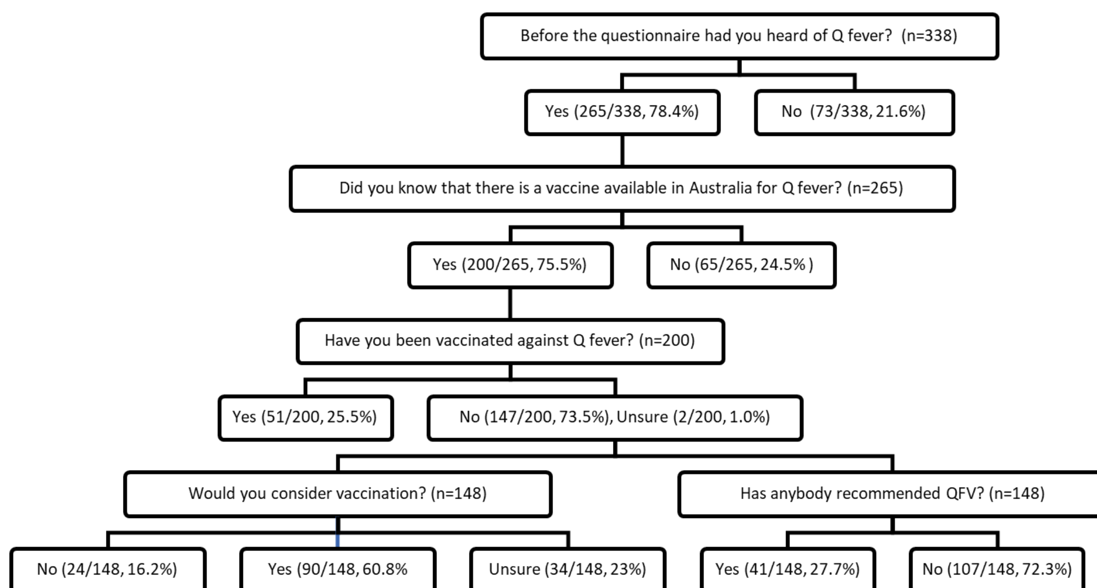


**Figure 4.1** Flow diagram of responses to questions exploring Q fever vaccine awareness and vaccination status of Australian wildlife rehabilitators participating in a nationwide online survey on Q fever knowledge, attitudes and practices conducted in 2018. The total number (n) of responses for some questions varies due to some participants not responding to every question.

### 4.4.2 Factors associated with Q fever vaccination status

#### *4.4.2.1 Univariable analysis*

Of the 16 explanatory variables investigated for association with QFV status amongst the entire cohort (n=338), 11 progressed to multivariable analysis and collinearity between variables was not demonstrated (Cramer's V <0.7).

#### *4.4.2.2 Multivariable analysis*

No significant interaction terms were identified. Multivariable modelling identified five variables significantly associated with the outcome variable QFV status (**Table 4.1**). After controlling for the other variables in the model, vaccinated AWR were more likely to be aged ≤ 50 years ($p < 0.001$), have university-level education ($p = 0.003$), have attended an animal birth ($p = 0.043$) and reside in New South Wales/Australian Capital Territory (NSW/ACT) and Queensland (QLD) than in other Australian jurisdictions ($p = 0.013$). Four variables [occupational contact with animals, number of animals rehabilitated per year, domestic ruminants living on the same property and primarily rehabilitated wildlife at an animal facility open to the public (e.g. zoo)], although non-significant ($p > 0.05$) were retained in the final model as confounders. The Hosmer and Lemeshow Goodness of Fit test indicated that the model was a good representation of the data ($p = 0.133$).

### 4.4.3 Q fever awareness

**Table 4.2** shows the descriptive and odds ratio results for variables significantly associated with Q fever awareness in the two subgroups: AWR with prior awareness of Q fever and prior awareness of QFV (n=200; classified as being 'aware of Q fever') and AWR who were either aware of Q fever but unaware of QFV or unaware of Q fever and QFV (n=138; classified as being 'unaware' of Q fever). The descriptive results of the other variables analysed for an association with Q fever awareness were 'similar' between the two subgroups (**Table S1 Appendix D**).

#### *4.4.3.1 Subgroup 'Unaware'*

Overall, 40.8% (138/338) of respondents were classified as being 'unaware' of Q fever, as defined. Around one quarter (32/125; 25.6%) of those who reported occupational animal contact and just under one third (59/180; 32.8%) who had been present at an animal birth were 'unaware' of Q fever.

**Table 4.1** Results of multivariable logistic regression analysis for variables associated with Q fever vaccination status among Australian wildlife rehabilitators participating in a nationwide online survey conducted in 2018 (n=338).

| Variable name and description | β | SE (β) | Adjusted odds ratio | 95% CI | *p*-value |
|---|---|---|---|---|---|
| Intercept | -5.73 | 0.85 | | | |
| Age | | | | | < 0.001 |
| >50 | | | Ref | | |
| ≤50 | 1.51 | 0.39 | 4.51 | 2.14-10.11 | |
| Education level | | | | | 0.003 |
| High School Level/TAFE or private college | | | Ref | | |
| University/Postgraduate | 1.02 | 0.36 | 2.78 | 1.39-5.73 | |
| State of residence | | | | | 0.013 |
| Other | | | Ref | | |
| NSW/ACT | 1.06 | 0.53 | 2.90 | 1.10-8.83 | 0.043 |
| Queensland | 1.57 | 0.57 | 4.82 | 1.64-16.0 | 0.006 |
| Present at an animal birth | | | | | 0.043 |
| No | | | Ref | | |
| Yes | 0.77 | 0.37 | 2.14 | 1.02-4.73 | |
| Occupational animal contact | | | | | 0.069† |
| No | | | Ref | | |
| Yes | 0.66 | 0.37 | 1.94 | 0.95-4.01 | |
| Number of animals rehabilitated per year | | | | | 0.138† |
| ≥ 50 | | | Ref | | |
| 1-50 | 0.67 | 0.47 | 1.95 | 0.81-5.28 | |
| Domestic ruminants living on the same property | | | | | 0.169† |
| No | | | Ref | | |
| Yes | 0.55 | 0.40 | 1.73 | 0.79-3.74 | |
| Primarily rehabilitated wildlife at a rescue facility open to the public eg zoo | | | | | 0.235† |
| No | | | Ref | | |
| Yes | 0.84 | 0.70 | 2.32 | 0.57-9.16 | |

TAFE Technical and Further Education, NSW New South Wales, ACT Australian Capital Territory, CI Confidence Interval, SE Standard error, β Beta coefficient, † **retained in the model as confounders,** Ref-Reference category

**Table 4.2** Descriptive (n, %, totals) and odds ratio (and Chi-square *p*-value) results for variables significantly associated with awareness of Q fever and Q fever vaccination among Australian wildlife rehabilitators participating in a nationwide online survey conducted in 2018 (n=338).

| Variable name and description | Awareness of Q fever and the Q fever vaccine n (%) | | | Odds ratio | *p*-value |
|---|---|---|---|---|---|
| | Aware (n=200) | Unaware (n=138) | Row total | | |
| State of residence* | | | | | 0.001 |
| Other | 31 (40.3) | 46 (59.7) | 77 (22.8) | Ref | |
| NSW/ACT | 121 (63.7) | 69 (36.3) | 190 (56.2) | 2.60 | |
| Queensland | 48 (67.6) | 23 (32.4) | 71 (21.0) | 3.10 | |
| Primary place of rehabilitating Australian wildlife | | | | | |
| Wildlife rescue facility open to the public eg zoo | | | | | 0.050* |
| No | 188 (58.0) | 136 (42.0) | 324 (95.9) | Ref | |
| Yes | 12 (85.7) | 2 (14.3) | 14 (4.1) | 4.30 | |
| Occupational animal contact* | | | | | <0.001 |
| No | 107 (50.2) | 106 (49.8) | 213 (63.0) | Ref | |
| Yes | 93 (74.4) | 32 (25.6) | 125 (37.0) | 2.88 | |
| Present at an animal birth* | | | | | 0.001 |
| No | 79 (50.0) | 79 (50.0) | 158 (46.7) | Ref | |
| Yes | 121 (67.2) | 59 (32.8) | 180 (53.3) | 2.05 | |

Rehabilitators were classified as 'aware' of Q fever if they had heard of Q fever and the Q fever vaccine and 'unaware' if they had not heard of Q fever or had heard of Q fever but had not heard of the Q fever vaccine. NSW New South Wales, ACT Australian Capital Territory, * Fisher's exact test, Ref-Reference category

*4.4.3.2 Subgroup 'Aware'*

Over three quarters (265/338; 78.4%) of the cohort had heard of Q fever before participating in the survey. The majority (200/265; 75.5%) of these respondents had also heard of the Q fever vaccine and were classified as being 'aware' of Q fever. Of these 25.5% (51/200) had been vaccinated. Participants who were ***'***aware***'*** of Q fever were significantly more likely to report occupational animal contact ($p < 0.001$), or to have attended an animal birth ($p < 0.001$), or to have come from NSW/ACT or QLD ($p = 0.001$) (**Table 4.2**).

**Table 4.3** provides a descriptive analysis of this subgroup of 'aware' AWR (n=200) relative to their QFV status. Vaccinated AWR were more likely to have heard about Q fever through employment/education sources ($p = 0.013$) and less likely to have heard about Q fever through media ($p < 0.001$), wildlife associated activities ($p = 0.001$) or a family member or a friend ($p = 0.019$). The descriptive results of the other variables analysed for an association with QFV status in 'aware' AWR were 'similar' between vaccinated and unvaccinated participants (**Table S2 Appendix D**).

### 4.4.4 Attitudes towards vaccines and Q fever vaccination

Participants were asked to indicate their level of agreement with five attitudinal statements regarding vaccination. Since some participants did not select a response for every statement the denominators in the description below vary. The majority (296/337; 87.8%) of the cohort agreed that, in general, vaccines are important for disease prevention and 70.4% (235/334) were not concerned about vaccines being harmful. Of the responses to the three specific QFV questions, presented only to the subgroup of 'aware' AWR (n=200), two-thirds agreed that the QFV was important (131/198; 65.8%) and effective (131/198; 66.2%) and three quarters considered it to be safe (148/198; 74.4%).

**Table 4.3** Descriptive (n, %, totals) and odds ratio (and Chi-square *p*-value) results for variables significantly associated with Q fever vaccination status among Australian wildlife rehabilitators who were aware of Q fever and the availability of Q fever vaccine (n=200). Results are from a nationwide online survey conducted in 2018.

| Variable name and description | Q fever vaccination status n (%) | | | Odds ratio | *p*-value |
|---|---|---|---|---|---|
| | Vaccinated (n=51)) | Not vaccinated (n=149) | Row total | | |
| Age | | | | | 0.000 |
| >50 | 13 (12.3) | 93 (87.7) | 106 (53.0) | Ref | |
| ≤50 | 38 (40.4) | 56 (59.6) | 94 (47.0) | 4.85 | |
| Education level | | | | | 0.002 |
| High School Level/TAFE or private college | 18 (16.7) | 90 (83.3) | 108 (54.0) | Ref | |
| University/Postgraduate | 33 (35.9) | 59 (64.1) | 92 (46.0 | 2.80 | |
| Number of animals rehabilitated per year | | | | | 0.044 |
| 1-50 | 43 (28.5) | 108 (71.5) | 151 (75.5) | Ref | |
| ≥ 50 | 7 (14.6) | 41 (85.4) | 48 (24.0) | 0.43 | |
| Occupational animal contact | | | | | 0.007 |
| No | 19 (17.8) | 88 (82.2) | 107 (53.5) | Ref | |
| Yes | 32 (34.4) | 61 (65.6) | 93 (46.5) | 2.43 | |
| Source of hearing about Q fever | | | | | |
| Media | | | | | <0.001 |
| No | 46 (31.9) | 98 (68.1) | 144 (72.0) | Ref | |
| Yes | 5 (8.9) | 51 (91.1) | 56 (28.0) | 0.21 | |
| Wildlife associated activities | | | | | 0.001 |
| No | 32 (37.2) | 54 (62.8) | 86 (43.0) | Ref | |
| Yes | 19 (16.7) | 95 (83.3) | 114 (57.0) | 0.34 | |
| Employment/education | | | | | 0.013 |
| No | 32 (21.1) | 120 (78.9) | 152 (76.0) | Ref | |
| Yes | 19 (39.6) | 29 (60.4) | 48 (24.0) | 2.46 | |
| Family member or friend | | | | | 0.019 |
| No | 47 (28.7) | 117 (71.3) | 164 (82.0) | Ref | |
| Yes | 4 (11.1) | 32 (88.9) | 36 (18.0) | 0.31 | |

TAFE-Technical and Further Education, Ref-Reference category

## 4.4.5 Q fever vaccination in 'aware' but non-vaccinated AWR

### *4.4.5.1 Attitudes towards Q fever vaccination*

Of 200 'aware' respondents, 73.5% (147/200) stated that they had not been vaccinated and 1% (2/200) were unsure of their vaccination status. Of the 148 responses to the question concerning their potential to receive the Q fever vaccine, 60.8% (90/148) stated that they would consider vaccination, 23.0% (34/148) were unsure and 16.2% (24/148) would not consider becoming vaccinated against Q fever (**Figure 4.1**).

**Figure 4.2** shows the level of agreement with five attitudinal statements regarding vaccination in 'aware' AWR who had not been vaccinated or were unsure of their vaccination status (149/200; 74.5%). The majority (141/149; 94.6%) agreed that, in general, vaccines are important for disease

prevention, 74.8% (110/147) were not concerned about vaccines being harmful and over two thirds agreed that the Q fever vaccine was important (93/148; 62.8%), effective (94/148; 63.5%) and safe (104/147; 70.7%).

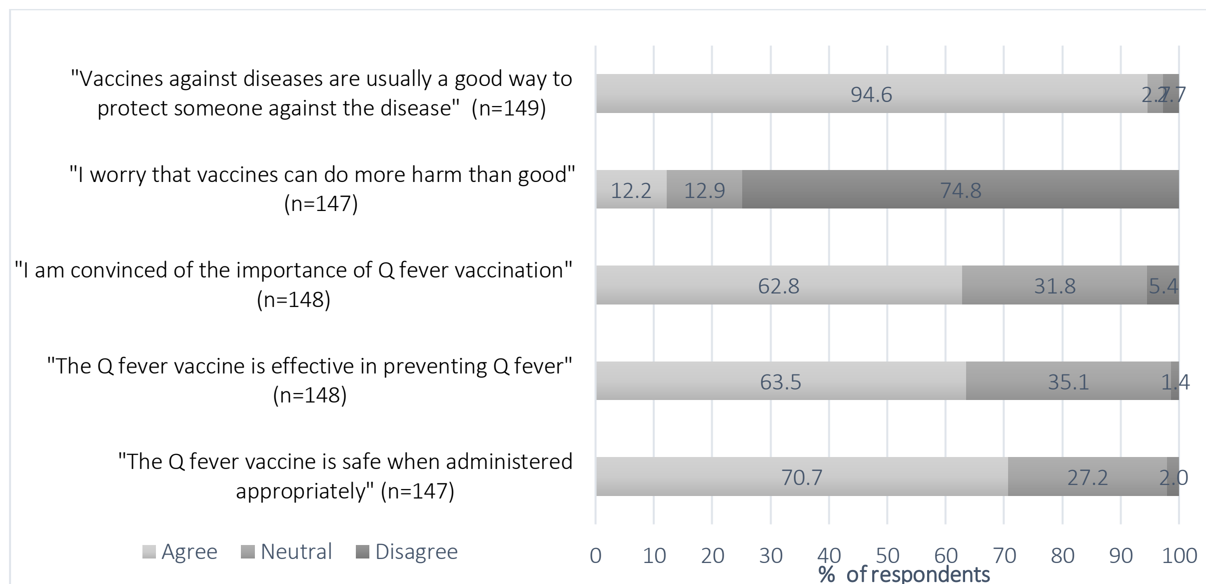


**Figure 4.2** shows the level of agreement with five attitudinal statements regarding vaccination in 'aware' AWR who had not been vaccinated or were unsure of their vaccination status. (149/200; 74.5%). The majority (141/149; 94.6%) agreed that, in general, vaccines are important for disease prevention, 74.8% (110/147) were not concerned about vaccines being harmful and over two thirds agreed that the Q fever vaccine was important (93/148; 62.8%), effective (94/148; 63.5%) and safe (104/147; 70.7%).

*4.4.5.2 Barriers to Q fever vaccination*

The reasons cited for not being vaccinated by the subgroup of unvaccinated respondents, and those unsure of their vaccination status (148/200; 74.0%) but who were aware of the Q fever vaccine, are presented in **Table 4.4**. Complacency towards vaccination and lack of Q fever knowledge (121/148; 81.8%), and issues surrounding convenience of vaccination (64/148; 43.2%) were cited as reasons for not having received QFV. Twenty (20/338; 5.9%) participants reported they were ineligible for vaccination due to having returned a positive pre-vaccination screening test or being previously medically diagnosed with Q fever.

*4.4.5.3 Q fever vaccination recommendations*

There were 148 responses to the question regarding vaccine recommendations, posed to 'aware' AWR who were non-vaccinates and participants unsure of their vaccination status (149/200; 74.5%). Almost three quarters (107/148; 72.3%) of these participants (148/200; 74.0%) had never had QFV recommended to them. Vaccination had been recommended to 27.7% (41/148; 27.7%) of participants by another AWR (21/41; 51.2%), followed by veterinary personnel (14/41; 34.1%), a doctor (9/41; 22.0%), employer or educational institution (8/41; 19.5%) and by a friend or family member (3/41; 7.3%).

**Table 4.4** Reasons cited for not being vaccinated against Q fever by 148 unvaccinated Australian wildlife rehabilitators participating in a nationwide online survey conducted in 2018. The reasons have been categorised into themes. Participants were able to select more than one response.

| Theme | Reasons for not getting vaccinated | Number (%) |
|---|---|---|
| Complacency/lack of knowledge | I don't think Q fever is serious enough to require vaccination<br>I haven't got around to doing it<br>I don't think I am at risk of acquiring Q fever<br>I was told I was not at risk<br>I was unaware I needed to | 121 (81.8) |
| Convenience | It is too difficult to find a medical practitioner who gives the vaccine<br>Vaccination is not provided by my employer or wildlife rehabilitation group<br>The cost of getting vaccinated is too expensive | 64 (43.2) |
| Hesitancy | I think the Q fever vaccine may harm my health<br>I think the Q fever vaccine may not be effective | 5 (3.4) |
| Pre-existing immunity | The pre-vaccination screening process indicated I should not have the vaccine<br>I have had medically diagnosed Q fever and was advised I am unable to be vaccinated | 20 (13.5) |

## 4.5 Discussion

This publication is the second from an online survey that investigated the knowledge, attitudes and practices (KAP) of AWR regarding Q fever (Mathews et al., 2022) and the Q fever vaccine. The first publication identified that AWR are at increased risk of developing Q fever compared to the general Australian population (as evidenced by self-reported medically diagnosed Q fever) The study also found that despite the Australian government recommending QFV for AWR (Australian Technical Advisory Group on Immunisation, 2021), only 15.1% of the survey respondents self-reported having received the Q fever vaccine available in Australia. These findings are in line with those reported in a

Q fever seroprevalence study in AWR attending a wildlife rehabilitator conference in 2018 (Mathews, Toribio, et al., 2021). While the percentage of those vaccinated in the online KAP survey was identified as low, this likely represents an over-estimate, consequent to the effects of sampling bias, where those with an interest in, or some knowledge of, Q fever are more likely to have participated in the survey. The actual percentage of the AWR population that remain unvaccinated is therefore likely to be higher than the 85% reported for this cohort. To understand the reasons for the lack of vaccine uptake identified in the companion paper, and to inform approaches for improvement in vaccine uptake, this paper provides the analysis of the data obtained in the online survey regarding AWR knowledge of Q fever and QFV, and their attitudes towards QFV and vaccination more generally.

Multivariable logistic regression analysis identified several significant explanatory factors associated with QFV status, providing insight into possible reasons for a failure in vaccine uptake in this cohort. After controlling for confounders, vaccinated AWR were more likely to be ≤50 years. This coincides with the finding that AWR >50 years (from the same study cohort in the companion publication) were greater than six times more likely to self-report medically diagnosed Q fever (Mathews et al., 2022). While increasing age is commonly associated with higher Q fever notifications (Sloan-Gardner et al., 2017) and increased *C. burnetii* seropositivity (Gidding et al., 2019; Gidding et al., 2020; Islam et al., 2011; Karki et al., 2015; Tozer et al., 2011), this result implies that younger AWR may be more aware of Q fever and the availability of the Q fever vaccine. With the advent of the availability of the Q-Vax® from 1989, some Australian universities began mandating QFV for students undertaking studies in animal and veterinary science from 1990 onwards (Sellens, Norris, et al., 2018), with the remaining Australian universities mandating vaccination at variable times after that. Such vaccination programs would not have been available to AWR aged >50 years who had undertaken animal associated studies before 1989, which may explain the higher vaccination uptake in AWR ≤50 years. While interventions aimed at increasing Q fever knowledge and awareness should target AWR of all ages, there may be value in more targeted programs for those AWR >50 years, especially considering the typical age

demographics of AWR cohorts (Englefield, Candy, et al., 2019; Guy & Banks, 2012; Mathews, Toribio, et al., 2021; Tribe & Brown, 2000).

Vaccinated rehabilitators were also three times more likely to have achieved higher levels of education (university/postgraduate). One explanation for this association is that many AWR may have undergone vaccination as a requirement of their university studies (in some instances this was achieved in mass vaccination clinics organised by the educational facility), whereas those employed in para-veterinary positions such as veterinary nursing staff, may not have been required, or offered vaccination as part of their TAFE level training (Sellens et al., 2016). As reported in the companion paper on this same AWR cohort (Mathews et al., 2022), those who self-reported medically diagnosed Q fever, were more than 10 times more likely to have lower education levels. The higher education levels reported by vaccinated AWR may also result in more opportunities to acquire knowledge regarding the safety (Carpiano et al., 2019), importance and efficacy (Larson et al., 2016) of the Q fever vaccine. This may be supported by the finding that vaccinated rehabilitators were more likely to have heard about Q fever through sources including employment and education and were less likely to report having heard about Q fever through a family member or friend, via wildlife associated activities or media. Unvaccinated AWR may therefore have encountered incomplete, misleading and/or false information about Q fever and QFV, which may have prevented or deterred them from seeking and/or receiving QFV. Social factors were also potentially at play, whereby AWR may be influenced by, and conform to, the expectations, values and ideas of others (Brewer et al., 2017) who may be misinformed or disagreeable to vaccination.

The Australian Immunisation Handbook recommends QFV for those working in high-risk occupations who may become exposed to infected animals that may shed the organism in high numbers, particularly via placental tissue and birth fluids (Australian Technical Advisory Group on Immunisation, 2021). Although occupation was not recorded in this study, a likely explanation for the finding that vaccinated AWR were more than twice as likely to have attended an animal birth is that many AWR

are working in environments that pose an increased likelihood of exposure to *C. burnetii* via known reservoir animal species, (such as ruminants) and their products of conception. Therefore, vaccinated AWR may have been vaccinated as a condition of their employment, or, as mentioned above, as a requirement of their university studies (Sellens et al., 2016). This may be supported by the findings that vaccinated AWR were more likely to have achieved university level education, and report hearing about Q fever through sources related to employment and education (**Table 4.3**).

The results of this analysis showed that vaccinated rehabilitators were significantly more likely to reside in NSW and QLD than in other Australian states and territories which is consistent with the findings of a Q fever seroprevalence study in AWR (Mathews, Toribio, et al., 2021). While Q fever is notifiable in all Australian states and territories, NSW and QLD consistently account for approximately 85% of notifications nationally (National Notifiable Diseases Surveillance System, 2021). Therefore a possible explanation for this finding is that due to the increased incidence of Q fever in NSW and QLD, there is a greater awareness of Q fever generally, which may have resulted in greater awareness and utilisation of the Q fever vaccine in these two states. Although the relative risk of contracting Q fever is lower in Australian jurisdictions which typically report fewer notifications; given the potential for Q fever to be misdiagnosed due to non-specific clinical symptoms, particularly in the absence of a high index of suspicion (Domingo et al., 1999), Q fever may be under-reported in these areas. These findings imply that interventions aimed at improving Q fever awareness in AWR, and subsequently QFV uptake, should not only be aimed at those in QLD and NSW but should follow a nationwide approach including those residing in other Australian states and territories.

In health behaviour models, engaging in vaccination behaviour requires some knowledge of the disease, the health risks it poses, and an awareness that a vaccine exists (Brewer et al., 2017). This study identified a significant knowledge gap regarding Q fever in AWR, with just over 40% of the cohort classified as unaware of Q fever, due to stating that they had either not heard of Q fever,, or had not heard of the Q fever vaccine. A lack of awareness regarding Q fever and/or QFV has also been

reported in other Australian high-risk groups including goat producers (Gunther et al., 2019), cat breeders (Shapiro, Norris, et al., 2017), livestock farmers (Rahaman, Marshall, et al., 2021) and veterinary nurses (Sellens et al., 2016). Furthermore, approximately one quarter of those unaware of Q fever reported occupational animal contact and around one third reported being present at animal births (Table 4.2). This is concerning given the potential for *C. burnetii* infection from direct or indirect exposure to infected animals and their products of conception, and that national guidelines recommend QFV for such individuals (Australian Technical Advisory Group on Immunisation, 2021). These findings highlight the need for the development and implementation of educational interventions that will raise awareness of Q fever and QFV and the factors associated with them among AWR.

A lack of confidence in the Q fever vaccine was evident among the unvaccinated respondents, who had knowledge of Q fever and the Q fever vaccine, with approximately 30% indicating that they were unsure about its importance, effectiveness and safety (Figure 4.2). Given that the majority of this subgroup agreed that vaccines, in general, are important for disease prevention, and were unconcerned about their potentially harmful effects, this lack of confidence in the Q fever vaccine likely reflects misperceptions regarding the safety and efficacy of the Q fever vaccine specifically. Confidence in the efficacy and safety of a vaccine has been shown to heavily impact the decision to receive vaccination in general (Kumar et al., 2016). Therefore, this observed lack of confidence in Q-Vax®, represents a significant barrier for many AWR. The vaccine has been demonstrated to be highly effective with an estimated efficacy ranging from 83-100% (Chiu & Durrheim, 2007; Gefenaite et al., 2011; Woldeyohannes et al., 2020). Although its administration to those with prior *C. burnetii* exposure is contraindicated due to the risk of hypersensitivity reactions (Marmion et al., 1990; Sellens, Bosward, et al., 2018), the safety of the vaccination process is greatly enhanced by potential vaccinees having to undergo stringent pre-vaccination screening protocols (via serological and intradermal skin testing one week before inoculation), to identify and exclude those with pre-existing immunity from receiving the Q fever vaccine (Australian Technical Advisory Group on Immunisation,

2021). Local (characterised by pain or swelling at the injection site) and systemic (including fever, headache and arthralgia) adverse events following immunisation with Q-Vax® have been reported (Gidding et al., 2009; Marmion et al., 1990; Schoffelen et al., 2014; Sellens, Bosward, et al., 2018), however, most are non-severe, few require medical attention, and no deaths have been reported as an outcome following QFV (Therapeutic Goods Administration Database of Adverse Event Notifications—Medicines, 2022). Thus concerns regarding Q-Vax's safety and efficacy appear to be largely unfounded. In addition, decision making regarding vaccination acceptance relies heavily on the perceived integrity, competence and trust in the authorities providing vaccine recommendations (Larson et al., 2015). Dialogue-based education specifically targetted at AWR such as webinars comprised of panellists who are experts in Q fever research, supported by esteemed individuals within the AWR community, could help to educate AWR about Q fever, and correct misperceptions about, and instil confidence in, the Q fever vaccine (World Health Organization, 2014), which in turn may boost uptake in those who are hesitant about QFV.

As reported in other Australian studies, (Gidding et al., 2019; Massey et al., 2009; Milazzo et al., 2005; Patel et al., 1997; Rahaman, Marshall, et al., 2021; Rahaman, Milazzo, et al., 2021; Sellens et al., 2016), the cost of QFV was a notable barrier to vaccination for around 20% of unvaccinated participants who were aware of the Q fever vaccine. Currently, Q-Vax® is not covered by the Australian Government Pharmaceutical Benefits Scheme (Archibald, 2019) and, consequently, vaccinees or employers must bear the associated cost, which can be more than AUD400 per vaccine (Hamilton Medical Centre, 2022). This is a considerable impost on AWR who are mostly unpaid volunteers, and who, due to their rehabilitation responsibilities, incur an average annual financial commitment estimated to be AUD5300 (Englefield, Candy, et al., 2019), which is in addition to their in-kind contribution of time. Factors associated with vaccine convenience in general, such as affordability, are known to significantly impact the decision as to whether or not to become vaccinated (World Health Organization, 2014). Reducing the out-of-pocket expenses associated with vaccination through subsidies or vaccination programmes has been shown to improve vaccine uptake

(Betsch et al., 2015). The Australian National Q fever vaccination program, which was implemented in Australia between 2001 and 2006, is an example of a successful vaccination program (Gidding et al., 2009). This scheme, funded by the Australian government, covered the costs associated with QFV and pre-vaccination screening for over 50,000 abattoir workers, sheep shearers, farm workers and their families. As well as increasing Q fever vaccine uptake in these at-risk groups, this scheme led to a substantial decline in Australian national Q fever notifications by over 50% (Gidding et al., 2009). However, since its cessation, the number of annual Q fever notifications have been steadily rising (Sloan-Gardner et al., 2017).

Another convenience-related barrier to vaccination identified among non-vaccinated AWR who were aware of the Q fever vaccine was the two step process required for vaccination with the currently available vaccine. While this process significantly improves the safety of the vaccine (as mentioned above), the requirement for training and experience in interpreting the skin test has meant that some potential vaccinees can encounter difficulty in accessing medical practitioners skilled in administering Q-Vax®. This situation is exacerbated for those residing in regional and remote areas of Australia (~70% of the AWR participants in this study), whereby potential vaccinees have to travel long distances to attend vaccination appointments. Difficulty accessing vaccination has also been recognised as a barrier to QFV vaccination in other Australian at-risk cohorts (Lower et al., 2017; Massey et al., 2009; Sellens et al., 2016). An online Q fever training module for Australian medical practitioners, which includes instruction in vaccine screening and administration, has been developed by the Australian College of Rural and Remote Medicine, and its wide implementation may result in improved vaccine accessibility and subsequent vaccine uptake (Australian College of Rural and Remote Medicine, 2018).

Complacency which ‘exists where perceived risks of vaccine-preventable diseases are low, and vaccination is not deemed a necessary preventive action’ (MacDonald, 2015) is also generally identified as a barrier to vaccination. It is possible that complacent AWR may perceive the risk of

contracting Q fever as low, due to a lack of knowledge and awareness (Betsch et al., 2018), and therefore do not regard vaccination as an important or necessary strategy for disease prevention (Kumar et al., 2016). Complacency can also occur when other life or health-related responsibilities are perceived as being more important, and thus take priority over vaccination (MacDonald, 2015). Informational interventions to enable AWR to accurately perceive their risk of acquiring Q fever, and interventions that facilitate vaccination by making it more convenient (Betsch et al., 2015), may help to increase vaccine uptake in complacent AWR.

Many of the interventions suggested in this discussion could be part of formalised training programs developed for AWR and included as an essential part of their induction and ongoing training. The challenges associated with accessing the entire AWR population for this study due to the lack of a unifying national AWR governing body and the representativeness of the study cohort have been discussed previously in the companion paper (Mathews et al., 2022). The formation of a centralised governing body and member database could provide a conduit for the efficient dissemination of accurate information regarding Q fever and other relevant topics potentially via online training modules to increase accessibility.

## 4.6 Conclusion

This study identified several factors associated with Q fever vaccine uptake in AWR. Just under half of the study population had not heard of Q fever and/or the Q fever vaccine. However, even for the respondents who were aware of the vaccine, only one quarter were vaccinated. Notable barriers to vaccination included lack of confidence in, and complacency towards, the Q fever vaccine, as well as factors negatively impacting convenience including limited accessibility and high cost of vaccination. To improve vaccine uptake in AWR, multicomponent intervention strategies are recommended. Educational interventions to improve Q fever vaccine uptake should focus on increasing awareness and knowledge of Q fever (particularly around risk perception) and building confidence in the vaccine. Strategies that ensure vaccination is affordable are likely to enhance vaccine uptake as are programs

that facilitate ease of access to practitioners trained in Q fever vaccination, particularly in regional areas during seasons when rehabilitation workloads are at their lowest and at convenient locations such as rehabilitator conferences, education and training sessions.

# Chapter 5 Detection of *Coxiella burnetii* DNA in Australian native wildlife

## 5.1 Abstract

*Coxiella burnetii* is the obligate intracellular bacterium responsible for the serious zoonotic disease, Q fever, with domestic ruminants (cattle, sheep, goats) considered the main source of infection. Australian native wildlife have been implicated as a source of *C. burnetii*, however their contribution to the Australian Q fever burden is poorly understood. The objective of this study was to determine the prevalence of *C. burnetii* DNA in tissues, swabs and secretions opportunistically obtained from Australian native wildlife species and identify potential shedding routes in these animals. A multiplex qPCR targeting three *C. burnetii* gene targets (IS*1111*, *com1*, *htpAB*) was optimised, with the limit of detection determined to be approximately 11 *C. burnetii* genome equivalents per reaction. Samples (n=366) were collected from macropods (n=126; 34.4%), koalas (n=226; 61.7%) and wombats (n=14; 3.8%) from different geographical locations across New South Wales and the Australian Capital Territory. Scat samples (n=103) were collected from the environment on St Helena Island, Queensland – an island heavily populated with Red-necked wallabies. Two (2/366; 0.5%) samples obtained from cloacal and urogenital swabs of an Eastern Grey kangaroo (EGK) and a koala respectively were positive for *C. burnetii* DNA, with copy numbers of approximately 11 *C. burnetii* genome equivalents per reaction. An additional five (5/366; 1.4%) animals including three EGK, a koala and a wombat, were classified as 'suspect', with amplification in their tissues below the designated lower limit of detection for the qPCR assays. None of the scat samples from St Helena Island were positive. The low *C. burnetii* DNA prevalence in samples examined in this study suggests that Australian native wildlife may not be a major source of *C. burnetii* for humans, however, given the low infectious dose, the aerosol transmission route and prolonged survival of the bacterium in the environment, people in close contact with Australian native wildlife and their habitats remain at risk. Genotyping of the *C. burnetii* DNA obtained from these positive animals and comparison with genotypes obtained from human Q

fever cases may demonstrate relevance to human disease. Cloacal or urogenital swabs may be useful sampling sites for future investigations into *C. burnetii* and Australian native wildlife.

## 5.2 Background

The results of the serological survey (Chapter 2) and the KAP online (Chapter 3) survey presented in previous chapters of this dissertation have demonstrated that AWR are at increased risk of exposure to *C. burnetii* and have a higher prevalence of self-reported medically diagnosed Q fever than the general Australian population, however an association between *C. burnetii* seropositivity or self-reported Q fever and direct contact with wildlife was not identified. The findings thus far suggest that AWR are at increased risk of acquiring Q fever through direct or indirect contact with traditional sources of *C. burnetii* such as domestic ruminants or through their wildlife associated activities, and not via contact with the wildlife that they rehabilitate. Although risk factors associated with wildlife have not been identified, given the serological (Banazis et al., 2010; Cooper, Barnes, et al., 2012; Pope et al., 1960; Potter et al., 2011) and molecular (Banazis et al., 2010; Bennett et al., 2011; Cooper et al., 2013; Potter et al., 2011; Shapiro et al., 2020; Tozer et al., 2014) evidence that wildlife may become infected with *C. burnetii* and potentially shed the organism into the environment, the possibility for Australian native wildlife species to act as reservoirs for *C. burnetii* cannot be discounted. Investigating the cycle of infection of *C. burnetii* in wildlife is crucial to gaining an understanding of the risk that wildlife may present for *C. burnetii* transmission to humans. Given that macropods have been implicated as potential infection sources, these species were the main focus of this study, however, other wildlife species have been suggested as potential sources of *C. burnetii* (Bennett et al., 2011; Tozer et al., 2014), therefore this investigation was extended to include wombats and koalas as the opportunity arose.

### 5.2.1 Study Aims

The objective of this study was to determine the prevalence of *C. burnetii* DNA in tissues, swabs and secretions opportunistically obtained from Australian native wildlife species (marsupial species) using an optimised multiplex qPCR assay with the secondary objective being to identify potential shedding routes in these animals.

## 5.3 Materials and Methods

The investigation is presented as four case studies with details shown in **Table 5.1**. The geographical location from which the animals were samples is shown in **Figure 5.1**.

**Table 5.1** Details of four case studies that were undertaken to investigate for the presence of *Coxiella burnetii* DNA in tissues obtained from Australian native wildlife species residing in New South Wales, Australian Capital Territory and Queensland, Australia. Scientific names of species are listed below table.

| Species | Case Study | Geographical region | Australian state | Local government area |
|---|---|---|---|---|
| Eastern Grey kangaroo | 1a | Dubbo | NSW | Dubbo Regional Council |
| | 1b | Canberra | ACT | Canberra |
| | 1c | Valla (Nambucca Heads region) | NSW | Nambucca Shire |
| Wombat, macropod species, koala | 2 | Camden | NSW | Camden Council |
| Koala | 3a | Lismore | NSW | City of Lismore |
| | 3b | Port Macquarie | NSW | Port Macquarie-Hastings Council |
| | 3c | Campbelltown | NSW | Campbelltown City |
| Red-necked wallaby | 4 | St Helena Island | QLD | Brisbane City Council |

Eastern grey kangaroo (*Macropus giganteus*), *Common wombat* (*Vombatus ursinus*), macropods (*Macropus* spp.), Koala (*Phascolarctos cinereus*), Red-necked wallaby (*Macropus rufogriseus*). NSW – New South Wales, QLD Queensland, ACT Australian Capital Territory

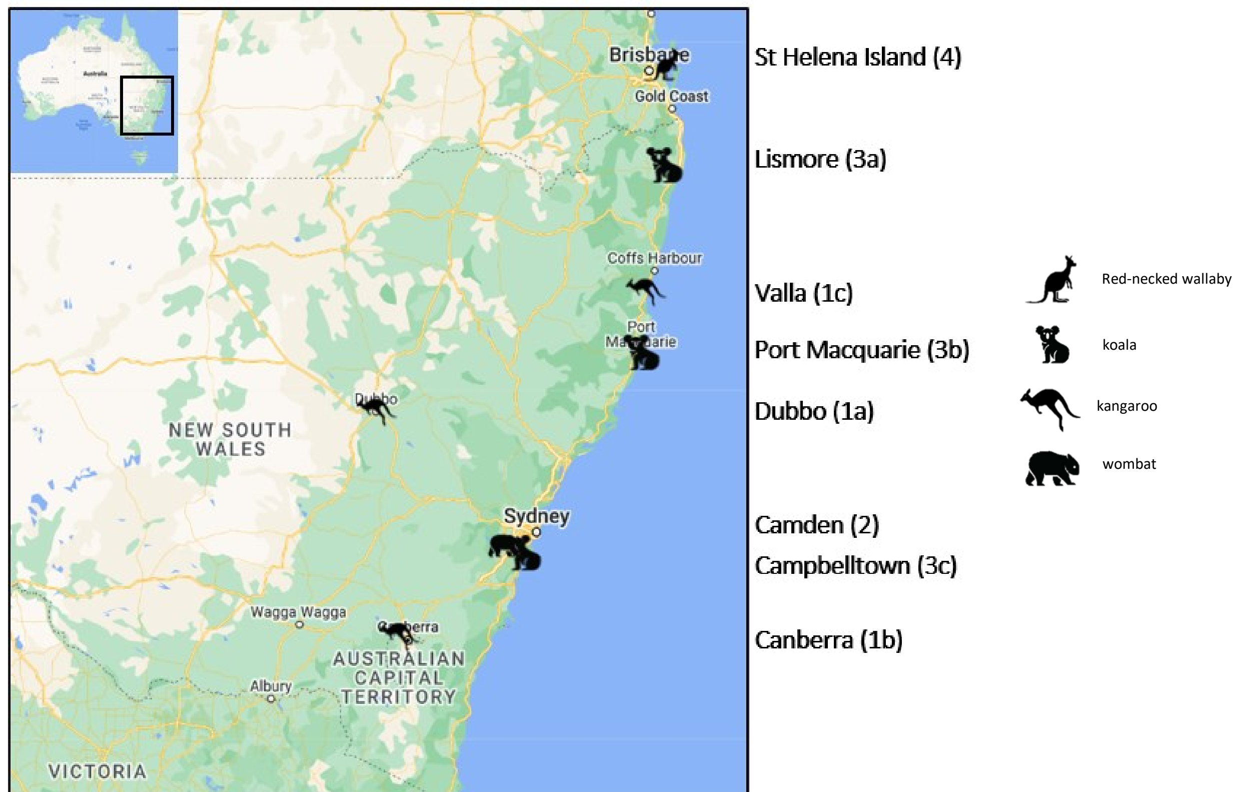


**Figure 5.1** The geographical locations in New South Wales and Queensland, Australia from which Australian native wildlife tissues were obtained and analysed for the presence of *Coxiella burnetii* DNA Inset shows the location of these states within Australia.

## 5.3.1 Animals and sample collection

Samples were collected from animals that were killed as part of a population control program for overabundant kangaroo populations. A scientific licence (SL102336) was issued by the Department of Planning, Industry and Environment for sample collection in this study.

### *5.3.1.1 Eastern Grey Kangaroos*

#### 5.3.1.1.1. Sample collection

The samples analysed in Case study 1 were obtained from Eastern Grey Kangaroos (EGK; *Macropus giganteus*) from three locations within New South Wales (NSW) and the Australian Capital Territory (ACT): Dubbo (1a), Canberra (1b) and Valla (Nambucca Heads region) (1c) **(Figure 5.2).**

Samples from Case study 1a were obtained opportunistically from commercially harvested EGK from the Dubbo region of NSW. Kangaroos were dressed in the field overnight by a licenced kangaroo

harvester, who was briefed on the purpose of the project and how to minimise environmental contamination during sample collection. The viscera of each animal were removed and stored in separate bags before being transported to the University of Sydney, Camden Campus on ice and stored at 4°C overnight until processing the following day at which urine, cloacal swab, spleen, bladder, lymph node, faeces, uterus and penis were collected. Samples were stored at -20°C until analysis. As these EGK were harvested for human consumption, bone marrow, kidney and lung samples were not available, as these tissues remain with the carcase for meat quality testing (Spiegel & Wynn, 2014).

**Figure 5.2** The geographical locations in the Australian Capital Territory and New South Wales, Australia from which Eastern Grey kangaroo (*Macropus giganteus*) tissues were obtained and analysed for the presence of *Coxiella burnetii* DNA in Case study 1. Samples were obtained from Dubbo, and Valla in NSW and Canberra in the ACT

Samples from Case studies 1b and 1c were obtained opportunistically in 2017 from EGK which were part of registered kangaroo culls in the Canberra (ACT) and Valla (NSW) regions respectively. Samples from Case study 1b were provided by Professor David Phalen from Sydney School of Veterinary Science, the University of Sydney and samples from Case study 1c were provided by Associate Professor Catherine Herbert and Dr Maquel Brandimarti, from the School of Life and Environmental

Science, the University of Sydney. Animals were necropsied in the field, before transportation to the University of Sydney and stored at -20°C until analysed.

5.3.1.1.2. Sample size calculation

Sample size determination was based on *C. burnetii* DNA prevalence values reported in surveys conducted in Western Grey kangaroos (WGK; *Macropus fuliginosus*) (Banazis et al., 2010; Potter et al., 2011). Assuming 7% *C. burnetii* DNA detection, this study would require 26 animals from each sampling location for estimating a *C. burnetii* DNA prevalence in EGK with 10% precision and 95% confidence (Dhand & Khatkar, 2014).

*5.3.1.2 Wombats/maropods/ koalas*

5.3.1.2.1. Sample collection

Case study 2 included wildlife species from the Camden region in NSW **(Figure 5.3).** All animals in this study presented to the University of Sydney's Avian and Reptile Exotic Pet Hospital at Camden in NSW either having died in a motor vehicle accident or were euthanased after arrival on humanitarian grounds. Animals were either necropsied immediately or stored at 4 °C and necropsied within 24 hours of death to obtain samples including spleen, bladder, liver, lung, bone marrow, lymph node, uterus, penis, urine, cloacal swab, faeces and pouch swabs. Samples were stored at -20°C until analysis.

5.3.1.2.2. Sample size calculation

As there are no studies previously conducted to assess for the presence of *C. burnetii* DNA in wombats, sample size determination was based on limited *C. burnetii* DNA prevalence values reported in surveys conducted in WGK (Banazis et al., 2010; Potter et al., 2011) and koalas (Tozer et al., 2014). Assuming 6% *C. burnetii* DNA detection, this study would require 22 animals for estimating *C. burnetii* DNA prevalence in wombats and other wildlife with 10% precision and 95% confidence (Dhand & Khatkar, 2014).

**Figure 5.3** Geographical location in New South Wales, Australia from which Australian native wildlife samples were obtained to investigate for the presence of *Coxiella burnetii* DNA in Case study 2. Samples were collected from wombats in the Camden Shire council area.

*5.3.1.3 Koalas*

5.3.1.3.1. Background and sample collection An anecdotal report of medically diagnosed Q fever in a person extensively involved in the care and rehabilitation of koalas (*Phascolarctos cinereus*) raised the question as to whether koalas are a source of *C. burnetii* for humans. In this case, exposures to a variety of animal sources were also reported by the patient and there have been no published cases to date of Q fever in humans where the source of infection was conclusively identified as koalas. There is one publication in the peer-reviewed literature reporting a 5.1% *C. burnetii* DNA detection rate in koalas (Tozer et al., 2014), whereby, of the 99 koala samples tested, *C. burnetii* DNA was detected in 3/26 (11.5%) blood, 1/43 (2.33%) faecal and 1/30 (3.3%) urine samples using PCR. The criteria for a positive result in this study was molecular amplification of one gene (IS*1111* or *com1*) and quantification data was not provided. Demonstration of the presence of *C. burnetii* DNA in koala samples is the first step in the process of determining whether koalas can become infected with and shed *C. burnetii* in their secretions and excretions, and so be a source of infection for humans. This work was conducted in collaboration with The Koala Health Hub (KHH) in the School of Veterinary Science, the University of Sydney, and utilised DNA previously extracted from koala urogenital swabs (UGT swabs) submitted to the KHH for chlamydia testing. Swabs were collected from koalas admitted

to wildlife rehabilitation facilities at Lismore (3a) and Port Macquarie (3b) and Campbelltown (3c) (Figure 5.4).

5.3.1.3.2. Sample size calculation

Sample size determination was based on *C. burnetii* DNA prevalence in koalas of 5% (Tozer et al., 2014). Assuming 5% *C. burnetii* DNA detection, a total of 225 samples (73 randomly selected from each location) are required for estimating *C. burnetii* DNA prevalence in koalas with 5% precision and 95% confidence (Dhand & Khatkar, 2014).

Figure 5.4 Geographical location, in New South Wales, Australia, from which urogenital swabs of koalas (*Phascolarctos cinereus*) were obtained to investigate for the presence of *Coxiella burnetii* DNA in Case study 3.

*5.3.1.4 Red-necked wallabies*

5.3.1.4.1. Background and sample collection

St. Helena Island is a National Park located in Queensland, 21 kilometres east of Brisbane and four kilometres east of the mouth of the Brisbane River in Moreton Bay (Figure 5.5). In 1867 it was declared a penal settlement and operated as a high security prison until 1932. The island is now heritage listed and attracts tourists and school children who travel from the mainland to the island for day trips (Department of Environment and Science, 2017-2018).

Figure 5.5 Geographical location, in Queensland, Australia, from which Red-necked wallaby (*Macropus rufogriseus*) environmental faecal (scat) samples were obtained to investigate for the presence of *Coxiella burnetii* DNA in Case study 4.

At the time of sampling, there were more than 1,000 Red-necked wallabies (RNW; *Macropus rufogriseus*) inhabiting the island, representing a significant overpopulation of that species and consequently, faecal contamination was extensive. Cattle have been continuously raised on the Island until approximately 2016, with only young animals raised for beef in recent times and no cattle have been born on this island for many years. Before this investigation there had been no evidence of human exposure to *C. burnetii* on the island, as all staff working on the island tested negative for previous exposure to *C. burnetii* at pre-Q fever-vaccination screening. However, because of the high faecal load on the island, and given the published evidence that macropods can become infected with *C. burnetii* (Banazis et al., 2010; Cooper, Barnes, et al., 2012; Pope et al., 1960), and that *C. burnetii* DNA has been detected in macropod faeces (Banazis et al., 2010; Potter et al., 2011), Queensland Parks and Wildlife Service determined it necessary to ascertain whether the faecal contamination on this island posed a risk to its staff and visitors in terms of acquiring Q fever.

A total of 103 environmental faecal samples (scat) were collected at random from three sites across the island (30 from site A, 40 from site B and 33 from site C) by Professor David Phalen in February 2018. Individual faecal samples were placed into zip-locked bags, transported back to Sydney on ice and stored at -20°C until required.

5.3.1.4.2. Sample size calculation

Sample size determination was based on *C. burnetii* DNA prevalence values reported in surveys conducted in Western Grey kangaroos (WGK) (Banazis et al., 2010; Potter et al., 2011). Assuming 7% *C. burnetii* DNA detection in faeces this study would require 101 faecal samples for estimating *C. burnetii* DNA prevalence in RNW faeces with 5% precision and 95% confidence (Dhand & Khatkar, 2014).

### 5.3.2 DNA extractions

A summary of the extraction methods used to detect host and *Coxiella burnetii* DNA for the four case studies is provided in **Table 5.2.**

*5.3.2.1 High throughput 96 well extractions (Case studies 1 and 2)*

For Case studies 1-3, DNA extractions were performed using a high throughput Biosprint® 96 One-For-All Vet Kit (AsureQuality, Australia) and a robotic workstation (Biosprint 96®, QIAGEN, Australia) utilising a 96 well plate format according to the manufacturer's instructions. Before extraction, the samples underwent pre-processing according to the methods below. Eight randomly distributed extraction controls (EC) were included in every 96 well extraction plate. Samples were eluted in 100µL elution buffer.

5.3.2.1.1. Tissues

For tissue samples (lymph node, spleen, liver, ileum, bladder, uterus, penis, lung, kidney), approximately 25mg of tissue was placed into a 2mL microcentifuge tube containing 500µL of sterile phosphate-buffered saline (PBS) and one 5 mm stainless steel bead (Aussie Sapphire, Glen Innes, Australia). Tissue samples were homogenised for 60 seconds at 25Hz using a bead beater (TissueLyser II; Qiagen, Australia) at 25 Hz, after which 160µL of the tissue homogenate was removed and added to tubes containing 40µL of Proteinase K.

**Table 5.2** Summary of DNA extraction and PCR methodologies utilised for the four case studies that were undertaken to investigate Australian native wildlife species for the presence of *Coxiella burnetii* DNA. Scientific names of species are listed below table.

| Species | Case Study | Australian geographical region | DNA extraction method | Endogenous control | *Coxiella burnetii* PCR |
|---|---|---|---|---|---|
| Eastern Grey Kangaroo | 1a | Dubbo | High throughput (Biosprint® 96 One-For-All Vet Kit) | Eastern Grey Kangaroo cytochrome b | Multiplex |
| | 1b | Canberra | | | |
| | 1c | Valla | | | |
| Wombat , macropod species, koala | 2 | Camden | | Eukaryotic 18S rRNA | |
| Koala | 3a | Lismore | High throughput (MagMAX™CORE Nucleic Acid Purification Kit) | Koala β-actin | |
| | 3b | Port Macquarie | | | |
| | 3c | Campbelltown | | | |
| Red-necked wallaby | 4 | St Helena Island* | Spin column (QIAGEN® DNeasy Blood & Tissue Kit) | Red-necked wallaby cytochrome b | Singleplex |

*All regions were in the Australian state of New South Wales apart from St Helena Island that is located in Queensland Eastern grey kangaroo (*Macropus giganteus*), *Common wombat* (*Vombatus ursinus*), macropods (*Macropus* spp.), Koala (*Phascolarctos cinereus*), Red-necked wallaby (*Macropus rufogriseus*).

5.3.2.1.2. Swabs

For swabs (pouch and cloacal), the tip was removed using sterile scissors and placed into a microcentifuge tube containing 500µL sterile PBS. The tips were vortexed for 10 seconds and incubated at room temperature for 5 minutes. Samples were then vortexed for 10 seconds, after which 160µL of the PBS suspension was added to tubes containing 40µL of Proteinase K.

5.3.2.1.3. Faeces and urine

Approximately 100mg of faeces was weighed into a 2mL polypropylene microcentrifuge tube and 1mL sterile PBS was added (1/10 dilution). Samples were shaken and vortexed vigorously to break up the faeces. The samples were centrifuged for 1min at 1,000xg to sediment the large debris and 500mL of the supernatant was transferred to a clean 1.5mL microcentrifuge tube. Following centrifugation at 5,000xg for 10 min the supernatant was discarded, and the pellet was resuspended in 160µL of sterile

PBS after which 40µL of Proteinase K was added to the suspension. For urine 160µL was added to tubes containing 40µL of Proteinase K.

5.3.2.1.4. Bone marrow

Following the removal of connective tissue and muscle from the rib bone using a sterile scalpel blade, the bone fragment was placed into a 2mL microcentrifuge tube containing 400µL sterile PBS and 40µL of Proteinase K. Samples were vortexed and incubated at 56°C in a heat block, and vortexed every 15 minutes for the next hour. Following overnight incubation (15-20 hours) at 56°C, samples were vortexed and centrifuged at 1,000xg for 30 seconds to remove cellular debris. The DNA was extracted from? 300µL of the clarified supernatant.

*5.3.2.2 Extraction of DNA from urogenital swabs (Case study 3)*

The DNA extractions from koala UGT swabs for Case study 3, were performed by the KHH at the University of Sydney using a MagMAX™CORE Nucleic Acid Purification Kit (Thermo Scientific™, Australia) and a robotic workstation (KingFisher™Flex, Thermo Scientific™, Australia). The tips of the swabs were removed using clean bleached sterile scissors and placed into a microcentrifuge tube containing 350µL of MagMAX CORE Lysis Solution and 10µL of Proteinase K, after which they were briefly vortexed and incubated at 56°C for at least 60 minutes. Following transfer of the lysate to a 96 deep well plate the samples were processed according to the manufacturer's instructions. DNA was eluted in 100 µL of elution buffer.

*5.3.2.3 Spin columns for extraction of DNA from faecal samples (Case study 4)*

Case study 4 was opportunistically undertaken in 2018 (at the start of this PhD), before the development and optimisation of the high throughput DNA extraction procedure. Therefore the 103 RNW faecal DNA extractions were performed using spin columns (QIAGEN® DNeasy Blood & Tissue Kit; QIAGEN, Australia) modified and optimised as follows. For each sample, approximately 1g of faeces was weighed into a 15mL centrifuge tube and 9mL sterile PBS was added (1/10 dilution). Following shaking and vigorous vortexing to break up the faeces, samples were centrifuged for 1min

at 1,000xg to sediment the large debris, and 2mL of the supernatant was transferred to a 2mL centrifuge tube. The 2mL of supernatant was centrifuged at 5000xg for 10 min to form a pellet; the supernatant was discarded. The pellet was then resuspended in 1mL ATL lysis buffer, incubated for 3 hours at 56°C, then centrifuged at 16 000xg for 3 min, then 200µL of the resultant supernatant was transferred to a 1.5mL tube containing 20µL Proteinase K and 200µL AL buffer. The samples were then vortexed and incubated at 70°C for 10 minutes followed by the addition of 200µL of 100% EtOH to each sample. Samples were vortexed then transferred to a spin column and purified according to the DNeasy Blood and Tissue Extraction protocol (Qiagen, Australia). The DNA was eluted in 100µL of elution buffer. A negative EC was included in each extraction round to enable the detection of any potential contamination during the extraction procedure.

### 5.3.3 PCRs

#### *5.3.3.1 Endogenous controls*

To reduce the chances of samples being wrongly classified as negative for *C. burnetii* DNA the presence and integrity of host DNA in the samples was confirmed using host specific endogenous control qPCR assays (Table 5.3).

5.3.3.1.1. <u>Eastern Grey Kangaroos</u>

Detection and quantification of EGK genomic DNA (gDNA) in extracted samples was performed using a qPCR assay targeting kangaroo (*Macropus giganteus)* cytochrome b (*cyt b*) gene (KangCytb) developed in house using Primerblast (Ye et al., 2012). Each reaction contained 5µL 1X SensiFAST Probe No-ROX Kit (BioLine, Australia), primers and probe (Table 5.3), 2µL DNA and nuclease free water in a total volume of 10µL. Amplification and fluorescence detection was performed in a Bio-Rad-CFX Real-Time PCR Thermocycler (Bio-Rad laboratories Pty Ltd, Gladesville, NSW, Australia) according to the following cycling parameters: initial denaturation at 95°C for 3 minutes followed by 40 cycles of denaturation at 95°C for 10 seconds and at 60°C for 40 seconds. Each qPCR run included a no

template control (NTC; with nuclease-free water in place of DNA). Positive controls were included in each PCR run.

Samples were tested for the presence of inhibitors to reduce the chances of them being falsely classified as negative for *C. burnetii* DNA. To test for possible inhibitory substances in the different test matrices in the automated 96 well plate extraction format used to extract DNA for Case studies 1 and 2, eight DNA extracts from each sample type underwent amplification in the KangCytB PCR as neat (undiluted) and 1/10 dilution (Bustin et al., 2009). Sample matrices demonstrating the presence of inhibitors were diluted 1/10 before testing for the presence of *C. burnetii* DNA.

5.3.3.1.2. Wombats and other wildlife (Case study 2)

Detection of host gDNA in extracted samples from Case study 2 (wombats and other wildlife) was performed using a qPCR assay targeting the eukaryotic 18S rRNA gene (Broackes-Carter et al., 2002). Each reaction contained 5µL 1X SensiFAST Probe No-ROX Kit (BioLine, Australia), forward and reverse primers (Table 5.3), 2µL DNA and nuclease free water in a total volume of 10µL. Amplification and fluorescence detection was performed in a Bio-Rad-CFX Real-Time PCR Thermocycler (Bio-Rad laboratories Pty Ltd, Gladesville, NSW, Australia) according to the following cycling parameters: initial denaturation at 95$^{o}$C for 3 minutes followed by 40 cycles of denaturation at 95$^{o}$C for 10 seconds and at 60$^{o}$C for 40 seconds. Positive controls and NTC were included in each PCR run. Samples were considered positive if amplification occurred at quantification cycle ($C_q$) <32.

5.3.3.1.3. Koalas

Detection and quantification of koala DNA in extracted samples were performed by KHH using a singleplex qPCR assay targeting the *Phascolarctos cinereus* (koala) β-actin gene (Hulse et al., 2018) (Koalaβ-actin). Each reaction contained 5µL 1X SensiFAST Probe No-ROX Kit (BioLine, Australia), primers and probe (Table 5.3), 2µL DNA and nuclease free water in a total volume of 20µL. Amplification and fluorescence detection was performed in a Bio-Rad-CFX Real-Time PCR Thermocycler (Bio-Rad laboratories Pty Ltd, Gladesville, NSW, Australia) according to the following

cycling parameters: initial denaturation at 95°C for 3 minutes followed by 40 cycles of denaturation at 95°C for 10 seconds and at 58°C for 40 seconds. Positive controls and NTC were included in each PCR run. Samples were classified as positive if amplification occurred at a $C_q$ <32. Koala UGT swab DNA extracts were assessed for the presence of inhibitors using Koalaβ-actin (analysis performed by the KHH). Samples demonstrating the presence of inhibitors were diluted 1/10 before testing for the presence of *C. burnetii* DNA. Primers and probes were synthesised by Macrogen Inc (South Korea)

5.3.3.1.4. Red-necked wallabies

A conventional PCR (cPCR) assay targeting the cytochrome b (*cyt b*) gene of *Macropus rufogriseus* (RNWCytb), was utilised to detect and verify the quality of RNW gDNA in the extracted samples. Each reaction contained 5µL 1X SensiFAST Probe No-ROX Kit (BioLine, Australia), forward and reverse primers Primers and probes (Table 5.3) were synthesised by Macrogen Inc (South Korea), 2µL DNA and nuclease free water in a total volume of 10µL. Amplification was performed in a BIO-RAD® CFX96 Touch™ Thermocycler (Bio-Rad Laboratories Pty Ltd, Australia) according to the following cycling parameters: initial denaturation at 95°C for 3 minutes followed by 40 cycles of denaturation at 95°C for 10 seconds and at 60°C for 30 seconds. Positive controls and NTC were included in each PCR run. Amplicons were run on a 2% agarose gel containing Redsafe™ nucleic acid staining solution (Scientifix, Australia) and visualised using a Biorad® Gel Doc™ XR+ imaging system (Bio-Rad Laboratories Pty Ltd, Australia). To test for the presence of inhibitory substances in the RNW faecal DNA extracts, the intensity of the RNWCytb PCR products from the neat and 1/10 dilutions for each sample were compared on a 2% agarose gel and visualised as described above. Inhibition was identified if the band for the 1/10 dilution was of a similar or greater intensity than that of the neat band. Samples demonstrating the presence of inhibitors were diluted 1/10 before testing for the presence of *C. burnetii* DNA.

*5.3.3.2 Coxiella burnetii PCRs*

5.3.3.2.1. Singleplex

Detection and quantification of *C. burnetii* DNA in RNW faecal samples (Case Study 4) was achieved using pre-existing and validated singleplex qPCR assays targeting *com*1 and the IS*1111* (**Table 5.3**). Primers and probes were synthesised by Integrated DNA Technologies (Baulkham Hills, Australia) and each reaction contained 5µL 1X SensiFAST Probe No-ROX Kit (BioLine, Australia), primers and probe**,** 2µL DNA and nuclease free water in a total volume of 20µL. Amplification and fluorescence detection was performed in a Bio-Rad-CFX Real-Time PCR Thermocycler (Bio-Rad laboratories Pty Ltd, Gladesville, NSW, Australia) according to the following cycling parameters: initial denaturation at 95°C for 3 minutes followed by 40 cycles of denaturation at 95°C for 10 seconds and annealing and extension at 60 °C for 40 seconds. Positive controls containing 1,450, 725 and 25 copies of the *C. burnetii* genome per reaction (Amplirun® Vircell, Granada, Spain) and NTC were included in each run. The lower limit of detection for these qPCR assays was determined to be 25 copies per reaction which corresponded to a quantification cycle (Cq) of ~32 for IS*1111* and a Cq of ~33 for *com*1.

5.3.3.2.2. Multiplex

A multiplex qPCR (CoxMP) was developed for this investigation using the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines (Bustin et al., 2009). The CoxMP assay contained two single copy genes: *groEL* (heat shock operon; *htpAB*) and *com1* (the outer membrane protein-coding gene) and the multicopy insertion sequence gene: IS*1111* (**Table 5.3**) was optimised and validated using commercially available *C. burnetii* control DNA (Nine Mile RSA493; Amplirun® Vircell, Granada, Spain). The lower limit of detection for these qPCR assays was determined to be 11 copies of the *C. burnetii* genome per reaction which corresponded to a Cq of ~34, ~36 and ~35 for IS*1111*, *com*1 and *htpAB* respectively (**Appendix E**).

Detection and quantification of *C. burnetii* DNA in extracted samples from Case studies 1-3 was performed using the CoxMP. Each reaction contained 5µL 1X SensiFAST Probe No-ROX Kit (BioLine,

Australia), primers and probe (Table 5.3), 2µL DNA and nuclease free water in a total volume of 10µL. Amplification and fluorescence detection was performed in a Bio-Rad-CFX Real-Time PCR Thermocycler (Bio-Rad laboratories Pty Ltd, Gladesville, NSW, Australia) according to the following cycling parameters: initial denaturation at 95°C for 3 minutes followed by 40 cycles of denaturation at 95°C for 10 seconds and annealing and extension at 60°C for 40 seconds. Each qPCR run included NTC and positive controls containing 1,100, 110 and 11 copies of the *C. burnetii* genome per reaction (Amplirun® Vircell, Granada, Spain) were included in each run. Samples were initially screened as a single qPCR reaction, and any sample producing amplification for any gene target was subsequently repeated in triplicate.

5.3.3.2.3. Sample classification criteria

To reduce the chances of falsely identifying a sample as positive for *C. burnetii* DNA, a stringent classification system was adopted whereby the overall classification of samples for the presence or absence of *C. burnetii* DNA was based on the number of gene targets amplified, the pre-determined cut-off Cqs for each target gene (corresponding to the limit of detection) and the reproducibility of the triplicate reactions as follows:

- Samples were classified as positive for *C. burnetii* DNA if they amplified reproducibly in triplicate reactions for all three gene targets and produced Cqs at or below the pre-determined Cq cut off for each assay.
- Samples were classified as suspect for *C. burnetii* DNA if they amplified reproducibly in triplicate reactions for all three gene targets and produced Cqs above the pre-determined Cq cut off for each assay, or, amplification across triplicates within each assay was not reproducible despite it being present for all three gene targets with Cqs at or below the pre-determined Cq cut off for each assay.

Samples were classified as negative for *C. burnetii* DNA if amplification was not observed for any gene target in the single or triplicate PCR reactions. Samples that reproducibly amplified only one gene target in the triplicate reactions were also classified as negative irrespective of the Cq.

**Table 5.3** Primer and probe sequences for endogenous control and *Coxiella burnetii* PCR assays. Scientific names of species are listed below table

| Species | Gene target | Primer/Probe | Sequence (5'-3') | Final Concentration (nM) | Amplicon size (bp) | Reference or accession number |
|---|---|---|---|---|---|---|
| Red-necked wallaby | cytochrome B | RNWCytb-F | TTCTCCACGTAGGACGAGGT | 150 | 249 | EF368026.1 |
| | | RNWCytb-R | ATCGTGTAAGGGTGGCCTTG | 150 | | |
| Eastern Grey Kangaroo | cytochrome B | KangCytb-F | CAGACAACTTCTCTCCTGCCAA | 300 | 174 | U87137.1 |
| | | KangCytb-R | TGGATGTATGGAGGAGTGGGAT | 300 | | |
| | | KangCytb-P | [a]CFO560-TGATACTTCCTATTTGCCTACGCCATCC-BHQ1[b] | 150 | | |
| Eukaryotes | 18S rRNA | 18S rRNA-F | CGGCTACCACATCCAAGGAA | 300 | 315 | (Broackes-Carter et al., 2002) |
| | | 18S rRNA-R | GCTGGAATTACCGCGGCT | 300 | | |
| | | 18S rRNA-P | [c]FAM-TGCTGGCACCAGACTTGCCCTC-BHQ1 | 150 | | |
| Koala | β-actin | Koalaβ-actin-F | CTCAGATTATGTTTGAGACCTTC | 400 | 144 | (Hulse et al., 2018) |
| | | Koalaβ-actin-R | CCTTCATAGATGGGCACA | 400 | | |
| | | Koalaβ-actin-P | FAM-ACCATCACCAGAGTCCATCACAAT-BHQ1 | 200 | | |
| | IS*1111*** | IS*1111*-F | CGCAGCACGTCAAACCG | 300 | 146 | (de Bruin et al., 2011) |
| | | IS*1111*-R | TATCTTTAACAGCGCTTGAACGTC | 300 | | |
| | | IS*1111*-P | FAM-ATGTCAAAAGTAACAAGAATGATCGTAAC-BHQ1 | 200 | | |
| *Coxiella burnetii* | *com1*** | *com1*-F | AAAACCTCCGCGTTGTCTTCA | 400 | 76 | (Lockhart et al., 2011) |
| | | *com1*-R | GCTAATGATACTTTGGCAGCGTATTG | 300 | | |
| | | *com1*-P | [d]Cy5-AGAACTGCCCATTTTTGGCGGCCA-BHQ2[e] | 200 | | |
| | *htpAB**** | *htpAB*-F | GTGGCTTCGCGTACATCAGA | 300 | 114 | (Bond et al., 2016) |
| | | *htpAB*-R | CATGGGGTTCATTCCAGCA | 300 | | |
| | | *htpAB*-P | [f]HEX-AGCCAGTACGGTCGCTGTTGTGGT-BHQ1 | 200 | | |

[a]CAL Flour Orange 560 Amidite , [b] Black Hole Quencher-1, [c]6-Carboxyfluorescein, [d]Cyanine Dye 5, [e]Black Hole Quencher-2, [f] HEX™ Dye Phosphoramidite, *Insertion sequence *1111* (IS*1111*), **Heat shock operon (*htpAB*), ***Outer membrane protein (*com1*), Eastern grey kangaroo (*Macropus giganteus*), *Common wombat* (*Vombatus ursinus*), macropods (*Macropus* spp.), Koala (*Phascolarctos cinereus*), Red-necked wallaby (*Macropus rufogriseus*).

## 5.4 Results

Of the 366 animals that were examined in this study two (2/366; 0.5%) were determined to be positive for the presence of *C. burnetii* DNA. These included an EGK (via a cloacal swab) and a koala (via a UGT swab), with copy numbers of approximately 11 *C. burnetii* genome equivalents per reaction. All Red-necked wallaby scat samples (n=103) collected from the environment on St Helena Island were negative.

A breakdown of the number of samples and sample types collected for Case studies 1-4 are presented in **Table 5.4**.

Details of the PCR results for the animals classified as positive or suspect for the presence of *C. burnetii* DNA are presented in **Table 5.5**. The PCR results for all the samples tested in the CoxMP in triplicate for Case studies 1-4 is provided in **Appendix E.**

A summary of the overall classification and *C. burnetii* DNA prevalence in Australian native wildlife species from different geographical locations in New South Wales and St Helena Island in Queensland is presented in **Table 5.6.**

### 5.4.1 Case study 1 Eastern Grey kangaroo

#### *5.4.1.1 Case Study 1a Dubbo*

Case study 1a included 21 male (21/24; 87.5%) and three female (3/24; 12.5%) EGK from the Dubbo region in NSW. Of the 178 samples collected from 24 animals (**Table 5.4**), all but one (177/178; 99.4%) produced positive amplification in the KangCytb PCR, thus verifying the presence and integrity of the DNA. When assayed in the CoxMP in triplicate (**Appendix E**) one (1/13; 7.3%) sample obtained from a cloacal swab of one (1/24; 4.2%) animal was classified as suspect. The remaining 164 (164/177; 92.7%) samples from 23 animals did not amplify any gene target in the CoxMP when assayed as single reactions and were classified as negative for the presence of *C. burnetii* DNA. All EC and NTC were determined to be negative in the KangCytb and CoxMP.

**Table 5.4** Breakdown of the number of samples and sample types collected from Australian native wildlife species in various regions in New South Wales and Australian Capital Territory, Australia and on St Helena Island, Queensland for Case studies 1-4 which underwent investigation for the presence of *Coxiella burnetii* DNA. Scientific names of species are listed below table.

| Case study | 1a | | 1b | | 1c | | 2 | | 3a | | 3b | | 3c | | 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Region | Dubbo | | Canberra | | Valla | | Camden | | Lismore | | Port Macquarie | | Campbelltown | | St Helena Island | |
| Species | Eastern grey kangaroo | | | | | | Wombats, macropods and koalas | | Koala | | | | | | Red-necked wallaby | |
| Sample type | n | % | n | % | n | % | n | % | n | % | n | % | n | % | n | % |
| Bladder | 24 | 13.5 | 26 | 9.9 | 42 | 11.0 | 20 | 9.2 | | | | | | | | |
| Bone marrow | | | 28 | 10.7 | 41 | 10.8 | 20 | 9.2 | | | | | | | | |
| Cloacal swab | 24 | 13.5 | 29 | 11.1 | 44 | 11.5 | 20 | 9.2 | | | | | | | 103 | 100 |
| Faeces | 24 | 13.5 | 28 | 10.7 | 44 | 11.5 | 21 | 9.6 | | | | | | | | |
| Ilium | 24 | 13.5 | 29 | 11.1 | 42 | 11.0 | 21 | 9.6 | | | | | | | | |
| Kidney | | | | | | | 12 | 5.5 | | | | | | | | |
| Liver | | | 29 | 11.1 | 41 | 10.8 | 22 | 10.1 | | | | | | | | |
| Lung | | | | | | | 15 | 6.9 | | | | | | | | |
| Lymph node | 24 | 13.5 | | | | | 21 | 9.6 | | | | | | | | |
| Pouch swab | | | | | 31 | 8.1 | 2 | 0.9 | | | | | | | | |
| Spleen | 24 | 13.5 | 29 | 11.1 | 42 | 11.0 | 21 | 9.6 | | | | | | | | |
| Urine | 24 | 13.5 | 28 | 10.7 | 44 | 11.5 | 15 | 6.9 | | | | | | | | |
| Uterus | 3 | 1.7 | 36 | 13.7 | 10 | 2.6 | 8 | 3.7 | | | | | | | | |
| Penis | 7 | 3.9 | | | | | | | | | | | | | | |
| Urogenital swab | | | | | | | | | 74 | 100 | 76 | 100 | 75 | 100 | | |
| Total | **178** | **100** | **262** | **100** | **381** | **100** | **218** | **100** | **74** | **100** | **103** | **100** | **75** | **100** | **103** | **100** |

Eastern grey kangaroo (*Macropus giganteus*), *Common wombat* (*Vombatus ursinus*), macropods (*Macropus* spp.), Koala (*Phascolarctos cinereus*), Red-necked wallaby (*Macropus rufogriseus*).

**Table 5.5** Summary PCR results for Australian native wildlife species classified as positive or suspect for the presence of *Coxiella burnetii* as determined using a multiplex qPCR assay. Animals were sampled from various regions in New South Wales, and Canberra, in the Australian Capital Territory, Australia. Scientific names of species are listed below table.

| | | | | | qPCR assay Cq's and cut-offs | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Target gene | | | |
| **Location** | Animal ID | Species | Sex | Sample type | Endogenous control | *Coxiella burnetii* multiplex | IS*1111* ≤34 | *com1* ≤ 36 | *htpAB* ≤ 35 | Sample classification |
| **Dubbo** | 7 | Eastern grey kangaroo | male | cloacal swab | 18.2 | Singles | 31.6 | - | 37.45 | suspect |
| | | | | | | Triplicates | 33.4 | - | - | |
| | | | | | | | 32.8 | 38.6 | 35.4 | |
| | | | | | | | 33.4 | 37.7 | - | |
| **Valla** | 33 | Eastern grey kangaroo | male | cloacal swab | 21.3 | Singles | 31.23 | 33.02 | 35.26 | positive |
| | | | | | | Triplicates | 30.4 | 36.0 | 34.4 | |
| | | | | | | | 30.4 | 34.6 | 33.4 | |
| | | | | | | | 30.4 | 35.3 | 32.8 | |
| **Canberra** | 10 | Eastern grey kangaroo | female | spleen | 16.1 | Singles | 33.9 | 35.4 | | suspect |
| | | | | | | Triplicates | 35.2 | 38.1 | - | |
| | | | | | | | 36.7 | 36.6 | 34.6 | |
| | | | | | | | 34.4 | 39.7 | 35.9 | |
| **Canberra** | 2 | Eastern grey kangaroo | female | bladder | 15.4 | Singles | 34.1 | 38.8 | 33.5 | suspect |
| | | | | | | Triplicates | 37.3 | 36.2 | 35.1 | |
| | | | | | | | 35.9 | 36.6 | 35.0 | |
| | | | | | | | 34.4 | 37.4 | 36.1 | |
| **Camden** | 21 | Wombat | male | spleen | 20.4 | Singles | - | 37.17 | 35.07 | suspect |
| | | | | | | Triplicates | - | 38.02 | 35.08 | |
| | | | | | | | - | 37 | 35.13 | |
| | | | | | | | - | 37.5 | 35.09 | |
| Port Macquarie | 3772-8 | Koala | male | urogenital swab | 21.6 | Singles | 30.1 | 34.1 | 32.5 | positive |
| | | | | | | Triplicates | 29.7 | 34.1 | 32.8 | |
| | | | | | | | 29.8 | 33.8 | 33.5 | |
| | | | | | | | 30 | 34.4 | 33.1 | |
| Port Macquarie | 18-10145 | Koala | unknown | urogenital swab | 29.4 | Singles | 33.4 | 36.2 | 34.9 | suspect |
| | | | | | | Triplicates | 34 | 38.3 | - | |
| | | | | | | | 34 | - | 37 | |
| | | | | | | | 33.4 | - | - | |

ID-identification, qPCR-quantitative PCR, Cq-quantification cycle, IS*1111*- Insertion sequence *1111*, *htpAB* - heat shock operon, *com1*-outer membrane protein. Eastern grey kangaroo (*Macropus giganteus*), Common wombat (*Vombatus ursinus*), Koala (*Phascolarctos cinereus*)

**Table 5.6** *Coxiella burnetii* DNA prevalence in Australian native wildlife species from different geographical locations in New South Wales, Australian Capital Territory and St Helena's Island in Queensland, Australia. Scientific names of species are listed below table

| Case study | Region | Wildlife species | Number of animals | Positive | | Suspect | | Negative | |
|---|---|---|---|---|---|---|---|---|---|
| | | | n | n | % | n | % | n | % |
| 1a | Dubbo | | **24** | - | - | 1 | 4.2 | 23 | 95.8 |
| 1b | Canberra | EGK | **51** | - | - | 2 | 3.9 | 49 | 96.1 |
| 1c | Valla | | **44** | 1 | 2.3 | - | - | 43 | 97.7 |
| 2 | Camden | Wombat | **14** | - | - | 1 | - | 13 | 92.9 |
| | | EGK | **4** | - | - | - | - | 4 | 100.0 |
| | | Koala | **1** | - | - | - | - | 1 | 100.0 |
| | | Swamp wallaby | **1** | - | - | - | - | 1 | 100.0 |
| | | Wallaroo | **2** | - | - | - | - | 2 | 100.0 |
| 3a | Lismore | | **74** | - | - | - | - | 74 | 100.0 |
| 3b | Port Macquarie | Koalas | **76** | 1 | 1.3 | 1 | | 74 | 97.4 |
| 3c | Campbelltown | | **75** | - | - | - | - | 75 | 100.0 |
| 4 | St Helena Island | Red-necked wallaby* | **103** | - | - | - | - | 103 | 100.0 |
| | | Total | **469** | 2 | 0.4 | 5 | 1.6 | 461 | 98.3 |

*scat samples were obtained from the environment not individual animals, n -number of animals, EGK -Eastern Grey Kangaroo (*Macropus* giganteus), wombat (*Vombatus ursinus*), macropods (*Macropus* spp.), Koala (*Phascolarctos cinereus*), Common wallaroo (*Macropus robustus*), Swamp wallaby (*Wallabia bicolour*),koala (*Phascolarctos cinereus*).

### *5.4.1.2 Case Study 1b Canberra*

Case study 1b included 15 male (15/51/24; 29.4%) and 36 female (36/51; 70.6%) EGK from the Canberra region in ACT. Of the 262 samples collected from 51 animals **(Table 5.4)**, all but four (258/262; 98.5%) produced positive amplification in the KangCytb PCR, thus verifying the presence and integrity of the DNA. When assayed in the CoxMP in triplicate one (1/20; 5.0%) sample obtained from the bladder of one animal (1/51; 2.0%) and a one (1/20; 5.0%) obtained from the spleen of another (1/51; 2.0%) were classified as suspect (**Appendix E**). The remaining 238 (238/258; 92.2%) samples from 49 animals did not amplify any gene target in the CoxMP when assayed as single reactions and were classified as negative for the presence of *C. burnetii* DNA. All EC and NTC were determined to be negative in the KangCytb and CoxMP.

*5.4.1.3 Case Study 1c Valla New South Wales*

Case study 3 included 21 male (21/44;47.7%) and 23 female (23/44; 52.3%) EGK from Valla in NSW (Section 5.9.3). Of the 381 samples collected from 44 animals (Table 5.1), 366 (366/381; 96.1%) produced positive amplification in the KangCytb PCR, thus verifying the presence and integrity of the DNA. When assayed in the CoxMP in triplicate one (1/22; 4.5%) sample obtained from a cloacal swab of one (1/44; 2.3%%) animal was classified as positive (**Appendix E**). The remaining 344 (344/366; 94.0%) samples from 43 animals did not amplify any gene target in the CoxMP when assayed as single reactions and were classified as negative for the presence of *C. burnetii* DNA. All EC and NTC were determined to be negative in the KangCytb and CoxMP.

## 5.4.2 Case study 2 Wombats/maropods/ koalas

The majority (14/22; 63.6%) of samples for this case study were obtained from wombats (*Vombatus ursinus*) of which six (6/14; 42.9%) were male and eight (8/14: 57.1%) were female (**Table 5.7**). Of the four EGK two (2/4; 50.0%) were female, one was male (1/4; 25.0%) with one other (1/4; 25.0%) being a pinky whose sex was unable to be determined.

**Table 5.7** Number and sex distribution of native wildlife species from the Camden region of New South Wales, Australia investigated for the presence of *Coxiella burnetii* DNA via qPCR in Case study 2. Scientific names of species are listed below the table.

| | | Sex | |
|---|---|---|---|
| | Number of animals | male | female |
| **Wildlife species** | n | n | n |
| Wombat | 14 | 6.0 | 8.0 |
| Eastern grey kangaroo* | 4 | 1.0 | 2.0 |
| Koala | 1 | 1.0 | - |
| Swamp wallaby | 1 | - | 1.0 |
| Wallaroo | 2 | 2.0 | - |
| Total | 22 | 10 | 11 |

* one animal was a pinky (immature) marsupial) whose sex was unable to be determined, - indicates no animals, Eastern grey kangaroo (*Macropus giganteus*), wombat (*Vombatus ursinus*), macropods (*Macropus* spp.), Koala (*Phascolarctos cinereus*), wallaroo (*Macropus robustus*), Swamp wallaby (*Wallabia bicolour*)

Of the 218 samples collected from 22 animals (**Table 5.4**), 196 (196/218; 89.9%) produced positive amplification in the 18S rRNA PCR, thus verifying the presence and integrity of the DNA. When assayed in the CoxMP in triplicate one (1/15; 6.7%) sample obtained from a cloacal swab of one (1/22; 4.5%) animal (wombat) was classified as suspect (**Appendix E**). The remaining 174 (174/196; 88.7%) samples from 21 animals did not amplify any gene target in the CoxMP when assayed as single reactions and were classified as negative for the presence of *C. burnetii* DNA. All EC, NTC and were determined to be negative in the18s rRNA PCR and the CoxMP.

### 5.4.3 Case study 3 Koalas

A total of 225 koala UGT swab DNA extracts collected from 225 individual animals (sampled once) were analysed in this study and the breakdown of number of animals and sex for each geographical location is provided in **Table 5.8**. All 225 koala UGT swab DNA extracts produced positive amplification in the Koalaβ-actin, verifying the presence and integrity of the DNA, and no inhibition was observed by comparison of the neat and 1/10 dilutions (analysis performed by the KHH as part of routine diagnostic testing). When assayed in the CoxMP in triplicate one (1/225; 0.44%) koala from Port Macquarie was classified as positive and one (1/225; 0.44%) also from Port Macquarie was classified as suspect (**Appendix E**). The remaining 221 (221/225; 94.0%) samples from 221 animals did not amplify any gene target in the CoxMP when assayed as single reactions and were classified as negative for the presence of *C. burnetii* DNA. All EC, NTC and were determined to be negative in the β-actin and CoxMP.

**Table 5.8** Details of koala (*Phascolarctos cinereus*), urogenital swabs in Case study 3 collected from individual animals and tested for the presence of *Coxiella burnetii* DNA using qPCR in New South Wales, Australia. The sex was not available for all animals as these details are not routinely supplied to the Koala Health Hub.

| | | | Sex | | | | | |
|---|---|---|---|---|---|---|---|---|
| Geographical location | Number of animals | | Female | | Male | | Unknown | |
| | n | % | n | % | n | % | n | % |
| Campbelltown | 75 | 33.3 | 30 | 47.6 | 33 | 52.4 | 12 | 16 |
| Lismore | 74 | 32.9 | 21 | 35 | 39 | 65 | 14 | 18.9 |
| Port Macquarie | 76 | 33.8 | - | - | - | - | 76 | 100 |

### 5.4.4 Case study 4 Red-necked wallabies

A total of 103 RNW faecal (scat) samples were analysed in this study. As these samples were collected from the environment it is unknown how many individual animals this represented. Evidenced by agarose gel electrophoresis all 103 RNW faecal DNA extracts yielded a ~249bp amplicon in the RNWCytb PCR verifying the presence and integrity of the DNA. The band for the 1/10 dilution of the RNWCytb PCR product was of a lower intensity than that of the neat, indicating that inhibition was unlikely to be occurring in the PCR reactions. All (103/103; 100.0%) scat samples were classified as negative for the presence of *C. burnetii* DNA (**Appendix E**). All EC, NTC and were determined to be negative in the RNWCytB and CoxMP.

## 5.5 Discussion

### 5.5.1 Summary of findings

This study successfully sampled a broad variety of sample matrices (tissues, swabs, secretions and excretions) collected from Australian native wildlife across different geographical locations for the presence of *C. burnetii* DNA using the CoxMP developed specifically for this investigation. A key finding of this study was the low *C. burnetii* DNA prevalence observed among the wildlife species examined in this study, with only two samples from two different animals (a cloacal swab from a kangaroo collected at Valla and a UGT swab from a koala collected at Port Macquarie) identified as being positive. *Coxiella burnetii* DNA detection in UGT swabs and cloacal swabs suggests that shedding may be occurring via the gastrointestinal, reproductive and/or urinary tract, and that these anatomical sampling sites would be the most useful for, and should be included in, future investigations into *C. burnetii* and Australian native wildlife.

### 5.5.2 Positive animals and infection risk

The two swab samples determined to be positive for *C. burnetii* DNA (a kangaroo via a cloacal swab and a koala via a UGT swab) amplified at concentrations of approximately 11 genome equivalents (GE) per reaction, which extrapolates to ≤ 1,000 organisms per swab (assuming ≤ 10 genome equivalents

per reaction and 100% yield from DNA extraction). These concentrations are considerably lower than those found in ruminants where up to $10^9$ organisms per gram have been reported for placental tissue of sheep (Hartwell et al., 1951) and goats (Roest et al., 2012). Although present at relatively low copy numbers, given the low dose of approximately 10-15 organisms required to infect humans (Brooke et al., 2013), secretions and excretions from the urogenital and gastrointestinal tract of kangaroos and koalas may still represent a risk for *C. burnetii* exposure in those in close contact with these animals. This may include activities such as cleaning of cages and caring for koalas with chlamydial disease. These findings reinforce the need for AWR to be vaccinated against Q fever as per national guidelines (Australian Technical Advisory Group on Immunisation, 2021) and as recommended in other studies described in this dissertation (Mathews et al., 2022; Mathews, Toribio, et al., 2021).

### 5.5.3 Shedding routes

Given that infected ruminants shed *C. burnetii* in their urine, faeces and products of parturition (Marrie, 1990), the detection of *C. burnetii* DNA in cloacal and UGT swabs from these anatomical sites suggests that *C. burnetii* may be shed similarly in kangaroos and koalas. The primary shedding route for *C. burnetii* in domestic ruminants is via the female reproductive tissues (Berri et al., 2000) and *C. burnetii* has been isolated from ruminant semen (Kruszewska & Tylewska-Wierzbanowska, 1997), therefore the *C. burnetii* DNA in these swabs could also reflect the shedding of *C burnetii* via the reproductive tract of these animals. Due to the cloaca being a common opening for the release of products from the digestive, reproductive and urinary tracts (Burke et al., 2018), the origin of the *C. burnetii* DNA in the cloacal swabs may reflect several possibilities. Firstly, it may represent shedding of *C. burnetii* from mucosal surfaces following infection of mucosal epithelium after *C. burnetii* bacteraemia. Secondly, it is possible the DNA represents the passive passage of the organism through the gastrointestinal lumen following ingestion, with contamination of ingested food being from the animal's own faecal excretions or the excretions of other animals. Thirdly, although all due care was taken with samples collected specifically for the purposes of this study, the two positive swabs were

collected by colleagues initially for purposes of other studies and, as such, it is also possible these sampling sites may have been contaminated with *C*. *burnetii* located in the environment of the animal, as the swabs sampled sites located close to the external orifices of these animals. Without specific animal infection studies conducted in tightly controlled Physical Containment Level 3 (PC3) facilities, it is impossible to differentiate the source of this DNA.

The finding that no individual animal was positive for more than one sample type, suggests that in wildlife, no one tissue is suitable for the detection of *C. burnetii*. However, future investigations into *C. burnetii* and Australian wildlife should consider using cloacal or urogenital swabs as part of their sampling strategy, as they were identified as the most promising sampling sites in this study, and are also relatively easy to access.

### 5.5.4 Characterisation of positive samples

While the CoxMP is sensitive and able to detect low concentrations of *C. burnetii* DNA, it cannot distinguish between live and dead cells, therefore the infectious potential of the cloacal and UGT swab samples remains unknown. Isolation by culture would be required to demonstrate viability and this is normally performed by inoculating samples onto Vero cells or into axenic growth media and monitoring growth using IFA or qPCR (Sahu et al., 2020). However, *C. burnetii* isolation requires PC3 facilities which were not available for this project. Given that cloacal and UGT swabs would be contaminated with other micro-organisms besides *C. burnetii,* guinea pig or mouse inoculation would have been the most effective isolation technique for this sample type, although this isolation method also requires animal PC3 facilites (World Organisation for Animal Health, 2018) and has significant associated ethical issues. The development of an ethidium monoazide qPCR assay which can distinguish viable from non-viable cells would circumvent the need for specialised biosafety facilities (Mori et al., 2013) and enable an understanding of the infectious potential. To confirm genetic identity, Sanger sequencing of *C. burnetii* gene target amplicons from the DNA extracts should also be performed. Genotyping of these samples via the multiple locus variable number tandem repeat

analysis (MLVA) methodology could also be undertaken, and comparisons made with genotypes obtained from human Q fever cases to help demonstrate relevance to human disease (Vincent et al., 2016).

### 5.5.5 *Coxiella burnetii* and kangaroos

An important objective of this thesis was to determine whether macropods specifically may be a source of *C. burnetii* infection for humans either via direct contact with the wildlife themselves or via spillover to domestic species. In the current study, only one kangaroo was determined to be positive for *C. burnetii* DNA. This is considerably lower than the number of animals classified as positive in other kangaroo study populations (Table 1.4). Two large studies conducted in Western Australia detected *C. burnetii* DNA in 12.3% and 4.1% of WGK faecal samples (Banazis et al., 2010; Potter et al., 2011). However, as acknowledged by the authors, these findings could reflect the ingestion of *C. burnetii* organisms from the environment and its subsequent passive passage through the gastrointestinal lumen, rather than infection of, and active shedding from, the GIT mucosal epithelium. Although *C. burnetii* DNA was detected by PCR in the GIT tissue samples obtained from experimentally infected pregnant does, the authors of this study were not able to demonstrate *C. burnetii* antigen in GIT histological sections via immunohistochemistry (IHC). This finding led the authors to conclude that the failure to detect *C. burnetii* presence via IHC, meant that active bacterial multiplication was not occuring in the GIT, as it was in the placenta where *C. burnetii* was evident on IHC (Roest et al., 2012).

*Coxiella burnetii* DNA has also been detected in Australian raw meat containing kangaroo sold for pet consumption, with 43% of samples determined to be positive, and MLVA genotyping of positive samples identifying three distinct genotypes that have been previously recovered from Australian Q fever patients (Shapiro et al., 2020; Vincent et al., 2016). As with the previous studies reported above, the specific pathogenesis within the kangaroo could not be determined in this study, because it could not be ascertained whether the *C. burnetii* DNA represented intracellular infection, or contamination

within the meat processing plant via a livestock source. More specific evidence of *C. burnetii* infection in EGK was obtained by Pope et al. (1960), who demonstrated exposure via CFT, and then isolated viable *C. burnetii* organisms via guinea pig inoculation from kangaroo blood and *Amblyomma triguttatum* (ornate kangaroo tick) ticks that were infesting kangaroos. More recent studies have detected *C. burnetii* DNA in the blood of EGK and also in *A. triguttatum* ticks collected from EGK (Cooper et al., 2013). These latter studies demonstrate that kangaroos can become infected with *C. burnetii* and provide evidence to support that a sylvatic tick- kangaroo cycle exists.

### 5.5.6 *Coxiella burnetii* and koalas

Rehabilitating koalas was not identified as a risk factor for *C. burnetii* exposure in previous epidemiological investigations (Mathews et al., 2022; Mathews, Toribio, et al., 2021), and koalas are not typically reported in association with Q fever notifications, however *C. burnetii* DNA detection in a koala UGT swab in this study suggests that koalas may represent a potential source of *C. burnetii* for humans. The only other study to investigate koalas as a source of *C. burnetii* reported detecting *C. burnetii* DNA in the blood, faeces and urine of koalas (Tozer et al., 2014), however comparing current findings to those reported by Tozer et al. (2014) is difficult due to the limitations associated with PCR methodology and the lack of important information regarding Cqs and cut offs.

Despite being subject to sample size limitations, the observation that koalas classified as positive or suspect for *C. burnetii* DNA, also tested positive for *Chlamydia pecorum* via PCR (personal communication Damian Higgins), is interesting. Coinfection with *C. burnetii* and Chlamydiales has been reported for ruminants (Zsuzsa Kreizinger et al., 2015). If the urogenital system of koalas is a potential shedding route for *C. burnetii*, koalas coinfected with *C. burnetii* and *C. pecorum* could pose an increased risk of Q fever for those rehabilitating koalas because of the need to clean the (potentially *C. burnetii*-contaminated) urogenital discharges clinically present in chlamydial infections (Hulse et al., 2020). Further studies are required to determine whether synergies between the two pathogens exist and if there is a link between coinfection with *C. pecorum* and *C. burnetii*. Given that

the koala determined to be positive, and another classified as suspect were both from the Port Macquarie region, prospective studies in koalas could focus on this location. A double swabbing technique should be utilised which enables a greater number of epithelial cells on the second swab after the removal of the surface exudate by the first (Wildlife Health Australia, 2014).

### 5.5.7 Location

The kangaroo samples in this study were opportunistically obtained from kangaroo culls which are not widely publicised due to sensitivity in the general public associated with culling of this species. Thus, in this study, being able to sample opportunistically from such events was challenging. As shown in **Figure 5.6,** the majority were obtained from NSW local government areas (LGA) where Q fever incidence ranged from >10 cases per 100,000 population to 40 cases per 100,000 population (Clutterbuck et al., 2018). Sampling of kangaroos from LGA with higher Q fever prevalence, such as Cobar or Bogan Shire may have resulted in a greater number of animals testing positive for *C. burnetii*. Similarly, the sampling of animals from Northern New South Wales and Southern Queensland may have yielded a greater number of positive animals given that the majority of human Q fever notifications in Australia originate from this region (Eastwood et al., 2018; National Notifiable Diseases Surveillance System, 2021). Kangaroos from the Walgett region may have been more likely to test positive given that a Q fever outbreak occurred in Lightning Ridge (located in the Walgett Shire) in which 63% of outbreak cases sighting kangaroos on their residential property (Archer et al., 2017). While the limitations associated with sample collection have been outlined, it is also important to acknowledge the challenges associated with sampling kangaroo populations from ‘best case scenario’ locations.

### 5.5.8 Samples classified as suspect

In this study a less stringent sample classification system, as have been used in other studies (**Table 5.9**), would have resulted in a greater number of samples being classified as positive for *C. burnetii* DNA. While several samples amplified in the single PCR reactions, most of these were ultimately

classified as negative, as they were not repeatable or failed to amplify reproducibly when assayed in triplicate. This lack of reproducibility is likely due to the Poisson effect and reflects the randomness of target molecules in samples containing low concentrations of target DNA (de Ronde et al., 2017). This phenomenon was also observed in some of the samples which were classified as suspect. For example, animal 7 from Dubbo amplified reproducibly for IS*1111* however non-reproducible amplification was observed for *com*1 and *htpAB*.

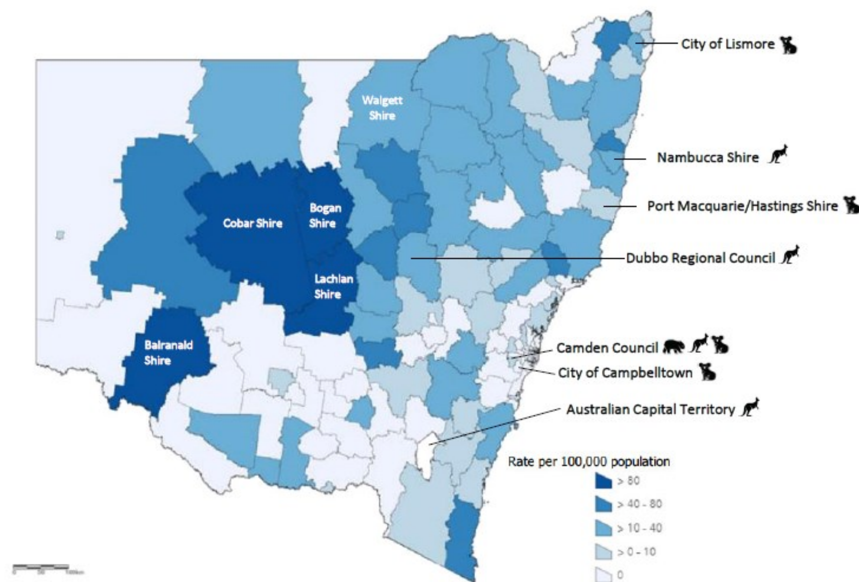


Figure 5.6 Australian geographical location of wildlife samples tested for the presence of *Coxiella burnetii* DNA, mapped to New South Wales, Q fever incidence rate (2018) by local government area (Clutterbuck et al., 2018).

Amplification at Cqs above the established Cq cut-off values were also observed in some of the samples classified as suspect. These high Cqs may reflect non-specific amplification of PCR product in these samples due to low levels of target DNA (Ruiz-Villalba et al., 2017). The absence of IS*1111* and the reproducible amplification of *com*1 and *htpAB* in DNA extracted from a wombat spleen, resulting in the animal being classified as suspect, is interesting. Similar findings have been reported for samples obtained from Australian native wildlife (Tozer et al., 2014) and ticks associated with Australian wildlife (Cooper et al., 2013), and the existence of *C. burnetii* strains devoid of IS*1111* has

been proposed as the cause of these findings (Marmion et al., 2005), although this has since been debated (Rolain et al., 2005)

### 5.5.9 Guidelines for qPCR assay development

Several studies have investigated Australian wildlife, for the presence of *C. burnetii* using qPCR (**Table 1.4**) (Banazis et al., 2010; Bennett et al., 2011; Cooper et al., 2013; Potter et al., 2011; Tozer et al., 2014). These studies reported *C. burnetii* DNA prevalence ranging from 4.1-25.0% which is considerably higher than the 0.5% observed in the current study. The variation in *C. burnetii* DNA prevalence between studies is likely due to several factors including the geographical location from which the animals were sampled (NSW vs QLD vs WA), the different sample matrices tested (tissues, blood, faeces and swabs), the variability molecular methodologies between studies, and the criteria by which samples are classified as positive or negative.

The MIQE guidelines (Bustin et al., 2009) describes the minimum information necessary for evaluating qPCR experiments and states full disclosure of analysis methods are required to enable readers to assess the validity of protocols. However, most of the other studies investigating *C. burnetii* DNA prevalence in Australian native wildlife, lack experimental detail concerning assay validation (particularly the limit of detection and associated Cq cut-offs for target genes), making the interpretation of their findings difficult. Another consideration in the previously published literature is the widespread use of qPCR assays targeting the multicopy insertion sequence IS*1111*. While IS*1111* offers increased sensitivity over single copy targets due to being present in multiple copies in the *C. burnetii* genome, it has recently been identified in *Coxiella*-like endosymbionts (CLE) of ticks via multi-locus DNA sequencing in some geographical regions (Duron, 2015) and qPCR assays targeting *C. burnetii* IS*1111* have been shown to amplify CLE IS*1111* (Elsa et al., 2015). Therefore, the classification of samples based on the detection of IS*1111* alone, could lead to false-positive identification of *C. burnetii* which could inflate DNA prevalence. Conversely, the existence of *C. burnetii* strains lacking IS*1111* has also been postulated (Marmion et al., 2005), therefore qPCR assays based on IS*1111* alone

may also increase the chances of false negative detection of *C. burnetii*. Furthermore, IS*1111* is unsuitable for quantification purposes due to the variability in copy number, which can range from 7 to 110 copies between strains (Klee et al., 2006). The amplification of single copy gene targets in conjunction with IS*1111* is recommended to enhance specificity, and thereby reduce the false-positive detection of *C. burnetii* in complex samples due to cross-reactivity of primer sequences with the DNA of CLE and the DNA of other organisms in the sample (Kuske et al., 2006; Luna et al., 2006). To build on the findings and address some of the limitations of previously published Australian studies, the CoxMP assay was developed for this investigation and a stringent sample classification was employed to minimise false positive detection of *C. burnetii.*

## 5.6 Conclusion

This chapter details an extensive molecular study to determine the presence of *C. burnetii* DNA in a variety of Australian native wildlife species with a particular focus on samples obtained from animals residing in NSW and the ACT and the testing of multiple tissues sourced from the same animal. A low *C. burnetii* DNA prevalence was observed with the two animals classified as positive for *C. burnetii* (a kangaroo via a cloacal swab and a koala via a UGT swab) amplifying at relatively low concentrations of around 11 *C. burnetii* GE per reaction. Potential shedding routes for *C. burnetii* in these positive animals include the GIT or UGT. These findings suggest that macropods and other Australian native wildlife species may not be a major source of *C. burnetii* for humans in comparison to livestock. However due to the low infectious dose, the aerosol transmission route and prolonged survival of *C. burnetii* in the environment, QFV should be recommended for people in close contact with Australian native wildlife and their habitats. There is a need for standardisation of molecular methodologies according to the MIQE guidelines. Further characterisation of positive samples via Sanger sequencing will confirm genetic identity; MLVA genotyping and comparison with genotypes obtained from humans and livestock will help to demonstrate relevance to human disease, while comparison to strains obtained from livestock residing in the same geographical region imay provide insight as to whether a transmission cycle or spillover exists between kangaroos and livestock.

# Chapter 6 Serological evidence of exposure to Spotted Fever Group and Typhus Group rickettsiae in Australian wildlife rehabilitators

## 6.1 Preface

Rickettsial infections, or rickettsioses are zoonotic diseases caused by Gram negative obligate intracellular bacteria, belonging to the $\alpha$ subdivision of proteobacteria, in the family *Rickettsiaceae.* Rickettsioses are transmitted to humans via arthropod vectors (ticks, lice, fleas and mites) (Walker, 1996). Humans are incidental hosts and become infected through bites of these vectors that have been feeding on infected animals (Graves & Stenos, 2017). Rickettsiae of clinical importance have been described in Australia, and several *Rickettsia* spp. have been associated with Australian wildlife species and their ticks (Graves & Stenos, 2009). Given that 43% of participants in the *C. burnetii* serological survey (Chapter 2) indicated that they had been bitten by a tick, this opportunistic study was undertaken using a subset of the AWR cohort in Chapter 2 to determine their level of exposure to *Rickettsia* spp..

Hereafter, the content of this chapter is published in an international peer reviewed scientific journal *Pathogens* (citation below). Only the format has been changed for consistency of style in this thesis.

**Mathews, K. O.,** Phalen, D., Norris, J. M., Stenos, J., Toribio, J.-A., Wood, N., Graves, S., Sheehy, P. A., Nguyen, C. & Bosward, K. L. (2021). Serological Evidence of Exposure to Spotted Fever Group and Typhus Group Rickettsiae in Australian Wildlife Rehabilitators. *Pathogens*, 10(6), 745.
https://doi:10.3390/pathogens10060745

.

## 6.2 Abstract

Rickettsioses are arthropod-borne zoonotic diseases, several of which occur in Australia. This study aimed to assess the exposure levels and risk factors for *Rickettsia* spp. among Australian wildlife rehabilitators (AWRs) using serology, PCR and a questionnaire. Antibody titres against Spotted Fever Group (SFG), Typhus Group (TG) and Scrub Typhus Group (STG) antigens were determined using an immunofluorescence assay. PCR targeting the *gltA* gene was performed on DNA extracts from whole blood and serum. Logistic regression was used to identify risk factors associated with seropositivity. Of the 27 (22.1%; 27/122) seropositive participants all were seropositive for SFG, with 5/27 (4.1%) also positive for TG. Of the 27 positive sera, 14.8% (4/27) were further classified as exposure to *R. australis*, 3.7% (1/27) to *R. honei*, 3.7% (1/27) to *R. felis* and 77.8% (21/27) were classified as 'indeterminate'— most of which (85.7%; 18/21) were indeterminate *R. australis*/*R. honei* exposures. Rickettsia DNA was not detected in whole blood or serum. Rehabilitators were more likely to be seropositive if more than one household member rehabilitated wildlife, were older than 50 years or had occupational animal contact. These findings suggest that AWRs are at increased risk of contracting rickettsia-related illnesses, however the source of the increased seropositivity remains unclear.

## 6.3 Introduction

Rickettsioses are among the oldest known diseases and are caused by bacteria from the genera *Rickettsia* and *Orientia*, which are transmitted to humans via arthropod vectors, including ticks, lice, fleas and mites (Walker, 1996). The genus *Rickettsia* is comprised of two main antigenic groups; the spotted fever group (SFG), which are primarily transmitted to vertebrate hosts by hard ticks (Ixodidae) (Blanton, 2019; Luce-Fedrow et al., 2015), and the typhus group (TG) transmitted by fleas and lice (Azad & Beard, 1998). Genus *Orientia* contains two known species; *O. tsutsugamushi* and *O. chuto*, transmitted by mites and together form the Scrub Typhus Group (STG) (Izzard et al., 2010). The salivary glands and faeces of these arthropod vectors may harbour large numbers of rickettsiae, and human infection can occur via bacterial injection during a blood meal, or through faecal contamination of the bite site (NSW Department of Health, 2016). The most common clinical presentations of rickettsiosis include headache, rash, fever, chills, muscle aches and an inoculation 'eschar' (scab) from the bite of a tick (McBride et al., 2007) or a mite (Xu et al., 2017).Severe cases of rickettsiosis can be fatal (Sexton & King, 1990; Stewart et al., 2019). The similarity of symptoms between rickettsioses and other diseases renders clinical diagnosis challenging. Therefore, many cases of human disease probably go unrecognised (Biggs et al., 2006). In Australia, rickettsial infection is not nationally notifiable, making it difficult to define the distribution of rickettsial diseases and understand the nationwide disease burden attributable to rickettsioses (National Notifiable Diseases Surveillance System, 2021).

Rickettsiae of clinical importance from both the STG, SFG and TG have been described in Australia, and several species of SFG rickettsia have been associated with Australian wildlife and their ticks (Graves & Stenos, 2009). Scrub typhus, caused by *O. tsutsugamushi* (STG) is endemic to tropical north Queensland (QLD) and the Torres strait Islands (Faa et al., 2003), the ‘top end’ of the Northern Territory (NT) (Whelan et al., 2004) and the Kimberley region of Western Australia (WA) (Graves et al., 1999). The main reservoir and vector of *O. tsutsugamushi* in Australia are the larvae of the mite species *Leptotrombidium deliense*, which parasitise rodents, marsupials, cattle, cats and dogs (Mullen & O'connor, 2019).

Queensland Tick Typhus (QTT), was the first tick-transmitted infection recognised in Australia and is predominantly seen along the eastern seaboard of Australia from Torres Strait to south-eastern Victoria (VIC) (Graves & Stenos, 2017). The causative agent of QTT, *R. australis* (member of SFG), is transmitted by the paralysis tick *Ixodes holocyclus*, and the common marsupial tick *I. tasmani*, whose respective vertebrate hosts are bandicoots and native rats (Raby et al., 2016). Flinders Island Spotted Fever (FISF), occurring on Flinders Island in Bass Strait, South Australia (SA) and north QLD is caused by *R. honei* (SFG) (Unsworth et al., 2005) and is transmitted by the reptile tick *Bothriocroton hydrosauri*, whose vertebrate hosts include snakes and blue-tongue lizards (Barker & Walker, 2014). The main arthropod vector of *R. felis* (also a member of the SFG) causing cat flea typhus (Williams et al., 2011) is the cat flea (*Ctenocephalides felis*), the reservoir host of which is yet to be determined,, but is thought likely to be the dog (Hii et al., 2011; Ng-Nguyen et al., 2020). Murine typhus is caused by *R. typhi*, which is currently the only member of the TG recognised in Australia. *Rickettsia typhi* is transmitted by the fleas of rodents and has been implicated in human disease in WA (Beaman & Marinovitch, 1999), QLD (Graves et al., 1992) and Victoria (Jones et al., 2004).

Over the past 20 years, several emerging rickettsioses have been reported in Australia (Parola et al., 2005). In 2007, a *Rickettsia* spp. was identified that was genetically related to *R. honei* (SFG) and produced similar symptoms to FISF (Unsworth et al., 2007). The agent, subsequently designated *Rickettsia honei* subsp. *marmionii* was detected in *Haemaphysalis novaeguineae* ticks, which typically infest macropods (Parola et al., 2013), and to date, it has not been found in *B. hydrosauri* (Graves & Stenos, 2009). The associated rickettsiosis was named Australian Spotted Fever owing to its different epidemiology compared to the parent strain *R. honei*. This subspecies has also been isolated from the blood of chronically ill patients (Unsworth et al., 2008). Several new rickettsia species of unknown pathogenicity have also been described in Australian ticks. *R. gravesii* (SFG) has been isolated from the ornate kangaroo tick *Amblyomma triguttatum* (Owen et al., 2006), and molecular methods have identified novel rickettsiae in ticks collected from Australian mammals including: koalas (Koala rickettsia from *B. concolor*) (Vilcins et al., 2008), Tasmanian devils (*Candidatus* Rickettsia tasmanensis from *I.*

*tasmani*) (Izzard et al., 2009) and the marsupial mouse (*R. antechini* from *I. antechini*) (Graves & Stenos, 2009). Although the pathogenicity of these recently described *Rickettsia* spp. is unknown, their potential to cause disease in humans cannot be discounted, particularly for those living in endemic areas and/or in regular contact with Australian wildlife and their ticks.

Although Australia is home to several rickettsia that are pathogenic to humans, the level of nationwide exposure to *Rickettsia* spp. within the Australian population is unknown. Clinical studies of chronically ill patients with suspected rickettsia-related illness have reported seroprevalence to SFG as high as 41% (Unsworth et al., 2008). A recent study on Australian veterinarians reported that 16.0% of participants were seropositive for *R. felis*, 4.6% for *R. typhi* and 35.1% were seropositive for both organisms (Teoh et al., 2017). Reports of frequent tick bites and low-grade illness amongst bushland recreationists, prompted a study into the seroprevalence to SFG rickettsia in rogainers, who may spend 6–24 h in the bush whilst participating in the sport (Abdad et al., 2014). The rogainer group in this study, who frequented areas of WA with a high *R. gravesii* prevalence in ticks, had a significantly higher SFG seroprevalence in comparison to the control group (23.1% and 2.1% respectively) and were 14 times more likely to be seropositive for SFG rickettsia.

Australian wildlife rehabilitators (AWRs) are potentially at risk of contracting rickettsioses because the wildlife for which they care may harbour ticks, fleas, lice and mites, all of which are rickettsial vectors, however the degree of rickettsia exposure amongst this population is unknown. In a study investigating the zoonotic disease Q fever in a cohort of AWRs, 43.8% of participants reported having been bitten by a tick (Mathews, Toribio, et al., 2021), indicating that AWRs are potentially at risk of rickettsioses. Therefore, the aim of this study was to: 1) determine the level of prior exposure to *Rickettsia* spp. in a population of AWRs attending a wildlife rehabilitation conference through measurement of SFG, TG and STG antibody titres, 2) investigate the association between seropositivity and risk factors for exposure to *Rickettsia* spp. to determine potential sources of exposure for wildlife rehabilitators, and 3) identify current infections in this AWR cohort using a PCR assay specific to SFG and TG rickettseae.

## 6.4 Methods

### 6.4.1 Study design and participant recruitment

The serum samples tested in this study were obtained opportunistically from a previous cross-sectional study investigating *Coxiella burnetii* seroprevalence in AWRs. To be eligible to participate in this study, AWRs were required to be >18 years and to have rehabilitated Australian mammals. Participants from the aforementioned study who elected to receive their Q fever serology results and provided their contact details for this purpose, were invited to participate in the current study via a hyperlink or web address to the secure online platform Research Electronic Data Capture (REDCap) (Harris et al., 2019; Harris et al., 2009) hosted at The University of Sydney, where they could access a detailed participant information statement (PIS). Willing participants provided online consent to have their blood sample tested for antibodies against *Rickettsiaceae* and provided their contact details if they wished to be notified of their individual serological results and/or a summary of the project outcomes. For participants supplying a postal address, hard copies of the PIS consent form and a stamped self-addressed envelope were included in the mailout with their Q fever serology results. This research was approved by the Human Research Ethics Committee of the University of Sydney (project number 2018/457).

### 6.4.2 Sample size calculation

The sample size for this study was calculated using Statulator software (Dhand & Khatkar, 2014). Assuming a nationwide average of 2% seroprevalence to SFG rickettsia (control group in the rogainer study; Abdad et al. (2014), an expected response rate of 15% (serosurvey of veterinary workers (Sellens et al., 2020)) and a national wildlife rehabilitator population size of 14,358 (Mathews, Toribio, et al., 2021), this study would require a sample size of 103 AWRs for estimating seroprevalence to *Rickettsia* spp. with 7.0% absolute precision and 95% confidence.

### 6.4.3 Questionnaire

The paper-based questionnaire (Appendix A) completed by each participant at the time of blood sample collection has been previously described (Mathews, Toribio, et al., 2021). Of relevance to the current

study were questions regarding: (i) demographics of the rehabilitator and where they rehabilitated wildlife, (ii) the type of wildlife they rehabilitated and other animals located on or nearby the caring residence (iii) their rehabilitation and husbandry practices which included a question regarding the frequency of tick bites.

### 6.4.4 Laboratory methods

*6.4.4.1 Blood sample collection*

Blood samples were collected from participants on each day of the conference. Approximately 8mL of blood was drawn from the median cubital vein of each participant and divided into serum separator tubes (Interpath, Victoria, Australia) and EDTA blood tubes (Interpath, Victoria, Australia) by a certified phlebotomist or registered doctor. The serum separator tubes were centrifuged at 4000×g for 10 min, after which the serum was removed and stored at −20°C until transportation to the laboratory. All blood samples were deidentified.

*6.4.4.2 Serology*

The serum samples were analysed at the Australian Rickettsial Reference Laboratory (ARRL), Geelong, Australia using an in-house indirect immunofluorescence assay (IFA) accredited by the National Association of Testing Authorities (accreditation No. 14342).

6.4.4.2.1. Screening of sera for antibodies to *Rickettsiaceae*

Serum samples were initially screened for reactivity to SFG, TG and STG. Antibodies against SFG were tested using a combined preparation of *R. australis*, *R. honei* and *R. felis* antigens; against TG using *R. typhi* antigen; and against STG using *O. tsutsugamushi* (Gilliam and Karp strains) antigen. Sera was diluted 1/128 in 2% casein then approximately 5µL was spotted in duplicate onto a glass slide coated with antigens (described above). After incubation at 35°C for 40 min, the slides were washed with PBS (diluted 1/10) and air-dried before adding a combined conjugate containing fluorescein labelled goat anti-human IgA + IgG + IgM (H+L). The incubation and wash steps were repeated, the slides were dried and mounted with a coverslip. Each well was visualised using fluorescence microscopy (400×; Axioskop

40; Zeiss). Sera was deemed positive if fluorescence was observed at a dilution of 1/128 and classified according to reactivity to antigenic group (SFG, TG, STG).

6.4.4.2.2. Titration of sera against R. *australis*, *R. honei*, *R. felis* and *R. typhi* antigens

Positive sera underwent doubling dilutions (1/128 to 1/1024) in 2% casein. Each dilution was spotted in duplicate onto glass slides coated with individual antigen preparations of *R. honei*, *R. australis*, *R. felis* and *R. typhi* after which the slides were processed as described above. A minimum titre of 1/256 was required to deem a sample as positive. Species specific seroreactivity within and between serological groups (SFG, TG) was defined when; sera was reactive to only one species, or, if sera was reactive to more than one species, a four-fold minimum difference between antigens of reactive species was required, and in such instances, the species with the highest titre was designated as the agent responsible for the infection. Serum from patients returning a titre within these limits was classified as 'indeterminate' as it is impossible to determine the causative agent of infection with such titres. All antigens and screening slides were prepared in-house at ARRL as described by Teoh et al. (2017), and antibodies were manufactured by KPL/ SeraCare (USA). Positive and negative human serum samples were included on each slide.

*6.4.4.3 DNA extraction*

Genomic DNA was extracted from whole blood and serum using the Biosprint® 96 One-For-All Vet Kit (Qiagen, Germany) with the following modifications. For whole blood, 200µL of EDTA blood and 40mL of Proteinase K was incubated at 56°C for 30 min. For serum, 160µL of sample and 40µL of Proteinase K was incubated at 56°C for 3 h. Following incubation, 240µL of each blood lysate and 140µL of each serum lysate was loaded into a 96 well plate and DNA extractions were performed using the Biosprint® 96 automated extraction system (Thermo Fisher Scientific, USA) in accordance with the manufacturer's instructions. Eight randomly distributed extraction controls (ECs) using PBS in place of serum or blood were included in every 96-well plate.

*6.4.4.4 Real-time PCR (qPCR)*

A qPCR assay (*gltA*-PCR) using primers targeting a highly conserved region of rickettsial citrate synthase gene *gltA* (Stenos et al., 2005) was used to detect SFG and TG DNA in the serum and whole blood DNA extracts, and human β-actin served as an internal reference gene to verify DNA quality (Mediannikov et al., 2010). Rickettsial DNA provided by the ARRL and DNA extracted from a human buccal swab in house served as positive controls for the *gltA*-PCR and the β-actin PCR, respectively. Both assays were performed in singleplex and each reaction contained 1X SensiFAST No-Rox (Bioline, Alexandria, Australia), primers and probe (concentrations and sequences listed in Table 6.1), 2µL of DNA (extracted from blood or serum) and nuclease-free water in a total volume of 10µL. Assays were performed using a Bio-Rad-CFX Real-Time PCR Thermocycler (Bio-Rad Laboratories Pty Ltd, Gladesville, NSW, Australia) and underwent an initial denaturation at 95°C for 3 min followed by 40 cycles of denaturation at 95°C for 10s, annealing at 60°C for 40s. No template controls with nuclease-free water were used in place of sample DNA, and positive control DNA were included in every PCR run. Primers and probes were synthesised by Integrated DNA Technologies (Baulkham Hills, NSW, Australia). Any sample with a quantification cycle (Cq) < 40 was considered positive for β-actin. Samples returning a Cq < 40 for the citrate synthase gene were repeated and deemed positive for *gltA*-PCR if the same result was reproducible in triplicate. A subset of samples was tested for inhibition by diluting the sample 1/10 and comparing the Cq values.

### 6.4.5 Statistical analysis

*6.4.5.1 Data management*

The serological results of participants were added to a Microsoft Excel (Microsoft Corporation, Washington, USA) spreadsheet alongside their molecular and serological results for processing and subsequently analysed using R statistical program (R Core Team).

**Table 6.1** Sequence and product lengths of target gene primers used to detect SFG and TG DNA (citrate synthase) and human β-actin DNA (internal reference gene to verify DNA quality) in the whole blood and serum DNA extracts of Australian wildlife rehabilitators participating in a survey at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018.

| Target Gene and Primers | Primer Sequences (5′-3′) | Product Length (bp) | Final Concentration (nM) | Reference/ Primer Source |
|---|---|---|---|---|
| Citrate synthase | | | | |
| Forward primer | TCGCAAATGTTCACGGTACTTT | 74 | 300 | (Stenos et al., 2005) |
| Reverse primer | TCGTGCATTTCTTTCCATTGTG | | 300 | |
| Probe | FAM[a]- TGCAATAGCAAGAACCGTAGGCTGGATG -BHQ1[b] | | 200 | |
| Human β-actin | | | | |
| Forward primer | CATGCCATCCTGCGTCTGGA | 172 | 300 | (Mediannikov et al., 2010) |
| Reverse primer | CCGTGGCCATCTCTTGCTCG | | 300 | |
| Probe | FAM[a]- CGGGAAATCGTGCGTGACATTAAG-BHQ1[b] | | 200 | |

a 6-Carboxyfluorescein, b Black Hole Quencher-1, SFG—Spotted fever group, TG—Typhus Group

*6.4.5.2 Variables and risk factors*

The primary outcome variable was whether the AWR was seropositive or seronegative for exposure to *Rickettsia* spp. (rickettsial serostatus) based on assignment to antigenic groups (SFG, TG, STG). The secondary outcome variable was the classification of species-specific rickettsia infections (*R. australis*, *R. honei*, *R. felis*, *R. typhi*) in the seropositive participants. Descriptive statistics (mean, median and range for continuous variables, proportions for categorical variables) were generated to obtain information regarding the distribution of each variable. Continuous variables and questions regarding animal exposure and postcode of residence were handled as previously described (Mathews, Toribio, et al., 2021). Categories with 10% missing data were excluded in the statistical analysis.

Biosecurity practices were based on two questions in which participants indicated how frequently (‘always’, ‘frequently’, ‘occasionally’, ‘rarely’ or ‘never’) they utilised the following infection control practices while handling animals and cleaning enclosures: overalls/protective outerwear, disposable gloves, safety glasses, face mask, and prompt hand washing. The assessment and classification of adequate and enhanced biosecurity in both situations has been previously described (Mathews, Toribio, et al., 2021) and were established by the authors, using recommendations from the Australian Veterinary Association Guidelines for Veterinary Personal Biosecurity (Australian Veterinary Association, 2017) in combination with the National Wildlife Biosecurity Guidelines (Wildlife Health Australia, 2018).

Biosecurity practices were considered inadequate if participants 'never' used any form of personal protective equipment (PPE) when handling animals or cleaning enclosures. The use of each type of infection control was considered adequate if 'always' or 'frequently' was selected. Biosecurity practices were considered adequate if participants 'always' or 'frequently' used overalls/protective outerwear and practiced prompt hand washing when handling animals, and additionally wore disposable gloves when cleaning enclosures. Biosecurity practices were considered to be enhanced if participants 'always' or 'frequently' used overalls/protective outerwear, practiced prompt hand washing and wore disposable gloves when handling animals, and if all five methods of infection control were practiced when cleaning enclosures.

Potential risk factors for the outcome variable rickettsia serostatus were age, state of residence, remoteness area, total years rehabilitating wildlife, total weeks per year rehabilitating wildlife, rehabilitating wildlife on own property, number of people in household rehabilitating wildlife, wildlife species rehabilitated during rehabilitation career, total number of animals rehabilitated per year, association with reptiles, tick bite, occupational animal contact, biosecurity practices when handling animals and when cleaning enclosures.

*6.4.5.3 Modelling*

Univariable logistic regression was undertaken to identify associations between potential risk factors and serostatus (positive, negative). Risk factors with $p < 0.3$ in the univariable analysis were progressed to multivariable analysis after evaluating the strength of association between these risk factors using the Cramer's V statistic. When the Cramer's V statistic for a pair of risk factors was >0.7 only the variable which was more biologically plausible was included in subsequent multivariable analysis. Multivariable modelling was performed using backward selection where the variable with the least significance (Wald test) was removed sequentially. Variables with p-values < 0.1 were retained in the final model.

## 6.5 Results

### 6.5.1 Responses and demographics of Australian wildlife rehabilitators

Of the 162 conference attendees who provided blood for the previous study (Mathews et al., 2021) and were subsequently invited to participate in this study, 122 (75.3%) gave consent for their blood sample to be tested for antibody against *Rickettsia* spp. The median age of the 120/122 participants who disclosed their age was 55 years (range 21–79; IQR 48–62), and the majority of the cohort were female (113/122; 92.6%). All respondents had been actively rehabilitating wildlife for the past five years, and just over half (62/122; 50.8%) had been rehabilitating wildlife for more than 10 years. Almost all participants (118/122; 96.9%) identified their association with wildlife as a rehabilitator; however, 26.3% (31/118) also performed other wildlife-associated roles. These additional roles included veterinary nursing (18/118; 14.8%), wildlife research (5/118; 4.1%) and one participant also worked as a veterinarian (1/118; 1%). Just over half of the cohort resided in the conference host state of New South Wales (NSW; 64/122; 52.5%) followed by VIC (18/122; 14.8%), WA (16/122; 13.1%), QLD (12/122; 9.8%), SA (4/122; 3.3%), Tasmania (TAS; 4/122; 3.3%), NT (2/122; 1.6%) and the Australian Capital Territory (ACT; 2/122; 1.6%). The proportion of AWRs residing in NSW was higher than those in VIC and QLD (52.5%, 14.8% and 9.8% respectively) compared to the available total national population estimates for these states (32.0%, 25.8% and 20.1% respectively). The proportions within the remaining jurisdictions of WA, SA, TAS, ACT and NT (combined 22%) were comparable to the Australian population distribution. According to the available data on population distribution via remoteness area (National Rural Health Alliance, 2011), the proportion of the cohort residing in major cities was lower (46.7% vs. 70% respectively), while the proportion residing in inner regional Australia was higher (42% vs. 18% respectively) than the distribution of the general Australian population. The proportion of participants residing in outer regional/remote areas (11.5%; 14/122) was comparable to the population distribution for these remoteness categories (11%).

### 6.5.2 Wildlife rehabilitating demographics and practices

The majority of rehabilitators (97/122; 79.5%) spent over 30 weeks per year rehabilitating wildlife and the number of animals (mammals, birds and reptiles) rehabilitated per year ranged from 2 to 1500. For most participants, the location at which the majority of wildlife rehabilitation was undertaken was in their home or someone else's home (108/122; 88.5%), followed by a wildlife rescue centre/dedicated wildlife hospital (27/122; 22.1%), a veterinary clinic that also treats wildlife (15/122; 12.3%) and a zoo (5/122; 4.1%). Of the 114 AWRs who rehabilitated animals on their own property, 17.5% (20/114) housed animals exclusively within their home, 10.5% (12/114) in outdoor enclosures, while 71.9% (82/122) practiced both housing arrangements. For 79% (97/122) of AWRS, possums and gliders were the most commonly and frequently rehabilitated species, followed by kangaroos and wallabies and flying-foxes which were rehabilitated by 51.6% (63/122), 50.0% (61/122) and 39.34% (48/122) of AWRs respectively. Of the 58.2% (71/122) of participants reporting occupational animal contact, 81.7% (58/71) had been exposed to domestic animals, 73.2% (52/71) to wildlife and 36.6% (26/71) to ruminants.

Biosecurity practices adopted by 120 participants when handling animals and cleaning enclosures are presented in Table 6.2 (no questionnaire responses for 2 participants). Almost all AWRs practiced prompt hand washing after handling animals (116/120; 96.7%) and cleaning enclosures (117/120; 97.5%); however, 2.5% (3/120) of respondents did not practice any form of biosecurity when performing either activity. The vast majority of AWRs did not meet 'adequate' biosecurity requirements in either situation, with only 5.8% (7/120) and 2.5% (3/120) practicing 'enhanced biosecurity' when handling animals and cleaning enclosures, respectively.

### 6.5.3 Serology

#### *6.5.3.1 Rickettsia screening*

Of the 122 participants, 27 (22.1%; 95% CI 15.1% – 30.5%) were seropositive for *Rickettsia* spp. Of these, just under half (13/27; 48.1%) resided in NSW followed by VIC (7/27; 25.9%), QLD (3/27; 11.1%) and SA (2/27; 7.4%) with TAS and WA returning one seropositive participant each (1/27; 3.7%) (**Figure 6.1**, **Table**

6.2). Of the 27 seropositive participants, occupational contact with animals (domestic, companion, and wildlife) was reported by 70.1% (19/27). Just under half (12/27; 48.1%) reported having been bitten by a tick. All (27/27; 100%) of the seropositive participants were reactive to SFG, 18.5% (5/27) were reactive to TG and all (27/27; 100%) were non-reactive to STG.

*6.5.3.2 Rickettsia spp. titration*

The results of the titration for *Rickettsia* spp. exposure are displayed in **Table 6.3**. Twenty-one (21/27; 77.8%) of the serum samples were classified as 'indeterminate' due to titres being within twofold of one another. Of these, 18 (18/21; 85.7%) were classified as indeterminate *R. australis*/*R. honei* infections, one (1/21; 4.8%) was indeterminate for all three SFG species tested (*R. australis*/*R. honei* /*R. felis*) and the remaining two (2/21; 9.5%) 'indeterminate' infections were reactive to both SFG and TG rickettsia. Four (14.8%) of the 27 initial screening seropositive participants were classified as having been exposed to *R. australis* (4/27; 14.8%), while one was classified as exposed to *R. honei* (1/27; 3.7%) and one to *R. felis*. (1/27; 3.7%).

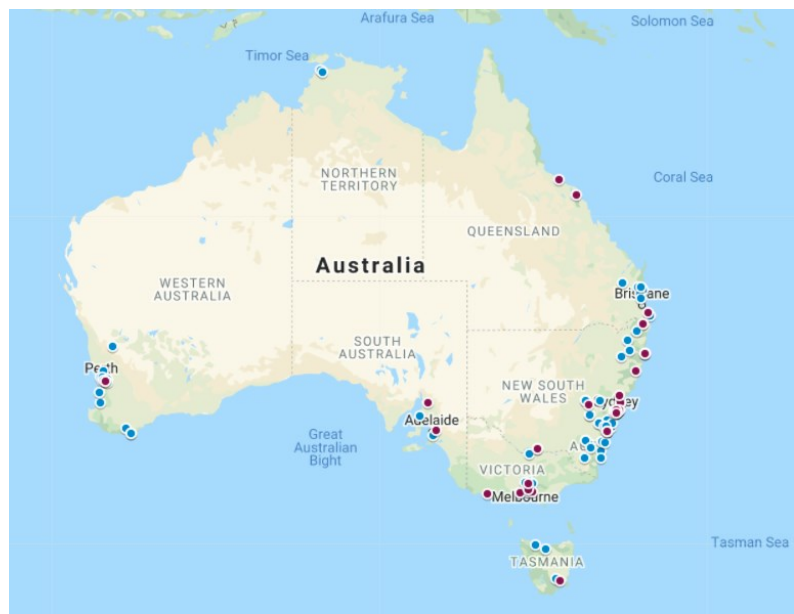


**Figure 6.1** Location of residence of 122 Australian wildlife rehabilitators participating in rickettsia seroprevalence survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. Maroon denotes seropositive and blue denotes seronegative for *Rickettsia* spp.

**Table 6.2** Biosecurity practices reported by 120 Australian wildlife rehabilitators when handling animals and cleaning enclosures. Results obtained from a survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018.

| Biosecurity Practice | Number (%) of Participants when Handling Animals | Number (%) of Participants when Cleaning Enclosures |
|---|---|---|
| Participant report of practice | | |
| No PPE | 4 (3.3) | 4 (3.3) |
| Prompt hand washing | 116 (96.7) | 117 (97.5) |
| Overalls/protective outerwear | 16 (13.3) | 25 (20.8) |
| Disposable gloves | 28 (23.3) | 47 (39.2) |
| Safety glasses | 5 (4.2) | 10 (8.3) |
| Face mask | 3 (2.5) | 7(5.8) |
| Level of biosecurity practice * | | |
| Inadequate | 104 (86.7) | 102 (85.0) |
| Adequate | 9 (7.5) | 15 (12.5) |
| Enhanced | 7 (5.8) | 3 (2.5) |

*Level of biosecurity practice was based on reported PPE (personal protection equipment) use and benchmarked against recommendations from the Australian Veterinary Association Guidelines for Veterinary Personal Biosecurity (Australian Veterinary Association, 2017) and National Wildlife Biosecurity Guidelines (Wildlife Health Australia, 2018).

.

**Table 6.3** Serological results (reciprocal titres) and antigenic classification of seropositive wildlife rehabilitators participating in a rickettsia seroprevalence survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018.

| | Spotted Fever Group (SFG) | | | Typhus Group (TG) | Sample Classification | | |
|---|---|---|---|---|---|---|---|
| Participant | *R. australis* | *R. honei* | *R. felis* | *R. typhi* | Antigenic Group | Species | State of Residence |
| 96 | ≥2048 | 256 | - | - | SFG | *R. australis* | VIC |
| 117 + | 1024 | 256 | - | - | SFG | *R. australis* | NSW |
| 147 | 512 | - | - | - | SFG | *R. australis* | NSW |
| 161 + | ≥2048 | 512 | - | 256 | SFG | *R. australis* | NSW |
| 110 + | 512 | ≥2048 | 512 | 256 | SFG | *R. honei* | NSW |
| 148 | - | - | 256 | - | SFG | *R. felis* | QLD |
| 6 + | ≥2048 | ≥2048 | 256 | - | SFG | *R. australis/R. honei* * | NSW |
| 13 | 1024 | 1024 | - | - | SFG | *R. australis/R. honei* * | VIC |
| 19 | 1024 | 1024 | - | - | SFG | *R. australis/R. honei* * | VIC |
| 20 + | ≥2048 | ≥2048 | - | - | SFG | *R. australis/R. honei* * | QLD |
| 27 + | 1024 | 1024 | - | - | SFG | *R. australis/R. honei* * | NSW |
| 34 | ≥2048 | 1024 | - | - | SFG | *R. australis/R. honei* * | SA |
| 36+ | 512 | 512 | - | - | SFG | *R. australis/R. honei** | QLD |
| 36 + | ≥2048 | ≥2048 | - | 256 | SFG | *R. australis/R. honei* * | NSW |
| 62 | 256 | 512 | - | - | SFG | *R. australis/R. honei* * | VIC |
| 83 + | 1024 | 1024 | - | - | SFG | *R. australis/R. honei* * | NSW |
| 86 + | ≥2048 | ≥2048 | 256 | - | SFG | *R. australis/R. honei* * | NSW |
| 87 | 512 | 512 | - | - | SFG | *R. australis/R. honei* * | NSW |
| 94 | 512 | 256 | - | - | SFG | *R. australis/R. honei* * | VIC |
| 113 | 256 | 256 | - | - | SFG | *R. australis/R. honei* * | SA |
| 115 | 512 | 512 | - | - | SFG | *R. australis/R. honei* * | WA |
| 138 + | 256 | 256 | - | - | SFG | *R. australis/R. honei* * | NSW |
| 158 | 512 | 512 | - | - | SFG | *R. australis/R. honei* * | VIC |
| 164 | 1024 | 512 | - | - | SFG | *R. australis/R. honei* * | VIC |
| 40+ | 512 | 1024 | 256 | - | SFG | *R. australis/R. honei/R. felis* * | NSW |
| 127+ | 512 | 256 | 256 | 256 | SFG/TG | *R. australis/R. honei/R. felis/R. typhi* * | NSW |
| 172 | 512 | 512 | - | 256 | SFG/TG | *R. australis/R. honei/R. typhi* * | TAS |

* Indeterminate rickettsial infections, + evidence of self-reported tick bite, Dash (-) = reciprocal antibody titre <256, VIC—Victoria, NSW—New South Wales, QLD—Queensland, WA—Western Australia, SA—South Australia, TAS—Tasmania.

### 6.5.4 *Rickettsia* spp. serostatus and investigated potential risk factors

Univariable logistic regression identified five risk factors (out of nine) that were associated with being serologically positive to *Rickettsia* spp. ($p < 0.3$) (Table 6.4), all of which were considered in the multivariable analysis. Three variables were retained in the final model ($p < 0.1$) (Table 6.5). Rehabilitators testing seropositive to *Rickettsia* spp. were 2.4 (95% CI = 0.89–7.32) times more likely to be >50 years of age, more than twice as likely to report occupational contact with animals compared to those without occupational animal contact (OR = 2.2; 95% CI = 0.88–6.16) and were 2.3 (95% CI = 0.95–5.90) times more likely to reside in homes where more than one household member rehabilitated wildlife.

### 6.5.5 Real-time PCR (qPCR)

All extraction controls and no template controls were negative for the β-actin gene ruling out the occurrence of cross contamination during DNA extraction and PCR set up. For each assay, amplification curves were observed for all positive control DNA samples indicating that the PCR assays were working appropriately. No inhibition was observed when comparing the human β-actin PCR assays of 1/10 diluted and neat whole blood or serum DNA extracts.

Of the 122 DNA samples extracted from whole blood, 121 (99.2%) were strongly positive for the β-actin endogenous control gene. Quantification cycles (Cqs) ranged from 19.41–29.25, indicating successful DNA extraction. Of these three (3/121; 2.4%) were positive in the *gltA*-PCR in the initial screen (Cqs~37), however, these amplifications were not reproducible when repeated in triplicate, and were subsequently considered negative. Of the 122 DNA samples extracted from serum, 91 (79.5%) amplified positive for the β-actin gene (Cq range 28.8–38.8). Of these four (4/91; 4.4%), were positive in the *gltA*-PCR in the initial screen (Cq's~38). This finding was not reproducible when these samples were assayed in triplicate, therefore these samples were subsequently considered negative.

**Table 6.4** Univariable logistic regression analysis of a positive serological result to *Rickettsia* spp. exposure among Australian wildlife rehabilitators participating in a survey at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. (p < 0.3).

| Variable Name and Description | Total Number | Seropositive | Seronegative | Odds Ratio | 95% Confidence Intervals | *p*-Value |
|---|---|---|---|---|---|---|
| State of residence | 122 | | | | | 0.365 |
| South West (WA + SA) | | 3 | 17 | 1 | | |
| Southeast (VIC + TAS) | | 8 | 14 | 3.24 | 0.77–16.99 | 0.125 |
| Northeast (QLD + NT) | | 3 | 11 | 1.55 | 0.25–9.74 | 0.63 |
| East (NSW + ACT) | | 13 | 53 | 1.39 | 0.39–6.58 | 0.637 |
| Age | 120 | | | | | 0.184 * |
| ≤50 | | 6 | 33 | 1 | | |
| >50 | | 21 | 60 | 1.93 | 0.74–5.67 | |
| Number of people in household rehabilitating wildlife | 121 | | | | | 0.145 * |
| 1 | | 13 | 60 | 1 | | |
| >1 | | 14 | 34 | 1.90 | 0.80–4.56 | |
| Total number of animals per year cared for per year | 119 | | | | | 0.226 * |
| 0–100 | | 18 | 75 | 1 | | |
| >100 | | 8 | 18 | 1.85 | 0.67–4.85 | |
| Occupational animal contact | 122 | | | | | 0.140 * |
| No | | 8 | 43 | 1 | | |
| Yes | | 19 | 52 | 1.96 | 0.81–5.17 | |
| Tick Bite | 122 | | | | | 0.577 |
| No | | 14 | 55 | 1 | | |
| Yes | | 13 | 40 | 1.27 | 0.56–3.43 | |
| Association with reptiles | 122 | | | | | 0.443 |
| No | | 23 | 86 | 1 | | |
| Yes | | 4 | 9 | 1.66 | 0.42–5.62 | |
| Biosecurity practices when handling animals | 120 | | | | | 0.220 * |
| None/handwash only | | 21 | 61 | 1 | | |
| Handwash and other | | 6 | 32 | 0.55 | 0.18–1.42 | |
| Biosecurity practices when cleaning enclosures | 120 | | | | | 0.973 |
| None/handwash only | | 15 | 52 | 1 | | |
| Handwash and other | | 12 | 41 | 1.02 | 0.42–2.40 | |

* p < 0.3, VIC—Victoria, NSW—New South Wales, ACT—Australian Capital Territory, QLD—Queensland, NT—Northern Territory WA—Western Australia, SA—South Australia, TAS—Tasmania.

**Table 6.5** Final multivariable logistic regression results for exposure to *Rickettsia* spp. among Australian wildlife rehabilitators participating in a survey at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. ($p < 0.1$).

| Variable Name and Description | Total Number | Seropositive | Seronegative | Adjusted Odds Ratio | 95% Confidence Intervals | *p*-Value |
|---|---|---|---|---|---|---|
| Age | 120 | | | | | 0.087 |
| ≤50 | | 6 | 33 | 1 | | |
| >50 | | 21 | 60 | 2.4 | 0.89–7.32 | |
| Number of people in household rehabilitating wildlife | 121 | | | | | 0.066 |
| 1 | | 12 | 60 | 1 | | |
| >1 | | 15 | 34 | 2.3 | 0.95–5.90 | |
| Occupational animal contact | 122 | | | | | 0.092 |
| No | | 8 | 43 | 1 | | |
| Yes | | 19 | 52 | 2.2 | 0.88–6.16 | |

## 6.6 Discussion

This is the first study to investigate rickettsia exposure in Australian wildlife rehabilitators, a population considered at risk of rickettsioses due to the numerous potential rickettsial species associated with Australian wildlife and their ticks (Graves et al., 1991; Graves & Stenos, 2009; Owen et al., 2006; Parola et al., 2013; Raby et al., 2016; Vilcins et al., 2009). This study reports an overall *Rickettsia* spp. seroprevalence of 22.1% (27/122) in this cohort of AWRs, with all positive sera reactive for SFG rickettsia, and the majority of infections (85.1%; 23/27) attributed to *R. australis* or *R. honei*, both of which are transmitted by ticks. All seropositive participants tested negative for *O. tsutsugamushi* (STG), however none of these participants resided in the tropical regions of WA, NT or QLD where scrub typhus is endemic (Faa et al., 2003; Graves et al., 1999; Whelan et al., 2004).

There are very few studies which have investigated exposure to *Rickettsia* spp. in Australian populations, however the 22.1% seroprevalence observed in the current study is comparable to the 23% SFG seroprevalence found in a study of Australian rogainers who are known to be at an increased risk of tick bites due to their bushland activities, and is considerably higher than the 2.1% SFG seroprevalence observed in the control group of the same study who had minimal tick exposure (Abdad et al., 2014). In contrast to the current study and the rogainer study in which participants were presumably healthy, another Australian study (using archived patient sera) reported a SFG seroprevalence of 39% and 41% in two cohorts of chronically ill patients (from Melbourne and Adelaide respectively) compared to <6% SFG seroprevalence in the control groups (Unsworth et al., 2008). The elevated SFG seroprevalence of these patients compared to the AWRs and rogainers could be due to sampling bias, in that the patient cohorts were selected on the basis that they were chronically ill with suspected rickettsia infection, whereas the rogainer and AWR populations were presumed healthy. Additionally, the high seroprevalence in the Adelaide patient cohort could be due to the Adelaide region of SA being endemic for spotted fever illnesses (Dyer et al., 2005; Unsworth et

al., 2005). However, the explanation for the Melbourne group is unclear because apart from Gippsland (Dwyer et al., 1991), there are no other known regions of rickettsia endemicity in Victoria.

Another Australian study on veterinarians attending a veterinary conference reported that overall 16% of participants were seropositive to *R. felis* (SFG), 4.6% to *R. typhi* (TG) and 35.1% were classified as 'indeterminate' *R felis* or *R. typhi* exposures (Teoh et al., 2017). These findings suggest that Australian veterinarians are at an increased risk of occupational exposure to rickettsia, primarily from exposure to fleas, however the authors were unable to demonstrate a significant association between seropositivity and contact with fleas or animals (companion, large and exotic). Similarly, in the current study, no association was found between seropositivity and exposure to animals (ruminants, domestic, wildlife). In contrast to veterinarians, the majority of rickettsial infections in AWRs were tick-associated, and although eight participants exhibited seroreactivity to *R. felis* and/or *R. typhi*, only one participant, (who did not identify as a veterinarian and had no occupational animal contact) was classified as having been exposed to *R. felis* (Table 6.3). A possible explanation for the greater *R. felis* and *R.typhi* seroprevalence in the veterinarian cohort is that this group worked in veterinary clinical practice. While the breakdown of type of animal exposure was not reported in this study, these veterinarians were more likely to be regularly exposed to larger numbers of companion animals than AWRs, in particular cats and dogs, which may act as potential hosts for fleas harbouring *R. felis* and *R typhi* (Barrs et al., 2010; Schloderer et al., 2006). Although the seropositivity in veterinarians was associated with flea-borne rickettsia and in the current study the majority of exposures were attributable to ticks, well over half of the seropositive participants in both studies (veterinarians 46/73, 63%; AWRs 21/27; 77.8%) were classified as 'indeterminate' rickettsial infections highlighting the difficulties in serodiagnosis due to cross reactivity between rickettsia species.

Quantitative PCR may be used to diagnose rickettsioses during the early stages of infection (Paris & Dumler, 2016), and has also been employed to detect rickettsia DNA in blood samples of chronically ill

patients (Unsworth et al., 2008). Given the elevated seroprevalence to SFG rickettsia in this cohort a highly specific *gltA*-PCR was performed (sensitivity of one copy per reaction (Cq = 35) (Stenos et al., 2005) on DNA extracted from whole blood and serum, to identify AWRs that may have been bacteraemic at the time of blood collection, or those who may have circulating organism due to long standing illness. Although a small number of DNA extracts (from both blood and serum) amplified positive for the *gltA*-PCR in the initial screen (producing Cqs~38), this amplification was not reproducible when the qPCR reactions on the same samples were repeated in triplicate, and so all samples were considered negative. Although details of participants' clinical history were not collected, and it is therefore unknown whether any had been clinically unwell and treated for or diagnosed with rickettsial disease, the absence of rickettsiaemic participants in this study is consistent with the presumption that they were healthy at the time of blood collection. Indeed, they were well enough to attend a wildlife rehabilitator conference, however the possibility of low levels of circulating rickettsiae and underlying illness in these participants cannot be discounted, particularly since estimates of rickettsia DNA concentration of as low as 8.40 × 101 ± 4.19 × 101 copies/mL of blood has been observed in patients with moderately severe disease (Kaplowitz et al., 1983). The assay for the β-actin gene was performed on DNA samples extracted from serum, with only 91 of these 122 samples (79.5%) amplifying positive for the β-actin gene (Cq range 28.8–38.8). The Cqs of these samples ranged from 28.82–38.8, and overall were considerably higher than those obtained from whole blood DNA extracts ($p = 0.007$). The higher Cqs and greater number of samples negative for the β-actin gene in the serum DNA extracts is expected, as the level of circulating DNA in the serum of healthy individuals is typically very low (Gal et al., 2004). Although for clinical diagnosis, whole blood and serum DNA extracts are considered suitable for PCR, DNA extracted from the buffy coat fraction may have improved the sensitivity of detection of rickettsial DNA, owing to the intracellular lifecycle of rickettisia and the higher concentration of leucocytes found in buffy coat (Bae et al., 2019). The samples in this study were collected at variable times between the hours of 9 am

and 2 pm. However, daily fluctuations in bacterial load have been observed in peripheral blood samples of patients infected with *Rickettsia rickettsia*, with peak bacteraemia occurring in early morning (Kato et al., 2016), therefore taking blood samples earlier in the day may have resulted in greater quantity of rickettsia DNA in the blood and serum.

Although 85.1% (23/27) of rickettsia infections in the current study were attributed *R. australis* or *R. honei*, which are both tick-transmitted, no association between reported prior tick bite and seropositivity was identified, and only 47% (11/23) of the seropositive participants reported having been bitten by a tick. Similarly, Abdad et al. (2014) found no association between SFG seroprevalence and tick bite in rogainers, and other studies have reported that ≤50% of patients with confirmed tick-transmitted rickettsial illness recalled being bitten by a tick (Stewart et al., 2019; Willis et al., 2019). These findings indicate that approximately 50% of bites go unrecognised, which may explain the observed lack of association between seropositivity and reported tick bite. The lack of tick bite awareness could be because the individual does not feel the tick attaching due to the local anaesthetic that ticks inject into the skin prior to biting (Graves & Stenos, 2017), or if the tick detaches before becoming engorged it may go unnoticed. It follows that the number of participants reporting tick bite in this study is likely an underestimation of the true exposure to tick bites.

Alternatively, it is also possible that participants who were seropositive for tick-borne rickettsiae may have been inoculated via means other than a tick bite. Excreta released by ticks during feeding contains high levels of rickettsiae (Reháçek, 1989) resulting in contamination of the skin and coat of the host animal with rickettsial organisms, hence the rehabilitator could become infected by inhaling aerosolised organisms while handling an animal on which ticks had fed (O'Connor et al., 1996). Although infection via the respiratory route is rarely described as a mode of transmission by ticks, infection in guinea pigs (Kenyon et al., 1979), monkeys (Saslaw & Carlisle, 1966; Wolf et al., 1967) and cases of aerosol transmission of *R. rickettsia* have also been reported in humans (Calia et al., 1970;

Johnson & Kadull, 1967; Oster et al., 1977). Indeed Murine Typhus caused by *R. typhi* can be acquired through the respiratory route (Saint et al., 1954) from infected flea faeces (Graves et al., 1992). Similarly, rickettsiae present on the skin and coat of animals may be transmitted via inoculation of skin abrasions and contamination of the conjunctiva.

This study utilised IFA methodology to titrate serum samples against antigen preparations from four rickettsia species (*R. australis*, *R. honei*, *R. felis* and *R. typhi*). Species specific seroreactivity was assigned to six (22.3%) participants, however the majority (21/27; 77.7%) of participants were classified as 'indeterminate' due to their lack of preferential reactivity to *R. australis* and *R. honei* antigens (Table 6.3). Although IFA is considered the gold standard reference method for rickettsia serodiagnosis (La Scola & Raoult, 1997), serological cross reactivity among the different rickettsial antigens is well documented, particularly between antigens of SFG rickettsia (Hechemy et al., 1989). Similarly, antigenic cross-reactivity is also displayed within the TG (La Scola et al., 2000) and between *R. felis* (SFG) and *R. typhi* (TG) (Teoh et al., 2016). This serologic cross-reactivity makes it difficult to infer the rickettsia species responsible for provoking the immune response (Delisle et al., 2016). Furthermore, extensive *R. australis* and *R. honei* serological cross-reactivity may preclude definitive speciation of the infecting rickettsia during clinical diagnosis (Baird et al., 1992). It is also possible that serological responses of the 'indeterminate' participants were from exposure to more than one species, or that these participants had been exposed to species of rickettsia that were not evaluated in this study, such as *R. honei* subsp. marmionii which is genetically related to *R. honei*. The high number of 'indeterminate' seropositive samples highlights the difficulties in diagnosing rickettsial infections and emphasises the importance of obtaining accurate details regarding a patient's clinical and epidemiological history to accompany diagnostic testing. Other methodologies offering greater specificity than IFA, such as Western blotting or cross-adsorption (La Scola et al., 2000), may result in

a more definitive determination of the species involved in the exposure. However, such analyses were beyond the scope of the current study and are not routinely undertaken.

In this study, a broad range of antibody titres were observed, with eight of the 27 (29.6%) seropositive AWRs displaying titres of 1/2048 (**Table 6.3**), which is eight-fold higher than the assigned 1/264 cut-off titre. Additional information regarding how recently these participants had been exposed could have been obtained by the collection of a second serum sample taken several weeks following the initial one to assess whether the antibody titres of these participants were rising, thus demonstrating recent infection, or through antibody subclass analysis including individual IgG and IgM titres (rather than the combined IgA, IgG and IgM conjugate used in this study). The sera in this study were opportunistically obtained from another study, for which the questionnaire accompanying the blood sample related to the zoonotic disease Q fever and did not specifically ask questions regarding symptoms of rickettsial illnesses and, therefore although they were well enough to attend a conference, it is unknown whether these wildlife conference participants were currently experiencing, or had previously suffered from, acute or chronic rickettsia related illnesses.

Multivariable logistic regression identified three risk factors suggestive of association a positive serostatus. Older participants (>50 years) were 2.4 (95% CI = 0.89–7.32) times more likely to be seropositive than rehabilitators <50 years. A similar association between age and SFG seropositivity was reported in the rogainer study by Abdad, Cook, Dyer, Stenos and Fenwick (Abdad et al., 2014). The positive association between seropositivity and age in these two studies is possibly due to an increased chance of exposure to rickettsia over time. In contrast Teoh et al. (2017) demonstrated that veterinarians >60 years had a decreased risk of exposure to *R. felis* and *R. typhi*, which was in line with older veterinarians reporting that they spent less time in clinical practice compared to their middle age and younger counterparts, and therefore had a reduced likelihood of exposure. Rehabilitators reporting occupational contact with animals were 2.2 (95% CI = 0.88–6.16) times more likely to be to

*Rickettsia* spp. seropositive. The source of exposure amongst the veterinarians in the study by Teoh et al. (2017) was thought to be from infected fleas located on companion animals, particularly cats and dogs. However, the AWRs in this study were exposed to a wide range of domestic and wildlife species and no association between seropositivity and any particular animal species was identified. The finding that rehabilitators residing in households where more than one person rehabilitated wildlife were more than twice as likely to be seropositive (OR = 2.3; 95% CI = 0.95–5.90) is interesting, and possibly suggestive of a link that could be explained by households with more than one rehabilitator in residence having greater exposure to larger numbers of animals, and therefore their ticks as rickettsial vectors.

Another possibility is that households with more than one rehabilitator are more likely to be involved in outdoor activities such as bushwalking or camping and therefore are more likely to be exposed to ticks. Further studies may indicate how it is that AWRs become exposed to rickettsiae. Future serological studies should focus on targeted questions that may allow for better understanding of how wildlife rehabilitators become exposed to ticks.

Rickettsia are emerging zoonoses and since first described by Ricketts in 1909 (Ricketts, 1909), the rickettsia genus has grown to comprise approximately 34 species (http://www.bacterio.cict.fr/qr/rickettsia.html; accessed February 7, 2021), and contains many novel species of unknown pathogenicity that are yet to be named. Given the recent emergence of *R. felis* in Australia (Schloderer et al., 2006), and the identification and characterisation of three novel rickettsiae over the past three decades including *R. gravesii* (Abdad et al., 2017), *R. honei* (Stenos et al., 1998) and *R. honei* subsp. marmionii (Unsworth et al., 2007), it is possible that the elevated seropositivity observed in this cohort of AWRs (particularly the participants classified as 'indeterminate' *R. australis*/*R. honei* infections) could be due to exposure to one or more novel

rickettsial species not yet discovered, or to a previously described species that is not known to be endemic in Australia.

## 6.7 Conclusion

This is the first study to investigate the level of exposure to *Rickettsia* spp. in rehabilitators of Australian wildlife. An elevated overall seroprevalence to *Rickettsia* spp. compared to control groups in other Australian studies was observed, with most exposures in the seropositive participants attributable to tick-borne SFG rickettsia. The activities associated with tick exposure in AWRs are unclear, nonetheless, these findings have significant health implications especially given that ticks can transmit a number of clinically important rickettsiae. The elevated seroprevalence to *Rickettisa* spp. observed in this cohort suggests that Australian wildlife rehabilitators would benefit from targeted education programs aimed at raising their awareness of arthropod-borne infections. Such programs should include information regarding potential exposure pathways, clinical symptoms of rickettsial disease, and, recommendations of appropriate precautionary measures that may be implemented to minimise exposure risk to arthropod-borne diseases. For example, rickettsial pathogens could be included as a key infectious disease of concern in the National Wildlife Biosecurity Guidelines issued by Wildlife Health Australia (Wildlife Health Australia, 2018).

# Chapter 7 General Discussion

## 7.1 Key findings, conclusions, and future directions

The research presented in this thesis has made an important contribution to the understanding of Q fever in AWR. This was accomplished by determining *C. burnetii* seroprevalence and levels of self-reported Q fever in AWR and exploring potential pathways of exposure. In addition, investigations were conducted to determine the level of awareness of Q fever and QFV and to identify factors associated with, and barriers to, Q fever vaccine uptake in AWR. To complement the investigations in AWR, a wide range of tissues were collected from a variety of Australian native wildlife species to determine potential *C. burnetii* transmission pathways. Finally, an opportunistic study was undertaken to determine the level of exposure of AWR to other zoonotic pathogens from the genus *Rickettsiaceae*. A summary of the key findings, conclusions, and suggestions for future research according to research chapters are presented in **Table 7.1**.

**Table 7.1** Summary of key findings, limitations, and suggestions for future research arising from the PhD thesis undertaken by Ms Karen Mathews at The University of Sydney School of Veterinary Science entitled 'The role of Australian native wildlife in Q fever'.

| | Contribution | Conclusions | Limitations | Future directions |
|---|---|---|---|---|
| Chapter 2 | First study to investigate C. burnetii exposure, Q fever and QFV in AWR and investigate potential risk factors associated with Q fever serostatus and vaccination status. | AWR have a higher *C. burnetii* seroprevalence and self-report a higher rate of Q fever compared to the general Australian population.<br><br>Low QFV uptake amongst the cohort.<br>Inadequate use of PPE when handling animals and cleaning enclosures. | Findings may not be generalisable to AWR across all Australian jurisdictions.<br><br>Q fever and QFV status were self-reported. | Larger longitudinal study in AWR to determine risk factors associated with Q fever and *C. burnetii* seroconversion. Questionnaire designed to differentially explore ruminant-associated and traditional risk factors versus wildlife associated risk factors.<br><br>Investigations into training programs in AWR regarding Q fever education and other zoonoses and infection control practices |
| Chapter 3 | Reports Q fever prevalence and risk factors associated with Q fever in AWR | AWR self-report a higher rate of Q fever compared to the general Australian population.<br><br>Risk factors were not associated with wildlife.<br><br>Low levels of QFV amongst the cohort.<br><br>Recommendation for 'wildlife' specific fields be added to the Q fever case report form to enhance wildlife-associated Q fever notification data.<br><br>Difficulties in accessing AWR population. | Difficulty in accessing the AWR population.<br><br>Occupation not measured.<br><br>Q fever and QFV status were self-reported.<br><br>There is a need for increased Q fever knowledge in AWR. | Construction of a 'one stop' online national database for registration of AWR which would serve as a portal through which they can be contacted and access information including WHA Biosecurity Guidelines and factsheets, training courses on zoonoses This could operate through WHA and include AWR QFV status, and the development of a government funded National QFV registry. |

| | Contribution | Conclusions | Limitations | Future directions |
|---|---|---|---|---|
| Chapter 4 | First study to investigate knowledge and attitudes towards Q fever and Q fever vaccination in AWR, and to explore factors associated with, and attitudes and barriers towards QFV in AWR. | There is a lack of awareness of Q fever and the Q fever vaccine.<br><br>A shortfall in vaccine uptake exists in AWR.<br><br>Barriers to vaccination include lack of knowledge, convenience and cost and uncertainty regarding the safety, efficacy, and importance of the Q fever vaccine<br>There is a need for increased Q fever knowledge in AWR.<br><br>There is a need for increased Q fever knowledge in AWR. | | Investigations into Q fever knowledge AWR to guide educational interventions.<br><br>Vaccination programs for AWR to reduce barriers to QFV.<br><br>Educational interventions to raise awareness of Q fever in AWR. |
| Chapter 5 | Multiple sample matrices collected from the same animal.<br><br>First extensive molecular study to determine the presence of *C. burnetii* DNA in a variety of Australian wildlife species in NSW | *C. burnetii* DNA prevalence was considerably lower than reported in other Australian studies -only two positive animals amplifying at low levels of ~10 *C. burnetii* genome equivalents per reaction.<br><br>Australian wildlife may not be a major source of *C. burnetii* for humans, however, given the low infectious dose, the aerosol transmission route and environmental persistence of the bacterium, people in close contact with Australian wildlife and their habitats remain at risk.<br><br>Urogenital swabs or cloacal swabs would be useful anatomical sampling sites for future studies. | Findings may not be generalisable to animals across all Australian jurisdictions as samples were mainly from NSW.<br><br>Difficulty in accessing wildlife populations to obtain samples.<br><br>Unequal numbers of male and female animals | Follow-up studies in wildlife sampled in regions with high rates of human infection e.g. Cobar shire<br><br>Molecular investigation of Australian tick species for the presence of *Coxiella*-like endosymbionts<br>Publication of the CoxMP qPCR assay for detecting *C. burnetii* DNA in Australian wildlife.<br>Longitudinal *C. burnetii* challenge experiments in macropods to follow course dissemination and shedding in tissues, secretions, and excretions. |

| | Contribution | Conclusions | Limitations | Future directions |
|---|---|---|---|---|
| | | Call for standardisation and validation of qPCR assays in studies investigating *C. burnetii* DNA prevalence in Australian native wildlife and transparency in reporting of results. | | |
| Chapter 6 | First study to investigate AWR for exposure to *Rickettsia* spp. and risk factors associated with exposure. | AWR are at risk of contracting Rickettsial-related illness mainly via tick-borne rickettsia.<br><br>The increased seropositivity was not associated with tick bites or contact with animal species (domestic or wildlife).<br><br>The source of increased seropositivity was unclear.<br><br>There is a need for increased rickettsia knowledge in AWR. | The questionnaire accompanying the blood sample was designed specifically for Q fever.<br><br>Findings may not be generalisable to AWR across all Australian jurisdictions. | Follow-up seroprevalence studies in AWR are required using a questionnaire designed for rickettsia to enable a detailed exploration of risk factors associated with exposure.<br><br>A large nationwide seroprevalence study is required to determine the exposure to *Rickettsia* spp. and associated risk factors in the general Australian population to quantify the disease burden.<br><br>It is recommended that rickettsial diseases be made nationally notifiable by the NNDSS. |

*C. burnetii* – *Coxiella burnetii*, AWR-Australian wildlife rehabilitator, NNDSS-National Notifiable Disease Surveillance System, QFV-Q fever vaccination, qPCR-quantitative PCR, NSW-New South Wales, CoxMP- multiplex qPCR assay targeting three *C. burnetii* genes, PPE-Personal Protective Equipment, WHA-Wildlife Health Australia.

## 7.2 Australian wildlife rehabilitators - an 'at-risk' group

### 7.2.1 *Coxiella burnetii* exposure and Q fever

Chapter 2 investigated *C. burnetii* exposure among AWR attending the AWRC in 2018. The 6.1% seroprevalence determined for AWR cohort was 70% greater than the 3.6% found in healthy Australian blood donors (Gidding et al., 2019), suggesting that AWR are almost twice as likely to be exposed to *C. burnetii* than the general Australian population. Three (3/147; 2.1%) participants also self-reported having had medically diagnosed Q fever providing further evidence of *C. burnetii* exposure in the cohort. These findings were corroborated by the online KAP survey (Chapter 3), in which 4.5% (13/287) of unvaccinated AWR self-reported medically diagnosed Q fever. This was estimated to be approximately 100-fold greater than the cumulative national Q fever notification rate over the 18 year period during which the AWR participants in this study reported having been diagnosed (4,530 cases of Q fever per 100,000 in AWR versus 43 notifications per 100,000 of population) (National Notifiable Diseases Surveillance System, 2021). Further evidence of *C. burnetii* infection in AWR was identified in the online KAP survey (Chapter 3), in which seven participants indicated their ineligibility for vaccination due to returning a positive pre-vaccination screening test. This higher level of clinical Q fever, coupled with the increased *C. burnetii* seroprevalence in AWR, reinforces the importance and need for QFV in this less recognised at-risk group.

### 7.2.2 Emerging zoonoses and AWR

Although not the focus of this research, Chapter 6 presented the findings of a second serosurvey that investigated exposure to *Rickettsia* spp. among AWR. This study utilised the sera obtained from conference delegates in Chapter 2. An elevated seroprevalence to *Rickettsia* spp. was observed in AWR compared to control groups in other Australian studies, with most infections attributable to tick-borne SFG *Rickettsia* (*R*. *australis* and/or *R. honei*). Although the activities associated with tick exposure were unclear, these findings have significant health implications, especially given that,

second to mosquitoes, ticks are currently considered to be the most common vectors for the transmission of arthropod-borne zoonoses (de la Fuente et al., 2017).

The exposure level to *Rickettsia* spp. and the incidence of rickettsioses in Australia is unknown, as rickettsial infection is not currently notifiable under the National Notifiable Disease Surveillance System (National Notifiable Diseases Surveillance System, 2021). Thus, the disease burden attributable to rickettsioses in Australia is difficult to define, however the burden and severity of SFG infections appear to be increasing, particularly in tropical Australia (Stewart et al., 2019). Evidence of tick-borne SFG rickettsia infections have been identified in chronically ill patients (Unsworth et al., 2008) and fatalities due to SFG rickettsioses have also been described (Sexton & King, 1990; Stewart et al., 2019).

More extensive cross-sectional seroprevalence studies (incorporating a well-developed questionnaire specifically for rickettsia, with larger sample sizes) are required, to determine exposure levels and risk factors associated with rickettsioses in AWR, and the general Australian population at large. To further understand the disease burden of rickettsioses in Australia, rickettsial infection could be listed as nationally notifiable under the National Notifiable Disease Surveillance System as it is in other parts of the world such as the United States (Nicholson & Paddock, 2019).

## 7.3 Factors contributing to elevated *C. burnetii* seroprevalence and higher rates of Q fever

### 7.3.1 Poor vaccine uptake

Despite national guidelines recommending QFV for wildlife and zoo workers (Australian Technical Advisory Group on Immunisation, 2021), substantial shortfalls in vaccine uptake were identified in both the serological (8.1%) and the online KAP (15.1%) survey (Chapter 2 and Chapter 3 respectively). These low levels of vaccine uptake are particularly concerning, especially given the findings from this thesis, which have demonstrated that AWR are at-risk of exposure to *C. burnetii* and developing Q

fever. The low levels of vaccination in AWR highlight the need for implementing interventions to improve vaccine uptake in this at-risk group.

In this study, a positive QFV status correlated with higher education levels, younger age and attending animal births, suggesting that vaccinated AWR may have undergone QFV in vaccination clinics as a requirement of their university studies, or may have been vaccinated due to occupational requirements. The NQFMP, funded by the Australian government from 2002 -2006, covered the costs associated with QFV and pre-vaccination screening in occupationally at-risk groups including abattoir workers and farmers, and is an example of a successful vaccination program (Gidding et al., 2009). This program increased Q fever vaccine uptake in these at-risk groups and reduced the Australian national Q fever notifications by over 50% (Gidding et al., 2009). Over two thirds of the unvaccinated AWR indicated a willingness to be vaccinated, therefore the implementation of a similar vaccination scheme for AWR may facilitate vaccination by making it more accessible and therefore address some of the key barriers to vaccination in AWR identified in Chapter 4.

### 7.3.2 The Q fever knowledge gap

Several reasons for vaccine uptake shortfalls were identified in the online KAP survey (Chapter 4). Firstly, approximately 40% of the cohort had not heard of Q fever and/or the Q fever vaccine, which represents a major barrier to seeking and/or receiving QFV. A notable proportion of these 'unaware' participants reported occupational contact (25.6%) with animals, or being present at animal births (32.8%); all activities known to pose an elevated risk of exposure to *C. burnetii*. Lack of knowledge and complacency were also identified as barriers to QFV among the subpopulation of unvaccinated AWR who had heard of Q fever, with the majority (81.8%) unaware of the risks associated with not being vaccinated, and around one third agreeing they were unsure about the safety, efficacy, and importance of the Q fever vaccine.

The National Wildlife Biosecurity Guidelines issued by Wildlife Health Australia (Wildlife Health Australia, 2018) state that AWR should be aware of, and implement, basic biosecurity practices at all times, irrespective of the animal species or perceived disease risk. These guidelines specifically recommend the use of P2/N95 particulate respirator, ventilation controls, dust management and QFV for AWR. The inadequate biosecurity practices identified in Chapter 2, and the low Q fever vaccination prevalence, suggest that many AWR are unaware of these guidelines, and therefore unaware of the importance of employing appropriate biosecurity practices to mitigate the risk of contracting Q fever, and other zoonotic diseases when caring for wildlife. This is cause for concern given that wildlife can serve as reservoirs of known and novel zoonotic pathogens which may be transmitted to humans through bites and scratches and contact with bodily secretions and excretions (Garland-Lewis et al., 2017). The COVID-19 global pandemic has demonstrated the risks and impact of diseases that emerge from the human-wildlife-environmental interface, emphasising the need for increased awareness of zoonoses, and the adoption of appropriate biosecurity practices by AWR.

The reasons for this large and multifaceted knowledge gap may be explored using responses to the knowledge and rehabilitation practices components of the online KAP survey (Appendix B). This will aid in the development and delivery of educational interventions that will raise awareness of Q fever, QFV and the risks associated with zoonotic diseases in general. Such interventions should employ a behavioural science approach to increase AWR engagement with appropriate biosecurity practices in general, combined with other approaches including stakeholder meetings and role-modelling to champion biosecurity practices (Lankford et al., (2003). It is important to recognise that while ensuring best practice biosecurity may mitigate the risk of many zoonoses, regarding Q fever, vaccination remains the main prevention strategy due to the environmental persistence and aerosol transmission route of *C. burnetii* (Maurin & Raoult, 1999; Raoult et al., 2005).

### 7.3.3 Animal contact

An extensive risk factor analysis did not identify any significant associations between *C. burnetii* seropositivity and demographic or animal-related risk factors including exposure to wildlife species, domestic ruminants, and other domestic animals (Chapter 2). Similarly, the analysis of the online KAP survey (Chapter 3) also did not identify any significant associations between self-reported medically diagnosed Q fever and direct contact with macropods or other wildlife species (Chapter 3). However, it was identified that AWR may be at increased risk of contracting Q fever via exposure to traditional infection sources including domestic ruminants at their property of residence (Chapter 3). Veterinary clinics were also flagged as a source of infection for unvaccinated AWR, and although occupation was not recorded in the online KAP survey, these AWR may be employed at veterinary clinics in support roles or as veterinary nurses and are not aware of, or do not have access to, vaccination. Indeed, it was identified in Chapter 2 that the majority of AWR working as veterinary nurses were not vaccinated which aligned with another study on Australian veterinary personnel (Sellens et al., 2016) whereby only 29% of veterinary nurses had been vaccinated. This underscores the need for veterinary clinics to exercise their legal responsibility to do whatever is 'reasonably practicable' to ensure the health and safety of all workers (New South Wales Government, 2011). This involves educating all employees, including support staff and AWR associated with veterinary clinics, about the risks and potential health consequences of Q fever and recommending QFV.

## 7.4 *Coxiella burnetii* in Australian native wildlife

### 7.4.1 *Coxiella burnetii* and Australian native wildlife

In Chapter 5 an optimised and validated multiplex qPCR assay (CoxMP) was used to screen a range of sample types collected from a variety of wildlife species for the presence of *C. burnetii* DNA. The samples from the two positive animals (a cloacal swab from a kangaroo and a UGT swab from a koala) were amplified at relatively low concentrations of approximately 10 GE per reaction. The low levels of

DNA detected in these samples, coupled with the low overall *C. burnetii* DNA prevalence in the animals examined, suggests that Australian native wildlife may not be a major source of *C. burnetii* for humans. These findings align with those from Chapter 2 and 3 where no association between exposure to wildlife species and *C. burnetii* seropositivity or Q fever was demonstrated. As discussed in section 1.3.1, most Q fever outbreaks in Australia are associated with domestic ruminants, which further supports the notion that Australian native wildlife are not a major source of infection. However, given the low infectious dose, aerosol transmission route and environmental persistence of *C. burnetii* these low concentrations may still pose a risk of infection to people, on occasion, especially those in close and regular contact with wildlife, confirming the need for QFV in AWR. Given that the urogenital tract of the koala was identified as a potential shedding route for *C. burnetii*, rehabilitators caring for koalas with chlamydial infections may be at increased risk of infection via exposure to *C. burnetii* contaminated urogenital secretions.

Only one male Eastern grey kangaroo was classified as positive in this study. Other Australian studies on macropods have reported *C. burnetii* DNA prevalence of up to 12% in faecal samples obtained from Western grey kangaroos (Banazis et al., 2010; Potter et al., 2011) however the authors of these studies acknowledge that it was not possible to distinguish between the passive passage of *C. burnetii* ingested from a contaminated environment (the kangaroos were co-grazing with ruminants), and active shedding of the bacterium from the gastrointestinal tract. Inhalation of infectious aerosols generated by mowing lawns heavily contaminated with kangaroo faeces has been considered a possible source of infection in human Q fever cases, however PCR did not detect *C. burnetii* DNA in any of the samples obtained from these local environments where these infections occurred (Flint et al., 2016). Nonetheless, even if the presence of *C. burnetii* in the faeces represents passive passage through the GIT, it is still a source of infection for those mowing heavy faecally-contaminated areas, therefore, people exposed to these situations should be vaccinated. Detection of *C. burnetii* DNA in

whole blood of Eastern grey kangaroos and other macropods has also been described (Cooper et al., 2013), however this could merely reflect transient bacteraemia in the animals during the early stages of infection. Seroprevalence studies in macropods indicate that they are susceptible to *C. burnetii* infection (Banazis et al., 2010; Cooper, Barnes, et al., 2012; Potter et al., 2011). A recent study on raw meat sold for pet consumption containing kangaroo reported that 29% of the packets tested positive for *C. burnetii*, with DNA most commonly detected in offal samples (Shapiro et al., 2020). While this finding suggests the existence of a sylvatic cycle in kangaroos and that kangaroos may be capable of amplifying *C. burnetii* in their tissues, the possibility of *C. burnetii* contamination occurring during the processing of the samples could not be ruled out. Nevertheless, the findings of *C. burnetii* in these products supports the notion that kangaroo harvesters are among those who should undergo Q fever vaccination.

### 7.4.2 Shedding routes

A major strength of this study was the sampling of a wide variety of tissue types from each animal to investigate potential shedding routes. It is well established that the birth products of infected ruminants may contain high levels of *C. burnetii*, and therefore exposure to these products represent a significant risk factor for Q fever in humans (Maurin & Raoult, 1999; Welsh et al., 1958). However, the pathobiology of *C. burnetii* in macropods (marsupial mammals) is likely to differ from that of ruminants (placental mammals), due to fundamental differences in reproductive strategies between these species. Although both taxa have placentas, the eutherian placenta is complex and functions to support the growth and development of the embryo/foetus during a prolonged gestation. Whereas in marsupials, the placenta is small, gestation is brief, and the developmentally immature marsupial neonates undergo most of their development externally in their mother's pouch (Abbot & Capra, 2017). Hence, *C. burnetii* may not localise in the reproductive tissue of macropods as it does in ruminants, and consequently, birth products and fluids from kangaroos may pose less of a risk for *C.*

*burnetii* infection in humans. This notion may be supported by the finding in this thesis, that handling joeys was not identified as a risk factor for *C. burnetii* seropositivity or medically diagnosed Q fever, nor was *C. burnetii* identified in the uterine tissue or cloacal swabs of female kangaroos. Banazis et al. (2010) also found no serological evidence of *C. burnetii* exposure in joeys despite seropositivity being demonstrated in the dams. Collectively these findings suggest that kangaroos may not shed *C. burnetii* in their birth products or fluids and exposure to joeys may have not been responsible for a case of human Q fever where the patient indicated handling joeys (Flint et al., 2016).

A better understanding of *C. burnetii* pathogenesis in macropods could be achieved by performing *C. burnetii* challenge experiments. Animals with no evidence of prior *C. burnetii* exposure could be identified using ELISA or IFA (bearing in mind that many animals and humans do not seroconvert or retain antibodies in their blood for prolonged periods following infection). Ideally, animals would be inoculated with *C. burnetii* intranasally and orally to investigate the efficiencies of these two potential natural infection routes. Following inoculation, animals would be systematically monitored for disease symptoms (e.g. fever) and temporal antibody response to infection monitored. The investigation of *C. burnetii* dissemination and excretion routes would be investigated by collecting tissues and excretions from infected animals at regular intervals. The presence of *C. burnetii* in tissues and excretions could be confirmed by immunohistochemical staining and PCR. Studies such as these would, however, be very costly as they require Animal Biosafety Level 3 containment facilities and would be subject to ethical considerations and are, therefore, very unlikely to be performed.

## 7.5 Gene targets and qPCR assay specificity

Another of the strengths of this study was the multiplex PCR (CoxMP) used to detect *C. burnetii* DNA in the samples. The CoxMP was developed in recognition of the limitations associated with the PCR methodology and assay specificity, identified in other studies investigating *C. burnetii* DNA prevalence in Australian native wildlife (Table 1.4). The identified limitations included the widespread use of the

multicopy insertion sequence IS*1111* for the detection of *C. burnetii*, and the classification of samples as positive, based on the amplification of this gene target alone. IS*1111* has been found in CLE therefore studies employing this detection strategy could have misidentified these bacteria as *C. burnetii*. Furthermore, in many studies, assay validation was not reported, and the limit of detection was not disclosed. Moreover, it is concerning that one study classified samples as positive based on an IS*1111* amplification of 50 cycles, given that, in the current study using the CoxMP, amplification of IS*1111* was not detected beyond ~34 cycles (corresponding to 11 GE per reaction).

Thus, there is a resounding need for the standardisation of molecular techniques and greater transparency in the reporting of molecular methodologies in studies investigating Australian native wildlife species as potential reservoirs of *C. burnetii*. It is recommended that when developing and reporting findings, researchers adhere to the MIQE guidelines to facilitate the interpretation of qPCR results within and between research groups. (Bustin et al., 2009). An objective of further research could encompass the molecular detection and characterisation of *Coxiella* spp. in the microbiome of ticks that infest Australian native wildlife. This may enable the development of qPCR assays with high specificity that will distinguish CLE from *C. burnetii*, therefore avoiding the misidentification of CLE as *C. burnetii*.

## 7.6 Closing comments

In 2015, the Australian federal government added 'wildlife and zoo workers who work with high-risk animals to the list of occupational groups who are at increased risk of contracting Q fever and for whom QFV is recommended. This addition was based on the increasing number of Q fever notifications reporting macropod exposure (kangaroos and wallabies), and studies from the peer-reviewed literature reporting evidence of *C. burnetii* exposure and infection in macropods, with some suggesting that they are reservoir species. Although AWR were declared an at-risk group, little was still known regarding the risk of *C. burnetii* exposure in AWR. Nor was it known if the wildlife

rehabilitator community in Australia were sufficiently aware of Q fever, and had knowledge of the availability of the Q fever vaccine, which remains the most effective means of preventing Q fever in humans. Increasing AWR knowledge about Q fever, through educational interventions and subsidised vaccination programs, is required to raise awareness of the potential short and long term health consequences of Q fever and other zoonotic diseases. Increased awareness of zoonoses, and the adoption of appropriate biosecurity practices, by the global wildlife rehabilitator community, will enable them to better protect themselves, and the greater community from known and unknown zoonotic pathogens.

.

# References


Abbot, P., & Capra, J. A. (2017). What is a placental mammal anyway? *eLife*, 6, e30994.https://doi.org/10.7554/eLife.30994

Abdad, M. Y., Abdallah, R. A., Karkouri, K. E., Beye, M., Stenos, J., Owen, H., Unsworth, N., Robertson, I., Blacksell, S. D., Nguyen, T.-T., Nappez, C., Raoult, D., Fenwick, S., & Fournier, P.-E. (2017). *Rickettsia gravesii* sp. nov.: A novel Spotted Fever Group rickettsia in Western Australian *Amblyomma triguttatum triguttatum* ticks. *International Journal of Systematic and Evolutionary Microbiology*, 67(9), 3156-3161.https://doi.org/10.1099/ijsem.0.001865

Abdad, M. Y., Cook, A., Dyer, J., Stenos, J., & Fenwick, S. G. (2014). Seroepidemiological study of outdoor recreationists' exposure to Spotted Fever Group rickettsia in Western Australia. *American Journal of Tropical Medicine and Hygiene*, 91(3), 584-588.https://doi.org/https://doi.org/10.4269/ajtmh.14-0102

Abdel-Moein, K. A., & Zaher, H. M. (2021). Parturient cat as a potential reservoir for *Coxiella burnetii*: A hidden threat to pet owners. *Vector Borne and Zoonotic Diseases*, 21(4), 264-268.https://doi.org/10.1089/vbz.2020.2714

Abinanti, F. R., Welsh, H. H., Winn, J. F., & Lennette, E. H. (1955). Q fever studies. XIX. Presence and epidemiologic significance of *Coxiella burnetii* in sheep wool. *American Journal of Hygiene*, 61(3), 362-370.https://doi.org/10.1093/oxfordjournals.aje.a119760

Ackland, J. R., Worswick, D. A., & Marmion, B. P. (1994). Vaccine prophylaxis of Q fever. a follow-up study of the efficacy of Q-Vax (CSL) 1985-1990. *Medical Journal of Australia*, 160(11), 704-708.https://doi.org/10.5694/j.1326-5377.1994.tb125909.x

Agerholm, J. S. (2013). *Coxiella burnetii* associated reproductive disorders in domestic animals-a critical review. *Acta Veterinaria Scandinavica*, 55(1), 13.https://doi.org/10.1186/1751-0147-55-13

Akman, L., Yamashita, A., Watanabe, H., Oshima, K., Shiba, T., Hattori, M., & Aksoy, S. (2002). Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nature Genetics*, 32(3), 402-407.https://doi.org/10.1038/ng986

Akter, R., Legione, A., Sansom, F. M., El-Hage, C. M., Hartley, C. A., Gilkerson, J. R., & Devlin, J. M. (2020). Detection of *Coxiella burnetii* and equine herpesvirus 1, but not *Leptospira* spp. or *Toxoplasma gondii*, in cases of equine abortion in Australia - a 25 year retrospective study. *PloS One*, 15(5), e0233100.https://doi.org/10.1371/journal.pone.0233100

Amit, S., Shinar, S., Halutz, O., Atiya-Nasagi, Y., & Giladi, M. (2014). Suspected person-to-person transmission of Q fever among hospitalized pregnant women. *Clinical Infectious Diseases*, 58(11), e146-e147.https://doi.org/10.1093/cid/ciu151

Anderson, A. D., Bijlmer, H., Fournier, P.-E., Graves, S., Hartzell, J., Kersh, G. J., Limonard, G., Marrie, T. J., Massung, R. F., & McQuiston, J. H. (2013). Diagnosis and management of Q fever—United States, 2013: Recommendations from CDC and the Q Fever Working Group. *Morbidity and Mortality Weekly Report: Recommendations and Reports*, 62(3), 1-29.https://www.cdc.gov/mmwr/preview/mmwrhtml/rr6203a1.htm

Angelakis, E., Mediannikov, O., Jos, S.-L., Berenger, J.-M., Parola, P., & Raoult, D. (2016). *Candidatus Coxiella* massiliensis infection. *Emerging Infectious Diseases*, 22(2), 285-288.https://doi.org/10.3201/eid2202.150106

Angelakis, E., Million, M., D'Amato, F., Rouli, L., Richet, H., Stein, A., Rolain, J. M., & Raoult, D. (2013). Q fever and pregnancy: Disease, prevention, and strain specificity. *European Journal of Clinical Microbiology & Infectious Diseases* 32(3), 361-368.https://doi.org/10.1007/s10096-012-1750-3

Angelakis, E., & Raoult, D. (2010). Q fever. *Veterinary Microbiology*, 140(3), 297-309.https://doi.org/10.1016/j.vetmic.2009.07.016

Arashima, Y., Kato, K., Komiya, T., Kumasaka, K., Matsukawa, Y., Murakami, M., Takahashi, K., Ikeda, T., & Arakawa, Y. (2004). Improvement of chronic nonspecific symptoms by long-term minocycline treatment in Japanese patients with *Coxiella burnetii* infection considered to have post-Q fever fatigue syndrome. *Internal Medicine*, 43(1), 49-54.

Archer, B. N., Hallahan, C., Stanley, P., Seward, K., Lesjak, M., Hope, K., & Brown, A. (2017). Atypical outbreak of Q fever affecting low-risk residents of a remote rural town in New South Wales. *Communicable Diseases Intelligence*, 41(2), E125-E133.http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi4102a.htm

Archibald, J. (2019). Disease in the dust: Experiences of Q fever during drought in Australia. *Perspectives in Public Health*, 139(2), 77-78.https://doi.org/10.1177/1757913918823423

Arricau-Bouvery, N., & Rodolakis, A. (2005). Is Q fever an emerging or re-emerging zoonosis? *Veterinary Research*, 36(3), 327-349.https://doi.org/10.1051/vetres:2005010

Arricau Bouvery, N., Souriau, A., Lechopier, P., & Rodolakis, A. (2003). Experimental *Coxiella burnetii* infection in pregnant goats: Excretion routes. *Veterinary Research*, 34(4), 423-433.https://doi.org/10.1051/vetres:2003017

Australian Bureau of Statistics. (2016). *Australian Demographic Statistics, Jun 2016*

Retrieved Accessed March 2017 from https://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/3101.0Jun%202016

Australian Bureau of Statistics. (2016, July 2016). *Australian Statistical Geography Standard (ASGS) Remoteness Structure*. Retrieved Accessed February 2020 from http://www.abs.gov.au/AUSSTATS/abs@.nsf/Lookup/1270.0.55.005Main+Features1July%202016?OpenDocument

Australian Bureau of Statistics. (2018). *Australian Demographic Statistics, Jun 2018*. Retrieved August 2019 from https://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/3101.0Jun%202018?OpenDocument

Australian College of Rural and Remote Medicine. (2018). *Q Fever - Early diagnosis and vaccination*. Retrieved January 20, 2022 from https://mycollege.acrrm.org.au/search/find-online-learning/details?id=11347&title=Q-Fever+-+Early+Diagnosis+and+Vaccination

Australian Red Cross Blood Service. (2019, June 2019). *Blood donation*. Retrieved Accessed February, 2020 from https://www.betterhealth.vic.gov.au/health/conditionsandtreatments/blood-donation

Australian Technical Advisory Group on Immunisation. (2021). The Australian Immunisation Handbook, . (March 4, 2022).https://immunisationhandbook.health.gov.au/vaccine-preventable-diseases/q-fever

Australian Veterinary Association. (2017). *Guidelines for veterinary personal biosecurity*. Retrieved July 31, 2018 from https://www.ava.com.au/library-resources/other-resources/veterinary-personal-biosecurity/

Azad, A. F., & Beard, C. B. (1998). Rickettsial pathogens and their arthropod vectors. *Emerging Infectious Diseases*, 4(2), 179-186.https://doi.org/10.3201/eid0402.980205

Baca, O. G., & Paretsky, D. (1983). Q fever and *Coxiella burnetii*: A model for host-parasite interactions. *Microbiological Reviews*, 47(2), 127-149.https://doi.org/10.1128/mr.47.2.127-149.1983

Bae, M., Jin, C. E., Park, J. H., Kim, M. J., Chong, Y. P., Lee, S.-O., Choi, S.-H., Kim, Y. S., Woo, J. H., Shin, Y., & Kim, S.-H. (2019). Diagnostic usefulness of molecular detection of *Coxiella burnetii* from blood of patients with suspected acute Q fever. *Medicine*, 98(23), e15724.https://doi.org/10.1097/md.0000000000015724

Baird, R. W., Lloyd, M., Stenos, J., Ross, B. C., Stewart, R. S., & Dwyer, B. (1992). Characterization and comparison of Australian human Spotted Fever Group rickettsiae. *Journal of Clinical Microbiology*, 30(11), 2896-2902.https://doi.org/10.1128/jcm.30.11.2896-2902.1992

Banazis, M. J., Bestall, A. S., Reid, S. A., & Fenwick, S. G. (2010). A survey of Western Australian sheep, cattle and kangaroos to determine the prevalence of *Coxiella burnetii*. *Veterinary Microbiology*, 143(2), 337-345.https://doi.org/10.1016/j.vetmic.2009.12.002
Barker, S. C., & Walker, A. R. (2014). Ticks of Australia. The species that infest domestic animals and humans. *Zootaxa*, (3816), 1-144.https://doi.org/10.11646/zootaxa.3816.1.1
Barrs, V. R., Beatty, J. A., Wilson, B. J., Evans, N., Gowan, R., Baral, R. M., Lingard, A. E., Perkovic, G., Hawley, J. R., & Lappin, M. R. (2010). Prevalence of *Bartonella* species, *Rickettsia felis*, haemoplasmas and the *Ehrlichia* group in the blood of cats and fleas in eastern Australia. *Australian Veterinary Journal*, 88(5), 160-165.https://doi.org/10.1111/j.1751-0813.2010.00569.x
Beaman, M. H., & Hung, J. (1989). Pericarditis associated with tick-borne Q fever. *Australian and New Zealand Journal of Medicine*, 19(3), 254-256.https://doi.org/10.1111/j.1445-5994.1989.tb00258.x
Beaman, M. H., & Marinovitch, N. (1999). Murine typhus in metropolitan Perth. *Medical Journal of Australia*, 170(2), 93-94.https://doi.org/10.5694/j.1326-5377.1999.tb126896.x
Beare, P. A., Unsworth, N., Andoh, M., Voth, D. E., Omsland, A., Gilk, S. D., Williams, K. P., Sobral, B. W., Kupko, J. J., 3rd, Porcella, S. F., Samuel, J. E., & Heinzen, R. A. (2009). Comparative genomics reveal extensive transposon-mediated genomic plasticity and diversity among potential effector proteins within the genus Coxiella. *Infection and Immunology*, 77(2), 642-656.https://doi.org/10.1128/iai.01141-08
Beech, M. D., Duxbury, A. E., & Warner, P. (1962). Q fever in South Australia: An outbreak in a meat-works. *Journal of Hygiene*, 60(1), 1-20.https://doi.org/10.1017/S0022172400039255
Bennett, M. D., Woolford, L., Banazis, M. J., O'Hara, A. J., Warren, K. S., Nicholls, P. K., Sims, C., & Fenwick, S. G. (2011). *Coxiella burnetii* in western barred bandicoots (*Perameles bougainville*) from Bernier and Dorre Islands in Western Australia. *EcoHealth*, 8(4), 519-524.https://doi.org/10.1007/s10393-011-0729-3
Benson, W. W., Brock, D. W., & Mather, J. (1963). Serologic analysis of a penitentiary group using raw milk from a Q fever infected herd. *Public Health Reports*, 78(8), 707-710.
Berón, W., Gutierrez, M. G., Rabinovitch, M., & Colombo, M. I. (2002). *Coxiella burnetii* localizes in a Rab7-Labeled Compartment with Autophagic Characteristics. 70(10), 5816-5821.https://doi.org/10.1128/IAI.70.10.5816-5821.2002 %J Infection and Immunity
Berri, M., Laroucau, K., & Rodolakis, A. (2000). The detection of *Coxiella burnetii* from ovine genital swabs, milk and fecal samples by the use of a single touchdown polymerase chain reaction. *Veterinary Microbiology*, 72(3-4), 285-293.https://doi.org/10.1016/s0378-1135(99)00178-9
Berri, M., Rousset, E., Champion, J. L., Arricau-Bouvery, N., Russo, P., Pepin, M., & Rodolakis, A. (2003). Ovine manure used as a garden fertiliser as a suspected source of human Q fever. *The Veterinary Record*, 153(9), 269.https://doi.org/https://doi.org/10.1136/vr.153.9.269
Betsch, C., Böhm, R., & Chapman, G. B. (2015). Using behavioral insights to increase vaccination policy effectiveness. *Policy Insights from the Behavioral and Brain Sciences*, 2(1), 61-73.https://doi.org/10.1177/2372732215600716
Betsch, C., Schmid, P., Heinemeier, D., Korn, L., Holtmann, C., & Böhm, R. (2018). Beyond confidence: Development of a measure assessing the 5C psychological antecedents of vaccination. *PloS One*, 13(12), e0208601.https://doi.org/10.1371/journal.pone.0208601
Biggs, H. M., Behravesh, C. B., Bradley, K. K., Dahlgren, F. S., Drexler, N. A., Dumler, J. S., Folk, S. M., Kato, C. Y., Lash, R. R., Levin, M. L., Massung, R. F., Nadelman, R. B., Nicholson, W. L., Paddock, C. D., Pritt, B. S., & Traeger, M. S. (2006). Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever and other Spotted Fever Group rickettsioses, ehrlichioses, and anaplasmosis. *Morbidity and Mortality Weekly Report*, 55(2), 1.https://doi.org/10.15585/mmwr.rr6502a1
Blanton, L. S. (2019). The Rickettsioses: A practical update. *Infectious Disease Clinics of North America*, 33(1), 213-229.https://doi.org/10.1016/j.idc.2018.10.010

Bond, K. A., Franklin, L. J., Sutton, B., & Firestone, S. M. (2017). Q-Vax Q fever vaccine failures, Victoria, Australia 1994–2013. *Vaccine*, 35(51), 7084-7087.https://doi.org/10.1016/j.vaccine.2017.10.088

Bond, K. A., Vincent, G., Wilks, C. R., Franklin, L., Sutton, B., Stenos, J., Cowan, R., Lim, K., Athan, E., Harris, O., Macfarlane-Berry, L., Segal, Y., & Firestone, S. M. (2016). One Health approach to controlling a Q fever outbreak on an Australian goat farm. *Epidemiology and Infection*, 144(6), 1129-1141.https://doi.org/10.1017/s0950268815002368

Boni, M., Davoust, B., Tissot-Dupont, H., & Raoult, D. (1998). Survey of seroprevalence of Q fever in dogs in the southeast of France, French Guyana, Martinique, Senegal and the Ivory Coast. *Veterinary Microbiology*, 64(1), 1-5.https://doi.org/https://doi.org/10.1016/S0378-1135(98)00247-8

Bosch, S. A., Musgrave, K., & Wong, D. (2013). Zoonotic disease risk and prevention practices among biologists and other wildlife workers: Results from a national survey, US National Park Service, 2009. *Journal of Wildlife Diseases*, 49(3), 475-585.https://doi.org/10.7589/2012-06-173

Brenner, A. E., Muñoz-Leal, S., Sachan, M., Labruna, M. B., & Raghavan, R. (2021). *Coxiella burnetii* and related tick endosymbionts evolved from pathogenic ancestors. *Genome Biology and Evolution*, 13(7).https://doi.org/10.1093/gbe/evab108

Brewer, N. T., Chapman, G. B., Rothman, A. J., Leask, J., & Kempe, A. (2017). Increasing vaccination: Putting psychological science Into action. *Psychological Science in the Public Interest*, 18(3), 149-207.https://doi.org/10.1177/1529100618760521

Broackes-Carter, F. C., Mouchel, N., Gill, D., Hyde, S., Bassett, J., & Harris, A. (2002). Temporal regulation of CFTR expression during ovine lung development: Implications for CF gene therapy. *Human Molecular Genetics*, 11(2), 125-131.https://doi.org/10.1093/hmg/11.2.125

Brooke, R. J., Kretzschmar, M. E., Mutters, N. T., & Teunis, P. F. (2013). Human dose response relation for airborne exposure to Coxiella burnetii. *BMC Infectious Diseases*, 13, 488.https://doi.org/10.1186/1471-2334-13-488

Brouqui, P., Dupont, H. T., Drancourt, M., Berland, Y., Etienne, J., Leport, C., Goldstein, F., Massip, P., Micoud, M., Bertrand, A., & et al. (1993). Chronic Q fever. Ninety-two cases from France, including 27 cases without endocarditis. *Archives of Internal Medicine*, 153(5), 642-648.https://doi.org/10.1001/archinte.153.5.642

Buckley, B. (1980). Q fever epidemic in Victorian general practice. *Medical Journal of Australia*, 1(12), 593-595.https://doi.org/10.5694/j.1326-5377.1980.tb135159.x

Buhariwalla, F., Cann, B., & Marrie, T. (1996). A dog-related outbreak of Q fever. *Clinical Infectious Diseases*, 23(4), 753-755.

Burke, C., Burnard, D., Polkinghorne, A., Webb, J., & Huston, W. M. (2018). Cloacal and ocular microbiota of the endangered Australian Northern Quoll. *Microorganisms*, 6(3), 68.https://doi.org/10.3390/microorganisms6030068

Burnet, F. M., & Freeman, M. (1937). Experimental studies on the virus of "Q" Fever. *Medical Journal of Australia*, (2), 299-305.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, 55(4), 611-622.https://doi.org/10.1373/clinchem.2008.112797

Cairns, K., Brewer, M., & Lappin, M. R. (2007). Prevalence of *Coxiella burnetii* DNA in vaginal and uterine samples from healthy cats of north-central Colorado. *Journal of Feline Medicine and Surgery*, 9(3), 196-201.https://doi.org/10.1016/j.jfms.2006.11.006

Calia, F. M., Bartelloni, P. J., & McKinney, R. W. (1970). Rocky Mountain spotted fever: Laboratory infection in a vaccinated individual. *Journal of the American Medical Association*, 211(12), 2012-2014.https://doi.org/10.1001/jama.1970.03170120056014

Campagna, S., Lévesque, B., Anassour-Laouan-Sidi, E., Côté, S., Serhir, B., Ward, B. J., Libman, M. D., Drebot, M. A., Makowski, K., Andonova, M., Ndao, M., & Dewailly, É. (2011). Seroprevalence of

10 zoonotic infections in 2 Canadian Cree communities. *Diagnostic Microbiology and Infectious Disease*, 70(2), 191-199.https://doi.org/10.1016/j.diagmicrobio.2011.01.009
Capo, C., Lindberg, F. P., Meconi, S., Zaffran, Y., Tardei, G., Brown, E. J., Raoult, D., & Mege, J. L. (1999). Subversion of monocyte functions by *Coxiella burnetii*: Impairment of the cross-talk between α(v)β3 integrin and CR3. *Journal of Immunology*, 163(11), 6078-6085.https://www.scopus.com/inward/record.uri?eid=2-s2.0-0033485430&partnerID=40&md5=ad72770ab6bb7ed7278be72737b0cec6
Carcopino, X., Raoult, D., Bretelle, F., Boubli, L., & Stein, A. (2007). Managing Q fever during pregnancy: The benefits of long-term cotrimoxazole therapy. *Clinical Infectious Diseases* 45(5), 548-555.https://doi.org/10.1086/520661
Carcopino, X., Raoult, D., Bretelle, F., Boubli, L., & Stein, A. (2009). Q Fever during pregnancy: a cause of poor fetal and maternal outcome. *Annals of the New York Academy of Sciences*, 1166, 79-89.https://doi.org/10.1111/j.1749-6632.2009.04519.x
Carpiano, R. M., Polonijo, A. N., Gilbert, N., Cantin, L., & Dubé, E. (2019). Socioeconomic status differences in parental immunization attitudes and child immunization in Canada: findings from the 2013 Childhood National Immunization Coverage Survey (CNICS). *Preventive Medicine*, 123, 278-287.https://doi.org/10.1016/j.ypmed.2019.03.033
Centres for Disease Control and Prevention. (2013). Diagnosis and management of Q fever - United States, 2013: Recommendations from CDC and the Q Fever working group. 62 (RR03), 1-23.https://www.cdc.gov/mmwr/preview/mmwrhtml/rr6203a1.htm?s_cid=rr6203a1_w
Centres for Disease Control and Prevention. (2021). *Epidemiology and statistics - Q fever in the United States*. Retrieved 13 March from http://www.cdc.gov/qfever/stats/index.html
Centres of Disease Control and Prevention. (2021). Retrieved 29 December, 2021 from https://emergency.cdc.gov/agent/agentlist.asp
Cerf, O., & Condron, R. (2006). *Coxiella burnetii* and milk pasteurization: an early application of the precautionary principle? *Epidemiology and Infection*, 134(5), 946-951.https://doi.org/10.1017/s0950268806005978
Chiu, C., Dey, A., Wang, H., Menzies, R., Deeks, S., Mahajan, D., Macartney, K., Brotherton, J., Jardine, A., Quinn, H., Jelfs, J., Booy, R., Lawrence, G., Jayasinghe, S., Roberts-Witteveen, A., Senanayake, S., Wood, N., & McIntyre, P. (2010). Vaccine preventable diseases in Australia, 2005 to 2007. *Communicable Diseases Intelligence Quarterly Report*, 34 Supp, S1-167.
Chiu, C. K., & Durrheim, D. N. (2007). A review of the efficacy of human Q fever vaccine registered in Australia. *New South Wales Public Health Bulletin*, 18(7-8), 133-136.https://doi.org/10.1071/nb07057
Chong, A. K. H., La Brooy, J., Norton, R., & Masson, J. (2003). Q fever: a recent 'outbreak' in Townsville. *Internal Medicine Journal*, 33(4), 208.https://doi.org/10.1046/j.1445-5994.2003.00335.x
Chou, C.-H., Yeh, T.-M., Lu, Y.-P., Shih, W.-L., Chang, C.-D., Chien, C.-H., Liu, S.-S., Wu, H.-Y., Tsai, F.-J., & Huang, H. H. (2014). Prevalence of zoonotic pathogens by molecular detection in stray dogs in Central Taiwan. *The Thai Journal of Veterinary Medicine*, 44(3), 363.
Clark, N. J., Tozer, S., Wood, C., Firestone, S. M., Stevenson, M., Caraguel, C., Chaber, A. L., Heller, J., & Soares Magalhães, R. J. (2020). Unravelling animal exposure profiles of human Q fever cases in Queensland, Australia, using natural language processing. *Ticks and Tick-Borne Diseases*.https://doi.org/10.1111/tbed.13565
Clutterbuck, H. C., Eastwood, K., Massey, P. D., Hope, K., & Mor, S. M. (2018). Surveillance system enhancements for Q fever in NSW, 2005-2015. *Communicable Diseases Intelligence* 42.https://pubmed.ncbi.nlm.nih.gov/30626297/
Clutterbuck, H. C., Eastwood, K., Massey, P. D., Hope, K., & Mor, S. M. (2018). Surveillance system enhancements for Q fever in NSW, 2005-2015. *Commun Dis Intell (2018)*, 42.
Coleman, S. A., Fischer, E. R., Howe, D., Mead, D. J., & Heinzen, R. A. (2004). Temporal analysis of Coxiella burnetii morphological differentiation. *Journal of Bacteriology*, 186(21), 7344-7352.https://doi.org/10.1128/JB.186.21.7344-7352.2004

Communicable Diseases Network Australia. (2018). *Q fever National Guidelines for Public Health Units.* Retrieved May, 2022 from https://www1.health.gov.au/internet/main/publishing.nsf/Content/cdna-song-q-fever.htm

Cooper, A. (2011). *Identification of potential reservoirs of Q fever in Queensland* James Cook University]. https://researchonline.jcu.edu.au/27726/1/27726-cooper-2011-thesis.pdf

Cooper, A., Barnes, T., Potter, A., Ketheesan, N., & Govan, B. (2012). Determination of Coxiella burnetii seroprevalence in macropods in Australia. *Veterinary Microbiology*, 155(2), 317-323.https://doi.org/10.1016/j.vetmic.2011.08.023

Cooper, A., Goullet, M., Mitchell, J., Ketheesan, N., & Govan, B. (2012). Serological evidence of *Coxiella burnetii* exposure in native marsupials and introduced animals in Queensland, Australia. *Epidemiology and Infection*, 140(7), 1304-1308.https://doi.org/10.1017/S0950268811001828

Cooper, A., Hedlefs, R., Ketheesan, N., & Govan, B. (2011). Serological evidence of *Coxiella burnetii* infection in dogs in a regional centre. *Australian Veterinary Journal*, 89(10), 385-387.https://doi.org/10.1111/j.1751-0813.2011.00819.x

Cooper, A., Stephens, J., Ketheesan, N., & Govan, B. (2013). Detection of *Coxiella burnetii* DNA in wildlife and ticks in Northern Queensland, Australia. *Vector Borne and Zoonotic Diseases*, 13(1), 12-16.https://doi.org/10.1089/vbz.2011.0853

Cox, H. R. (1938). A Filter-Passing Infectious Agent Isolated from Ticks. III Description of organism and cultivation experiments. *Public Health Reports (1896-1970)*, 53(52), 2270-2276.https://doi.org/10.2307/4582746

Cox, H. R., & Bell, E. J. (1939). The cultivation of *Rickettsia diaporica* in tissue culture and in the tissues of developing chick embryos. *Public Health Reports*, 54(49), 2171-2178.https://doi.org/Doi 10.2307/4583112

Cyr, J., Turcotte, M. È., Desrosiers, A., Bélanger, D., Harel, J., Tremblay, D., Leboeuf, A., Gagnon, C. A., Côté, J. C., & Arsenault, J. (2021). Prevalence of *Coxiella burnetii* seropositivity and shedding in farm, pet and feral cats and associated risk factors in farm cats in Quebec, Canada. *Epidemiology and Infection*, 149, e57-e57.https://doi.org/10.1017/S0950268821000364

Dal Pozzo, F., Martinelle, L., Leonard, P., Renaville, B., Renaville, R., Thys, C., Smeets, F., Czaplicki, G., Van Esbroeck, M., & Saegerman, C. (2017). Q Fever serological survey and associated risk factors in veterinarians, Southern Belgium, 2013. *Transboundary and Emerging Diseases*, 64(3), 959-966.https://doi.org/10.1111/tbed.12465

Davis, G. E., Cox, H. R., Parker, R. R., & Dyer, R. E. (1938). A filter-passing infectious agent isolated from tcks. Isolation from *Dermcentor andersoni,* reactions in animals and filtration experiments. *Public Health Reports (1896-1970)*, 53(52), 2259-2267.https://doi.org/10.2307/4582746

de Bruin, A., de Groot, A., de Heer, L., Bok, J., Wielinga, P. R., Hamans, M., van Rotterdam, B. J., & Janse, I. (2011). Detection of *Coxiella burnetii* in complex matrices by using multiplexquantitative PCR during a major Q fever outbreak in The Netherlands. *Applied and Environmental Microbiology*, 77(18), 6516-6523.https://doi.org/10.1128/AEM.05097-11

de la Fuente, J., Antunes, S., Bonnet, S., Cabezas-Cruz, A., Domingos, A. G., Estrada-Peña, A., Johnson, N., Kocan, K. M., Mansfield, K. L., Nijhof, A. M., Papa, A., Rudenko, N., Villar, M., Alberdi, P., Torina, A., Ayllón, N., Vancova, M., Golovchenko, M., Grubhoffer, L., Caracappa, S., Fooks, A. R., Gortazar, C., & Rego, R. O. M. (2017). Tick-pathogen interactions and vector competence: Identification of molecular drivers for tick-borne diseases. *Frontiers in Cellular and Infection Microbiology*, 7, 114.https://doi.org/10.3389/fcimb.2017.00114

de Ronde, M. W. J., Ruijter, J. M., Lanfear, D., Bayes-Genis, A., Kok, M. G. M., Creemers, E. E., Pinto, Y. M., & Pinto-Sietsma, S. J. (2017). Practical data handling pipeline improves performance of qPCR-based circulating miRNA measurements. *RNA*, 23(5), 811-821.https://doi.org/10.1261/rna.059063.116

Delisle, J., Mendell, N. L., Stull-Lane, A., Bloch, K. C., Bouyer, D. H., & Moncayo, A. C. (2016). Human infections by multiple spotted fever group rickettsiae in Tennessee. *American Journal of*

*Tropical Medicine and Hygiene*, 94(6), 1212-1217.https://doi.org/https://doi.org/10.4269/ajtmh.15-0372
Department of Environment and Science. (2017-2018). *St Helena Island National Park*. Queensland Government. Retrieved 6th November from https://www.npsr.qld.gov.au/parks/st-helena-island/culture.html
Derrick, E. H. (1937). "Q" Fever, a new fever entity: Clinical features, diagnosis and laboratory investigation. *Medical Journal of Australia*, 2(8), 281-299.https://doi.org/10.5694/j.1326-5377.1937.tb43743.x
Derrick, E. H. (1939). *Rickettsia burneti*: the cause of "Q " fever. *Medical Journal of Australia*, 1.
Derrick, E. H., Smith, D. J. W., Brown, H. E., & Freeman, M. (1939). The role of the bandicoot in the epidemiology of "Q" fever: a preliminary study. *Medical Journal of Australia*, 1(4), 150-155.https://doi.org/10.5694/j.1326-5377.1939.tb98484.x
Desjardins, I., Joulie, A., Pradier, S., Lecollinet, S., Beck, C., Vial, L., Dufour, P., Gasqui, P., Legrand, L., Edouard, S., Sidi-Boumedine, K., Rousset, E., Jourdain, E., & Leblond, A. (2018). Seroprevalence of horses to *Coxiella burnetii* in an Q fever endemic area. *Veterinary Microbiology*, 215, 49-56.https://doi.org/10.1016/j.vetmic.2017.11.012
Deutsch, D. L., & Peterson, E. T. (1950). Q fever: Transmission from one human being to others. *Journal of the American Medical Association*, 143(4), 348-350.https://doi.org/10.1001/jama.1950.02910390020006
Dhand, N. K., & Khatkar, M. S. (2014). *Statulator: An online statistical calculator. Sample size calculator for estimating a single proportion.* Retrieved May 7, 2019 from http://statulator.com/SampleSize/ss1P.html
Domingo, P., Muñoz, C., Franquet, T., Gurguí, M., Sancho, F., & Vazquez, G. (1999). Acute Q fever in adult patients: Report on 63 sporadic cases in an urban area. *Clinical Infectious Diseases*, 29(4), 874-879.https://doi.org/10.1086/520452
Dowd, K., Taylor, M., Toribio, J. A., Hooker, C., & Dhand, N. K. (2013). Zoonotic disease risk perceptions and infection control practices of Australian veterinarians: Call for change in work culture. *Preventative Veterinary Medicine*, 111(1-2), 17-24.https://doi.org/10.1016/j.prevetmed.2013.04.002
Duncan, C., Kersh, G. J., Spraker, T., Patyk, K. A., Fitzpatrick, K. A., Massung, R. F., & Gelatt, T. (2012). *Coxiella burnetii* in northern fur seal (*Callorhinus ursinus*) placentas from St. Paul Island, Alaska. *Vector Borne Zoonotic Diseases*, 12(3), 192-195.https://doi.org/10.1089/vbz.2011.0715
Dupont, H. T., Thirion, X., & Raoult, D. (1994). Q fever serology: Cutoff determination for microimmunofluorescence. *Clinical and Diagnostic Laboratory Immunology*

1(2), 189-196.https://doi.org/10.1128/cdli
Dupuis, G., Petite, J., Péter, O., & Vouilloz, M. (1987). An important outbreak of human Q fever in a Swiss alpine valley. *International Journal of Epidemiology*, 16(2), 282-287.https://doi.org/10.1093/ije/16.2.282
Duron, O. (2015). The IS*1111* insertion sequence used for detection of *Coxiella burnetii* is widespread in Coxiella-like endosymbionts of ticks. *FEMS Microbiology Letters*, 362(17), fnv132.https://doi.org/10.1093/femsle/fnv132
Duron, O., Noël, V., McCoy, K. D., Bonazzi, M., Sidi-Boumedine, K., Morel, O., Vavre, F., Zenner, L., Jourdain, E., Durand, P., Arnathau, C., Renaud, F., Trape, J.-F., Biguezoton, A. S., Cremaschi, J., Dietrich, M., Léger, E., Appelgren, A., Dupraz, M., Gómez-Díaz, E., Diatta, G., Dayo, G.-K., Adakal, H., Zoungrana, S., Vial, L., & Chevillon, C. (2015). The recent evolution of a maternally-inherited endosymbiont of ticks led to the emergence of the Q fever pathogen, *Coxiella burnetii*. *PLoS Pathogens*, 11(5), e1004892.https://doi.org/10.1371/journal.ppat.1004892
Dwyer, B. W., Graves, S. R., McDonald, M. I., Yung, A. P., Doherty, R. R., & McDonald, J. K. (1991). Spotted fever in East Gippsland, Victoria: A previously unrecognised focus of rickettsial

infection. *Medical Journal of Australia*, 154(2), 121-125.https://doi.org/10.5694/j.1326-5377.1991.tb120998.x
Dyer, J. R., Einsiedel, L., Ferguson, P. E., Lee, A. S., Unsworth, N. B., Graves, S. R., & Gordon, D. L. (2005). A new focus of *Rickettsia honei* spotted fever in South Australia. *Medical Journal of Australia*, 182(5), 231-234.https://onlinelibrary.wiley.com/doi/full/10.5694/j.1326-5377.2005.tb06673.x?sid=nlm%3Apubmed
Dyer, R. E. (1938). A flterpassinginfectious agent isolated from ticks. IV Human iInfection. *Public Health Reports (1896-1970)*, 53(52), 2277-2282.https://doi.org/10.2307/4582746
Eastwood, K., Graves, S. R., Massey, P., Bosward, K., van den Berg, D., & P., H. (2018). Q Fever: a rural disease with potential urban consequences. *Australian Journal for General Practitioners*, 47(3), 5555-5555.https://doi.org/10.31128/AFP-08-17-4299
Ebani, V. V. (2020). Retrospective study on the occurrence of antibodies against *Coxiella burnetii* in dogs from central Italy. *Pathogens*, 9(12), 1068.https://doi.org/10.3390/pathogens9121068
Eklund, C. M., Parker, R. R., & Lackman, D. B. (1947). A case of Q Fever probably contracted by exposure to ticks in nature. *Public Health Reports (1896-1970)*, 62(39), 1413-1416.https://doi.org/10.2307/4586287
Eldin, C., Angelakis, E., Renvoisé, A., & Raoult, D. (2013). *Coxiella burnetii* DNA, but not viable bacteria, in dairy products in France. *American Journal of Tropical Medicine and Hygiene*, 88(4), 765-769.https://doi.org/10.4269/ajtmh.12-0212
Eldin, C., Mahamat, A., Demar, M., Abboud, P., Djossou, F., & Raoult, D. (2014). Q fever in French Guiana. *The American Journal of TropicalMedicine and Hygiene*, 91(4), 771-776.https://doi.org/10.4269/ajtmh.14-0282
Eldin, C., Melenotte, C., Mediannikov, O., Ghigo, E., Million, M., Edouard, S., Mege, J.-L., Maurin, M., & Raoult, D. (2017). From Q Fever to *Coxiella burnetii* infection: a paradigm change. *Clinical Microbiology Reviews*, 30(1), 115-190.https://doi.org/10.1128/cmr.00045-16
Elliman, J., & Owens, L. (2020). Confirmation that *Candidatus* Coxiella cheraxi from redclaw crayfish (*Cherax quadricarinatus*) is a close relative of *Coxiella burnetii*, the agent of Q-fever. *Society for Applied Microbiology*, n/a(n/a).https://doi.org/10.1111/lam.13336
Elsa, J., Duron, O., Séverine, B., González-Acuña, D., & Sidi-Boumedine, K. (2015). Molecular methods routinely used to detect *Coxiella burnetii* in ticks cross-react with *Coxiella*-like bacteria. *Infection Ecology and Epidemiology*, 5, 29230.https://doi.org/10.3402/iee.v5.29230
Englefield, B., Blackman, S. A., Starling, M., & McGreevy, P. D. (2019). A review of Australian animal welfare legislation, regulation, codes of practice, and policy, and their Influence on stakeholders caring for wildlife and the animals for whom they care. *Animals*, 9(6), 335.https://doi.org/10.3390/ani9060335
Englefield, B., Candy, S., Starling, M., & McGreevy, P. (2019). The demography and practice of Australians caring for native wildlife and the psychological, physical and financial effects of rescue, rehabilitation and release of wildlife on the welfare of carers. *Animals* 9(12), 1127.https://doi.org/10.3390/ani9121127
Englefield, B., Starling, M., & McGreevy, P. (2018). A review of roadkill rescue: who cares for the mental, physical and financial welfare of Australian wildlife carers? *Wildlife Research* 45(2), 103.https://doi.org/10.1071/WR17099
Enright, J. B., Sadler, W. W., & Thomas, R. C. (1957). Pasteurization of milk containing the organism of Q fever. *American Journal of Public Health and the Nation's Health*, 47(6), 695-700.https://doi.org/10.2105/ajph.47.6.695
European Centre for Disease Prevention and Control. (2021). *Q fever - annual epidemiological report for 2019*. Retrieved January 3, 2022 from https://www.ecdc.europa.eu/en/publications-data/q-fever-annual-epidemiological-report-2019
Faa, A. G., McBride, W. J., Garstone, G., Thompson, R. E., & Holt, P. (2003). Scrub typhus in the Torres Strait islands of north Queensland, Australia. *Emerging Infectious Diseases*, 9(4), 480-482.https://doi.org/10.3201/eid0904.020509

Fenollar, F., Fournier, P.-E., Carrieri, M. P., Habib, G., Messana, T., & Raoult, D. (2001). Risks factors and prevention of Q Fever endocarditis. *Clinical Infectious Diseases*, 33(3), 312-316.https://doi.org/10.1086/321889

Fiset, P., Wisseman Jr, C. L., & Batawi, Y. E. (1975). Immunologic evidence of human fetal infection with Coxiella burneti. *American Journal of Epidemiology*, 101(1), 65-69.

Flint, J., Dalton, C. B., Merritt, T. D., Graves, S., Ferguson, J. K., Osbourn, M., Eastwood, K., & Durrheim, D. N. (2016). Q fever and contact with kangaroos in New South Wales. *Communicable Diseases Intelligence*, 40(2), 202-203.http://www.health.gov.au/internet/main/publishing.nsf/content/cda-cdi4002b.htm

Fournier, P.-E., Marrie, T. J., & Raoult, D. (1998). Diagnosis of Q Fever. *Journal of Clinical Microbiology*, 36(7), 1823-1834.https://doi.org/doi:10.1128/JCM.36.7.1823-1834.1998

Fournier, P. E., Marrie, T. J., & Raoult, D. (1998). Diagnosis of Q fever. *Journal of Clinical Microbilogy*, 36(7), 1823-1834.https://doi.org/10.1128/jcm.36.7.1823-1834.1998

Fujishiro, M. A., Scorza, A. V., Gookin, J. L., & Lappin, M. R. (2016). Evaluation of associations among *Coxiella burnetii* and reproductive abnormalities in cats. *Journal of Feline Medicine and Surgery*, 18(4), 344-347.https://doi.org/10.1177/1098612x15584693

Gal, S., Fidler, C., Lo, Y., Taylor, M., Han, C., Moore, J., Harris, A., & Wainscoat, J. (2004). Quantitation of circulating DNA in the serum of breast cancer patients by real-time PCR. *British Journal of Cancer*, 90(6), 1211-1215.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2409649/pdf/90-6601609a.pdf

Gale, M., Ketheesan, N., Govan, B., Kennedy, R. L., & Norton, R. (2007). Q fever cases at a North Queensland centre during 1994–2006. *Internal Medicine Journal*, 37(9), 644-646.https://doi.org/10.1111/j.1445-5994.2007.01441.x

Gale, P., Kelly, L., Mearns, R., Duggan, J., & Snary, E. L. (2015). Q fever through consumption of unpasteurised milk and milk products – a risk profile and exposure assessment. *Journal of Applied Microbiology*, 118(5), 1083-1095.https://doi.org/10.1111/jam.12778

Garland-Lewis, G., Whittier, C., Murray, S., Trufan, S., & Rabinowitz, P. M. (2017). Occupational risks and exposures among wildlife health professionals. *EcoHealth*, 14(1), 20-28.https://doi.org/10.1007/s10393-017-1208-2

Garner, M. G., Longbottom, H. M., Cannon, R. M., & Plant, A. J. (1997). A review of Q fever in Australia 1991-1994. *Australian and New Zealand Journal of Public Health*, 21(7), 722-773.https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1467-842X.1997.tb01787.x

Gefenaite, G., Munster, J. M., van Houdt, R., & Hak, E. (2011). Effectiveness of the Q fever vaccine: A meta-analysis. *Vaccine*, 29(3), 395-398.https://doi.org/10.1016/j.vaccine.2010.11.008

Ghanem-Zoubi, N., & Paul, M. (2020). Q fever during pregnancy: A narrative review. *Clinical Microbiology and Infection*, 26(7), 864-870.https://doi.org/10.1016/j.cmi.2019.10.024

Gibbons, G. C., & White, P. J. (2012). Q fever in a veterinary hospital; an unusual epidemiology. ASID Zoonoses, Sydney, Australia 27-28 July 2012. https://www.asid.net.au/documents/item/216

Gidding, H. F., Faddy, H. M., Durrheim, D. N., Graves, S. R., Nguyen, C., Hutchinson, P., Massey, P., & Wood, N. (2019). Seroprevalence of Q fever among metropolitan and non-metropolitan blood donors in New South Wales and Queensland, 2014-2015. *Medical Journal of Australia*, 210(7), 309-315.https://doi.org/10.5694/mja2.13004

Gidding, H. F., Peng, C. Q., Graves, S., Massey, P. D., Nguyen, C., Stenos, J., Quinn, H. E., McIntyre, P. B., Durrheim, D. N., & Wood, N. (2020). Q fever seroprevalence in Australia suggests one in twenty people have been exposed. *Epidemiology and Infection*, 148, e18.https://doi.org/10.1017/S0950268820000084

Gidding, H. F., Wallace, C., Lawrence, G. L., & McIntyre, P. B. (2009). Australia's national Q fever vaccination program. *Vaccine*, 27(14), 2037-2041.https://doi.org/10.1016/j.vaccine.2009.02.007

Gimenez, D. F. (1964). Staining of rickettsiae in yolk-sac cultures. *Stain Technology*, 39, 135-140.

Glazunova, O., Roux, V., Freylikman, O., Sekeyova, Z., Fournous, G., Tyczka, J., Tokarevich, N., Kovacava, E., Marrie, T. J., & Raoult, D. (2005). *Coxiella burnetii* genotyping. *Emerging Infectious Diseases*, 11(8), 1211-1217.https://doi.org/10.3201/eid1108.041354

Gonder, J. C., Kishimoto, R. A., Kastello, M. D., Pedersen, C. E., Jr., & Larson, E. W. (1979). Cynomolgus monkey model for experimental Q fever infection. *The Journal of Infectious Diseases*, 139(2), 191-196.https://doi.org/10.1093/infdis/139.2.191

González-Barrio, D., Maio, E., Vieira-Pinto, M., & Ruiz-Fons, F. (2015). European rabbits as reservoir for *Coxiella burnetii*. *Emerging Infectious Diseases*, 21(6), 1055-1058.https://doi.org/10.3201/eid2106.141537

González-Barrio, D., & Ruiz-Fons, F. (2019). *Coxiella burnetii* in wild mammals: A systematic review. *Transboundary and Emerging Diseases*, 66(2), 662-671.https://doi.org/10.1111/tbed.13085

Graves, S. R., Banks, J., Dwyer, B., & King, G. K. (1992). A case of murine typhus in Queensland. *Medical Journal of Australia*, 156(9), 650-651.https://doi.org/10.5694/j.1326-5377.1992.tb121464.x

Graves, S. R., Dwyer, B., McColl, D., & McDade, J. (1991). Flinders Island spotted fever: A newly recognised endemic focus of tick typhus in Bass Strait: Part 2. Serological investigations. *Medical Journal of Australia*, 154(2), 99-104.https://onlinelibrary.wiley.com/doi/abs/10.5694/j.1326-5377.1991.tb120994.x?sid=nlm%3Apubmed

Graves, S. R., Gerrard, J., & Coghill, S. (2020). Q fever following a tick bite. *Australian Journal of General Practice*, 49(12), 823-825.https://doi.org/10.31128/ajgp-01-20-5195

Graves, S. R., & Islam, A. (2016). Endemic Q Fever in New South Wales, Australia: A case series (2005-2013). *American Journal of Tropical Medicine and Hygiene*, 95(1), 55.https://doi.org/10.4269/ajtmh.15-0828

Graves, S. R., Jackson, C., Hussain-Yusuf, H., Vincent, G., Nguyen, C., Stenos, J., & Webster, M. (2016). *Ixodes holocyclus* tick-transmitted human pathogens in North-Eastern New South Wales, Australia. *Tropical Medicine and Infectious Disease*, 1(1), 4.https://doi.org/10.3390/tropicalmed1010004

Graves, S. R., & Stenos, J. (2009). Rickettsioses in Australia. *Annals of the New York Academy of Sciences*, 1166, 151-155.https://doi.org/10.1111/j.1749-6632.2009.04530.x

Graves, S. R., & Stenos, J. (2017). Tick-borne infectious diseases in Australia. *Medical Journal of Australia*, 206(7), 320.https://onlinelibrary.wiley.com/doi/full/10.5694/mja17.00090?sid=nlm%3Apubmed

Graves, S. R., Wang, L., Nack, Z., & Jones, S. (1999). *Rickettsia* serosurvey in Kimberley, Western Australia. *American Journal of Tropical Medicine and Hygiene*, 60(5), 786-789.https://doi.org/10.4269/ajtmh.1999.60.786

Guatteo, R., Seegers, H., Taurel, A.-F., Joly, A., & Beaudeau, F. (2011). Prevalence of *Coxiella burnetii* infection in domestic ruminants: A critical review. *Veterinary Microbiology*, 149(1-2), 1-16.https://doi.org/10.1016/j.vetmic.2010.10.007

Guimard, T., Amrane, S., Elsa, P., El Karkouri, K., Raoult, D., & Angelakis, E. (2017). Case report: scalp eschar and neck lymphadenopathy associated with bacteremia due to *Coxiella*-like bacteria. *American Journal of Tropical Medicine and Hygiene*, 97(5), 1319-1322.https://doi.org/10.4269/ajtmh.17-0251

Gunther, M. J., Heller, J., Hayes, L., & Hernandez-Jover, M. (2019). Dairy goat producers' understanding, knowledge and attitudes towards biosecurity and Q-fever in Australia. *Preventive Veterinary Medicine*, 170, 104742-104742.https://doi.org/10.1016/j.prevetmed.2019.104742

Guy, A. J., & Banks, P. (2012). A survey of current rehabilitation practices for native mammals in Eastern Australia. *Australian Mammalogy*, 34, 108-118.https://doi.org/10.1071/AM10046

Hackert, V. H., van der Hoek, W., Dukers-Muijrers, N., de Bruin, A., Al Dahouk, S., Neubauer, H., Bruggeman, C. A., & Hoebe, C. J. P. A. (2012). Q Fever: single-point source outbreak withhigh

attack rates and massivenumbers of undetected infections across an entire region. *Clinical Infectious Diseases*, 55(12), 1591-1599.https://doi.org/10.1093/cid/cis734
Hackstadt, T. (1988). Steric hindrance of antibody binding to surface proteins of *Coxiella burnetii* by phase I lipopolysaccharide. *Infection and Immunity*, 56(4), 802-807.https://doi.org/10.1128/iai.56.4.802-807.1988
Hackstadt, T. (1990). The role of lipopolysaccharides in the virulence of *Coxiella burnetii*. *Annals of the New York Academy of Sciences*, 590, 27-32.https://doi.org/10.1111/j.1749-6632.1990.tb42203.x
Hackstadt, T., & Williams, J. C. (1981). Biochemical stratagem for obligate parasitism of eukaryotic cells by *Coxiella burnetii*. *Proceedings of the National Academy of Sciences of the United States of America*, 78(5), 3240-3244.https://doi.org/10.1073/pnas.78.5.3240
Haering, R., Wilson, V., Zhuo, A., & Stathis, P. (2020). Towards a more effective model of wildlife care and rehabilitation: A survey of volunteers in New South Wales, Australia. *Australian zoologist*, 40, 605-627.https://doi.org/10.7882/AZ.2019.018
Hamilton Medical Centre. (2022). *Q fever vaccination*. Retrieved March 6, 2022 from https://hamiltonmedicalcentre.com.au/q-fever-vaccine
Harman, J. B. (1949). Q fever in Great Britain; clinical account of eight cases. *The Lancet*, 2(6588), 1028-1030.https://doi.org/10.1016/s0140-6736(49)91600-1
Harris, P. A., Taylor, R., Minor, B. L., Elliott, V., Fernandez, M., O'Neal, L., McLeod, L., Delacqua, G., Delacqua, F., Kirby, J., & Duda, S. N. (2019). The REDCap consortium: Building an international community of software platform partners. *Journal of Biomedical Informatics*, 95, 103208.https://doi.org/10.1016/j.jbi.2019.103208
Harris, P. A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N., & Conde, J. G. (2009). Research electronic data capture (REDCap)—A metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics*, 42(2), 377-381.https://doi.org/10.1016/j.jbi.2008.08.010
Hartwell, H. W., Lennette, E. H., Francis, R. A., & Winn, J. F. (1951). Q Fever in California: IV. Occurrence of *Coxiella burneti*i in the placenta of naturally infected sheep. *Public Health Reports (1896-1970)*, 66(45), 1473-1477.https://doi.org/10.2307/4587909
Harvey, M. S., Forbes, G. B., & Marmion, B. P. (1951). An outbreak of Q fever in East Kent. *Lancet*, 1152-1157.
Hatchette, T. F., Hayes, M., Merry, H., Schlech, W. F., & Marrie, T. J. (2003). The effect of *C. burnetii* infection on the quality of life of patients following an outbreak of Q fever. *Epidemiology and Infection*, 130(3), 491-495.https://doi.org/10.1017/s0950268803008343
Havas, K. A., & Burkman, K. (2011). A comparison of the serological evidence of *Coxiella burnetii* exposure between military working dogs andferal canines in Iraq. *Military Medicine*, 176(10), 1101-1103.https://doi.org/10.7205/milmed-d-11-00025
Hawker, J. I., Ayres, J. G., Blair, I., Evans, M. R., Smith, D. L., Smith, E. G., Burge, P. S., Carpenter, M. J., Caul, E. O., Coupland, B., Desselberger, U., Farrell, I. D., Saunders, P. J., & Wood, M. J. (1998). A large outbreak of Q fever in the West Midlands: windborne spread into a metropolitan area? *Communicable Disease and Public Health*, 1(3), 180-187.
Healy, B., van Woerden, H., Raoult, D., Graves, S., Pitman, J., Lloyd, G., Brown, N., & Llewelyn, M. (2011). Chronic Q fever: Different serological results in three countries-results of a follow-up study 6 years after a point source outbreak. *Clinical Infectious Diseases*, 52(8), 1013-1019.https://doi.org/10.1093/cid/cir132
Hechemy, K. E., Raoult, D., Fox, J., Han, Y., Elliott, L. B., & Rawlings, J. (1989). Cross-reaction of immune sera from patients with rickettsial diseases. *Journal of Medical Microbiology* 29(3), 199-202.https://doi.org/https://doi.org/10.1099/00222615-29-3-199
Hendrix, L. R., Samuel, J. E., & Mallavia, L. P. (1991). Differentiation of *Coxiella burnetii* isolates by analysis of restriction-endonuclease-digested DNA separated by SDS-PAGE. *Journal of General Microbiology*, 137(2), 269-276.https://doi.org/10.1099/00221287-137-2-269

Hickie, I., Davenport, T., Wakefield, D., Vollmer-Conna, U., Cameron, B., Vernon, S. D., Reeves, W. C., & Lloyd, A. (2006). Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: Prospective cohort study. *British Medical Journal*, 333(7568), 575.https://doi.org/10.1136/bmj.38933.585764.AE

Hii, S. F., Kopp, S. R., Abdad, M. Y., Thompson, M. F., O'Leary, C. A., Rees, R. L., & Traub, R. J. (2011). Molecular evidence supports the role of dogs as potential reservoirs for *Rickettsia felis*. *Vector Borne and Zoonotic Diseases*, 11(8), 1007-1012.

Hilbink, F., Penrose, M., Kovacova, E., & Kazar, J. (1993). Q fever is absent from New Zealand. *International Journal of Epidemiology*, 22(5), 945-949.https://doi.org/10.1093/ije/22.5.945

Honstettre, A., Ghigo, E., Moynault, A., Capo, C., Toman, R., Akira, S., Takeuchi, O., Lepidi, H., Raoult, D., & Mege, J.-L. (2004). Lipopolysaccharide from *Coxiella burnetii* Is Involved in bacterial phagocytosis, filamentous actinreorganization, and inflammatory responses through Toll-Like Receptor 4. *The Journal of Immunology*, 172(6), 3695-3703.https://doi.org/10.4049/jimmunol.172.6.3695

Hoover, T. A., Culp, D. W., Vodkin, M. H., Williams, J. C., & Thompson, H. A. (2002). Chromosomal DNA deletionsexplain phenotypic characteristics of two antigenic variants, Phase II and RSA 514 (Crazy), of the *Coxiella burnetii* Nine Mile strain. *Infection and Immunity*, 70(12), 6726-6733.https://doi.org/10.1128/iai.70.12.6726-2733.2002

Hosokawa, T., Koga, R., Kikuchi, Y., Meng, X. Y., & Fukatsu, T. (2010). Wolbachia as a bacteriocyte-associated nutritional mutualist. *Proceedings of the National Academy of Sciences of the United States of America*, 107(2), 769-774.https://doi.org/10.1073/pnas.0911476107

Hotta, A., Kawamura, M., To, H., Andoh, M., Yamaguchi, T., Fukushi, H., & Hirai, K. (2002). Phase variation analysis of *Coxiella burnetii* during serial passage in cell culture by use of monoclonal antibodies. *Infection and Immunity*, 70(8), 4747-4749.https://doi.org/10.1128/IAI.70.8.4747-4749.2002

Howe, D., Shannon, J. G., Winfree, S., Dorward, D. W., & Heinzen, R. A. (2010). *Coxiella burnetii* phase I and II variants replicate with similar kinetics in degradative phagolysosome-like compartments of human macrophages. *Infection and Immunity*, 78(8), 3465-3474.https://doi.org/10.1128/iai.00406-10

Huang, H. H. H., Power, R. I., Mathews, K. O., Ma, G. C., Bosward, K. L., & Šlapeta, J. (2021). Cat fleas (*Ctenocephalides felis* clade 'Sydney') are dominant fleas on dogs and cats in New South Wales, Australia: Presence of flea-borne *Rickettsia felis*, *Bartonella* spp. but absence of *Coxiella burnetii* DNA. *Current Research in Parasitology and Vector-Borne Diseases*, 1, 100045.https://doi.org/10.1016/j.crpvbd.2021.100045

Hulse, L. S., Beagley, K. W., Ellis, W., Fitzgibbon, S., Gillett, A., Barth, B., Robbins, A., Pyne, M., Larkin, R., & Johnston, S. D. (2020). Epidemiology of chlamydia-induced reproductive disease in male koalas (*Phascolarctos cinereus*) from southeast Queensland, Australia as assessed from penile urethral swabs and semen. *Journal of Wildlife Diseases*, 56(1), 82-92, .https://doi.org/10.7589/2019-03-062

Hulse, L. S., Hickey, D., Mitchell, J. M., Beagley, K. W., Ellis, W., & Johnston, S. D. (2018). Development and application of two multiplex real-time PCR assays for detection and speciation of bacterial pathogens in the koala. *Journal of Veterinary Diagnostic Investigation*, 30(4), 523-529.https://doi.org/10.1177/1040638718770490

International Society for Infectious Diseases. (2007). *Q fever - Australia (South Australia) (02): abattoir*. Retrieved June 3, 2021 from https://promedmail.org/promed-post/?id=20070713.2244

Irwin, M., Massey, P., & Durrheim, D. N. (2009). Enhanced Q fever risk exposure surveillance may permit better informed vaccination policy. *Communicable Diseases Intelligence*, Quarterly Report 33(1), 41-45.

Islam, A., Ferguson, J., Givney, R., & Graves, S. (2011). Seroprevalence to *Coxiella burnetii* among residents of the Hunter New England region of New South Wales, Australia. *American Journal of Tropical Medicine and Hygiene*, 84(2), 318-320.https://doi.org/10.4269/ajtmh.2011.10-0268

Iwakami, E., Arashima, Y., Kato, K., Komiya, T., Matsukawa, Y., Ikeda, T., Arakawa, Y., & Oshida, S. (2005). Treatment of chronic fatigue syndrome with antibiotics: Pilot study assessing the involvement of *Coxiella burnetii* infection. *Internal Medicine*, 44(12), 1258-1263.

Izzard, L., Fuller, A., Blacksell, S. D., Paris, D. H., Richards, A. L., Aukkanit, N., Nguyen, C., Jiang, J., Fenwick, S., Day, N. P. J., Graves, S., & Stenos, J. (2010). Isolation of a novel *Orientia* species (*Orientia chuto* sp. nov.) from a patient infected in Dubai. *Journal of Clinical Microbiology*, 48(12), 4404.https://doi.org/10.1128/JCM.01526-10

Izzard, L., Graves, S., Cox, E., Fenwick, S., Unsworth, N., & Stenos, J. (2009). Novel rickettsia in ticks, Tasmania, Australia. *Emerging Infectious Diseases*, 15(10), 1654-1656.https://doi.org/10.3201/eid1510.090799

Jager, M. M., Weers-Pothoff, G., Hermans, M. H. A., Meekelenkamp, J. C. E., Schellekens, J. J. A., Renders, N. H. M., Leenders, A. C. A. P., Schneeberger, P. M., & Wever, P. C. (2011). Evaluation of a diagnostic algorithm for acute Q fever in an outbreak setting. *Clinical and Vaccine Immunology*, 18(6), 963-968.https://doi.org/doi:10.1128/CVI.00009-11

Johnson, J. E., & Kadull, P. J. (1967). Rocky Mountain spotted fever acquired in a laboratory. *New England Journal of Medicine*, 277(16), 842-847.https://doi.org/10.1056/nejm196710192771603

Jones, R. M., Nicas, M., Hubbard, A. E., & Leingold, A. L. (2006). The infectious dose of C*oxiella burnetii* (Q fever). *Applied Biosafety*, 11(1), 32-41.https://doi.org/10.1177/153567600601100106

Jones, S. L., Athan, E., O'Brien, D., Graves, S. R., Nguyen, C., & Stenos, J. (2004). Murine typhus: The first reported case from Victoria *Medical Journal of Australia*, 180(9), 482.https://doi.org/10.5694/j.1326-5377.2004.tb06032.x

Kanfer, E., Farrag, N., Price, C., MacDonald, D., Coleman, J., & Barrett, A. (1988). Q fever following bone marrow transplantation. *Bone Marrow Transplantation*, 3(2), 165-166.

Kaplowitz, L. G., Lange, J. V., Fischer, J. J., & Walker, D. H. (1983). Correlation of rickettsial titers, circulating endotoxin, and clinical features in Rocky Mountain spotted fever. *Archives of Internal Medicine* 143(6), 1149-1151.https://jamanetwork.com/journals/jamainternalmedicine/articlepdf/603247/archinte_143_6_012.pdf

Karagiannis, I., Schimmer, B., Van Lier, A., Timen, A., Schneeberger, P., Van Rotterdam, B., De Bruin, A., Wijkmans, C., Rietveld, A., & Van Duynhoven, Y. (2009). Investigation of a Q fever outbreak in a rural area of The Netherlands. *Epidemiology and Infection*, 137(9), 1283-1294.https://doi.org/10.1017/S0950268808001908

Karki, S., Gidding, H., McIntyre, P., & Liu, B. (2015). Risk factors and burden of acute Q fever in older adults in New South Wales: A prospective cohort study. *Medical Journal of Australia*, 203(11).https://doi.org/10.5694/mja15.00391

Kato, C., Chung, I., & Paddock, C. (2016). Estimation of *Rickettsia rickettsii* copy number in the blood of patients with Rocky Mountain spotted fever suggests cyclic diurnal trends in bacteraemia. *Clinical Microbiology and Infection* 22(4), 394-396.https://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(15)01086-1/pdf

Kaufman, H. W., Chen, Z., Radcliff, J., Batterman, H. J., & Leake, J. (2018). Q fever: An under-reported reportable communicable disease. *Epidemiology and Infection*, 146(10), 1240-1244.https://doi.org/10.1017/S0950268818001395

Kazar, J. (2005). *Coxiella burnetii* infection. *Annals of the New York Academy of Sciences*, 1063(1), 105-114.https://doi.org/10.1196/annals.1355.018

Kenyon, R. H., Kishimoto, R. A., & Hall, W. C. (1979). Exposure of guinea pigs to *Rickettsia rickettsii* by aerosol, nasal, conjunctival, gastric, and subcutaneous routes and protection afforded by an experimental vaccine. *Infection and Immunity*, 25(2), 580-582.https://doi.org/10.1128/iai.25.2.580-582.1979

Kermode, M., Yong, K., Hurley, S., & Marmion, B. (2003). An economic evaluation of increased uptake in Q fever vaccination among meat and agricultural industry workers following implementation of the National Q Fever Management Program. *Australian and New Zealand Journal of Public Health*, 27(4), 390-398.https://doi.org/10.1111/j.1467-842x.2003.tb00415.x

Kermode, M., Yong, K., Hurley, S., & Marmion, B. (2003). An economic evaluation of increased uptake in Q fever vaccination among meat and agricultural industry workers following implementation of the National Q Fever Management Program. *Australian and New Zealand Journal of Public Health*, 27(4), 390-398.https://doi.org/10.1111/j.1467-842X.2003.tb00415.x

Kersh, G. J., Fitzpatrick, K. A., Self, J. S., Biggerstaff, B. J., & Massung, R. F. (2013). Long-term immune responses to *Coxiella burnetii* after vaccination. *Clinical and Vaccine Immunology*, 20(2), 129-133.https://doi.org/10.1128/CVI.00613-12

Kersh, G. J., Fitzpatrick, K. A., Self, J. S., Priestley, R. A., Kelly, A. J., Lash, R. R., Marsden-Haug, N., Nett, R. J., Bjork, A., Massung, R. F., & Anderson, A. D. (2013). Presence and persistence of *Coxiella burnetii* in the environments of goat farms associated with a Q fever outbreak. *Applied Environmental Microbiology*, 79(5), 1697-1703.https://doi.org/10.1128/aem.03472-12

Kersh, G. J., Lambourn, D. M., Raverty, S. A., Fitzpatrick, K. A., Self, J. S., Akmajian, A. M., Jeffries, S. J., Huggins, J., Drew, C. P., Zaki, S. R., & Massung, R. F. (2012). Coxiella burnetii infection of marine mammals in the Pacific Northwest, 1997-2010. *Journal of Wildlife Diseases*, 48(1), 201-206.https://doi.org/10.7589/0090-3558-48.1.201

Kersh, G. J., Oliver, L. D., Self, J. S., Fitzpatrick, K. A., & Massung, R. F. (2011). Virulence of pathogenic *Coxiella burnetii* strains after growth in the absence of host cells. *Vector Borne and Zoonotic Diseases*, 11(11), 1433-1438.https://doi.org/10.1089/vbz.2011.0670

Kishimoto, R. A., & Burger, G. T. (1977). Appearance of cellular and humoral immunity in guinea pigs after infection with *Coxiella burnetii* administered in small-particle aerosols. *Infection and Immunity*, 16(2), 518-521.https://doi.org/10.1128/iai.16.2.518-521.1977

Kittelberger, R., Mars, J., Wibberley, G., Sting, R., Henning, K., Horner, G. W., Garnett, K. M., Hannah, M. J., Jenner, J. A., Piggott, C. J., & O'Keefe, J. S. (2009). Comparison of the Q-fever complement fixation test and two commercial enzyme-linked immunosorbent assays for the detection of serum antibodies against *Coxiella burneii* (Q-fever) in ruminants : Recommendations for use of serological tests on imported animals in New Zealand. *New Zealand Veterinary Journal*, 57(5), 262-268.https://doi.org/10.1080/00480169.2009.58619

Klee, S. R., Tyczka, J., Ellerbrok, H., Franz, T., Linke, S., Baljer, G., & Appel, B. (2006). Highly sensitive real-time PCR for specific detection and quantification of *Coxiella burnetii*. *BMC Microbiology*, 6(1), 2-2.https://doi.org/10.1186/1471-2180-6-2

Komiya, T., Sadamasu, K., Kang, M., Tsuboshima, S., Fukushi, H., & Hirai, K. (2003). Seroprevalence of *Coxiella burnetii* infections among cats in different living environments. *Journal of Veterinary Medical Science*, 65(9), 1047-1048.

Komiya, T., Sadamasu, K., Toriniwa, H., Kato, K., Arashima, Y., Fukushi, H., Hirai, K., & Arakawa, Y. (2003). Epidemiological survey on the route of *Coxiella burnetii* infection in an animal hospital. *Journal of Infection and Chemotherapy*, 9(2), 151-155.https://doi.org/10.1007/s10156-003-0237-7

Kopecny, L., Bosward, K. L., Shapiro, A. J., & Norris, J. M. (2013). Investigating *Coxiella burnetii* infection in a breeding cattery at the centre of a Q fever outbreak. *Journal of Feline Medicine and Surgery*, 15(12), 1037-1045.https://doi.org/10.1177/1098612x13487360

Körner, S., Makert, G. R., Mertens-Scholz, K., Henning, K., Pfeffer, M., Starke, A., Nijhof, A. M., & Ulbert, S. (2020). Uptake and fecal excretion of *Coxiella burnetii* by Ixodes ricinus and

*Dermacentor marginatus* ticks. *Parasites and Vectors*, 13(1), 75.https://doi.org/10.1186/s13071-020-3956-z

Kosatsky, T. (1984). Household outbreak of Q-fever pneumonia related to a parturient cat. *Lancet*, 2(8417-8418), 1447-1449.https://doi.org/10.1016/s0140-6736(84)91633-7

Kreizinger, Z., Szeredi, L., Bacsadi, Á., Nemes, C., Sugár, L., Varga, T., Sulyok, K. M., Szigeti, A., Ács, K., Tóbiás, E., Borel, N., & Gyuranecz, M. (2015). Occurrence of *Coxiella burnetii* and Chlamydiales species in abortions of domestic ruminants and in wild ruminants in Hungary, Central Europe. *Journal of Veterinary Diagnostic Investigation*, 27(2), 206-210.https://doi.org/10.1177/1040638714563566
Kreizinger, Z., Szeredi, L., Bacsadi, Á., Nemes, C., Sugár, L., Varga, T., Sulyok, K. M., Szigeti, A., Ács, K., Tóbiás, E., Borel, N., & Gyuranecz, M. (2015). Occurrence of Coxiella burnetii and Chlamydiales species in abortions of domestic ruminants and in wild ruminants in Hungary, Central Europe. *Journal of Veterinary Diagnostic Investigation*, 27(2), 206-210.https://doi.org/10.1177/1040638714563566
Krumbiegel, E. R., & Wisniewski, H. J. (1970). Q fever in the Milwaukee area. II. Consumption of infected raw milk by human volunteers. *Archives of Environmental and Occupational Health*, 21, 63-65.

Kruse, H., Kirkemo, A.-M., & Handeland, K. (2004). Wildlife as source of zoonotic infections. *Emerging Infectious Diseases*, 10(12), 2067-2072.https://doi.org/10.3201/eid1012.040707

Kruszewska, D., & Tylewska-Wierzbanowska, S. (1997). Isolation of *Coxiella burnetii* from bull semen. *Research in Veterinary Science*, 62(3), 299-300.https://doi.org/10.1016/s0034-5288(97)90210-1

Kruszewska, D., & Tylewska-Wierzbanowska, S. K. (1993). *Coxiella burnetii* penetration into the reproductive system of male mice, promoting sexual transmission of infection. *Infection and Immunity*, 61(10), 4188-4195.https://www.scopus.com/inward/record.uri?eid=2-s2.0-0027485142&partnerID=40&md5=1293f6f2a44aa811509e5df27304fef7

Kumar, D., Chandra, R., Mathur, M., Samdariya, S., & Kapoor, N. (2016). Vaccine hesitancy: Understanding better to address better. *Israel Journal of Health Policy Research*, 5(1), 2.https://doi.org/10.1186/s13584-016-0062-y

Kuske, C. R., Barns, S. M., Grow, C. C., Merrill, L., & Dunbar, J. (2006). Environmental survey for four pathogenic bacteria and closely related species using phylogenetic and functional genes. *Journal of Forensic Sciences*, 51(3), 548-558.https://doi.org/10.1111/j.1556-4029.2006.00131.x

Lankford, M. G., Zembower, T. R., Trick, W. E., Hacek, D. M., Noskin, G. A., & Peterson, L. R. (2003). Influence of role models and hospital design on hand hygiene of healthcare workers. Emerging infectious diseases, 9(2), 217–223. https://doi.org/10.3201/eid0902.020249

La Scola, B., & Raoult, D. (1997). Laboratory diagnosis of rickettsioses: Current approaches to diagnosis of old and new rickettsial diseases. *Journal of Clinical Microbiology*, 35(11), 2715-2727.https://doi.org/10.1128/jcm.35.11.2715-2727.1997

La Scola, B., Rydkina, L., Ndihokubwayo, J. B., Vene, S., & Raoult, D. (2000). Cross serological differentiation of murine typhus and epidemic typhus using cross-adsorption and Western blotting. *Clinical and Diagnostic Laboratory Immunology*, 7(4), 612-616.https://doi.org/10.1128/CDLI.7.4.612-616.2000

Langley, J. M., Marrie, T. J., Covert, A., Waag, D. M., & Williams, J. C. (1988). Poker players' pneumonia. An urban outbreak of Q fever following exposure to a parturient cat. *New England Journal of Medicine*, 319(6), 354-356.https://doi.org/10.1056/nejm198808113190607

Larson, H. J., de Figueiredo, A., Xiahong, Z., Schulz, W. S., Verger, P., Johnston, I. G., Cook, A. R., & Jones, N. S. (2016). The state of vaccine confidence 2016: Global insights through a 67-country survey. *EBioMedicine*, 12, 295-301.https://doi.org/10.1016/j.ebiom.2016.08.042

Larson, H. J., Schulz, W. S., Tucker, J. D., & Smith, D. M. D. (2015). Measuring vaccine confidence: Introducing a global vaccine confidence index. *PLoS currents*, 7, ecurrents.outbreaks.ce0f6177bc97332602a97332608e97332603fe97332607d97332607f97332

607cc97332604.https://doi.org/10.1371/currents.outbreaks.ce0f6177bc97332602a8e3fe7d7f7cc4

Lautenschläger, S., Willems, H., Jäger, C., & Baljer, G. (2000). Sequencing and characterization of the cryptic plasmid QpRS from *Coxiella burnetii*. *Plasmid*, 44(1), 85-88.https://doi.org/10.1006/plas.2000.1470

Ledina, D., Bradarić, N., Milas, I., Ivić, I., Brncić, N., & Kuzmicić, N. (2007). Chronic fatigue syndrome after Q fever. *Medical Science Monitor*, 13(7), CS88-CS92.

Lee, H. J., Woo, Y., Hahn, T. W., Jung, Y. M., & Jung, Y. J. (2020). Formation and maturation of the phagosome: A key mechanism in innate immunity against intracellular bacterial infection. *Microorganisms*, 8(9).https://doi.org/10.3390/microorganisms8091298

Leon, A., Richard, E., Fortier, C., Laugier, C., Fortier, G., & Pronost, S. (2012). Molecular detection of *Coxiella burnetii* and *Neospora caninum* in equine aborted foetuses and neonates. *Preventive Veterinary Medicine*, 104(1-2), 179-183.https://doi.org/10.1016/j.prevetmed.2011.11.001

Lillie, R. D. (1942). Pathologic histology in guinea pigs following intraperitoneal inoculation with the virus of" Q" fever. *Public Health Reports (1896-1970)*, 296-306.

Lockhart, M. G., Graves, S. R., Banazis, M. J., Fenwick, S. G., & Stenos, J. (2011). A comparison of methods for extracting DNA from *Coxiella burnetii* as measured by a duplex qPCR assay. *Letters in Applied Microbiology*, 52(5), 514-520.https://doi.org/10.1111/j.1472-765X.2011.03034.x

Long, C. M., Beare, P. A., Cockrell, D. C., Larson, C. L., & Heinzen, R. A. (2019). Comparative virulence of diverse *Coxiella burnetii* strains. *Virulence*, 10(1), 133-150.https://doi.org/10.1080/21505594.2019.1575715

Lord, H., Fletcher-Lartey, S., Weerasinghe, G., Chandra, M., Egana, N., Schembri, N., & Conaty, S. (2016). A Q fever cluster among workers at an abattoir in south-western Sydney, Australia, 2015. *Western Pacific Surveillance and Response Journal* 7(4), 21-27.https://doi.org/10.5365/WPSAR.2016.7.2.012

Lowbridge, C. P., Tobin, S., Seale, H., & Ferson, M. J. (2012). EpiReview: notifications of Q fever in NSW, 2001-2010. *New South Wales  Public Health Bulletin*, 23(1-2), 31-35.https://doi.org/10.1071/nb11037

Lower, T., Corben, P., Massey, P., Depczynski, J., Brown, T., Stanley, P., Osbourn, M., & Durrheim, D. (2017). Farmers' knowledge of Q fever and prevention approaches in New South Wales. *Australian Journal of Rural Health*, 25(5), 306-310.https://doi.org/10.1111/ajr.12346

Luce-Fedrow, A., Mullins, K., Kostik, A. P., St John, H. K., Jiang, J., & Richards, A. L. (2015). Strategies for detecting rickettsiae and diagnosing rickettsial diseases. *Future Microbiology*, 10(4), 537-564.https://doi.org/10.2217/fmb.14.141

Lukácová, M., Barák, I., & Kazár, J. (2008). Role of structural variations of polysaccharide antigens in the pathogenicity of Gram-negative bacteria. *Clinical Microbiology and Infection*, 14(3), 200-206.https://doi.org/10.1111/j.1469-0691.2007.01876.x

Luna, V. A., King, D. S., Peak, K. K., Reeves, F., Heberlein-Larson, L., Veguilla, W., Heller, L., Duncan, K. E., Cannons, A. C., Amuso, P., & Cattani, J. (2006). *Bacillus anthracis* virulent plasmid pX02 genes found in large plasmids of two other *Bacillus* species. *Journal of Clinical Microbiology*, 44(7), 2367-2377.https://doi.org/10.1128/jcm.00154-06

Ma, G. C., Norris, J. M., Mathews, K. O., Chandra, S., Šlapeta, J., Bosward, K. L., & Ward, M. P. (2020). New insights on the epidemiology of *Coxiella burnetii* in pet dogs and cats from New South Wales, Australia. *Acta Tropica*, 205, 105416.https://doi.org/10.1016/j.actatropica.2020.105416

MacDonald, N. E. (2015). Vaccine hesitancy: Definition, scope and determinants. *Vaccine*, 33(34), 4161-4164.https://doi.org/10.1016/j.vaccine.2015.04.036

Mahamat, A., Edouard, S., Demar, M., Abboud, P., Patrice, J. Y., La Scola, B., Okandze, A., Djossou, F., & Raoult, D. (2013). Unique clone of Coxiella burnetii causing severe Q fever, French Guiana. *Emerging Infectious Diseases*, 19(7), 1102-1104.https://doi.org/10.3201/eid1907.130044

Malo, J. A., Colbran, C., Young, M., Vasant, B., Jarvinen, K., Viney, K., & Lambert, S. B. (2018). An outbreak of Q fever associated with parturient cat exposure at an animal refuge and veterinary

clinic in southeast Queensland. *Australian and New Zealand Journal of Public Health*, 42(5), 451-455.https://doi.org/10.1111/1753-6405.12784

Mamatkulov, A., Sekler, D., Yarmukhamedova, N., Mamatova, R., Vafakulov, S., & Musabaev, E. (2019). Detection of Q-fever in Samarkand region of Uzbekistan: Mite surveillance study. *International Journal of Infectious Diseases*, 79, 141.https://doi.org/10.1016/j.ijid.2018.11.345

Marenzoni, M. L., Stefanetti, V., Papa, P., Casagrande Proietti, P., Bietta, A., Coletti, M., Passamonti, F., & Henning, K. (2013). Is the horse a reservoir or an indicator of *Coxiella burnetii* infection? Systematic review and biomolecular investigation. *Veterinary Microbiology*, 167(3-4), 662-669.https://doi.org/10.1016/j.vetmic.2013.09.027

Marmion, B. P., Ormsbee, R. A., Kyrkou, M., Wright, J., Worswick, D. A., Izzo, A. A., Esterman, A., Feery, B., Shapiro, R. A., & Infection. (1990). Vaccine prophylaxis of abattoir-associated Q fever: Eight years' experience in Australian abattoirs. *Epidemiology and Infection*, 104(2), 275-287.https://doi.org/10.1017/s0950268800059458

Marmion, B. P., Shannon, M., Maddocks, I., Storm, P., & Penttila, I. (1996). Protracted debility and fatigue after acute Q fever [23]. *Lancet*, 347(9006), 977-978.https://doi.org/10.1016/S0140-6736(96)91469-5

Marmion, B. P., & Stoker, M. G. (1958). The epidemiology of Q fever In Great Britain: An analysis of the findings and some conclusions. *British Medical Journal*, 2(5100), 809-816.

Marmion, B. P., Storm, P. A., Ayres, J. G., Semendric, L., Mathews, L., Winslow, W., Turra, M., & Harris, R. J. (2005). Long-term persistence of *Coxiella burnetii* after acute primary Q fever. *QJM: An International Journal of Medicine*, 98(1), 7-20.https://doi.org/10.1093/qjmed/hci009

Marmion, B. P., Sukocheva, O., Storm, P. A., Lockhart, M., Turra, M., Kok, T., Ayres, J., Routledge, H., & Graves, S. (2009). Q fever: Persistence of antigenic non-viable cell residues of *Coxiella burnetii* in the host—implications for post Q fever infection fatigue syndrome and other chronic sequelae. *QJM: An International Journal of Medicine*, 102(10), 673-684.https://doi.org/10.1093/qjmed/hcp077

Marrie, T. J. (1990). Q fever -A review. *The Canadian Veterinary Journal*, 31(8), 555-563.http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1480833/

Marrie, T. J., Durant, H., Williams, J. C., Mintz, E., & Waag, D. M. (1988). Exposure to parturient cats: Arisk factor for acquisition of Q fever in Maritime Canada. *Journal of Infectious Diseases*, 158(1), 101-108.https://doi.org/10.1093/infdis/158.1.101

Marrie, T. J., Stein, A., Janigan, D., & Raoult, D. (1996). Route of infection determines the clinical manifestations of acute Q fever. *The Journal of infectious diseases*, 173(2), 484-487.https://doi.org/10.1093/infdis/173.2.484

Massey, P. D., Irwin, M., & Durrheim, D. N. (2009). Enhanced Q fever risk exposure surveillance may permit better informed vaccination policy. *Communicable Diseases Intelligence Quarterly Report*, 33(1), 41-45. .http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3301i.htm

Mathews, K. O., Phalen, D., Norris, J. M., Stenos, J., Toribio, J.-A., Wood, N., Graves, S., Sheehy, P. A., Nguyen, C., & Bosward, K. L. (2021). Serological evidence of exposure to Spotted Fever Group and Typhus Group rickettsiae in Australian wildlife rehabilitators. *Pathogens* 10(6), 745.https://doi.org/10.3390/pathogens10060745

Mathews, K. O., Savage, C., Norris, J. M., Phalen, D., Malikides, N., Sheehy, P. A., & Bosward, K. L. (2022). Risk factors associated with self-reported Q fever in Australian wildlife rehabilitators: Findings from an online survey *Zoonoses and Public Health*, (currently under review).

Mathews, K. O., Toribio, J.-A., Norris, J. M., Phalen, D., Wood, N., Graves, S. R., Sheehy, P. A., & Bosward, K. L. (2021). *Coxiella burnetii* seroprevalence and Q fever in Australian wildlife rehabilitators. *One Health*, 12, 100197.https://doi.org/10.1016/j.onehlt.2020.100197

Maurin, M., & Raoult, D. (1999). Q Fever. *Clinical Microbiology Reviews*, 12(4), 518-553.https://doi.org/10.1128/CMR.12.4.518

Mboussou, Y., Jaubert, J., Larrieu, S., Atiana, L., Naze, F., Folio, C., Randrianaivo, H., Bertolotti, A., Picot, S., Robillard, P.-Y., Boukerrou, M., & Gérardin, P. (2019). Pregnancy outcomes of Q fever: Prospective follow-up study on Reunion island. *BMC Infectious Diseases*, 19(1), 1001.https://doi.org/10.1186/s12879-019-4619-6

McBride, W. J., Hanson, J. P., Miller, R., & Wenck, D. (2007). Severe spotted fever group rickettsiosis, Australia. *Emerging Infectious Diseases*, 13(11), 1742-1744.https://doi.org/10.3201/eid1311.070099

McCaul, T. F., & Williams, J. C. (1981). Developmental cycle of Coxiella burnetii: Structure and morphogenesis of vegetative and sporogenic differentiations. *Journal of Bacteriology*, 147(3), 1063-1076.https://www.scopus.com/inward/record.uri?eid=2-s2.0-0019771657&partnerID=40&md5=5e669e848175bfa9c7826e9d975f0f27

https://jb.asm.org/content/jb/147/3/1063.full.pdf

Mediannikov, O., Fenollar, F., Socolovschi, C., Diatta, G., Bassene, H., Molez, J.-F., Sokhna, C., Trape, J.-F., & Raoult, D. (2010). *Coxiella burnetii* in humans and ticks in rural Senegal. *PLoS Neglected Tropical Diseases*, 4(4), e654.https://doi.org/10.1371/journal.pntd.0000654

Melenotte, C., Million, M., Audoly, G., Gorse, A., Dutronc, H., Roland, G., Dekel, M., Moreno, A., Cammilleri, S., Carrieri, M. P., Protopopescu, C., Ruminy, P., Lepidi, H., Nadel, B., Mege, J. L., Xerri, L., & Raoult, D. (2016). B-cell non-Hodgkin lymphoma linked to *Coxiella burnetii*. *Blood*, 127(1), 113-121.https://doi.org/10.1182/blood-2015-04-639617

Meredith, A. L., Cleaveland, S. C., Denwood, M. J., Brown, J. K., & Shaw, D. J. (2015). *Coxiella burnetii* (Q-Fever) seroprevalence in prey and predators in the United Kingdom: Evaluation of infection in wild rodents, foxes and domestic cats using a modified ELISA. *Transboundary and Emerging Diseases*, 62(6), 639-649.https://doi.org/10.1111/tbed.12211

Merhej, V., Tattevin, P., Revest, M., Le Touvet, B., & Raoult, D. (2012). Q fever osteomyelitis: A case report and literature review. *Comparative Immunology, Microbiology and Infectious Diseases*, 35(2), 169-172.https://doi.org/10.1016/j.cimid.2011.12.008

Milazzo, A., Featherstone, K. B., & Hall, R. G. (2005). Q fever vaccine uptake in South Australian meat processors prior to the introduction of the National Q Fever Management Program. *Communicable Diseases Intelligence*, 29(4), 400-406. https://pubmed.ncbi.nlm.nih.gov/16465932/.https://pubmed.ncbi.nlm.nih.gov/16465932/

Milazzo, A., Hall, R., Storm, P. A., Harris, R. J., Winslow, W., & Marmion, B. P. (2001). Sexually transmitted Q fever. *Clinical Infectious Diseases*, 33(3), 399-402.https://doi.org/10.1086/321878

Miller, M., Roche, P., Yohannes, K., Spencer, J., Bartlett, M., Brotherton, J., Hutchinson, J., Kirk, M., McDonald, A., & Vadjic, C. (2005). Australia's notifiable diseases status, 2003 annual report of the National Notifiable Diseases Surveillance System. *Communicable Diseases Intelligence*, 29(1), 1-61.https://www.scopus.com/inward/record.uri?eid=2-s2.0-22844451970&partnerID=40&md5=6abed4f447cf4b1e271c197d81581afc

Million, M., & Raoult, D. (2015). Recent advances in the study of Q fever epidemiology, diagnosis and management. *Journal of Infection*, 71, S2-S9.https://doi.org/doi.org/10.1016/j.jinf.2015.04.024

Million, M., Roblot, F., Carles, D., D'Amato, F., Protopopescu, C., Carrieri, M. P., & Raoult, D. (2014). Reevaluation of the risk of fetal death and malformation after Q Fever. *Clinical Infectious Diseases*, 59(2), 256-260.https://doi.org/10.1093/cid/ciu259

Mori, M., Boarbi, S., Michel, P., Bakinahe, R., Rits, K., Wattiau, P., & Fretin, D. (2013). *In vitro* and i*n vivo* Infectious potential of *Coxiella burnetii:*: A study on Belgian livestock isolates. *PloS One*, 8(6).https://doi.org/10.1371/journal.pone.0067622

Morroy, G., Peters, J. B., van Nieuwenhof, M., Bor, H. H. J., Hautvast, J. L. A., van der Hoek, W., Wijkmans, C. J., & Vercoulen, J. H. (2011). The health status of Q-fever patients after long-term follow-up. *BMC Infectious Diseases*, 11.https://doi.org/10.1186/1471-2334-11-97

Mousapour, M., Oveisi, A., Key, Y. A., Mikaeili, E., Rahimi, F., Shademan, B., Bedoustani, A. B., Fattahi, S., Fasaei, M. S., & Abbasnezhad, A. D. (2020). First serological & molecular study of *Coxiella burnetii* in stray, domestic cats, and their owners in Iran. *Topics in Companion Animal Medicine*, 41, 100471.https://doi.org/10.1016/j.tcam.2020.100471

Muleme, M., Stenos, J., Vincent, G., Campbell, A., Graves, S., Warner, S., Devlin, J. M., Nguyen, C., Stevenson, M. A., Wilks, C. R., Firestone, S. M., & Pasetti, M. F. (2016). Bayesian validation of the indirect immunofluorescence assay and Its superiority to the enzyme-linked immunosorbent assay and the complement fixation test for detecting antibodies against *Coxiella burnetii* in goat serum. *Clinical and Vaccine Immunology*, 23(6), 507-514.https://doi.org/doi:10.1128/CVI.00724-15

Mullen, G. R., & O'connor, B. M. (2019). Mites (Acari). In G. R. Mullen & L. A. Durden (Eds.), *Medical and Veterinary Entomology* (Third ed., pp. 533-602). Academic Press. https://doi.org/10.1016/B978-0-12-814043-7.00026-1

Nagaoka, H., Akiyama, M., Sugieda, M., Nishio, T., Akahane, S., Hattori, H., Ho, T., Fukushi, H., & Hirai, K. (1996). Isolation of *Coxiella burnetii* from children with influenza-like symptoms in Japan. *Microbiology and Immunology*, 40(2), 147-151.

Nagaoka, H., Sugieda, M., Akiyama, M., Nishina, T., Akahane, S., & Fujiwara, K. (1998). Isolation of *Coxiella burnetii* from the vagina of feline clients at veterinary clinics. *Journal of Veterinary Medical Science*, 60(2), 251-252.

National Centre for Disease Control. (1998). Q fever outbreak in an abattoir in Cooma, NSW. *Communicable Diseases Intelligence*, 22(10), 222.https://www.scopus.com/inward/record.uri?eid=2-s2.0-84921427371&partnerID=40&md5=d4235932d531722a1adee6a22b593132

National Notifiable Diseases Surveillance System. (2016). Australia's notifiable disease status, 2016: Annual report of the national notifiable diseases surveillance system https://doi.org/10.33321/cdi.2021.45.28

National Notifiable Diseases Surveillance System. (2021). *Disease notification rates, Australia, 1991 to 2020 and year-to-date notifications for 2021*. Retrieved November, 10 2021 from http://www9.health.gov.au/cda/source/rpt_3.cfm

National Notifiable Diseases Surveillance System. (2021). *Notifications of selected diseases by state territory and year*. Australian Government Department of Health. Retrieved Accessed 17 August, 2021 from http://www9.health.gov.au/cda/source/cda-index.cfm

National Rural Health Alliance. (2011). *The Little book of rural health numbers*. Retrieved February 17, 2020 from https://www.ruralhealth.org.au/book/demography

[Record #1065 is using a reference type undefined in this output style.]

[Record #851 is using a reference type undefined in this output style.]

Ng-Nguyen, D., Hii, S.-F., Hoang, M.-T. T., Nguyen, V.-A. T., Rees, R., Stenos, J., & Traub, R. J. (2020). Domestic dogs are mammalian reservoirs for the emerging zoonosis flea-borne spotted fever, caused by *Rickettsia felis*. *Scientific Reports*, 10(1), 4151.https://doi.org/10.1038/s41598-020-61122-y

Nicholson, W. L., & Paddock, C. D. (2019). *Travel-related infectious diseases*. Retrieved June 17, 2022 from https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/rickettsial-including-spotted-fever-and-typhus-fever-rickettsioses-scrub-typhus-anaplasmosis-and-ehr

Nielsen, S. Y., Mølbak, K., Henriksen, T. B., Krogfelt, K. A., Larsen, C. S., & Villumsen, S. (2014). Adverse pregnancy outcomes and *Coxiella burnetii* antibodies in pregnant women, Denmark. *Emerging Infectious Diseases*, 20(6), 925-931.https://doi.org/10.3201/eid2006.130584

Noda, H., Munderloh, U. G., & Kurtti, T. J. (1997). Endosymbionts of ticks and their relationship to *Wolbachia* spp. and tick-borne pathogens of humans and animals. *Journal of Applied Environmental Microbiology*, 63(10), 3926-3932.https://aem.asm.org/content/aem/63/10/3926.full.pdf

NSW Department of Health. (2016, 7th September 2016). *Typhus (epidemic, murine and other rickettsial diseases) fact sheet*. Retrieved 4th February from https://www.health.nsw.gov.au/Infectious/factsheets/Pages/Typhus.aspx#

O'Connor, B. A., Tribe, I. G., & Givney, R. (2015). A windy day in a sheep saleyard: An outbreak of Q fever in rural South Australia. *Epidemiology and Infection*, 143(2), 391-398.https://doi.org/10.1017/S0950268814001083

O'Connor, L. F., Kelly, H. A., Lubich, J. M., Lindsey, R. J., & McComish, M. J. (1996). A cluster of murine typhus cases in Western Australia. *Medical Journal of Australia*, 165(1), 24-26.https://doi.org/10.5694/j.1326-5377.1996.tb124812.x

Oliphant, J. W., Gordon, D. A., Meis, A., & Parker, R. R. (1949). Q fever in laundry workers, presumably transmitted from contaminated clothing. *American Journal of Epidemiology*, 49(1), 76-82.https://doi.org/10.1093/oxfordjournals.aje.a119261

Omsland, A. (2012). Axenic growth of *Coxiella burnetii*. *Advances in Experimental Medicine and Biology*, 984, 215-229.https://doi.org/10.1007/978-94-007-4315-1_11

Ormsbee, R. A. (1952). The growth of *Coxiella burnetii* in embryonated eggs. *Journal of Bacteriology*, 63(1), 73-86.https://doi.org/10.1128/jb.63.1.73-86.1952

Ormsbee, R. A., Bell, E. J., Lackman, D. B., & Tallent, G. (1964). The Influence of phase on the protective potency of Q fever vaccine. *Journal of Immunology* 92, 404-412.https://pubmed.ncbi.nlm.nih.gov/14128986/

Orr, B., Malik, R., Westman, M. E., & Norris, J. M. (2022). Seroprevalence of *Coxiella burnetii* in pig-hunting dogs from north Queensland, Australia. *Australian Veterinary Journal*, 100(6), 230-235.https://doi.org/10.1111/avj.13151

Oskam, C. L., Gofton, A. W., Greay, T. L., Yang, R., Doggett, S., Ryan, U. M., & Irwin, P. J. (2017). Molecular investigation into the presence of a *Coxiella* sp. in *Rhipicephalus sanguineus* ticks in Australia. *Veterinary Microbiology*, 201, 141-145.https://doi.org/10.1016/j.vetmic.2017.01.021

Oster, C. N., Burke, D. S., Kenyon, R. H., Ascher, M. S., Harber, P., & Pedersen, C. E. (1977). Laboratory-acquired Rocky Mountain spotted fever: The hazard of aerosol transmission. *New England Journal of Medicine*, 297(16), 859-863.https://doi.org/10.1056/NEJM197710202971604

Owen, H., Clark, P., Stenos, J., Robertson, I., & Fenwick, S. (2006). Potentially pathogenic spotted fever group rickettsiae present in Western Australia. *Australian Journal of Rural Health*, 14(6), 284-285.https://doi.org/10.1111/j.1440-1584.2006.00831.x

Oyston, P. C., & Davies, C. (2011). Q fever: the neglected biothreat agent. *Journal of Medical Microbiology*, 60(Pt 1), 9-21.https://doi.org/10.1099/jmm.0.024778-0

Paris, D. H., & Dumler, J. S. (2016). State of the art of diagnosis of rickettsial diseases: The use of blood specimens for diagnosis of scrub typhus, spotted fever group rickettsiosis, and murine typhus. *Current Opinion in Infectious Diseases*, 29(5), 433-439.https://doi.org/10.1097/qco.0000000000000298

Parker, N., Robson, J., & Bell, M. (2010). A serosurvey of *Coxiella burnetii* infection in children and young adults in South West Queensland. *Australian and New Zealand Journal of Public Health*, 34(1), 79-82.https://doi.org/10.1111/j.1753-6405.2010.00478.x

Parker, N. R., Barralet, J. H., & Bell, A. M. (2006). Q fever. *The Lancet*, 367(9511), 679-688.https://doi.org/https://doi.org/10.1016/S0140-6736(06)68266-4

Parola, P., Paddock, C. D., & Raoult, D. (2005). Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clinical Microbiology Reviews*, 18(4), 719.https://doi.org/10.1128/CMR.18.4.719-756.2005

Parola, P., Paddock, C. D., Socolovschi, C., Labruna, M. B., Mediannikov, O., Kernif, T., Abdad, M. Y., Stenos, J., Bitam, I., Fournier, P. E., & Raoult, D. (2013). Update on tick-borne rickettsioses around the world: A geographic approach. *Clinical Microbiology Reviews*, 26(4), 657-702.https://doi.org/10.1128/cmr.00032-13

Patel, M., Sheridan, J., & Bell, M. (1997). Q fever vaccination in Queensland abattoirs. *Communicable Diseases Intelligence*, 21(3), 29-31. .https://pubmed.ncbi.nlm.nih.gov/9079588/.https://search-informit-org.ezproxy.library.sydney.edu.au/doi/abs/10.3316/informit.446937116403340

Penttila, I. A., Harris, R. J., Storm, P., Haynes, D., Worswick, D. A., & Marmion, B. P. (1998). Cytokine dysregulation in the post-Q-fever fatigue syndrome. *QJM: Monthly Journal of the Association of Physicians*, 91(8), 549-560.https://doi.org/10.1093/qjmed/91.8.549

Perrin, T. L., & Bengtson, I. A. (1942). The histopathology of experimental" Q" fever in mice. *Public Health Reports*, 790-798.

Philip, C. B. (1948). Observations on experimental Q fever. *The Journal of Parasitology*, 34(6), 457-464.https://doi.org/10.2307/3273312

Pickard, N. (2016, 29th June, 2016). St Georges Basin resident urges others to get Q fever vaccination. *South Coast Register (online)*. https://www.southcoastregister.com.au/story/3998834/q-fever-brings-st-georges-basin-man-close-to-death/

Pinsky, R. L., Fishbein, D. B., Greene, C. R., & Gensheimer, K. F. (1991). An outbreak of cat-associated Q Fever in the United States. *The Journal of Infectious Diseases*, 164(1), 202-204.https://doi.org/10.1093/infdis/164.1.202

Pommier de Santi, V., Briolant, S., Mahamat, A., Ilcinkas, C., Blanchet, D., de Thoisy, B., Reynaud, Y., Hyvert, G., Marié, J. L., Edouard, S., Davoust, B., & Raoult, D. (2018). Q fever epidemic in Cayenne, French Guiana, epidemiologically linked to three-toed sloth. *Comparative Immunology, Microbiology and Infectious Diseases*, 56, 34-38.https://doi.org/10.1016/j.cimid.2017.12.004

Pope, J. H., Scott, W., & Dwyer, R. (1960). *Coxiella burnetii* in kangaroo and kangaroo ticks in Western Queensland. *Australian Journal of Experimental Biology and Medical Science*, 38(1), 17-27.http://www.nature.com.ezproxy1.library.usyd.edu.au/icb/journal/v38/n1/pdf/icb19603a.pdf

Porter, S. R., Czaplicki, G., Mainil, J., Guattéo, R., & Saegerman, C. (2011). Q Fever: Currenstate oknowledge and perspectives of research of a neglected zoonosis. *International Journal of Microbiology*, 2011, 22.https://doi.org/10.1155/2011/248418

Potter, A. S., Banazis, M. J., Yang, R., Reid, S. A., & Fenwick, S. G. (2011). Prevalence of *Coxiella burnetii* in Western Grey Kangaroos (*Macropus fuliginosus*) in Western Australia. *Journal of Wildlife Diseases*, 47(4), 821-828.https://doi.org/10.7589/0090-3558-47.4.821

Psaroulaki, A., Chochlakis, D., Ioannou, I., Angelakis, E., & Tselentis, Y. (2014). Presence of *Coxiella burnetii* in fleas in Cyprus. *Vector Borne and Zoonotic Diseases*, 14(9), 685-687.https://doi.org/10.1089/vbz.2013.1399

R Core Team. *R: A language and environment for statistical computing. R foundation for statistical computing. Retrieved from https://www.R-project.org/*

Raby, E., Pearn, T., Marangou, A. G., Merritt, A. J., Murray, R. J., Dyer, J. R., & Graves, S. R. (2016). New foci of spotted fever group rickettsiae Including *Rickettsia honei* in Western Australia. *Tropical Medicine and Infectious Disease*, 1(1), 5.https://doi.org/10.3390/tropicalmed1010005

Rahaman, Md R., Marshall, H., Milazzo, A., Crabb, D., & Bi, P. (2021). Q fever prevention and vaccination: Australian livestock farmers' knowledge and attitudes to inform a One Health approach. *One Health*, 12, 100232.https://doi.org/10.1016/j.onehlt.2021.100232

Rahaman, Md R., Milazzo, A., Marshall, H., Chaber, A. L., & Bi, P. (2021). Q fever vaccination: Australian animal science and veterinary students' One Health perspectives on Q fever prevention. *Human Vaccines & Immunotherapeutics*, 17(5), 1374-1381.https://doi.org/10.1080/21645515.2020.1829900

Raijmakers, R. P. H., Jansen, A. F. M., Keijmel, S. P., ter Horst, R., Roerink, M. E., Novakovic, B., Joosten, L. A. B., van der Meer, J. W. M., Netea, M. G., & Bleeker-Rovers, C. P. (2019). A possible role for mitochondrial-derived peptides humanin and MOTS-c in patients with Q fever fatigue

syndrome and chronic fatigue syndrome. *Journal of Translational Medicine*, 17(1), 157.https://doi.org/10.1186/s12967-019-1906-3
Raoult, D., Brouqui, P., Marchou, B., & Gastaut, J. A. (1992). Acute and chronic Q fever in patients with cancer. *Clinical Infectious Diseases*, 14(1), 127-130.https://doi.org/10.1093/clinids/14.1.127
Raoult, D., Fenollar, F., & Stein, A. (2002). Q fever during pregnancy: diagnosis, treatment, and follow-up. *Archives of Internal Medicine*, 162(6), 701-704.https://doi.org/10.1001/archinte.162.6.701
Raoult, D., Marrie, T., & Mege, J. (2005). Natural history and pathophysiology of Q fever. *Lancet Infectious Diseases*, 5(4), 219-226.https://doi.org/10.1016/s1473-3099(05)70052-9
Raoult, D., Sexton, D. J., & Mitty, J. (2018). Clinical manifestations and diagnosis of Q fever. *UpToDate. Waltham, MA: UpToDate Inc. com (Accessed on Decenber 16, 2018.)*.
Raoult, D., Tissot-Dupont, H., Foucault, C., Gouvernet, J., Fournier, P. E., Bernit, E., Stein, A., Nesri, M., Harle, J. R., & Weiller, P. J. (2000). Q fever 1985-1998. Clinical and epidemiologic features of 1,383 infections. *Journal of Medicine*, 79(2), 109-123.
Reháçek, J. (1989). Ecological relationships between ticks and rickettsiae. *European Journal of Epidemiology*, 5(4), 407-413.https://doi.org/10.1007/BF00140130
Ricketts, H. T. (1909). A micro-organism which apparently has a specific relationship to Rocky Mountain spotted fever: A preliminary report. *Journal of the American Medical Association* 52(5), 379-380.
Rodolakis, A. (2009). Q Fever in Dairy Animals. *Annals of the New York Academy of Sciences*, 1166(1), 90-93.https://doi.org/10.1111/j.1749-6632.2009.04511.x
Roest, H.-J., Bossers, A., van Zijderveld, F. G., & Rebel, J. M. (2013). Clinical microbiology of *Coxiella burnetii* and relevant aspects for the diagnosis and control of the zoonotic disease Q fever. *Veterinary Quarterly*, 33(3), 148-160.https://doi.org/10.1080/01652176.2013.843809
Roest, H.-J., Post, J., van Gelderen, B., van Zijderveld, F. G., & Rebel, J. M. J. (2013). Q fever in pregnant goats: Humoral and cellular immune responses. *Veterinary Research*, 44(1), 67.https://doi.org/10.1186/1297-9716-44-67
Roest, H.-J., van Gelderen, B., Dinkla, A., Frangoulidis, D., van Zijderveld, F., Rebel, J., & van Keulen, L. (2012). Q fever in pregnant goats: Pathogenesis and excretion of Coxiella burnetii. *PloS One*, 7(11), e48949.https://doi.org/10.1371/journal.pone.0048949
Rolain, J. M., Raoult, D., Marmion, B. P., Harris, R. J., Storm, P., & Ayres, J. (2005). Molecular detection of *Coxiella burnetii* in blood and sera during Q fever - Response. *QJM: An International Journal of Medicine*, 98.https://doi.org/10.1093/qjmed/hci099
Rudi, K., Moen, B., Drømtorp, S. M., & Holck, A. L. (2005). Use of ethidium monoazide and PCR in combination for quantification of viable and dead cells incomplex samples. *Applied and Environmental Microbiology*, 71(2), 1018-1024.https://doi.org/doi:10.1128/AEM.71.2.1018-1024.2005
Ruiz-Villalba, A., van Pelt-Verkuil, E., Gunst, Q. D., Ruijter, J. M., & van den Hoff, M. J. (2017). Amplification of nonspecific products in quantitative polymerase chain reactions (qPCR). *Biomolecular Detection and Quantification*, 14, 7-18.https://doi.org/10.1016/j.bdq.2017.10.001
Saengsawang, P., Pangjai, D., Kaewmongkol, G., & Inpankaew, T. (2022). Detection of antibodies against three zoonotic *Bartonella* spp. and cross-reactivity among species and *Coxiella burnetii* in dogs and cats from Central Thailand. *Comparative Immunology, Microbiology and Infectious Diseases*, 81.https://doi.org/10.1016/j.cimid.2021.101743
Safe Work NSW. (2022). *Q fever* https://www.safework.nsw.gov.au/hazards-a-z/diseases/q-fever
Sahu, R., Rawool, D. B., Vinod, V. K., Malik, S. V. S., & Barbuddhe, S. B. (2020). Current approaches for the detection of *Coxiella burnetii* infection in humans and animals. *Journal of Microbiological Methods*, 179, 106087.https://doi.org/https://doi.org/10.1016/j.mimet.2020.106087
Saint, E. G., Drummond, A. F., & Thorburn, I. O. (1954). Murine typhus in Western Australia. *Medical Journal of Australia*, 2(19), 731-737.https://doi.org/10.5694/j.1326-5377.1954.tb66921.x

Samuel, J. E., Frazier, M. E., & Mallavia, L. P. (1985). Correlation of plasmid type and disease caused by *Coxiella burnetii*. *Infection and Immunity*, 49(3), 775-779.https://doi.org/10.1128/iai.49.3.775-779.1985

Sánchez, C. A., & Baker, M. L. (2016). Disease risk perception and safety practices: A survey of Australian flying fox rehabilitators. *PLoS Neglected Tropical Diseases*, 10(2), e0004411.https://doi.org/10.1371/journal.pntd.0004411

Sánchez, J., Souriau, A., Buendía, A. J., Arricau-Bouvery, N., Martínez, C. M., Salinas, J., Rodolakis, A., & Navarro, J. A. (2006). Experimental *Coxiella burnetii* infection in pregnant goats: A histopathological and immunohistochemical study. *Journal of Comparative Pathology*, 135(2-3), 108-115.https://doi.org/10.1016/j.jcpa.2006.06.003

Saslaw, S., & Carlisle, H. N. (1966). Aerosol infection of monkeys with *Rickettsia rickettsii*. *Bacteriological Reviews*, 30(3), 636-645.https://doi.org/10.1128/mmbr.30.3.636-645.1966

Savage, C. (2017). *Knowledge,attitude and practices of Australian wildlife rehanilitators in relation to Q fever* The University of Sydney].

Savinelli, E. A., & Mallavia, L. P. (1990). Comparison of *Coxiella burnetii* plasmids to homologous chromosomal sequences present in a plasmidless endocarditis-causing isolate. *Annals of the New York Academy of Sciences*, 590, 523-533.https://doi.org/10.1111/j.1749-6632.1990.tb42262.x

Schloderer, D., Owen, H., Clark, P., Stenos, J., & Fenwick, S. G. (2006). *Rickettsia felis* in fleas, Western Australia. *Emerging Infectious Diseases*, 12(5), 841-843.https://doi.org/10.3201/eid1205.051458

Schneeberger, P. M., Hermans, M. H., van Hannen, E. J., Schellekens, J. J., Leenders, A. C., & Wever, P. C. (2010). Real-time PCR with serum samples is indispensable for early diagnosis of acute Q fever. *Clinical and Vaccine Immunology*, 17(2), 286-290.https://doi.org/10.1128/cvi.00454-09

Schoch, C. L., Ciufo, S., Domrachev, M., Hotton, C. L., Kannan, S., Khovanskaya, R., Leipe, D., McVeigh, R., O'Neill, K., Robbertse, B., Sharma, S., Soussov, V., Sullivan, J. P., Sun, L., Turner, S., & Karsch-Mizrachi, I. (2020). NCBI Taxonomy: A comprehensive update on curation, resources and tools. *Database: The Journal of Biological Databases and Curation*, 2020.https://doi.org/10.1093/database/baaa062

Schoffelen, T., Joosten, L., Herremans, T., de Haan, A., Ammerdorffer, A., Rümke, H., Wijkmans, C., Roest, H., Netea, M., van Der Meer, J., Sprong, T., & van Deuren, M. (2013). Specific interferon [gamma] detection for the diagnosis of previous Q fever. *Clinical Infectious Diseases*, 56(12), 1742.https://doi.org/10.1093/cid/cit129

Schoffelen, T., Wong, A., Rümke, H. C., Netea, M. G., Timen, A., van Deuren, M., & Vermeer-de Bondt, P. E. (2014). Adverse events and association with age, sex and immunological parameters of Q fever vaccination in patients at risk for chronic Q fever in the Netherlands 2011. *Vaccine*, 32(49), 6622-6630.https://doi.org/10.1016/j.vaccine.2014.09.061

Schramek, S., Radziejewska-Lebrecht, J., & Mayer, H. (1985). 3-C-branched aldoses in lipopolysaccharide of phase I *Coxiella burnetii* and their role as immunodominant factors. *European Journal of Biochemistry*, 148(3), 455-461.https://doi.org/10.1111/j.1432-1033.1985.tb08861.x

Sellens, E., Bosward, K. L., Norris, J. M., Wood, N., Heller, J., Graves, S., & Gidding, H. F. (2020). *Coxiella burnetii* seroprevalence in unvaccinated veterinary workers in Australia: Evidence to support Q fever vaccination. *Zoonoses and Public Health*, 67(1), 79-88.https://doi.org/10.1111/zph.12658

Sellens, E., Bosward, K. L., Willis, S., Heller, J., Cobbold, R., Comeau, J. L., Norris, J. M., Dhand, N. K., & Wood, N. (2018). Frequency of adverse events Following Q fever immunisation in young adults. *Vaccines*, 6(4), 83.https://doi.org/10.3390/vaccines6040083

Sellens, E., Norris, J., Dhand, N. K., Heller, J., Hayes, L., Gidding, H., Willaby, H., Wood, N., & Bosward, K. (2018). Willingness of veterinarians in Australia to recommend Q fever vaccination in veterinary personnel: Implications for workplace health and safety compliance. *PloS One*, 13(6), e0198421.https://doi.org/10.1371/journal.pone.0198421

Sellens, E., Norris, J. M., Dhand, N. K., Heller, J., Hayes, L., Gidding, H. F., Willaby, H., Wood, N., & Bosward, K. L. (2016). Q Fever knowledge, attitudes and vaccination status of Australia's veterinary workforce in 2014. *PloS One*, 11(1), e0146819.https://doi.org/10.1371/journal.pone.0146819

Seo, M.-G., Lee, S.-H., VanBik, D., Ouh, I.-O., Yun, S.-H., Choi, E., Park, Y.-S., Lee, S.-E., Kim, J. W., & Cho, G.-J. J. P. O. (2016). Detection and genotyping of Coxiella burnetii and Coxiella-like bacteria in horses in South Korea. 11(5), e0156710.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4886966/pdf/pone.0156710.pdf

Seqirus. (2019). *Q-VAX® Q Fever Vaccine and Q-VAX® Skin Test, Skin test Product Information (R100517 & R100518)*. Retrieved November,20 2021 from https://labeling.seqirus.com/PI/AU/Q-Vax/EN/Q-Vax-Product-Information.pdf

Seqirus. (2021). A concise guide to Q fever and Q fever vaccination. https://www.qfeverfacts.com.au/wp-content/uploads/2021/06/Q-Fever-Facts-A-Concise-Guide-to-Q-fever-and-Q-fever-Vaccination.pdf

Serbezov, V., Kazár, J., Novkirishki, V., Gatcheva, N., Kováčová, E., & Voynova, V. (1999). Q fever in Bulgaria and Slovakia. *Emerging Infectious Diseases*, 5(3), 388-394.https://doi.org/10.3201/eid0503.990309

Seshadri, R., Paulsen, I. T., Eisen, J. A., Read, T. D., Nelson, K. E., Nelson, W. C., Ward, N. L., Tettelin, H., Davidsen, T. M., Beanan, M. J., Deboy, R. T., Daugherty, S. C., Brinkac, L. M., Madupu, R., Dodson, R. J., Khouri, H. M., Lee, K. H., Carty, H. A., Scanlan, D., Heinzen, R. A., Thompson, H. A., Samuel, J. E., Fraser, C. M., & Heidelberg, J. F. (2003). Complete genome sequence of the Q-fever pathogen *Coxiella burnetii*. *Proceedings of the National Academy of Sciences of the United States of America*, 100(9), 5455-5460.https://doi.org/10.1073/pnas.0931379100

Sexton, D. J., & King, G. (1990). Fatal Queensland Tick Typhus. *Journal of Infectious Diseases*, 162(3), 779-780.https://doi.org/10.1093/infdis/162.3.779

Shapiro, A. J., Bosward, K. L., Heller, J., & Norris, J. M. (2015). Seroprevalence of *Coxiella burnetii* in domesticated and feral cats in eastern Australia. *Veterinary Microbiology*, 177(1-2), 154-161.https://doi.org/10.1016/j.vetmic.2015.02.011

Shapiro, A. J., Bosward, K. L., Mathews, K. O., Vincent, G., Stenos, J., Tadepalli, M., & Norris, J. M. (2020). Molecular detection of *Coxiella burnetii* in raw meat intended for pet consumption. *Zoonoses and Public Health*, 67(4), 443-452.https://doi.org/10.1111/zph.12707

Shapiro, A. J., Brown, G., Norris, J. M., Bosward, K. L., Marriot, D. J., Balakrishnan, N., Breitschwerdt, E. B., & Malik, R. (2017). Vector-borne and zoonotic diseases of dogs in North-west New South Wales and the Northern Territory, Australia. *BMC Veterinary Research*, 13(1), 238.https://doi.org/10.1186/s12917-017-1169-2

Shapiro, A. J., Norris, J. M., Bosward, K. L., & Heller, J. (2017). Q Fever (*Coxiella burnetii*) Knowledge and attitudes of Australian cat breeders and their husbandry practices. *Zoonoses and Public Health*, 64(4), 252-261.https://doi.org/10.1111/zph.12305

Shapiro, A. J., Norris, J. M., Heller, J., Brown, G., Malik, R., & Bosward, K. L. (2016). Seroprevalence of *Coxiella burnetii* in Australian dogs. *Zoonoses and Public Health*, 63(6), 458-466.https://doi.org/10.1111/zph.12250

Shivaprasad, H., Cadenas, M., Diab, S. S., Nordhausen, R., Bradway, D., Crespo, R., & Breitschwerdt, E. J. A. d. (2008). Coxiella-like infection in psittacines and a toucan. 52(3), 426-432.

Sivabalan, P., Saboo, A., Yew, J., & Norton, R. (2017). Q fever in an endemic region of North Queensland, Australia: A 10 year review. *One Health*, 3, 51-55.https://doi.org/10.1016/j.onehlt.2017.03.002

Sloan-Gardner, T. S., Massey, P. D., Hutchinson, P., Knope, K., & Fearnley, E. (2017). Trends and risk factors for human Q fever in Australia, 1991-2014. *Epidemiology and Infection*, 145(4), 787-795.https://doi.org/10.1017/S0950268816002843

Smith, T. A., Driscoll, T., Gillespie, J. J., & Raghavan, R. (2015). A Coxiella-like endosymbiontis a potential vitamin source for the lone star tick. *Genome Biology and Evolution*, 7(3), 831-838.https://doi.org/10.1093/gbe/evv016

Spiegel, N. B., & Wynn, P. C. (2014). Promoting kangaroo as a sustainable option for meat production on the rangelands of Australia. *Animal Frontiers*, 4(4), 38-45.https://doi.org/10.2527/af.2014-0032

Standards Australia. (2010). Safety in laboratories. Part 3, Microbiological safety and containment (6th ed. ed.). Standards Australia. Available at https://www.nzms.org.nz/uploads/9/9/5/6/99562762/102745_as_nzs_2243.3_public commenting-draft.pdf

Stein, A., Kruszewska, D., Gouvernet, J., & Raoult, D. J. E. j. o. e. (1997). Study of the 16S–23S ribosomal DNA internal spacer of Coxiella burnetii. 13(4), 471-475.

Stein, A., & Raoult, D. (1993). Lack of pathotype specific gene in human *Coxiella burnetii* isolates. *Microbial Pathogenesis*, 15(3), 177-185.https://doi.org/10.1006/mpat.1993.1068

Stenos, J., Graves, S. R., & Unsworth, N. B. (2005). A highly sensitive and specific real-time PCR assay for the detection of spotted fever and typhus group rickettsiae. *American Journal of Tropical Medicine and Hygiene*, 73(6), 1083-1085.

Stenos, J., Roux, V., Walker, D., & Raoult, D. (1998). *Rickettsia honei* sp. nov., the aetiological agent of Flinders Island spotted fever in Australia. *International Journal of Systematic Bacteriology*, 48 1399-1404.https://doi.org/10.1099/00207713-48-4-1399

Stevenson, S., Gowardman, J., Tozer, S., & Woods, M. (2015). Life-threatening Q fever infection following exposure to kangaroos and wallabies. *BMJ Case Reports*, 2015.https://doi.org/10.1136/bcr-2015-210808

Stewart, A. G. A., Smith, S., Binotto, E., McBride, W. J., & Hanson, J. (2019). The epidemiology and clinical features of rickettsial diseases in North Queensland, Australia: Implications for patient identification and management. *PLoS Neglected Tropical Diseases*, 13(7), e0007583.https://doi.org/10.1371/journal.pntd.0007583

Stoker, M. G. P., & Fiset, P. (1956). Phase variation of the Nine Mile and other strains of *Rickettsia burnetii*. *Canadian Journal of Microbiology*, 2(3), 310-321.https://doi.org/10.1139/m56-036

Tellis, A. N., Rowe, S. M., Coilparampil, R., Jenkins, C., Dart, A., Zadoks, R. N., Regnerus, C. D., & Bosward, K. L. (2022). Evaluation of three immunological assays to mitigate the risk of transboundary spread of *Coxiella burnetii* by alpacas. *Transboundary and Emerging Diseases*, 69(2), 793-804.https://doi.org/10.1111/tbed.14051

Teoh, Y. T., Hii, S. F., Graves, S., Rees, R., Stenos, J., & Traub, R. J. (2016). Evidence of exposure to *Rickettsia felis* in Australian patients. *One Health* 2, 95-98.https://doi.org/10.1016/j.onehlt.2016.06.001

Teoh, Y. T., Hii, S. F., Stevenson, M. A., Graves, S., Rees, R., Stenos, J., & Traub, R. J. (2017). Serological evidence of exposure to *Rickettsia felis* and *Rickettsia typhi* in Australian veterinarians. *Parasites and Vectors*, 10(1), 129.https://doi.org/10.1186/s13071-017-2075-y

Therapeutic Goods Administration Database of Adverse Event Notifications—Medicines. (2022). *Australian Government Department of Health; Canberra, Australia: 2022*. Retrieved February 10, 2022 from https://apps.tga.gov.au/PROD/DAEN/daen-report.aspx

[Record #435 is using a reference type undefined in this output style.]

Thompson, M., Mykytczuk, N., Gooderham, K., & Schulte-Hostedde, A. (2012). Prevalence of the bacterium *Coxiella burnetii* in wild rodents from a Canadian natural environment park. *Zoonoses and Public Health*, 59(8), 553-560.https://doi.org/10.1111/j.1863-2378.2012.01493.x

Tissot-Dupont, H., Amadei, M.-A., Nezri, M., & Raoult, D. (2004). Wind in November, Q fever in December. *Emerging infectious diseases*, 10(7), 1264-1269.https://doi.org/10.3201/eid1007.030724

Tissot-Dupont, H., & Raoult, D. (2008). Q Fever. *Infectious Disease Clinics of North America*, 22(3), 505-514.https://doi.org/https://doi.org/10.1016/j.idc.2008.03.002

Tissot-Dupont, H., Vaillant, V., Rey, S., & Raoult, D. (2007). Role of sex, age, previous valve lesion, and pregnancy in the clinical expression and outcome of Q fever after a large outbreak. *Clinical Infectious Diseases*, 44(2), 232-237.https://doi.org/10.1086/510389

To, H., Sakai, R., Shirota, K., Kano, C., Abe, S., Sugimoto, T., Takehara, K., Morita, C., Takashima, I., Maruyama, T., Yamaguchi, T., Fukushi, H., & Hirai, K. (1998). Coxiellosis in domestic and wild birds from Japan. *Journal of Wildlife Diseases*, 34(2), 310-316.https://doi.org/10.7589/0090-3558-34.2.310

Toman, R., Skultety, L., & Ihnatko, R. (2009). *Coxiella burnetii* glycomics and proteomics—tools for linking structure to function. *Annals of the New York Academy of Sciences*, 1166(1), 67-78.

Tozer, S., Lambert, S., Sloots, T., & Nissen, M. (2011). Q fever seroprevalence in metropolitan samples is similar to rural/remote samples in Queensland, Australia. *European Journal of Clinical Microbiology and Infectious Diseases*, 30(10), 1287-1293.https://doi.org/10.1007/s10096-011-1225-y

Tozer, S., Lambert, S., Strong, C., Field, H., Sloots, T., & Nissen, M. (2014). Potential animal and environmental sources of Q Fever infection for humans in Queensland. *Zoonoses and Public Health*, 61(2), 105-112.https://doi.org/10.1111/zph.12051

Tozer, S., Wood, C., Si, D., Nissen, M., Sloots, T., & Lambert, S. (2020). The improving state of Q fever surveillance. A review of Queensland notifications, 2003–2017. *Communicable Diseases Intelligence*, 44.https://doi.org/10.33321/cdi.2021.45.28

Tribe, A., & Brown, P. R. (2000). The role of wildlife rescue groups in the care and rehabilitation of Australian fauna. *Human Dimensions of Wildlife*, 5(2), 69-85.https://doi.org/10.1080/10871200009359180

Tripepi, G., Jager, K. J., Dekker, F. W., & Zoccali, C. (2010). Selection bias and information bias in clinical research. *Nephron Clinical Practice*, 115(2), c94-c99.https://doi.org/10.1159/000312871

Unsworth, N. B., Graves, S., Nguyen, C., Kemp, G., Graham, J., & Stenos, J. (2008). Markers of exposure to spotted fever rickettsiae in patients with chronic illness, including fatigue, in two Australian populations. *Quarterly Journal of Medicine*, 101(4), 269-274.https://doi.org/10.1093/qjmed/hcm149

Unsworth, N. B., Stenos, J., Graves, S. R., Faa, A. G., Cox, G. E., Dyer, J. R., Boutlis, C. S., Lane, A. M., Shaw, M. D., Robson, J., & Nissen, M. D. (2007). Flinders Island spotted fever rickettsioses caused by "marmionii" strain of *Rickettsia honei*, eastern Australia. *Emerging Infectious Diseases*, 13(4), 566-573.https://doi.org/10.3201/eid1304.050087

Unsworth, N. B., Stenos, J., McGregor, A. R., Dyer, J. R., & Graves, S. R. (2005). Not only 'Flinders Island' spotted fever. *Pathology*, 37(3), 242-245.

Valková, D., & Kazár, J. (1995). A new plasmid (QpDV) common to *Coxiella burnetii* isolates associated with acute and chronic Q fever. *FEMS Microbiology Letters*, 125(2-3), 275-280.https://doi.org/10.1111/j.1574-6968.1995.tb07368.x

Van den Brom, R., Schimmer, B., Schneeberger, P. M., Swart, W. A., van der Hoek, W., & Vellema, P. (2013). Seroepidemiological survey for *Coxiella burnetii* antibodies and associated risk factors in Dutch livestock veterinarians. *PloS One*, 8(1), e54021.https://doi.org/10.1371/journal.pone.0054021

Van den Brom, R., van Engelen, E., Roest, H. I. J., van der Hoek, W., & Vellema, P. (2015). *Coxiella burnetii* infections in sheep or goats: an opinionated review. *Veterinary Microbiology*, 181(1-2), 119-129.https://doi.org/10.1016/j.vetmic.2015.07.011

van der Hoek, W., Schneeberger, P. M., Oomen, T., Wegdam-Blans, M. C., Dijkstra, F., Notermans, D. W., Bijlmer, H. A., Groeneveld, K., Wijkmans, C. J., Rietveld, A., Kampschreur, L. M., & van Duynhoven, Y. (2012). Shifting priorities in the aftermath of a Q fever epidemic in 2007 to 2009 in the Netherlands: From acute to chronic infection. *Eurosurveillance*, 17(3).https://www.scopus.com/inward/record.uri?eid=2-s2.0-84856272360&partnerID=40&md5=00255b584ba62bfa4ced5bd3b75f7f59

Vapniarsky, N., Barr, B., & Murphy, B. (2012). Systemic Coxiella-like infection with myocarditis and hepatitis in an eclectus parrot (*Eclectus roratus*). *Veterinary Pathology*, 49(4), 717-722.
Vilcins, I. E., Old, J. M., & Deane, E. M. (2008). Detection of a spotted fever group *Rickettsia* in the tick *Ixodes tasmani* collected from koalas in Port Macquarie, Australia. *Journal of Medical Entomology*, 45(4), 745-750.https://doi.org/10.1603/0022-2585(2008)45[745:DOASFG]2.0.CO;2
Vilcins, I. E., Old, J. M., & Deane, E. M. (2009). Molecular detection of Rickettsia, Coxiella and *Rickettsiella* DNA in three native Australian tick species. *Experimental and Applied Acarology*, 49, 229-242.https://doi.org/10.1007/s10493-009-9260-4
Vincent, G., Stenos, J., Latham, J., Fenwick, S., & Graves, S. (2016). Novel genotypes of *Coxiella burnetii i*dentified in isolates from Australian Q fever patients. *International Journal of Medical Microbiology*, 306(6), 463-470.https://doi.org/https://doi.org/10.1016/j.ijmm.2016.05.014
Wade, A. J., Cheng, A. C., Athan, E., Molloy, J. L., Harris, O. C., Stenos, J., & Hughes, A. J. (2006). Q fever outbreak at a cosmetics supply factory. *Clinical Infectious Diseases* 42(7), e50-52.https://doi.org/10.1086/501127
Walker, D. H. (1996). Rickettsiae. In S. Baron (Ed.), *Medical Microbiology*. University of Texas www.ncbi.nlm.nih.gov/books/NBK7624/
Wegdam-Blans, M., Vainas, T., van Sambeek, M. R., Cuypers, P. W., Tjhie, H. T. J., van Straten, A. H. M., & Teijink, J. A. (2011). Vascular complications of Q-fever infections. *European Journal of Vascular and Endovascular Surgery*, 42(3), 384-392.https://doi.org/https://doi.org/10.1016/j.ejvs.2011.04.013
Weisburg, W., Dobson, M., Samuel, J., Dasch, G., Mallavia, L., Baca, O., Mandelco, L., Sechrest, J., Weiss, E., & Woese, C. (1989). Phylogenetic diversity of the Rickettsiae. *Journal of Bacteriology*, 171(8), 4202-4206.https://jb.asm.org/content/jb/171/8/4202.full.pdf
Weiskopf, D., Weinberger, B., & Grubeck-Loebenstein, B. (2009). The aging of the immune system. *Transplant International*, 22(11), 1041-1050.https://doi.org/10.1111/j.1432-2277.2009.00927.x
Welsh, H. H., Lennette, E. H., Abinanti, F. R., & Winn, J. F. (1958). Air-borne transmission of Q fever: The role of parturition in the generation of aerosols. *Annals of the New York Academy of Sciences*, 70(3), 528-540.https://doi.org/10.1111/j.1749-6632.1958.tb35409.x
Whelan, P. I., Ralph, A., Currie, B. J., & Raines, M. (2004). Scrub typhus in the Northern Territory: Exceeding the boundaries of Litchfield National Park. *Communicable Diseases Intelligence Quarterly Report*, 28.
Wielders, C., Morroy, G., Wever, P., Coutinho, R., Schneeberger, P., & van der Hoek, W. (2013). Strategies for early detection of chronic Q-fever: a systematic review. *European Journal of Clinical Investigation*, 43(6), 616-639.https://doi.org/https://doi.org/10.1111/eci.12073
Wildlife Health Australa. (2014). Chlamydia in koalas.https://wildlifehealthaustralia.com.au/Portals/0/Documents/FactSheets/Mammals/Chlamydia%20in%20Koalas%20Feb%202014%20(1.1).pdf
Wildlife Health Australia. (2017). Chlamydia in Australian wild birds - Fact sheet.https://www.wildlifehealthaustralia.com.au/Portals/0/Documents/FactSheets/Avian/Chlamydia%20in%20Australian%20Wild%20Birds.pdf
Wildlife Health Australia. (2018). *National wildlife biosecurity guidelines*. Wildlife Health Australia. Retrieved December 10, 2018 from https://www.wildlifehealthaustralia.com.au/Portals/0/Documents/ProgramProjects/National_Wildlife_Biosecurity_Guidelines.PDF
Wildlife Health Australia. (2018). Salmonella infection in Australian reptiles fact sheet.https://wildlifehealthaustralia.com.au/Portals/0/Documents/FactSheets/Reptiles/Salmonella_infection_in_Australian_Reptiles.pdf
Wildlife Health Australia. (2019). Australian bat lyssavirus factsheet.https://wildlifehealthaustralia.com.au/Portals/0/Documents/FactSheets/mammals/Australian_Bat_Lyssavirus.pdf

Wildlife Health Australia. (2020). Tullaraemia and Australian wildlife-Fact sheet.https://wildlifehealthaustralia.com.au/Portals/0/Documents/FactSheets/Public%20health/Tularaemia_and_Australian_Wildlife.pdf

Wildman, M. J., Smith, E. G., Groves, J., Beattie, J. M., Caul, E. O., & Ayres, J. G. (2002). Chronic fatigue following infection by *Coxiella burnetii* (Q fever): Ten-year follow-up of the 1989 UK outbreak cohort. *QJM - Monthly Journal of the Association of Physicians*, 95(8), 527-538.https://doi.org/10.1093/qjmed/95.8.527

Williams, J. C., Thomas, L. A., & Peacock, M. G. (1986). Humoral immune response to Q fever: Enzyme-linked immunosorbent assay antibody response to *Coxiella burnetii* in experimentally infected guinea pigs. *Journal of Clinical Microbiology*, 24(6), 935-939.https://doi.org/10.1128/jcm.24.6.935-939.1986

Williams, M., Izzard, L., Graves, S. R., Stenos, J., & Kelly, J. J. (2011). First probable Australian cases of human infection with *Rickettsia felis* (cat-flea typhus). *Medical Journal of Australia*, 194(1), 41-43.https://onlinelibrary.wiley.com/doi/full/10.5694/j.1326-5377.2011.tb04145.x?sid=nlm%3Apubmed

Willis, G., Lodo, K., McGregor, A., Howes, F., Williams, S., & Veitch, M. (2019). New and old hotspots for rickettsial spotted fever acquired in Tasmania, 2012–2017. *Australian and New Zealand Journal of Public Health*, 43(4), 389-394.https://doi.org/10.1111/1753-6405.12918

Woc-Colburn, A., Garner, M., Bradway, D., West, G., D'Agostino, J., Trupkiewicz, J., Barr, B., Anderson, S., Rurangirwa, F., & Nordhausen, R. (2008). Fatal coxiellosis in Swainson's Blue Mountain rainbow lorikeets (*Trichoglossus haematodus moluccanus*). *Veterinary Pathology*, 45(2), 247-254.https://journals.sagepub.com/doi/pdf/10.1354/vp.45-2-247

Woldehiwet, Z. (2004). Q fever (coxiellosis): Epidemiology and pathogenesis. *Research in Veterinary Science*, 77(2), 93-100.https://doi.org/10.1016/j.rvsc.2003.09.001

Woldeyohannes, S. M., Perkins, N. R., Baker, P., Gilks, C. F., Knibbs, L. D., & Reid, S. A. (2020). Q fever vaccine efficacy and occupational exposure risk in Queensland, Australia: A retrospective cohort study. *Vaccine*, 38(42), 6578-6584.https://doi.org/10.1016/j.vaccine.2020.08.006

Wolf, G. L., Cole, C. R., Carlisle, H. N., & Saslaw, S. (1967). The pathogenesis of Rocky Mountain spotted fever in monkeys, infected by inhalation. *Archives of Pathology*, 84(5), 486-494.https://www.scopus.com/inward/record.uri?eid=2-s2.0-0014145482&partnerID=40&md5=07c41c426b3af21ab855620e3e6bd467

WorkSafe QLD. (2022). *Q fever*. Retrieved May 12, 2022 from https://www.worksafe.qld.gov.au/safety-and-prevention/hazards/hazardous-exposures/biological-hazards/diseases-from-animals/q-fever

World Health Organization. (2014). *Report of the SAGE working group on vaccine hesitancy.* Retrieved January 12, 2022 from http://www.who.int/immunization/sage/meetings/2014/october/1_Report_WORKING_GROUP_vaccine_hesitancy_final.pdf.

World Organisation for Animal Health. (2018). Q fever - *OIE Terrestrial manual* (3.1.16), 560-577.https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.01.16_Q_FEVER.pdf

Wright, K. B. (2017). Researching internet-based populations: Advantages and disadvantages of online survey research, online questionnaire authoring software packages, and web survey services. *Journal of Computer-Mediated Communication*, 10(3).https://doi.org/10.1111/j.1083-6101.2005.tb00259.x

Xu, G., Walker, D. H., Jupiter, D., Melby, P. C., & Arcari, C. M. (2017). A review of the global epidemiology of scrub typhus. *PLoS Neglected Tropical Diseases*, 11(11), e0006062.https://doi.org/10.1371/journal.pntd.0006062

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics*, 13, 134.https://doi.org/10.1186/1471-2105-13-134

Yon, L., Duff, J. P., Ågren, E. O., Erdélyi, K., Ferroglio, E., Godfroid, J., Hars, J., Hestvik, G., Horton, D., Kuiken, T., Lavazza, A., Markowska-Daniel, I., Martel, A., Neimanis, A., Pasmans, F., Price, S. J., Ruiz-Fons, F., Ryser-Degiorgis, M.-P., Widén, F., & Gavier-Widén, D. (2019). Recent changes in infectious diseases in European wildlife. *Journal of Wildlife Diseases*, 55(1), 3-43, 41.https://doi.org/10.7589/2017-07-172

Zhang, G., Russell-Lodrigue, K. E., Andoh, M., Zhang, Y., Hendrix, L. R., & Samuel, J. E. (2007). Mechanisms of vaccine-induced protective immunity against *Coxiella burnetii* infection in BALB/c mice. *Journal of Immunology* 179(12), 8372-8380.https://doi.org/10.4049/jimmunol.179.12.8372

Zhong, J., Jasinskas, A., & Barbour, A. G. (2007). Antibiotic treatment of the tick vector *Amblyomma americanum* reduced reproductive fitness. *PloS One*, 2(5).https://doi.org/10.1371/journal.pone.0000405

## Appendix A Questionnaire for Serological Survey

THE UNIVERSITY OF SYDNEY

# Developing a Better Understanding of Q fever and Bartonellosis in Wildlife Rehabilitators – A Serosurvey

**Investigators: Karen Mathews (PhD Candidate) and Associate Professor Katrina Bosward**

We are interested in gaining further information on the immune status for Q fever and bartonellosis within the wildlife rehabilitator community of Australia.

This survey forms part of a PhD investigating the role of macropods in Q fever and bartonellosis which is being undertaken to advance our understanding of the potential role that macropods may play in the transmission of these diseases to wildlife rehabilitators.

**Thank you for taking the time to participate in our survey.**

On average this survey should take about 7 minutes to complete.

* Indicates a question is must be answered to proceed with the survey.

**Personal information:**

If you would like to receive your blood test results, please indicate below and provide your name and email or postal address. Results will be analysed and published as de-identified data, so your personal information will be kept confidential.

Please understand that if you do not wish to supply your personal details, your results will still be included in the study, but you will not have access to your blood results.

Lab ID Number: *Attach label here*

**Yes I would like to receive my Q fever blood test results** ☐

Name: ______________________________________________

Email OR postal address: (*This is the preferred address for correspondence of your lab results*)

______________________________________________

______________________________________________

## Section 1: Questions about you and where you look after wildlife.

1.1 *In the past 5 years in what capacity have you been associated with Australian mammals?

*Please check all that apply*

☐ Wildlife Carer/rehabilitator ☐ Veterinarian ☐ Wildlife researcher

☐ Veterinary nurse or technician ☐ Other - *Please specify* [ ]

☐ I have not looked after Australian mammals <u>End of survey</u> Thank you for your interest, however you are ineligible to participate in this survey.

1.2 *Gender ☐ Female ☐ Male ☐ Prefer not to say

1.3 *Age in years [ ]

1.4 *Postcode where you live? [ ]

1.5 *What is your occupation? [ ]

1.6 *<u>Within the past 5 years</u> have you been an active wildlife rehabilitator?

☐ Yes

☐ No → if no, which year did you last look after wildlife?

Please estimate if unsure (e.g. 1999) [ ]

1.7 *How many <u>years in total</u> have you been /were you a wildlife rehabilitator directly working with Australian mammals?

☐ 1 - 5 ☐ 5 – 10 ☐ more than 10

1.8 *Where do/did you <u>primarily</u> look after wildlife? *Please check all boxes that apply*

☐ Wildlife hospital

☐ Wildlife rescue centre or dedicated wildlife hospital

☐ Veterinary clinic that also sees wildlife

☐ Your home or someone else's home

☐ Zoo

☐ Other – *please specify*

1.9 *What is the postcode at which most of your wildlife rehabilitation is/was undertaken?

## Section 2: This section is about where you care for wildlife

2.1 *Do/did you look after wildlife at <u>your own property</u>?

☐ Yes ☐ No → Please proceed to **Question 2.4**

2.2 * If you rehabilitate wildlife on your own property, where are the animals housed?

*Please check each box that applies*

☐ In the house where you live

☐ In a separate building or outside enclosure (e.g. cage, shed, yard, aviary)

☐ Other - *Please specify*

2.3 *How many people in your household care for or rehabilitate wildlife?

2.4 *Please indicate approximately how many weeks a year, on average, you actively look(ed) after wildlife

☐ less than 10 ☐ 11-30 ☐ more than 30

2.5 *What is your best estimation of the number of animals that you have cared for over the past year?

2.6 ***Over the course of your wildlife rehabilitation career**, which species of wildlife have you looked after, and **how often** would they have been in your care?

*Please check all that apply*

| **Wildlife Species** | **How often would this species be in your care?** | | | |
|---|---|---|---|---|
| | Frequently | Occasionally | Rarely | Never |
| **Kangaroos** | ☐ | ☐ | ☐ | ☐ |
| **Wallabies** | ☐ | ☐ | ☐ | ☐ |
| **Wallaroo** | ☐ | ☐ | ☐ | ☐ |
| **Pademelon** | ☐ | ☐ | ☐ | ☐ |
| **Bandicoots** | ☐ | ☐ | ☐ | ☐ |
| **Possums and Gliders** | ☐ | ☐ | ☐ | ☐ |
| **Koalas** | ☐ | ☐ | ☐ | ☐ |
| **Wombats** | ☐ | ☐ | ☐ | ☐ |
| **Flying-foxes** | ☐ | ☐ | ☐ | ☐ |
| **Echidnas** | ☐ | ☐ | ☐ | ☐ |
| ***Other - please specify species below**** | ☐ | ☐ | ☐ | ☐ |
| * | ☐ | ☐ | ☐ | ☐ |

2.7 *__In the past year__ which species of wildlife have you rehabilitated and __how often__ would these species have been in your care? *Please check all that apply*

| Wildlife Species | How often would this species be in your care? | | | |
|---|---|---|---|---|
| | Frequently | Occasionally | Rarely | Never |
| **Kangaroos** | ☐ | ☐ | ☐ | ☐ |
| **Wallabies** | ☐ | ☐ | ☐ | ☐ |
| **Wallaroo** | ☐ | ☐ | ☐ | ☐ |
| **Pademelon** | ☐ | ☐ | ☐ | ☐ |
| **Bandicoots** | ☐ | ☐ | ☐ | ☐ |
| **Possums and Gliders** | ☐ | ☐ | ☐ | ☐ |
| **Koalas** | ☐ | ☐ | ☐ | ☐ |
| **Wombats** | ☐ | ☐ | ☐ | ☐ |
| **Flying-foxes** | ☐ | ☐ | ☐ | ☐ |
| **Echidnas** | ☐ | ☐ | ☐ | ☐ |
| ***Other* – *please specify species below**** | | | | |
| * | ☐ | ☐ | ☐ | ☐ |

2.8 *Please indicate in the list below the animals that reside on your property or __within a 2km radius__ of your property. *Please check all that apply*

| | | | |
|---|---|---|---|
| Cattle | ☐ | Kangaroos | ☐ |
| Sheep | ☐ | Bandicoots | ☐ |
| Goats | ☐ | Possums | ☐ |
| Horses | ☐ | Flying-foxes | ☐ |
| Pigs | ☐ | Koalas | ☐ |
| Poultry | ☐ | Wombats | ☐ |
| Cats | ☐ | *Other - Please check & specify species below** | |
| Dogs | ☐ | * | ☐ |

2.9 *__In the past 5 years__ have you had contact with any of the species listed in the table below __as part of your employment?__ *Please check all that apply*

If you don’t have contact with animals in any other occupation, please check this box

☐ and proceed to **Question 2.10**

| Wildlife Species | How often would this species be in your care? | | | |
|---|---|---|---|---|
| | Frequently | Occasionally | Rarely | Never |
| **Cattle** | ☐ | ☐ | ☐ | ☐ |
| **Sheep** | ☐ | ☐ | ☐ | ☐ |
| **Goats** | ☒ | ☐ | ☐ | ☐ |
| **Horses** | ☐ | ☐ | ☐ | ☐ |
| **Pigs** | ☐ | ☐ | ☐ | ☐ |
| **Poultry** | ☐ | ☐ | ☐ | ☐ |
| **Cats** | ☐ | ☐ | ☐ | ☐ |
| **Dogs** | ☐ | ☐ | ☐ | ☐ |
| **Kangaroos** | ☐ | ☐ | ☐ | ☐ |
| **Bandicoots** | ☐ | ☐ | ☐ | ☐ |
| **Possums and Gliders** | ☐ | ☐ | ☐ | ☐ |
| **Koalas** | ☐ | ☐ | ☐ | ☐ |
| **Wombats** | ☐ | ☐ | ☐ | ☐ |
| ***Other –*** ***please specify species below**** | | | | |
| * | ☐ | ☐ | ☐ | ☐ |

2.10 *Have you ever been present or assisted with the birth of any animal species other than humans?

☐ Frequently ☐ Occasionally ☐ Rarely ☐ Never

If you have assisted with the birth of which animal species?

***In this section, please remember your responses are anonymous and indicate the usual practices you undertake rather than the ideal.***

## Section 3: This section is about your rehabilitation practices

3.1 *In the past 5 years have you handled or reared orphaned joeys (kangaroos, wallabies, koalas, bandicoots, possums and wombats) or juvenile flying-foxes?

☐ Yes - *Please specify type*

☐ No

3.2 *Within the past year how often have you rehabilitated macropods?

☐ Frequently ☐ Occasionally ☐ Rarely ☐ Never

3.3 *When handling animals how often do you use:

| ***Check all boxes that apply*** | Always | Frequently | Occasionally | Rarely | Never |
|---|---|---|---|---|---|
| Disposable gloves | ☐ | ☐ | ☐ | ☐ | ☐ |
| Safety glasses | ☐ | ☐ | ☐ | ☐ | ☐ |
| Face mask | ☐ | ☐ | ☐ | ☐ | ☐ |
| Overalls / protective outerwear | ☐ | ☐ | ☐ | ☐ | ☐ |
| Prompt hand washing | ☐ | ☐ | ☐ | ☐ | ☐ |

3.4 *When cleaning enclosures how often do you use:

| ***Check all boxes that apply*** | Always | Frequently | Occasionally | Rarely | Never |
|---|---|---|---|---|---|
| Disposable gloves | ☐ | ☐ | ☐ | ☐ | ☐ |
| Safety glasses | ☐ | ☐ | ☐ | ☐ | ☒ |
| Face mask | ☐ | ☐ | ☐ | ☐ | ☐ |
| Overalls / protective outerwear | ☐ | ☐ | ☐ | ☐ | ☐ |
| Prompt hand washing | ☐ | ☐ | ☐ | ☐ | ☐ |

3.5 * How often do you usually wash the following items?

| Check all boxes that apply | I don't do this activity | Frequently | Occasionally | Rarely | Never |
|---|---|---|---|---|---|
| Clothes you wear when handling wildlife | ☐ | ☐ | ☐ | ☐ | ☐ |
| Clothes you wear when cleaning enclosures | ☐ | ☐ | ☐ | ☐ | ☐ |
| Dirty washable bedding | ☐ | ☐ | ☐ | ☐ | ☐ |
| Dirty pouch liners | ☐ | ☐ | ☐ | ☐ | ☐ |

3.6 *In the past 5 years have you ever been bitten by a tick?

☐ Frequently ☐ Occasionally ☐ Rarely ☐ Never

## Section 4: This section is about Q fever vaccination status and disease

4.1 *Which of the following best describes your Q fever vaccination status?

☐ Yes, I have been vaccinated → Go to Q 4.3

☐ No, I have NOT been vaccinated → Go to Q 4.2

☐ I cannot recall if I have been vaccinated → Go to Q 4.5

4.2 *Was the reason you have **NOT** been vaccinated for Q fever because you were ineligible as a result of pre-vaccination screening process?

☐ Yes → Go to Q 4.3

☐ No → Go to Q 4.5

4.3 *In what year did you receive your Q fever screening +/- vaccination?
Please estimate if unsure or enter "don't recall" if unable to estimate

4.4 *Where did you receive your Q fever screening +/- vaccination?

☐ University provided health service

☐ Workplace Screening and Vaccination Program

☐ At a private general practitioner

☐ Other - *Please Specify*

☐ I don't recall

4.5 *Have you ever had Q fever disease?

***Note: A positive skin or blood test on pre-vaccination screening is not confirmation of Q fever illness.***

☐ No – END OF QUESTIONAIRRE. Thank you for your participation

☐ Yes – go to Q 4.6

4.6 *In what year did you have Q fever disease? *Please estimate if unsure* ☐

4.7 *How was the diagnosis made?

☐ University provided health service

☐ Medical practitioner - no laboratory testing

☐ Medical practitioner - laboratory testing

☐ I suspect I have had Q fever but have not had it medically diagnosed

☐ I don't recall

END OF QUESTIONNAIRE

***THANK YOU, WE APPRECIATE YOUR WILLINGNESS TO COMPLETE THIS QUESTIONNAIRE.***

# Appendix B Knowledge, Attitudes and Practices online questionnaire

## Questionnaire for Australian Wildlife Rehabilitators About Knowledge, Attitudes and Practices regarding Q fever

You are invited to take part in an important public health survey of **Australian wildlife rehabilitators** being conducted by The School of Veterinary Science at the University of Sydney. This questionnaire has been designed to help us understand the knowledge, attitudes and practices of Australian wildlife rehabilitators with regard to Q fever disease and the Q fever vaccination. The findings from this survey may assist with developing recommendations and guidelines for Australian rehabilitators regarding Q fever disease and vaccination as well as identify areas for further research.

Please click on **next** is you wish to participate.

Next

## Important information before you start the questionnaire

- For each question, please click on the box(es) that best applies and follow instructions for questions where more than one box can be checked.
- Boxes can be un-checked if you want to change your answer.
- In this survey **'wildlife'** refers only to **Australian mammals.**

* Indicates a question is compulsory

## Section 1: Questions about you and where you look after wildlife

1.1 *In your role as a wildlife rehabilitator have you looked after Australian mammals?

☐ Yes

☐ No → skip to page notifying the respondent they are ineligible to do the survey

1.2 *Gender

☐ Female

☐ Male

☐ Prefer not to say

☐ Prefer to self-describe ______

1.3 *What is your age? ______

1.4 *Highest level of education that you have completed

*Please check one box only*

☐ High school level

☐ TAFE or Private Colleges

☐ University

☐ Postgraduate studies (PhD, Masters etc.)

1.5 *Postcode where you live? ______

1.6 *Are you currently an active wildlife rehabilitator?

☐ Yes

☐ No → if no, which year did you last look after wildlife? Please estimate if unsure (e.g. 1999) ☐

1.7 *How many years in total have you been /were you a wildlife rehabilitator, directly working with Australian mammals?

☐ 1 - 5

☐ 5 – 10

☐ more than 10

1.8 *Are/were you associated with a licensed wildlife rehabilitation group?

☐ Yes If yes, which one(s)? Please indicate in table below

☐ No

*Please check all boxes that apply*

| | | | |
|---|---|---|---|
| WIRES | ☐ | Native Arc (WA) | ☒ |
| Wildlife Victoria | ☐ | Fourth Crossing Wildlife | ☐ |
| Wildlife Rescue | ☐ | FAUNA | ☒ |
| Wildlife Rescuers (VIC) | ☐ | Brisbane Area Rescue Network | ☐ |
| Wildlife ARC | ☐ | Native Animal Rescue (WA | ☐ |
| Wildcare NT | ☐ | Native Animal Network Inc. (SA | ☐ |
| Wildcare Australia | ☐ | LAOKO | ☐ |
| Wildlife Aid | ☐ | AWARE | ☐ |
| WAWRC | ☐ | Australian Animal Rescue | ☐ |

| Tweed Valley Wildlife Rehabilitators | ☐ | ACT Wildlife | ☐ |
|---|---|---|---|
| SMWS | ☐ | North Queensland Wildlife Care | ☐ |
| Snowy Mountains Wildlife Rescue | ☐ | AWARE | ☐ |
| ONARR | ☐ | Northern Rivers Wildlife Carers | ☐ |
| Northern Tablelands Wildlife Carers | ☐ | Other Please specify below | ☐ |
| Qld Wildlife Carers & Volunteers Assoc | ☐ | | |

1.9 *Where do/did you primarily look after Australian wildlife?

*Check all boxes that apply*

☐ private animal care facility not associated with a private residence, that is not open to the general public (eg. wildlife rescue and rehabilitation facility)

☐ animal care and housing facility that is open to the general public (eg. zoo)

☐ veterinary clinic

☐ other please specify below

1.10 *What is the postcode at which most of your wildlife caring is/was undertaken?

1.11 *Do/did you look after wildlife at your own property?

☐ Yes

☐ No → Please proceed to **Question 1.14**

1.12 * If you rehabilitate wildlife on your own property, where are the animals housed?

*Please check each box that applies*

☐ In the house where you live

☐ In a separate building or outside enclosure (e.g. cage, shed, yard, aviary)

1.13 *How many people live in your household?

1.14 *Please indicate, how many animals you look after per year.

☐ 1 - 10
☐ 10-30
☐ 30-50
☐ more than 50

## Section 2: Questions about the wildlife you look after and nearby animals

2.1 *What type of Australian mammal do you, or have you, look(ed) after in your role as a wildlife rehabilitator?

*Please check boxes for **all** animal types and species that apply*

| | | | |
|---|---|---|---|
| **Kangaroos** | ☐ | **Possums** | ☐ |
| **Wallabies** | ☐ | **Wombats** | ☐ |
| **Wallaroo** | ☐ | **Koalas** | ☐ |
| **Pademelon** | ☐ | **Flying-foxes** | ☐ |
| **Bandicoots** | ☐ | **Echidnas** | ☐ |
| **Other native mammals Please specify** | ☐ | | |

.2 *The following table of questions relates to animals **living on the same property where you look after wildlife.** This may be as pets, farmed animals or animals passing through.

Please answer to the best of your knowledge and check **YES** if the situation applies.

| **Animal species** | **Living on same property as the wildlife in your care** | **Have direct contact with the wildlife in your care** | **Have direct contact with you** |
|---|---|---|---|
| **Wildlife species** | **YES** | **YES** | **YES** |
| Kangaroos | ☒ | ☐ | ☐ |
| Wallabies | ☐ | ☐ | ☐ |
| Possums | ☐ | ☐ | ☐ |
| Bandicoots | ☐ | ☐ | ☐ |
| Flying-foxes | ☐ | ☐ | ☐ |
| Koalas | ☐ | ☐ | ☐ |
| Wombats | ☐ | ☐ | ☐ |
| Echidnas | ☐ | ☐ | ☐ |
| Birds | ☐ | ☐ | ☐ |
| Reptiles | ☐ | ☐ | ☐ |
| Rabbits | ☐ | ☐ | ☐ |
| Mice and rats | ☐ | ☐ | ☐ |
| Other Please specify | ☐ | ☐ | ☐ |
| None of the above | ☐ | ☐ | ☐ |
| **Domestic species** | **YES** | **YES** | **YES** |
| Cattle | ☐ | ☐ | ☐ |
| Sheep | ☐ | ☐ | ☐ |
| Goats | ☐ | ☐ | ☐ |
| Horses | ☐ | ☐ | ☐ |
| Pigs | ☐ | ☐ | ☐ |
| Poultry | ☐ | ☐ | ☐ |
| Cats | ☐ | ☐ | ☐ |
| Dogs | ☐ | ☐ | ☐ |
| Pet rabbits | ☐ | ☐ | ☐ |
| Pet mice or rats | ☐ | ☐ | ☐ |
| Other Please specify | ☐ | ☐ | ☐ |
| None of the above | ☐ | ☐ | ☐ |

2.3 *The following table of questions relates to **animals living outside of the property boundaries, but within 2km of that property where you look after wildlife**

Please answer to the best of your knowledge and click on **YES** if the situation applies. If you live in a suburban area you may only be able to answer regarding close neighbours.

| **Animal species** | **Living outside of but within 2km of the property where you look after wildlife** | **Have direct contact with the wildlife in your care** | **Have direct contact with you** |
|---|---|---|---|
| **Wildlife species** | **YES** | **YES** | **YES** |
| Kangaroos | ☐ | ☐ | ☐ |
| Wallabies | ☐ | ☐ | ☐ |
| Possums | ☐ | ☐ | ☐ |
| Bandicoots | ☐ | ☐ | ☐ |
| Flying-foxes | ☐ | ☐ | ☐ |
| Koalas | ☐ | ☐ | ☐ |
| Wombats | ☐ | ☐ | ☐ |
| Echidnas | ☐ | ☐ | ☐ |
| Birds | ☐ | ☐ | ☐ |
| Reptiles | ☐ | ☐ | ☐ |
| Rabbits | ☐ | ☐ | ☐ |
| Mice and rats | ☐ | ☐ | ☐ |
| Other<br>Please Specify | ☐ | ☐ | ☐ |
| None of the above | ☐ | ☐ | ☐ |
| **Domestic species** | **YES** | **YES** | **YES** |
| Cattle | ☐ | ☐ | ☐ |
| Sheep | ☐ | ☐ | ☐ |
| Goats | ☐ | ☐ | ☐ |
| Horses | ☐ | ☐ | ☐ |
| Pigs | ☐ | ☐ | ☐ |
| Poultry | ☐ | ☐ | ☐ |
| Cats | ☐ | ☐ | ☐ |
| Dogs | ☐ | ☐ | ☐ |
| Pet rabbits | ☐ | ☐ | ☐ |
| Pet mice or rats | ☐ | ☐ | ☐ |
| Other<br>Please Specify | ☐ | ☐ | ☐ |
| None of the above | ☐ | ☐ | ☐ |

2.4 *If you have another occupation as well as being a wildlife rehabilitator, **in that occupation** do you have contact with any of the animals listed below?

☐ I don't have contact with animals in any other occupation

*Check all boxes that apply*

☐ Cattle
☐ Sheep
☐ Goats
☐ Horses
☐ Pigs
☐ Poultry
☐ Cats
☐ Dogs
☐ Horses
☐ Other

☐ Kangaroos
☐ Bandicoots
☐ Possums
☐ Flying-foxes
☐ Koalas
☐ Wombats
☐ Birds
☐ Rabbits
☐ Rodents

If your answer was 'other' please specify

**2.5** *Have you ever been present or assisted with the birth of any animal species other than humans?

☐ Yes - If yes, which animal species
☐ No

2.6 *Have you ever been bitten by a tick while in your role as a wildlife rehabilitator?

☐ Yes

☐ No

☐ Unsure

## Section 3: Questions about your rehabilitation practices

3.1 *Do you hand rear orphaned joeys (kangaroos, wallabies, koalas, bandicoots, possums and wombats) or juvenile flying-foxes?

☐ Yes if yes, please indicate which type of joey or pup (e.g. kangaroo, possum)

☐ No

*Please indicate the **personal protection** you typically use in the following scenarios.

3.2 * When handling animals how often do you use:

| *Check all boxes that apply* | Always | Frequently | Occasionally | Rarely | Never |
|---|---|---|---|---|---|
| **Disposable gloves** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Safety glasses** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Face mask** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Overalls / protective outerwear** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Prompt hand washing** | ☐ | ☐ | ☐ | ☐ | ☐ |

3.3 *When cleaning enclosures how often do you use:

| *Check all boxes that apply* | Always | Frequently | Occasionally | Rarely | Never |
|---|---|---|---|---|---|
| **Disposable gloves** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Safety glasses** | ☐ | ☐ | ☐ | ☐ | ☒ |
| **Face mask** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Overalls / protective outerwear** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Prompt hand washing** | ☐ | ☐ | ☐ | ☐ | ☐ |

3.4 *How do you dispose of the following materials and wastes?

| *Check all boxes that apply* | **I don't do this activity** | **In the rubbish bin** | **In an uncovered compost heap** | **In a covered compost bin** | **Burn** | **Bury** | **Take to tip** | **Flush down the toilet** | **Other – please specify below** |
|---|---|---|---|---|---|---|---|---|---|
| **Dead animals** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Urine and faeces** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Pouch liners** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Soiled bedding if discarding** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Lawn clippings from animal living areas** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

3.5 *How often do you clean enclosures – that is, remove faeces, uneaten food and bedding?

*Please check one box only*

☐ Once a day

☐ More often than once a day

☐ Every second day

☐ Twice weekly

☐ Weekly

☐ Not applicable

3.6 *Please check all the activities you undertake when cleaning enclosures, cages and aviaries etc.

*Please check all the activities you do*

☐ Roll any enclosure paper linings to capture faeces, urine and uneaten food?

☐ Hose out cages/enclosure

☐ Sweep or rake out faeces and urine in cages/enclosures

☐ Mow lawns or disturb the soil around cages/enclosures

☐ Wear the same clothes into your home afterwards

☐ Change your clothes before doing other activities

3.7 *Do you use a disinfectant when cleaning enclosures or anywhere the wildlife under your care is living?

☐ Yes -> proceed to 3.8

☐ No → proceed to **Question 3.9**

3.8 *When do you use the disinfectant?

☐ Before cleaning/hosing down

☐ After cleaning/hosing down

3.9 *How do you usually wash the following items?

| *Check all boxes that apply* | **I don't do this activity** | **Cold water & detergent** | **Hot water & detergent** | **Soak in sanitizer before washing (e.g. Milton)** | **Other** |
|---|---|---|---|---|---|
| **Clothes you wear when handling wildlife** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Clothes you wear when cleaning enclosures** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Dirty washable bedding** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Dirty pouch liners** | ☐ | ☐ | ☐ | ☐ | ☐ |

3.10 *Please select the statement that describes your washing practices

| *Check all boxes that apply* | Yes | No | Sometimes |
|---|---|---|---|
| The clothes that I wear while caring for wildlife and other clothing are washed in the same machine. | ☐ | ☐ | ☐ |
| The clothes that I wear while caring for wildlife are washed in the same machine but in different loads. | ☐ | ☐ | ☐ |
| I do not always separate my wildlife clothing from my other clothing | ☐ | ☐ | ☐ |
| Other family members are also involved in washing clothes and bedding. | ☐ | ☐ | ☐ |
| I care for wildlife in my day to day clothing. | ☐ | ☐ | ☐ |

## 4: Questions about what you know and how you feel about Q fever

4.1 *Before this questionnaire, had you heard of Q fever?

☐ Yes

☐ No → proceed to **Section 4.6 -**

4.2 *Where have you heard about Q fever?

*Please check all boxes that apply*

☐ Social media

☐ Web-based article

☐ Newspaper or magazines

☐ TV

☐ Radio

☐ Doctor

☐ Veterinarian or Vet nurse

☐ Wildlife rehabilitation group

☐ Wildlife conference

☐ Family or friend

☐ Other wildlife rehabilitator

☐ Government agency

☐ Training session

☐ Other – please specify below

4.3 *What do you think are the most common way(s) a person can acquire Q fever?

*Check all the ways you think apply*

☐ Contact with other infected people

☐ Contact with infected animals, animal tissues or body fluids

☐ Inhalation of Infected dust, including dust spread by the wind

☐ Contact with environments and objects contaminated by infected animals

☐ Tick bites

☐ Mosquito bites

☐ Animal bites

☐ Drinking contaminated raw (un-pasteurised) dairy products such as milk and cheese

☐ Unsure

**Please answer true or false to the following questions: (Knowledge measure questions)**

4.4 *Animals that are infected with the causative agent of Q fever are usually obviously sick

☐ True ☐ False ☐ Unsure

4.5 *Q fever in people is easily recognised and diagnosed

☐ True ☐ False ☐ Unsure

4.6 *Q fever can cause serious long-term illness in people

☐ True ☐ False ☐ Unsure

4.7 *The organism that causes Q Fever is a

☐ fungus ☐ parasite ☐ virus ☐ bacteria

4.8 *The main route of infection for the organism that causes Q fever is

☐ through cuts and open wounds on the skin

☐ inhaling the airborne organism

☐ eating contaminated food

☐ directly across the skin surface

4.9 *Please select one response that describes your feelings about the statement:

"*Wildlife rehabilitators are at risk of Q fever from contact with Australian native mammals*".

*Please check only one box*

☐ Strongly disagree

☐ Disagree

☐ Neither agree nor disagree

☐ Agree

☐ Strongly agree

## Section 5. Questions about Q fever vaccination

5.1 *Before this questionnaire, did you know that there is a vaccine available in Australia that protects people against Q fever?

☐ Yes

☐ No → if no, please proceed to **Question 5.75**

5.2 *Have you ever been vaccinated against Q fever?

☐ Yes → if yes, please proceed to **Question 5.3**

☐ No → if no, please proceed to **Question 5.4**

☐ Can't recall → please proceed to **Question 5.5**

5.3 *In which year were you vaccinated? Please estimate if unsure (e.g. 1999) ☐

→ Please proceed now to **Question 5.7**

5.4 *Please indicate any reasons for you ***not*** getting vaccinated to date.

*Check as many boxes as apply*

☐ The pre-vaccination screening process indicated I should not have the vaccine

☐ I have had medically diagnosed Q fever disease and was advised I am unable to be vaccinated

☐ The cost of getting vaccinated is too expensive

☐ It is too difficult to find a medical practitioner who gives the vaccine

☐ Vaccination is not provided by my employer or wildlife rehabilitation group

☐ Pre-screening and vaccination is too time consuming

☐ I think the Q fever vaccine may harm my health

☐ I think the Q fever vaccine may not be effective

☐ I don't think Q fever is serious enough to require vaccination

☐ I don't think I am at risk of acquiring Q fever

☐ I was told I was not at risk – please specify by whom

☐ Haven't got around to doing it

☐ I was unaware I needed to

☐ Other – please specify below

5.5 *Would you consider being vaccinated against Q fever in the future?

☐ Yes

☐ No

☐ Unsure

5.6 *Has anyone recommended that you should be vaccinated for Q fever?

☐ Yes →If yes, please indicate who made the recommendation:

☐ No → if no, please proceed to **Question5.7**

*Check as many boxes as apply*

☐ Doctor

☐ Veterinarian

☐ Veterinary Nurse

☐ Friend

☐ Other wildlife rehabilitator

☐ Speaker at a wildlife conference/workshop or training session

☐ Government

☐ Employer

☐ University/college

☐ Wildlife rehabilitation group

☐ Other Please specify below

5.7 *For the following 5 statements, we are interested in your ***feelings*** and would like you to indicate your level of agreement:

| *Check only one box* | Strongly disagree | Disagree | Neither agree nor disagree | Agree | Strongly Agree | Unsure |
|---|---|---|---|---|---|---|
| 1. “Vaccines against diseases are usually a good way to protect someone against the disease” | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 2. “I worry that vaccines can do more harm than good” | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 3.”I am convinced of the importance of Q fever vaccination” | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 4. “The Q fever vaccine is effective in preventing Q fever” | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 5.“The Q fever vaccine is safe when administered appropriately” | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

5.8 *Are there any other comments or observations you’d like to make regarding Q fever and the Q fever vaccination?

## Section 6: Questions about Q fever exposure and disease

6.1 *In the time you have been rehabilitating wildlife, on average, what do you think your level of exposure to the Q fever causative agent may have been?

*Please check one box only*

☐ Unsure

☐ No Exposure

☐ Low exposure

☐ Moderate exposure

☐ High exposure

6.2 *Have you ever had Q fever disease?

***A positive skin or blood test on pre-vaccination screening is not confirmation of Q fever illness.***

☐ Yes

☐ No → if no, please proceed to **Question 6.9**

6.3 In what **year** did you have Q fever disease? Please estimate if unsure ☐

6.4 *How were you diagnosed with Q fever disease? *Please check only **one** box*

☐ Medical Practitioner, with **no** laboratory testing

☐ Medical Practitioner, **confirmed with laboratory testing**

☐ I suspect I have had Q fever but have not had it medically diagnosed

☐ Other - Please specify

6.5 *Were you hospitalised during your Q fever illness?

☐ Yes

☐ No → if no, please proceed to **Question 5.7**

6.6 *For how many days were you hospitalised? ☐ days

6.7 *To what extent did you experience each of the following when you had Q fever disease?

| *Please check one box in each horizontal row* | **Did not experience** | **Mild** | **Moderate** | **Severe** |
|---|---|---|---|---|
| **Fever and chills** | ☐ | ☐ | ☐ | ☐ |
| **Sweats** | ☐ | ☐ | ☐ | ☐ |
| **Headaches** | ☐ | ☐ | ☐ | ☐ |
| **Muscle and joint pains** | ☐ | ☐ | ☐ | ☐ |
| **Endocarditis (heart valve disease)** | ☐ | ☐ | ☐ | ☐ |
| **Hepatitis (liver disease)** | ☐ | ☐ | ☐ | ☐ |
| **Fatigue** | ☐ | ☐ | ☐ | ☐ |
| **Pneumonia** | ☐ | ☐ | ☐ | ☐ |

| Other – please describe below and indicate severity | ☐ | ☐ | ☐ | ☐ |
|---|---|---|---|---|

Please specify 'Other' as indicated above

| |
|---|
| |

6.8 *Have you experienced pregnancy complications that were attributed to Q fever?

☐ Yes

☐ No

6.9 *Have you been medically diagnosed as having Post Q fever fatigue syndrome?

☐ Yes

☐ No

6.10 * How many people living in your household, apart from yourself, have been diagnosed with Q fever disease?

☐ 0

☐ 1

☐ 2

☐ 3

☐ ≥ 4

## Section 7: Survey completion

We greatly appreciate your help with this study and thank you for taking the time to complete this questionnaire.

After you submit the questionnaire you will be redirected to a link that will give you the option to

register to receive a summary report of the findings and/or enter the prize draw for an iPad Air 2

In addition to this survey, we are conducting a serosurvey to gain further information on the immune status for Q fever within the Australian wildlife rehabilitator community. The survey will involve giving a blood sample which will be tested to determine if you have been previously exposed to Q fever and these blood test results will be confidentially available to participants. There will be an opportunity to participate in the serosurvey at the National Wildlife Rehabilitators Conference in Sydney in July 2018 however if you are unable to attend the conference but would like to participate please supply your email address in the link provided and we will contact you to advise how you may participate in this important study.

Please note that this is done via separate links which will not be linked to your responses to this questionnaire to ensure that your responses remain anonymous.

Thank you once again for your participation in this important work.

SUBMIT

## Section 8: Additional links

Thankyou your questionnaire has been submitted.

Please indicate your preference by checking the box(es)

☐ Yes I would like to enter the draw to win an iPAD

☐ Yes I would like to receive the summary of findings of this study

☐ Yes I am interested in participating in the serosurvey please provide me with further details

If you answered **yes** to any of the above, please provide your contact details below. Please note your personal details will be separated and de-identified from your survey responses, preserving your confidentiality and anonymity.

Please indicate how you would like to be contacted

☐ Email: [ ]

☐ Postal: 

If you would like to obtain further information about Q fever we recommend the following websites:

Health NSW Fact sheet

http://www.health.nsw.gov.au/infectious/factsheets/pages/q-fever.aspx

SafeWork NSW Factsheet

http://www.safework.nsw.gov.au/health-and-safety/safety-topics-a-z/diseases/q-fever

Australian Immunisation Handbook 10th Edition 2013 – Chapter on Q fever

http://www.immunise.health.gov.au/internet/immunise/publishing.nsf/Content/Handbook10-home~handbook10part4~handbook10-4-15

Wildlife Health Australia – Q fever in Australian wildlife fact sheet

https://www.wildlifehealthaustralia.com.au/Portals/0/Documents/FactSheets/Mammals/Q%20Fever%20in%20Australian%20Wildlife%20Jun%202013%20(1.4).pdf

*If you have any concerns surrounding Q fever infection, with regards to yourself or someone you may know, please contact your GP as soon as possible to discuss these concerns and potential Q fever testing.

# Appendix C Univariable analysis for risk factors associated with self-reported Q fever

**Table C1.** Results of univariable analysis for risk factors associated with self-reported Q fever among 287 unvaccinated Australian wildlife rehabilitators participating in a nationwide online survey conducted in 2018.

| | | Self-reported Q fever | | | | |
|---|---|---|---|---|---|---|
| Variable Name and Description | Total number | Yes | No | Odds ratio | 95% LCL | *P*-value |
| **Age** | 287 | | | | | 0.185 |
| ≤50 | | 3 | 112 | 1 | | |
| >50 | | 10 | 162 | 2.30 | 0.687 - 10.448 | |
| **Education level** | 287 | | | | | 0.036 |
| University/Postgraduate | | 2 | 118 | 1 | | |
| High School Level/TAFE or private college | | 11 | 156 | 4.16 | 1.090 - 27.213 | |
| **Primary rehabilitated Australian wildlife at a veterinary clinic** | 287 | | | | | 0.011 |
| No | | 9 | 256 | 1 | | |
| Yes | | 4 | 18 | 6.32 | 1.592 - 21.567 | |
| **Rehabilitate kangaroos or wallabies** | 287 | | | | | 0.185 |
| No | | 2 | 87 | 1 | | |
| Yes | | 11 | 187 | 2.56 | 0.657 - 16.486 | |
| **No or non-ruminant occupational animal contact** | 287 | | | | | 0.014 |
| No | | 8 | 243 | 1 | | |
| Yes | | 5 | 31 | 4.90 | 1.407 - 15.647 | |
| **Present at non-human mammalian births** | 287 | | | | | 0.167 |
| No | | 5 | 137 | 1 | | |
| Have attended ruminant birth | | 6 | 61 | 2.69 | 0.783- 9.675 | 0.113 |
| Have not attended ruminant birth | | 2 | 76 | 0.712 | 0.102 - 3.433 | 0.700 |
| **Kangaroos and/or wallabies living on the same property where wildlife is rehabilitated** | 287 | | | | | |
| No | | 4 | 141 | 1 | | 0.140 |
| Yes | | 9 | 133 | 2.39 | 0.757 - 8.973 | |
| **Domestic ruminants living on the same property where wildlife is rehabilitated** | 287 | | | | | 0.006 |
| No | | 6 | 223 | 1 | | |
| Yes | | 7 | 51 | 5.10 | 1.629 - 16.468 | |

TAFE - Technical and Further Education, CI – confidence intervals

# Appendix D Factors associated with Q fever vaccination

**Table D1.** Descriptive (n, %, totals) and odds ratio (and Chi-square *p*-value) results for variables tested for association with awareness of Q fever and Q fever vaccination among Australian wildlife rehabilitators participating in a nationwide online survey conducted in 2018 (n=338).

| Variable name and description | Awareness of Q fever and the Q fever vaccine n (%) | | | Odds ratio | *p*-value |
|---|---|---|---|---|---|
| | Aware (n=200) | Unaware (n=138) | Row total | | |
| **Age** | | | | | 0.440 |
| >50 | 106 (57.3) | 79 (42.7) | 185 (54.7) | Ref | |
| ≤50 | 94 (61.4) | 59 (38.6) | 153 (45.3) | 1.19 | |
| **Education level** | | | | | 0.744 |
| High School Level/TAFE or private college | 108 (58.4) | 77 (41.6) | 185 (54.7) | Ref | |
| University/Postgraduate | 92 (60.1) | 61 (39.9) | 153 (45.3) | 1.08 | |
| **State of residence*** | | | | | 0.001 |
| Other | 31 (40.3) | 46 (59.7) | 77 (22.8) | Ref | |
| NSW/ACT | 121 (63.7) | 69 (36.3) | 190 (56.2) | 2.60 | |
| Queensland | 48 (67.6) | 23 (32.4) | 71 (21.0) | 3.10 | |
| **Primary place of rehabilitating Australian wildlife** | | | | | |
| Wildlife rescue facility closed to the public | | | | | 0.321 |
| No | 177 (60.2) | 117 (39.8) | 294 (87.0) | Ref | |
| Yes | 23 (52.3) | 21 (47.7) | 44 (13.0) | 0.72 | |
| Wildlife rescue facility open to the public eg zoo | | | | | 0.050 |
| No | 188 (58.0) | 136 (42.0) | 324 (95.9) | Ref | |
| Yes | 12 (85.7) | 2 (14.3) | 14 (4.1) | 4.30 | |
| Veterinary clinic | | | | | 0.153 |
| No | 178 (58.0) | 129 (42.0) | 307 (90.8) | Ref | |
| Yes | 22 (71.0) | 9 (29.0) | 31 (9.2) | 1.77 | |
| Private residence | | | | | 0.536 |
| No | 23 (54.8) | 19 (45.2) | 42 (12.4) | Ref | |
| Yes | 177 (59.8) | 119 (40.2) | 296 (87.6) | 1.23 | |
| **Number of animals rehabilitated per year** | | | | | 0.426 |
| 1-50 | 151 (58.1) | 109 (41.9) | 260 (76.9) | Ref | |
| ≥ 50 | 48 (63.2) | 28 (36.8) | 76 (22.5) | 1.24 | |
| **Number of years rehabilitating Australian wildlife** | | | | | 0.707 |
| 1-10 | 106 (58.2) | 76 (41.8) | 182 (53.8) | Ref | |
| ≥ 10 | 94 (60.3) | 62 (39.7) | 156 (46.2) | 1.09 | |
| **Occupational animal contact*** | | | | | <0.001 |
| No | 107 (50.2) | 106 (49.8) | 213 (63.0) | Ref | |
| Yes | 93 (74.4) | 32 (25.6) | 125 (37.0) | 2.88 | |
| **Present at an animal birth*** | | | | | 0.001 |
| No | 79 (50.0) | 79 (50.0) | 158 (46.7) | Ref | |
| Yes | 121 (67.2) | 59 (32.8) | 180 (53.3) | 2.05 | |
| **Domestic ruminants living on the same property** | | | | | |
| No | 150 (56.8) | 114 (43.2) | 264 (78.1) | Ref | 0.432 |
| Yes | 50 (67.6) | 24 (32.4) | 74 (21.9) | 0.83 | |

Rehabilitators were classified as 'aware' of Q fever if they had heard of Q fever and the Q fever vaccine and 'unaware' if they had not heard of Q fever or had heard of Q fever but had not heard of the Q fever vaccine. NSW New South Wales, ACT Australian Capital Territory, * Fisher's exact test, Ref-Reference category

**Table D2.** Descriptive (n, %, totals) and odds ratio (and Chi-square *p*-value) results for variables analysed for association with Q fever vaccination status among Australian wildlife rehabilitators who were aware of Q fever and the availability of Q fever vaccine (n=200). Results are from a nationwide online survey conducted in 2018.

| Variable name and description | Q fever vaccination status n (%) | | | Odds ratio | *p*-value |
|---|---|---|---|---|---|
| | Vaccinated (n=51)) | Not vaccinated (n=149) | Row total | | |
| Age | | | | | 0.000 |
| >50 | 13 (12.3) | 93 (87.7) | 106 (53.0) | Ref | |
| ≤50 | 38 (40.4) | 56 (59.6) | 94 (47.0) | 4.85 | |
| Education level | | | | | 0.002 |
| High School Level/TAFE or private college | 18 (16.7) | 90 (83.3) | 108 (54.0) | Ref | |
| University/Postgraduate | 33 (35.9) | 59 (64.1) | 92 (46.0 | 2.80 | |
| State of residence | | | | | 0.097 |
| Other | 6 (19.4) | 25 (80.6) | 31 (15.5) | Ref | |
| NSW/ACT | 27(22.3) | 94 (77.7) | 121 (60.5) | 1.20 | |
| Queensland | 18 37.5) | 30 (62.5) | 48 (24.0) | 2.50 | |
| Primary place of rehabilitating Australian wildlife | | | | | |
| Wildlife rescue facility closed to the public | | | | | 0.945 |
| No | 45 (25.4) | 132 (74.6) | 177 (88.5) | Ref | |
| Yes | 6 (26.1) | 17 (73.9) | 23 (11.5) | 1.04 | |
| Wildlife rescue facility open to the public eg zoo | | | | | 0.060 |
| No | 45 (23.9) | 143 (76.1) | 188 (94.0) | Ref | |
| Yes | 6 (50.0) | 6 (50.0) | 12 (6.0) | 1.24 | |
| Veterinary clinic | | | | | 0.093 |
| No | 42 (23.6) | 136 (76.4) | 178 (89.0) | Ref | |
| Yes | 9 (40.9) | 13 (59.1) | 22 (11.0) | 1.24 | |
| Private residence | | | | | 0.570 |
| No | 7 (30.4) | 16 (69.6) | 23 (11.5) | Ref | |
| Yes | 44 (24.9) | 133 (75.1) | 177 (88.5) | 0.76 | |
| Number of animals rehabilitated per year | | | | | 0.044 |
| 1-50 | 43 (28.5) | 108 (71.5) | 151 (75.5) | Ref | |
| ≥ 50 | 7 (14.6) | 41 (85.4) | 48 (24.0) | 0.43 | |
| Number of years rehabilitating Australian wildlife | | | | | 0.051 |
| 1-10 | 33 (31.1) | 73 (68.9) | 106 (53.0) | Ref | |
| ≥ 10 | 18 (19.1) | 76 (80.9) | 94 (47.0) | 0.52 | |
| Occupational animal contact | | | | | 0.007 |
| No | 19 (17.8) | 88 (82.2) | 107 (53.5) | Ref | |
| Yes | 32 (34.4) | 61 (65.6) | 93 (46.5) | 2.43 | |
| Present at an animal birth | | | | | 0.165 |
| No | 16 (20.3) | 63 (79.7) | 79 (39.5) | Ref | |
| Yes | 35 (28.9) | 86 (71.1) | 121 (60.5) | 1.60 | |
| Domestic ruminants living on the same property | | | | | 0.231 |
| No | 35 (23.3) | 115 (76.7) | 150 (75.0) | Ref | |
| Yes | 16 (32.0) | 34 (68.0) | 50 (25.0) | 1.55 | |

| Variable name and description | Q fever vaccination status n (%) | | | Odds ratio | *p*-value |
|---|---|---|---|---|---|
| | Vaccinated (n=51)) | Not vaccinated (n=149) | Row total | | |
| Source hearing about Q fever | | | | | |
| Media | | | | | <0.001 |
| No | 46 (31.9) | 98 (68.1) | 144 (72.0) | Ref | |
| Yes | 5 (8.9) | 51 (91.1) | 56 (28.0) | 0.21 | |
| Medical practitioner | | | | | 0.2 |
| No | 39 (23.6) | 126 (76.4) | 165 (82.5) | Ref | |
| Yes | 12 (34.3) | 23 (65.7) | 35 (17.5) | 1.69 | |
| Veterinarian or veterinary nurse | | | | | 0.101 |
| No | 31 (22.1) | 109 (79.9) | 140 (70.0) | Ref | |
| Yes | 20 (33.3) | 40 (66.7) | 60 (30.0) | 1.76 | |
| Wildlife associated activities | | | | | 0.001 |
| No | 32 (37.2) | 54 (62.8) | 86 (43.0) | Ref | |
| Yes | 19 (16.7) | 95 (83.3) | 114 (57.0) | 0.34 | |
| Employment/education | | | | | 0.013 |
| No | 32 (21.1) | 120 (78.9) | 152 (76.0) | Ref | |
| Yes | 19 (39.6) | 29 (60.4) | 48 (24.0) | 2.46 | |
| Family member or friend | | | | | 0.019 |
| No | 47 (28.7) | 117 (71.3) | 164 (82.0) | Ref | |
| Yes | 4 (11.1) | 32 (88.9) | 36 (18.0) | 0.31 | |

TAFE-Technical and Further Education, Ref-Reference category

# Appendix E CoxMP assay development and wildlife PCR results

Optimisation of *Coxiella burnetii* multiplex PCR

Due to the large number of samples requiring analysis a multiplex qPCR assay targeting two single copy genes: *groEL* (heat shock operon; *htpAB*) and *com1* the outer membrane protein-coding gene and the multicopy insertion sequence gene IS*1111* (Bond et al., 2016; de Bruin et al., 2011; Lockhart et al., 2011) was optimised for the detection and quantification of *C. burnetii* DNA in extracted samples. Characterisation of the multiplex assay was guided by the MIQE (minimum information for publication of quantitative real-time PCR experiments) guidelines (Bustin et al., 2009).

The multiplex assay was optimised using a standard curve generated from tenfold serial dilutions of commercially available *C. burnetii* DNA of known copy number (Amplirun® Vircell, Granada, Spain). Standards between 1 and 11 000 copies per reaction were used to generate standard curves from which amplification efficiencies were calculated for the three target genes. To investigate reproducibility, mean Cq values and standard deviations were calculated for each dilution. The limit of detection was defined as the lowest DNA concentrations at which all of the positive samples were detected. Each standard was performed in triplicate and each reaction contained 5µL 1X SensiFAST Probe No-ROX Kit (BioLine, Australia), primers and probe (**Table 5.3**), 2µl *C. burnetii* DNA standard and nuclease free water in a total volume of 10µl. Reactions were also performed in singleplex using the same reaction conditions described above. Amplification and fluorescence detection was performed in a Bio-Rad-CFX Real-Time PCR Thermocycler (Bio-Rad laboratories Pty Ltd, Gladesville, NSW, Australia) according to the following cycling parameters: initial denaturation at 95 $^{o}$C for 3 minutes followed by 40 cycles of denaturation at 95$^{o}$C for 10 seconds and annealing and extension at 60$^{o}$C for 40 seconds. Each qPCR run included a NTC (with nuclease-free water in place of DNA). Primers and

probes for the optimisation of the multiplex qPCR reactions were purchased from Integrated DNA Technologies (Baulkham Hills, Australia).

**Table E1**. Results of singleplex and multiplex *Coxiella burnetii* qPCR assays using three gene targets

| IS*1111* | | | | | | |
|---|---|---|---|---|---|---|
| | Singleplex | | | Multiplex | | |
| Copies/reaction | Mean Cq | SD | Co Var | Mean Cq | SD | Co Var |
| 11000.0 | 24.2 | 0.036 | 0.149 | 24.1 | 0.085 | 0.354 |
| 1100.0 | 27.6 | 0.083 | 0.299 | 27.5 | 0.105 | 0.381 |
| 110.0 | 31.3 | 0.075 | 0.238 | 31.2 | 0.034 | 0.110 |
| 11.0 | 34.8 | 0.163 | 0.468 | 35.4 | 0.183 | 0.517 |
| 1.1 | - | - | - | - | - | - |
| Efficiency | 92.4 | | | 93.9 | | |
| $R^2$ | 0.999 | | | 0.989 | | |
| *com1* | | | | | | |
| | Singleplex | | | Multiplex | | |
| Copies/reaction | Mean Cq | SD | Co Var | Mean Cq | SD | Co Var |
| 11000.0 | 26.0 | 0.031 | 0.117 | 25.8 | 0.056 | 0.216 |
| 1100.0 | 29.7 | 0.066 | 0.223 | 29.4 | 0.010 | 0.036 |
| 110.0 | 33.5 | 0.330 | 0.985 | 33.3 | 0.040 | 0.121 |
| 11.0 | 37.5 | 0.609 | 1.621 | 37.6 | 0.719 | 1.914 |
| 1.1 | - | - | - | - | - | - |
| Efficiency | 94.2 | | | 91 | | |
| $R^2$ | 0.967 | | | 0.977 | | |
| *htpAB* | | | | | | |
| | Singleplex | | | Multiplex | | |
| Copies/reaction | Mean Cq | SD | Co Var | Mean Cq | SD | Co Var |
| 11000.0 | 24.9 | 0.017 | 0.069 | 24.9 | 0.120 | 0.483 |
| 1100.0 | 28.4 | 0.120 | 0.423 | 28.4 | 0.152 | 0.535 |
| 110.0 | 32.2 | 0.164 | 0.511 | 32.0 | 0.126 | 0.394 |
| 11.0 | 35.4 | 0.828 | 2.342 | 37.0 | 1.515 | 4.089 |
| 1.1 | - | - | - | - | - | - |
| Efficiency | 99.2 | | | 94.2 | | |
| $R^2$ | 0.985 | | | ` | | |

*Insertion sequence 1111 (*IS1111*), **Heat shock operon (*htpAB*), ***Outer membrane protein (*com1*)

SD-standard deviation, Cq-quantification cycle, Co-Var-coefficient of variation

**Table E2.** Cut-off quantification cycles (Cq) for multiplex *Coxiella burnetii* qPCR assays

| | *IS1111* | *com1* | *htpAB* |
|---|---|---|---|
| Positive | Cq ≤ 34 | Cq ≤ 36 | Cq ≤ 35 |
| Negative | Cq >34 | Cq >36 | Cq >35 |

*Insertion sequence 1111 (*IS1111*), **Heat shock operon (*htpAB*), ***Outer membrane protein (*com1*)

## Testing DNA extracts for the presence of inhibitors

**Table E3.** Quantification cycle (Cq), change and mean change in Cq for the different sample types.

| Sample type | Sample ID | Cq (neat) | Cq (1/10) | Δ Cq | Mean Δ Cq |
|---|---|---|---|---|---|
| urine | D1 | 28.6 | 31.6 | 3.0 | 3.1 |
| | D2 | 24.7 | 28.0 | 3.3 | |
| | D3 | 31.0 | 34.0 | 3.0 | |
| | D4 | 22.5 | 25.5 | 3.0 | |
| | D5 | 25.3 | 28.4 | 3.1 | |
| | D6 | 26.6 | 29.1 | 2.5 | |
| | D7 | 27.2 | 30.4 | 3.2 | |
| | D8 | 28.5 | 32.2 | 3.7 | |
| cloacal swab | D1 | 21.5 | 25.2 | 3.7 | 3.5 |
| | D2 | 34.8 | 37.4 | 2.6 | |
| | D3 | 27.8 | 33.7 | 5.9 | |
| | D4 | 19.4 | 23.0 | 3.6 | |
| | D5 | 23.1 | 26.4 | 3.3 | |
| | D6 | 19.1 | 22.1 | 3.0 | |
| | D7 | 18.7 | 21.8 | 3.1 | |
| | D8 | 21.6 | 24.5 | 2.9 | |
| faeces | D1 | 30.2 | 39.9 | 9.7 | 4.3 |
| | D2 | 25.4 | 29.5 | 4.1 | |
| | D3 | 24.5 | 28.1 | 3.6 | |
| | D4 | 24.0 | 27.4 | 3.4 | |
| | D5 | 32.3 | 35.9 | 3.6 | |
| | D6 | 29.2 | 32.6 | 3.4 | |
| | D7 | 28.4 | 31.8 | 3.4 | |
| | D8 | 27.2 | 30.5 | 3.3 | |
| ilium | D1 | 16.1 | 18.7 | 2.6 | 3.5 |
| | D2 | 15.9 | 19.8 | 3.9 | |
| | D3 | 15.4 | 18.7 | 3.3 | |
| | D4 | 16.9 | 20.4 | 3.5 | |
| | D5 | 14.9 | 18.3 | 3.4 | |
| | D6 | 16.6 | 20.3 | 3.7 | |
| | D7 | 17.6 | 21.4 | 3.8 | |
| | D8 | 15.8 | 19.9 | 4.1 | |
| spleen | D1 | 20.9 | 23.1 | 2.2 | 3.2 |
| | D2 | 17.1 | 20.3 | 3.2 | |
| | D3 | 20.1 | 23.6 | 3.5 | |
| | D4 | 16.3 | 19.5 | 3.2 | |
| | D5 | 16.8 | 20.3 | 3.5 | |
| | D6 | 16.0 | 19.3 | 3.3 | |
| | D7 | 21.4 | 24.7 | 3.3 | |
| | D8 | 20.9 | 24.3 | 3.4 | |

| Sample type | Sample ID | Cq (neat) | Cq (1/10) | Δ Cq | Mean Δ Cq |
|---|---|---|---|---|---|
| bladder | D1 | 20.4 | 23.7 | 3.3 | 3.5 |
| | D2 | 30.6 | 33.8 | 3.2 | |
| | D3 | 27.3 | 30.6 | 3.3 | |
| | D4 | 26.3 | 29.8 | 3.5 | |
| | D5 | 21.2 | 24.9 | 3.7 | |
| | D6 | 27.1 | 30.6 | 3.5 | |
| | D7 | 28.1 | 31.4 | 3.3 | |
| | D8 | 23.5 | 27.4 | 3.9 | |
| lymph node | D1 | 16.2 | 19.8 | 3.6 | 3.4 |
| | D2 | 15.9 | 19.1 | 3.2 | |
| | D3 | 16.1 | 19.5 | 3.4 | |
| | D4 | 15.2 | 18.8 | 3.6 | |
| | D5 | 14.3 | 17.7 | 3.4 | |
| | D6 | 15.6 | 18.9 | 3.3 | |
| | D7 | 16.4 | 19.6 | 3.2 | |
| | D8 | 20.3 | 23.5 | 3.2 | |
| liver | EF2 | 22.5 | 25.8 | 3.3 | 3.4 |
| | EF3 | 27.8 | 31.2 | 3.4 | |
| | EF4 | 22.0 | 25.5 | 3.5 | |
| | EF5 | 21.7 | 25.0 | 3.3 | |
| | EF6 | 26.1 | 29.6 | 3.5 | |
| | EF7 | 29.9 | 33.1 | 3.2 | |
| | EF8 | 17.7 | 21.1 | 3.4 | |
| | EF9 | 20.3 | 23.7 | 3.4 | |
| bone marrow | C2 | 21.28 | 23.4 | 2.1 | 2.0! |
| | C3 | 20.6 | 22.1 | 1.5 | |
| | C4 | 21.9 | 22.7 | 0.7 | |
| | C5 | 18.9 | 22.3 | 3.4 | |

Δ Cq = Cq (neat)- Cq (1/10)

## Case study 1a (Dubbo) PCR results

**Table E4.** Summary of samples producing amplification in a multiplex qPCR assay targetting *Coxiella burnetii*. Samples were collected from Eastern Grey kangaroos residing in the Dubbo region of New South Wales, Australia.

| Animal ID | Species | Sex | Sample type | qPCR assay Cqs and cut-offs | | | | | Sample classification |
|---|---|---|---|---|---|---|---|---|---|
| | | | | KCB | *Coxiella burnetii* multiplex | IS*1111* ≤34 | *com1* ≤36 | *htpAB* ≤35 | |
| 4 | Eastern Grey | female | cloacal swab | 19.7 | Singles | 35.7 | - | - | negative |
| | | | | | Triplicates | 35.7 | - | - | |
| | | | | | | 34.6 | - | - | |
| | | | | | | 36.7 | - | - | |
| 7 | Eastern Grey | male | cloacal swab | 18.2 | Singles | 31.6 | - | 37.45 | suspect |
| | | | | | Triplicates | 33.4 | - | - | |
| | | | | | | 32.8 | 38.6 | 35.4 | |
| | | | | | | 33.4 | 37.7 | - | |
| 14 | Eastern Grey | male | cloacal swab | 16.9 | Singles | 36.4 | - | - | negative |
| | | | | | Triplicates | 35.4 | 35.4 | 35.4 | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 9 | Eastern Grey | male | lymph node | 16.8 | Singles | 35.6 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 1 | Eastern Grey | male | bladder | 23.8 | Singles | 37.1 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 3 | Eastern Grey | male | gut | 15.7 | Singles | 37.2 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 11 | Eastern Grey | male | gut | 18.4 | Singles | 37.2 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 12 | Eastern Grey | male | spleen | 19.3 | Singles | 37.6 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 15 | Eastern Grey | male | spleen | 22.1 | Singles | 36.1 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 2 | Eastern Grey | male | spleen | 23.3 | Singles | 37.2 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 24 | Eastern Grey | male | spleen | 22.5 | Singles | - | 38.3 | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 15 | Eastern Grey | male | faeces | 25.0 | Singles | 39.6 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 22 | Eastern Grey | male | faeces | 24.2 | Singles | - | 35.0 | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |

## Case study 1b (Canberra) PCR results

**Table E5.** Summary of samples producing amplification in a multiplex qPCR assay targetting *Coxiella burnetii*. Samples were collected from Eastern Grey kangaroos residing in the Canberra region of New South Wales, Australia.

| Animal ID | Species | Sex | Sample type | qPCR assay Cqs and cut-offs | | | | | Sample classification |
|---|---|---|---|---|---|---|---|---|---|
| | | | | KCB | *Coxiella burnetii* multiplex | IS*1111* ≤34 | *com1* ≤36 | *htpAB* ≤35 | |
| 2 | Eastern Grey | female | liver | 19.6 | Singles | 38.1 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 21 | Eastern Grey | male | liver | 19.2 | Singles | 38.1 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 10 | Eastern Grey | female | spleen* | 16.1 | Singles | 33.9 | 35.4 | | suspect |
| | | | | | Triplicates | 35.2 | 38.1 | - | |
| | | | | | | 36.7 | 36.6 | 34.6 | |
| | | | | | | 34.4 | 39.7 | 35.9 | |
| 11 | Eastern Grey | female | spleen* | 17.3 | Singles | 36.2 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 12 | Eastern Grey | female | spleen* | 17.5 | Singles | 34.3 | - | - | negative |
| | | | | | Triplicates | 35.7 | - | - | |
| | | | | | | 35.2 | - | - | |
| | | | | | | 34.5 | - | - | |
| 16 | Eastern Grey | female | spleen* | 14.5 | Singles | 35.5 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | 38.5 | | | |
| | | | | | | - | - | - | |
| 2 | Eastern Grey | female | bladder* | 15.4 | Singles | 34.1 | 38.8 | 33.5 | suspect |
| | | | | | Triplicates | 37.3 | 36.2 | 35.1 | |
| | | | | | | 35.9 | 36.6 | 35.0 | |
| | | | | | | 34.4 | 37.4 | 36.1 | |
| 3 | Eastern Grey | female | bladder* | 15.4 | Singles | 37.3 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 2 | Eastern Grey | female | urine | 29.7 | Singles | - | - | 37.2 | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 23 | Eastern Grey | male | cloacal swab | 21.5 | Singles | 35.3 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 28 | Eastern Grey | male | faeces | 22.9 | Singles | 35.6 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 29 | Eastern Grey | male | faeces | 26.0 | Singles | 36.8 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 59 | Eastern Grey | female | faeces | 25.8 | Singles | - | 34.9 | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |

| Animal ID | Species | Sex | Sample type | KCB | qPCR assay Cqs and cut-offs | | | | Sample classification |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Coxiella burnetii* multiplex | IS*1111* ≤34 | *com1* ≤36 | *htpAB* ≤35 | |
| 8 | Eastern Grey | female | gut | 19.9 | Singles | 37.1 | - | - | negative |
| | | | | | Triplicates | 36.8 | - | - | |
| | | | | | | - | - | - | |
| | | | | | | 37.1 | - | - | |
| 17 | Eastern Grey | female | gut | 18.8 | Singles | 37.2 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 17 | Eastern Grey | female | uterus* | 12.5 | Singles | 34.8 | - | 36.6 | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | 36.6 | - | - | |
| | | | | | | - | - | - | |
| 23 | Eastern Grey | female | uterus* | 14.1 | Singles | 35.8 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | 39.5 | |
| | | | | | | 39.1 | - | - | |
| 48 | Eastern Grey | female | uterus* | 15.7 | Singles | 36.4 | - | - | negative |
| | | | | | Triplicates | 34.1 | - | - | |
| | | | | | | - | - | - | |
| | | | | | | 33.9 | - | - | |
| 43 | Eastern Grey | female | uterus* | 17.2 | Singles | - | - | 37.1 | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | 36.33 | - | - | |
| | | | | | | 38.77 | - | - | |
| 45 | Eastern Grey | female | uterus* | 16.4 | Singles | 36.8 | - | 36.0 | negative |
| | | | | | Triplicates | 33.59 | - | - | |
| | | | | | | 34.21 | - | - | |
| | | | | | | 33.79 | - | - | |

## Case study 1c (Valla) PCR results

**Table E6.** Summary of samples producing amplification in a multiplex qPCR assay targetting *Coxiella burnetii*. Samples were collected from Eastern Grey kangaroos residing in Valla New South Wales, Australia.

| Animal ID | Species | Sex | Sample type | KCB | qPCR assay Cqs and cut-offs | | | | Sample classification |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Coxiella burnetii* multiplex | IS*1111* ≤34 | *com1* ≤36 | *htpAB* ≤35 | |
| 29 | Eastern Grey | female | urine | 26.7 | Singles | 39.82 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 33 | Eastern Grey | male | cloacal swab | 21.3 | Singles | 31.23 | 33.02 | 35.26 | positive |
| | | | | | Triplicates | 30.4 | 36.0 | 34.4 | |
| | | | | | | 30.4 | 34.6 | 33.4 | |
| | | | | | | 30.4 | 35.3 | 32.8 | |
| 6 | Eastern Grey | male | liver | 23.5 | Singles | 38.03 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |

| Animal ID | Species | Sex | Sample type | KCB | qPCR assay Cqs and cut-offs | | | | Sample classification |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Coxiella burnetii* multiplex | IS*1111* ≤34 | *com1* ≤36 | *htpAB* ≤35 | |
| 4 | Eastern Grey | male | spleen | 19.0 | Singles | 37.37 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | 35.9 | - | 36.9 | |
| | | | | | | - | - | - | |
| 32 | Eastern Grey | female | spleen | 21.7 | Singles | 34.67 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | 34.0 | - | - | |
| | | | | | | 34.8 | 35.4 | - | |
| 40 | Eastern Grey | male | spleen | 21.0 | Singles | 37.42 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 47 | Eastern Grey | male | spleen | 18.3 | Singles | 36.6 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 28 | Eastern Grey | female | faeces | 27.7 | Singles | 36.66 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 31 | Eastern Grey | male | faeces | 26.4 | Singles | 39.96 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 34 | Eastern Grey | female | faeces | 30.9 | Singles | 37.79 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 35 | Eastern Grey | female | faeces | 29.6 | Singles | 36.24 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 19 | Eastern Grey | male | bladder | 22.5 | Singles | 36.18 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | 37.2 | - | - | |
| | | | | | | 37.3 | - | - | |
| 27 | Eastern Grey | female | bladder | 22.2 | Singles | 37.25 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | 36.7 | - | - | |
| 33 | Eastern Grey | male | bladder | 20.3 | Singles | 36.61 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | 36.2 | - | - | |
| 41 | Eastern Grey | male | bladder | 21.5 | Singles | 37.02 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | 36.2 | |
| | | | | | | - | - | - | |
| 45 | Eastern Grey | female | bladder | 20.8 | Singles | 35.33 | 36.75 | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 1 | Eastern Grey | female | gut | 18.7 | Singles | 36.36 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 24 | Eastern Grey | male | gut | 20.3 | Singles | 36.53 | - | - | negative |
| | | | | | Triplicates | - | - | - | |

| Animal ID | Species | Sex | Sample type | KCB | *Coxiella burnetii* multiplex | IS*1111* ≤34 | *com1* ≤36 | *htpAB* ≤35 | Sample classification |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | qPCR assay Cqs and cut-offs | | | |
| | | | | | | 35.5 | - | - | |
| | | | | | | - | - | 35.3 | |
| 26 | Eastern Grey | male | gut | 18.6 | Singles | 36.24 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 27 | Eastern Grey | female | gut | 21.0 | Singles | 36.26 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 34 | Eastern Grey | female | gut | 20.9 | Singles | 37.28 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | 35.2 | |
| 45 | Eastern Grey | female | gut | 19.5 | Singles | 37.56 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |

## Case study 2 (Camden) PCR results

**Table E7.** Summary of samples producing amplification in a multiplex qPCR assay targetting *Coxiella burnetii*. Samples were collected from wildlife residing in the Camden region of New South Wales, Australia.

| Animal ID | Species | Sex | Sample type | 18S | *Coxiella burnetii* multiplex | IS*1111* ≤34 | *com1* ≤36 | *htpAB* ≤35 | Sample classification |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | qPCR assay Cqs and cut-offs | | | |
| 11 | Eastern Grey | female | lymph node | 17.9 | Singles | 36.8 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 21 | wombat | male | spleen | 20.4 | Singles | - | 37.17 | 35.07 | suspect |
| | | | | | Triplicates | - | 38.02 | 35.08 | |
| | | | | | | - | 37 | 35.13 | |
| | | | | | | - | 37.5 | 35.09 | |
| 1 | wombat | female | liver | 25.0 | Singles | 39.4 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 7 | wombat | male | liver | 15.9 | Singles | - | - | 36.7 | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 17 | wombat | male | liver | 18.5 | Singles | 38.4 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 1 | wombat | female | bladder | 15.1 | Singles | 36.84 | 38.3 | - | negative |
| | | | | | Triplicates | 35.4 | - | - | |
| | | | | | | - | - | - | |

| Animal ID | Species | Sex | Sample type | 18S | *Coxiella burnetii* multiplex | IS*1111* ≤34 | *com1* ≤36 | *htpAB* ≤35 | Sample classification |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | - | - | - | |
| 18 | wombat | male | bladder | 27.6 | Singles | 37.7 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 13 | wombat | female | lung | 15.8 | Singles | 36.7 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | 36.1 | |
| | | | | | | - | - | 36.2 | |
| 15 | wallaroo | male | lung | 12.7 | Singles | 36.9 | - | - | negative |
| | | | | | Triplicates | - | - | 36.1 | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 18 | wombat | male | lung | 18.5 | Singles | 37.7 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 19 | wombat | male | kidney | 25.5 | Singles | 39.6 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 22 | wombat | male | kidney | 20.7 | Singles | 38.2 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 1 | wombat | female | urine | 19.2 | Singles | 38.4 | - | - | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |
| 9 | wombat | female | uterus | 17.9 | Singles | - | 37.1 | - | negative |
| | | | | | Triplicates | 35.97 | - | - | |
| | | | | | | 37.05 | - | - | |
| | | | | | | 36.17 | - | - | |
| 8 | wombat | female | bone marrow | 13.2 | Singles | - | - | 36.47 | negative |
| | | | | | Triplicates | - | - | - | |
| | | | | | | - | - | - | |
| | | | | | | - | - | - | |

## Case study 3 (3a-Lismore, 3b Port Macquarie and 3c Campbelltown) PCR results

**Table E8.** Summary of urogenital swab samples producing amplification in a multiplex qPCR assay targetting *Coxiella burnetii*. Samples were collected  from koala feom the Lismore, Port Macquarie and Campbelltown regions of New South Wales, Australia.

| Animal ID | Species | Sex | Sample type | Region | qPCR assay Cqs and cut-offs | | | | | Sample classification |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | β-actin | *Coxiella burnetii* multiplex | IS*1111*≤34 | *com1* ≤36 | *htpAB* ≤ 35 | |
| 3772-8 | koala | male | urogenital swab | Port Macquarie | 21.6 | Singles | 30.1 | 34.1 | 32.5 | positive |
| | | | | | | Triplicates | 29.7 | 34.1 | 32.8 | |
| | | | | | | | 29.8 | 33.8 | 33.5 | |
| | | | | | | | 30 | 34.4 | 33.1 | |
| 18-10145 | koala | unknown | urogenital swab | Port Macquarie | 29.4 | Singles | 33.4 | 36.2 | 34.9 | suspect |
| | | | | | | Triplicates | 34 | 38.3 | - | |
| | | | | | | | 34 | | 37 | |
| | | | | | | | 33.4 | | - | |
| 17-00050 | koala | male | urogenital swab | Campbelltown | 26 | Singles | 33.6 | - | - | negative |
| | | | | | | Triplicates | - | - | - | |
| | | | | | | | - | - | - | |
| | | | | | | | - | - | - | |
| 17-04944 | koala | male | urogenital swab | Northern Rivers | 22.7 | Singles | - | - | 36 | negative |
| | | | | | | Triplicates | - | - | - | |
| | | | | | | | - | - | - | |
| | | | | | | | - | - | - | |

## Case study 4 (St Helena Island) PCR results

**Table E9.** Summary of Red-necked wallaby scat samples producing amplification in a multiplex qPCR assay targetting *Coxiella burnetii*. Samples were collected from the environment on St Helena Island, Queensland.

| Sample ID | DNA dilution | PCR assay Cq's and cut-offs | | Sample classification |
|---|---|---|---|---|
| | | IS*1111* ≤ 32 | *com1* ≤ 33 | |
| C31 | Neat | 36.2 | | negative |
| | Neat | 35.1 | 36.8 | |
| | 1/5 | 37.8 | | |
| | 1/5 | 39.0 | | |
| C36 | Neat | 32.7 | 36.9 | negative |
| | Neat | 32.1 | | |
| | 1/5 | 36.5 | | |
| | 1/5 | 36.3 | 38.0 | |