Q fever and Australia's veterinary workforce:

Research to inform vaccine policy.

© Emily Sellens

BAppSc BSc BVMS

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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.

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List of Peer Reviewed Publications

1. **Sellens E**, Norris JM, Dhand NK, Heller J, Hayes L, Gidding HF, Willaby H, Wood N, Bosward KL. Q Fever Knowledge, Attitudes and Vaccination Status of Australia's Veterinary Workforce in 2014. *PLoS One*. 2016 Jan 12;11(1):e0146819. doi: 10.1371/journal.pone.0146819.

2. **Sellens E**, Norris JM, Dhand NK, Heller J, Hayes L, Gidding HF, Willaby H, Wood N, Bosward KL. Willingness of veterinarians in Australia to recommend Q fever vaccination in veterinary personnel: Implications for workplace health and safety compliance. *PLoS One*. 2018 Jun 1;13(6):e0198421. doi: 10.1371/journal.pone.0198421.

3. **Sellens E**, Bosward KL, Norris JM, Wood N, Heller J, Graves S, Gidding HF. *Coxiella burnetii* seroprevalence in unvaccinated veterinary workers in Australia: Evidence to support Q fever vaccination. Zoonoses Public Health. 2020 Feb;67(1):79-88. doi: 10.1111/zph.12658. Epub 2019 Nov 2.

4. **Sellens E**, Bosward KL, Willis S, Heller J, Cobbold R, Comeau JL, Norris JM, Dhand NK, Wood N. Frequency of Adverse Events Following Q Fever Immunisation in Young Adults. Vaccines (Basel). 2018 Dec 13;6(4):83. doi: 10.3390/vaccines6040083.

Contributions to Conference Proceedings

Authors	*Title*	*Contribution*	*Conference details*
***Emily Sellens**, Katrina L. Bosward, Jacqueline M. Norris, Nicholas Wood, Jane Heller, Stephen Graves, Heather F. Gidding*	*Coxiella burnetii* seroprevalence in Australian Veterinary Workers	Invited speaker and conference abstract	AVA Annual Conference, 5th – 10th May 2019, Perth, Australia
***Emily Sellens**, Jeannette L Comeau, Katrina L Bosward, Jane Heller, Jacqueline M Norris, Lynne Hayes, Rowland Cobbold, Susan Willis, Nicholas Wood*	Adverse Events following Q fever vaccination in young Australian adults	Conference abstract and poster	One Health Eco Health Conference, 3rd - 7th December 2016, Melbourne, Australia
***Emily Sellens**, Jacqueline M. Norris, Navneet K. Dhand, Jane Heller, Lynne Hayes, Heather F. Gidding, Nicholas Wood, Katrina L. Bosward*	Q fever vaccine: A call for increased vaccine uptake among Australia's veterinary workforce	Conference abstract and poster	One Health Eco Health Conference, 3rd - 7th December 2016, Melbourne, Australia
***Emily Sellens**, Jacqueline M. Norris, Navneet K. Dhand, Jane Heller, Lynne Hayes, Heather F. Gidding, Nicholas Wood, Katrina L. Bosward*	Willingness of Australian veterinarians to recommend Q fever vaccination to veterinary colleagues	Conference abstract and poster	Marie Bashir Colloquium, 6th November 2015, the University of Sydney, Australia
***Emily Sellens**, Navneet K Dhand, Katrina L Bosward, Jacqueline M Norris, Jane Heller, Lynne Hayes, N Wood.*	Knowledge and attitudes towards Q fever disease and vaccination: a national survey of Australia's veterinary workforce	Conference abstract and oral presentation (presented by Navneet Dhand)	International Society for Veterinary Epidemiology and Economics Conference, 3rd-7th November 2015, Merida, Yucutan, Mexico.
***Emily Sellens**, Navneet Dhand, Katrina L Bosward, Jacqueline M Norris, Jane Heller, Lynne Hayes, Heather F Gidding, Nicholas Wood.*	Attitudes and barriers to Q fever vaccination in the Australian veterinary workforce	Invited speaker and conference abstract	Pan Pacific Veterinary Conference, 24th - 29th May 2015, Brisbane, Australia.
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Chapter 3: **Sellens E**, Norris JM, Dhand NK, Heller J, Hayes L, Gidding HF, Willaby H, Wood N, Bosward KL. Willingness of veterinarians in Australia to recommend Q fever vaccination in veterinary personnel: Implications for workplace health and safety compliance. *PLoS One*. 2018 Jun 1;13(6):e0198421. doi: 10.1371/journal.pone.0198421.

Chapter 4: **Sellens E**, Bosward KL, Norris JM, Wood N, Heller J, Graves S, Gidding HF. *Coxiella burnetii* seroprevalence in unvaccinated veterinary workers in Australia: Evidence to support Q fever vaccination. Zoonoses Public Health. 2020 Feb;67(1):79-88. doi: 10.1111/zph.12658. Epub 2019 Nov 2.

Chapter 6: **Sellens E**, Bosward KL, Willis S, Heller J, Cobbold R, Comeau JL, Norris JM, Dhand NK, Wood N. Frequency of Adverse Events Following Q Fever Immunisation in Young Adults. Vaccines (Basel). 2018 Dec 13;6(4):83. doi: 10.3390/vaccines6040083.

I, *Emily Sellens*, collaborated with co-authors on the final study design, methodology, and project administration and was primarily responsible for investigation, data curation, formal analysis, visualization, and writing original draft manuscripts. I am very grateful to Associate Professor Katrina Bosward, Professor Jacqueline Norris, Associate Professor Nicholas Wood, Associate Professor Navneet Dhand, Associate Professor Heather Gidding, Professor Stephen Graves, Associate Professor Jane Heller, and Lynne Hayes for their assistance with investigation, data analysis, and reviewing and editing of manuscripts. I am also very grateful to Chelsea Nguyen and John Stenos at the Australian Rickettsial Reference Laboratory for performing serological analysis on blood samples, and to Dr Sarah Tozer for her laboratory work at the Queensland Paediatric and Infectious Diseases Laboratory.

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who proved quite a disruption when she arrived in 2016, thank you for not accidentally (or purposefully) deleting this thesis off the computer. I am looking forward to getting you a Q fever vaccination one day, which will eliminate one parenting anxiety. Especially since you have declared your love of animals over people, and your desire to own a menagerie of species, great and small.

To my Mum, Dad and brother, please stop asking if I will ever finish my PhD – it is done now.

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List of Abbreviations and Acronyms

18F-FDG PET/CT	fluorodeoxyglucose positron emission tomography/computed tomography
ABS	Australian Bureau of Statistics
ACT	Australian Capital Territory
AEFI	Adverse events following immunisation
ARRL	Australian Rickettsial Reference Laboratory
ASGS	Australian Statistical Geography Standard
AVA	Australian Veterinary Association
CDC	USA Centers for Disease Control and Prevention
CFT	Complement fixation test
CI	Confidence interval
CLB	*Coxiella*-like bacteria
CMI	Cell mediated immunity
DAEN	Australian Government Therapeutic Goods Administration National Database of Adverse Event Notifications
ELISA	Enzyme-linked immunosorbent assays
FcR	Fc receptor
GIT	gastrointestinal tract
HI-FCS	Heat Inactivated Filter Sterilised Foetal Calf Serum
IAP	integrin-associated protein
IFA	Immunofluorescence assay
IFN-γ	Interferon gamma
Ig	Immunoglobulin
IGRA	Interferon gamma release assay
IL	Interleukin
IQR	Interquartile range
ISR	Injection site reaction
LCL	Lower confidence limit
LCV	Large cell variant
LHD	Local health district
LPS	Lipopolysaccharide
LSI	Lymphocyte stimulation index
NQFMP	National Q fever Management Program
NSW	New South Wales
NT	Northern Territory
OR	Odds Ratio
OR	Odds ratio
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PPE	Personal protective equipment
QFS	Q fever fatigue syndrome

RR	Relative Response
RIA	Radioimmunoassay
SA	South Australia
SCV	Small cell variant
Th1	T-helper cell type 1
TLR	toll like receptor
TNF	Tumour necrosis factor
UCL	Upper confidence limit
USA	United States of America
VNCA	Veterinary Nursing Council of Australia
WA	Western Australia
WH&S	Workplace health and safety

Abstract

Q fever is a zoonotic disease of worldwide importance, capable of causing significant and prolonged morbidity, low mortality, and burdening health systems with an enhanced requirement for diagnostics, prolonged treatment regimes, and disease surveillance. Most infections are attributed to livestock, though other species may harbour the causative pathogen, *Coxiella burnetii*. Australia is currently the only country with a licensed Q fever vaccine, where it is recommended for workers considered to have a high risk for *C. burnetii* exposure. Veterinarians are routinely vaccinated, usually at the commencement of university studies, however, veterinary support staff including nurses and kennel hands are not. Outbreaks in small animal clinics in Sydney raised questions about the risk of exposure to *C. burnetii* via non-ruminant species in Australia, along with highlighting the potential under-vaccination of veterinary support workers. This thesis subsequently sought to examine the epidemiology of *C. burnetii* and Q fever vaccination in Australia's veterinary workforce.

Chapter 1 presents an in-depth literature review of *C. burnetii* epidemiology, Q fever disease and vaccination, which draws upon the global literature but focuses on the Australian setting. This review identifies gaps in our knowledge of Q fever disease and vaccination in Australia, particularly regarding the veterinary workforce. Chapters 2 and 3 investigate these knowledge gaps through a national survey of veterinary workers to understand their knowledge, awareness, and attitudes regarding Q fever disease and vaccination. These chapters also determine Q fever vaccine uptake by veterinarians and veterinary nurses separately, and explore the drivers of, and barriers to, vaccine uptake. Chapter 4 measures *C. burnetii* seroprevalence in unvaccinated veterinary workers and identifies demographic and work characteristics associated with seropositivity. Similarly, seroprevalence is measured in Chapter 5 in workers previously vaccinated for Q fever to examine the longevity of serological responses post-vaccination. Finally, Chapter 6 gathers important data for adverse events following immunisation [AEFI]

for the Q fever vaccine, with greater representation of younger adults and females than previously described.

Collectively, these studies demonstrate a deficiency in vaccine uptake among veterinary nurses and in veterinarians graduating internationally, despite seroprevalence confirming an increased risk of exposure to *C. burnetii* across all practice types. Seroprevalence data collected in unvaccinated workers highlighted the importance of vaccination upon entering the veterinary workforce, but also demonstrated that most unvaccinated workers are potentially eligible for Q fever vaccination despite many years working with animals. The greatest opportunity to improve vaccine uptake appears to be via strict workplace vaccination policies and greater compliance with workplace health and safety law. Financial assistance from the government could aid the implementation of such policies, for example subsidising the vaccination process. Serological profiles described in vaccinated veterinary workers up to 25 years post-vaccination differed from those previously described in abattoir, raising questions about the longevity of immunity; clinical trial data may not translate from abattoir workers to other at-risk professions where recurrent exposure may be less frequent. Though, no instances of Q fever disease were reported in vaccinated workers and further studies are recommended to explore cell mediated immune responses post-vaccination. Finally, AEFI data collected inform the wider use of the current Q fever vaccination beyond occupational settings and into at-risk communities, with a higher frequency of AEFI, and AEFI of greater severity expected in females than in males. Though, the vaccine remained safe in this cohort and these data have since supported further vaccine research in younger cohorts (<15 years old). While this research is intended to provide veterinary specific insights for the continued improvement of workplace health and safety practices in Australia, the findings have implications for the wider community both within Australia and abroad.

1 Background and Literature Review

1.1 History of Q fever

Investigations into “Query Fever” were first published in 1937 following an outbreak of a febrile illness in abattoir workers in Brisbane, Queensland, Australia. The illness, abbreviated to Q fever, was initially reported in Brisbane in 1933 and the routine failure to isolate a causative agent from blood cultures and agglutination tests warranted a thorough investigation (Derrick, 1937). Derrick (1937) documented the clinical disease in people and successfully replicated illness in guinea pigs following their inoculation with blood or urine from infected human patients. Unable to isolate the aetiological agent through axenic culture, Derrick (1937) proposed the pathogen to be a virus. Tissue samples of experimentally infected guinea pigs were forwarded to Dr. F. M. Burnet at the Walter and Eliza Hall Institute of Medical Research, Melbourne, where rickettsia-like bodies were identified within splenic samples (Burnet & Freeman, 1937). Around the same time, Gordon Davis and Herald Rea Cox were investigating the rickettsial disease “Rocky Mountain Fever” in Montana, USA. Ticks collected from Nine Mile were found to transmit an infective organism that caused fever but lacked other pathognomonic signs of Rocky Mountain Fever. The team concluded that the pathogen was new, displaying both viral and rickettsial properties, and the associated illness was termed “Nine Mile Fever” (Cox, 1939; Davis, 1938).

In 1938 a member of the Montana team acquired an infection manifesting as a febrile illness. The illness was reproducible in guinea pigs through inoculation with the patient’s blood and was found to be clinically identical to Nine Mile Fever. Rickettsia-like organisms were identified in splenic samples from the infected guinea pigs, directly connecting the work of Derrick (1937) and Burnet (1937) in Australia. Although the organisms resembled rickettsia in many ways, they were able to pass through filters normally impermeable to other rickettsia and it was proposed that the causative agent of Q fever be classified as a separate genus (Cox, 1939). The organism was given the classification of *Coxiella burnetii* in honour of Cox

and Burnet and was placed in the *Rickettsiaceae* family of the order Rickettsiales. This classification became redundant in later years following 16S rRNA gene sequencing analysis, when *C. burnetii* was reclassified into the order Legionellales within the Gamma subdivision of Proteobacteria (Stein, Saunders, Taylor, & Raoult, 1993).

Coxiella burnetii has since been described in nearly every country in which it has been investigated. Q fever most often presents as sporadic cases, though hyperendemic foci also exist in some communities and small outbreaks can occur following exposure to a common source, while larger region-wide outbreaks may occur under unique circumstances (Million & Raoult, 2015). Due to the considerable impact on public health, Q fever is a notifiable disease in some countries including Australia and the United States of America [USA] (Anderson et al., 2013; Australian Government Department of Health, 2019). The USA Centers for Disease Control and Prevention [CDC] classified the pathogen as a category B bioterrorism agent reflecting moderately easy pathogen dissemination, moderate morbidity and low mortality, and the burden of an enhanced requirement for diagnostics and disease surveillance (National Center for Emerging and Zoonotic Infectious Diseases, 2018).

1.2 Pathobiology

1.2.1 Classification

Coxiella burnetii is a small obligately intracellular bacterium. The cell membrane is similar to that of Gram-negative bacteria, though staining using the Gram technique is variable, and bacteria are on occasions acid-fast (McCaul & Williams, 1981). *Coxiella* species generally stain using the Gimenez method (Gimenez, 1964; McCaul & Williams, 1981).

Two species within the genus *Coxiella* have been formally identified: *C. burnetii*, the agent of Q fever, and *C. cheraxi*, a pathogen of crayfish (Duron et al., 2015; Tan & Owens, 2000). Additionally, many strains of tick-borne *Coxiella*-like bacteria [CLB] are described, which are divided into four genetically diverse clades

(Duron et al., 2015). *Coxiella burnetii* is described within clade A, suggesting the recent evolutionary transition of a symbiont of soft ticks to a vertebrate pathogen, which may have been facilitated by the transference of virulence genes from co-infecting pathogens (Duron et al., 2015). Although *C. burnetii* is the only formally identified species confirmed to be pathogenic in vertebrates, cases of severe clinical illness in birds have been attributed to CLB (Shivaprasad et al., 2008; Vapniarsky, Barr, & Murphy, 2012) . In humans, *Candidatus* Coxiella mudrowiae and *Ca.* C. massiliensis, have recently been described as possible agents of human infection following their PCR detection in tick-bite patients exhibiting skin eschars in France, though the bacteria were unable to be cultured (Angelakis et al., 2016; Guimard et al., 2017). *Candidatus* C. massiliensis has also been described in *Rhipicephalus sanguineus* ticks in Australia (Oskam, Owens, Codello, Gofton, & Greay, 2018), with DNA sequencing exhibiting 100% similarity to *Ca.* C. massiliensis isolated from ticks removed from the aforementioned French patients (Angelakis et al., 2016). However, transmission to people has not been described in Australia (Oskam et al., 2018). In other species, CLB were detected by PCR at low prevalence (0.7%) in horses in South Korea, though their clinical relevance is not known (Seo et al., 2016).

1.2.2 Phase variation

Two antigenically distinct variants of *C. burnetii* are described: phase I and phase II. Phase I reflects the bacterium in its virulent form and can be isolated from infected animals and the environment. Phase II reflects the avirulent form, which emerges via repeated passage of phase I bacteria through embryonated eggs (Hotta et al., 2002; Mege, Maurin, Capo, & Raoult, 1997) or within cell culture over time (Baca et al., 1981; Burton, Stueckemann, Welsh, & Paretsky, 1978). Both phases are morphologically identical when examined with electron microscopy (Jerrels, Hinrichs, & Mallavia, 1974). However, differences become apparent when studying the lipopolysaccharide [LPS] of the cell wall (Amano & Williams, 1984). In a similar manner to Gram-negative bacteria, *C. burnetii* exhibits smooth-to-rough mutational variation of the LPS chains (Amano & Williams, 1984; Hackstadt, Peacock, Hitchcock, & Cole, 1985). Virulent phase I bacteria

are ‘smooth’ with full-length LPS, while avirulent phase II bacteria are ‘rough’ with truncated LPS (Hackstadt et al., 1985; Vishwanath & Hackstadt, 1988). The transition from phase I to phase II reflects an irreversible loss of the O-antigen polysaccharide and an outer core, with antigenically intermediate phases recognised during the transition (Hotta et al., 2002). The mechanisms of antigenic shift over time are not fully understood, though phase II bacteria may be the product of chromosomal deletions or the suppression or upregulation of genes under certain biological conditions (Toman, Heinzen, Samuel, & Mege, 2012). Phase II bacteria of the Nine Mile strain for example, exhibit a large chromosomal deletion and base changes across a number of chromosomal regions (Thompson, Hoover, Vockin, & Shaw, 2003). However, these deletions and changes are not consistent across all strains exhibiting phase variation (Thompson et al., 2003).

The constituents of phase I LPS are important for *C. burnetii* pathogenicity and immunogenicity. Phase I LPS sterically inhibits the binding of antibodies (Vishwanath & Hackstadt, 1988) and masks recognition by toll-like receptors [TLR], the latter allowing for infection of dendritic cells while interfering with their activation and production of inflammatory cytokines (Gorvel et al., 2015; Shannon, Howe, & Heinzen, 2005). Conversely, phase II bacteria are more susceptible to antibody binding and readily activate dendritic cells, resulting in enhanced innate and adaptive immune responses against bacteria in phase II (Shannon et al., 2005). Consequently, virulent phase I bacteria are capable of evading host immune responses while avirulent phase II bacteria are readily killed. This susceptibility to host defences explains the inability to isolate phase II *C. burnetii* from natural infections or experimentally infected immunocompetent host animals (Arricau-Bouvery & Rodolakis, 2005; Baca & Paretsky, 1983; Vishwanath & Hackstadt, 1988). Phase II may only emerge in laboratory settings as a strategy of efficiency where energy-demanding mechanisms of host immune evasion, including the synthesis of full-length phase I LPS, are no longer required (Toman et al., 2012).

Antibodies against both phase I and phase II LPS are produced in response to natural infection or to vaccination with phase I antigen, and the antibody profile exhibited over time provides useful diagnostic information (Kersh, Fitzpatrick, Self, Biggerstaff, & Massung, 2013; Teunis et al., 2013; Wegdam-Blans et al., 2012; Worswick & Marmion, 1985). Phase variation is also consequential for vaccine development as exposure to phase II antigen results in the exclusive production of phase II antibodies, and in animal models phase II vaccines have been ineffective for the prevention of Q fever (Arricau-Bouvery et al., 2005; Zhang et al., 2007). Investigations into virulence factors and protective immune responses have therefore focused on comparing various properties of phase I and phase II bacteria. The use of phase II *C. burnetii* for laboratory studies is also advantageous, as biosafety level-2 facilities may be used instead of level-3 facilities required for handling phase I strains. In particular, a phase II variant of the Nine Mile strain has proven to be a suitable *ex vivo* model for the study of pathogen–host interactions (Larson et al., 2016).

1.2.3 Host cell infection

Mononuclear phagocytic cells (monocytes and macrophages) are the primary target cells for *C. burnetii* infection, with alveolar macrophages of the lung and Kupffer cells of the liver the most common sites of primary infection (Khavkin & Tabibzadeh, 1988; Stein et al., 2005). Bacteria enter host phagocytic cells passively, following attachment with a host-cell surface receptor complex of leukocyte response integrin LRI$\alpha_v\beta_3$ and integrin-associated protein [IAP] (Capo et al., 1999). Virulent phase I bacteria interfere with the coupling of the LRI$\alpha_v\beta_3$-IAP complex, which impairs IAP-dependant activation of complement receptor CR3 and results in inappropriate activation of macrophages and cytoskeleton reorganisation to permit intracellular survival (Capo et al., 1999).

The pathogen has also been confirmed in other cells following infection, including pneumocytes, fibroblasts, epithelial and endothelial cells, and adipocytes (Bechah et al., 2014; Khavkin & Tabibzadeh, 1988; Sobotta, Bonkowski, et al., 2017). However, the ability to replicate outside of phagocytic cells varies

between anatomical sites and between species (Sobotta, Bonkowski, et al., 2017). *In vitro*, *C. burnetii* is capable of infecting and replicating within a variety of cell lines, including primary macrophages, macrophage-like cells, epithelial cells and fibroblasts (Sobotta et al., 2016; Voth & Heinzen, 2007), while genetic studies have been further advanced by axenic growth in synthetic medium (Larson et al., 2016).

1.2.4 Lifecycle in vertebrate hosts

Coxiella burnetii exhibits a biphasic lifecycle, involving large and small cell variants [LCVs and SCVs respectively]. The pleomorphic nature of these variants is responsible for the variable Gram-stain and acid-fast responses of *C. burnetii* (McCaul & Williams, 1981). The LCVs are the replicative form (Coleman, Fischer, Howe, Mead, & Heinzen, 2004) which are pleomorphic in nature but generally rounded with a diameter up to 2 µm (Amano & Williams, 1984; Maurin & Raoult, 1999; McCaul & Williams, 1981). When viewed under electron microscopy, the LCVs exhibit a simple cell wall composed of a trilaminar cytoplasmic membrane separated from a three layered outer membrane by a periplasmic space (McCaul & Williams, 1981). The SCVs are the stationary, non-replicating form (Coleman et al., 2004) which are rod shaped, 0.2 to 0.5 µm in size, and exhibit condensed chromatin and a thick cell envelope with very dense material within the periplasmic space composed of proteins and peptidoglycan (Amano & Williams, 1984; Maurin & Raoult, 1999; McCaul & Williams, 1981). The SCVs exhibit increased resilience to oxidative and nutritional stress, and the fortified cell wall promotes both intracellular survival and survival within the environment where they are resistant to chemical agents and adverse physical insults (McCaul & Williams, 1981; Sandoz et al., 2016).

Both variants are capable of infecting host cells with equal efficiency in cell culture, though this has not been confirmed in animal models (Sandoz, Sturdevant, Hansen, & Heinzen, 2014). Following phagocytosis, *C. burnetii* mediates maturation of the phagosome into an acidic parasitophorous vacuole, which vastly increases in size over time (Baca & Paretsky, 1983; Hussain, Broederdorf, Sharma, & Voth, 2010; Voth &

Heinzen, 2007). Within this vacuole, SCVs undergo morphogenesis into LCVs, and the LCVs then exponentially replicate via simple binary fission (Coleman et al., 2004; Handley, 1967; Stelzner, 1968). It was initially thought that LCVs also underwent sporulation, as small dense spore-like bodies were observed in the poles of LCVs during apparent asymmetrical cell division, which were proposed to then differentiate into SCVs (McCaul & Williams, 1981). However, it has since been demonstrated that SCVs are instead a result of morphological differentiation of LCVs to SCVs, with the latter re-emerging and predominating within infected cells over time as the pathogen enters a stationary phase of its lifecycle (Coleman et al., 2004; Heinzen, Hackstadt, & Samuel, 1999). The role of the spore-like bodies described by McCaul and Williams (1981) remains unclear, though it has been suggested that these may contribute to the persistence of *C. burnetii* antigen following infection (Harris, Storm, Lloyd, Arens, & Marmion, 2000).

In mammals, *C. burnetii* displays a tropism for reproductive tissues and mammary gland epithelial cells, allowing for the excretion of bacteria into the environment during parturition and lactation and hence, the lifecycle is complete (Roest et al., 2012; Sanchez et al., 2006; Sobotta, Bonkowski, et al., 2017). The mechanism by which viable bacteria are distributed within hosts is not well described and *C. burnetii* was thought not to be directly cytopathic (Coleman et al., 2004; Heinzen et al., 1999). However, replicating *C. burnetii* Nine Mile phase II have been demonstrated to actively induce apoptosis *in vitro* in THP-1 cells, which could facilitate the spread of infection to further sites if exhibited during natural infection (Zhang, Zhang, Hendrix, Tesh, & Samuel, 2012). Indeed, a transient bacteraemia occurs towards the end of the incubation period in humans, which is usually two to three weeks after initial infection but may be prolonged in cases with a low inoculum dose (Angelakis & Raoult, 2010).

Stationary SCVs may also persist within the host in various tissues, occasionally seeding viable bacteria into the bloodstream or episodically recrudescing into metabolically active infection during pregnancy and with immune suppression (Harris et al., 2000; Marmion et al., 2005). In humans, persistent infection of

prosthetic or diseased heart valves, and of vascular or osteoarticular lesions or prostheses are well described, though tropism towards these sites is not well understood (Million & Raoult, 2015). Persistence of infection within the reproductive tract of women infected during pregnancy is presumed to be the source of recrudescence during subsequent pregnancies (Anderson et al., 2013), and adipose tissue has been suggested as possible site of persistence in humans based on studies in mouse models (Bechah et al., 2014). Bone marrow is another proposed site of cryptic infection (Harris et al., 2000; Marmion et al., 2005) and hypermetabolic lesions in the bone marrow and spleen are reported in both acute and persistently infected Q fever patients, reflecting the lymphoid tropism of the pathogen (Eldin, Melenotte, et al., 2016).

1.3 Reservoirs of *Coxiella burnetii*

1.3.1 Domestic ruminants

Domestic ruminants are considered the major source of *C. burnetii* infection for people, with most Q fever outbreaks being attributed to sheep, goats, and cattle (Angelakis & Raoult, 2010). Serological evidence of *C. burnetii* exposure in sheep, goats and cattle has been widely established, with positive results obtained in every region sampled, with the exception of New Zealand (Eldin et al., 2017; Guatteo, Seegers, Taurel, Joly, & Beaudeau, 2011). Shedding of bacteria in ruminants is most pronounced in the reproductive tissues and fluids at the time of parturition or abortion. In goats, the greatest numbers of bacteria are found in the foetal trophoblasts of the placentomes (Sanchez et al., 2006). Shedding also occurs to a lesser degree in the vaginal mucus, milk and faeces of ruminants (Berri, Souriau, Crosby, & Rodolakis, 2002; Guatteo et al., 2006; Rodolakis et al., 2007; Roest et al., 2012). Persistent shedding in asymptomatic ruminants has been attributed to the establishment of *C. burnetii* infection within intestinal and mammary epithelial cells without inciting a significant host immune response (Sobotta, Bonkowski, et al., 2017).

1.3.2 Companion animals

Kosatsky (1984) was the first to describe an outbreak of Q fever related to a parturient cat, which occurred in 1982 in Nova Scotia, Canada. Thirteen adults were affected with pneumonia, of which nine were hospitalised. The outbreak was subsequently traced to one household in which a cat had given birth and was nursing kittens in the entryway thereafter (Kosatsky, 1984). Further cases of Q fever associated with parturient cats have been reported in the USA and Australia (Kopecny, Bosward, Shapiro, & Norris, 2013; Malo et al., 2018; Pinsky, Fishbein, Greene, & Gensheimer, 1991), and the pathogen has been isolated from vaginal swabs of pet cats in Japan (Nagaoka et al., 1998) and detected by PCR in uterine biopsies from both reproductively healthy and abnormal pet cats in the USA (Cairns, Brewer, & Lappin, 2007; Fujishiro, Scorza, Gookin, & Lappin, 2016). Parturient cats and new-born kittens are now considered a significant source of *C. burnetii* infection of people in maritime Canada (Higgins & Marrie, 1990) and Japan (Komiya, Sadamasu, Kang, et al., 2003) and a possible source of infection elsewhere, with biosafety precautions, such as wearing gloves and a mask, recommended whilst attending to aborting or parturient cats and neonatal kittens (Fujishiro et al., 2016).

The first outbreak of dog-associated Q fever was also described in Nova Scotia following exposure of a family to a parturient dog (Buhariwalla, Cann, & Marrie, 1996). While dog-associated Q fever remains less commonly reported than cats, Q fever has been confirmed in veterinary workers in a small animal veterinary hospital in suburban Sydney, Australia, following exposure to a parturient dog (Gibbons & White, 2014). Asymptomatic transmission from dogs to humans was also described in veterinary workers in Japan (Komiya, Sadamasu, Toriniwa, et al., 2003), and *C. burnetii* DNA has been detected by PCR in canine placentas in the Netherlands (Roest et al., 2013).

Serological studies provide further evidence that cats and dogs may be a reservoir for *C. burnetii*. Seropositivity is described in pet cats in Japan, Korea, South Africa, Zimbabwe, and Australia (Komiya,

Sadamasu, Kang, et al., 2003; Ma et al., 2020; Matthewman et al., 1997; Shapiro, Bosward, Heller, & Norris, 2015) and in dogs in Australia and in countries in Europe, Africa, South America, the Middle East and Asia (Boni, Davoust, Tissot-Dupont, & Raoult, 1998; Cooper, Hedlefs, Ketheesan, & Govan, 2011; Dejan, Tamara, Katarina, Dragan, & Sonja, 2019; Havas & Burkman, 2011; Ma et al., 2020; Shapiro, Norris, Heller, et al., 2016; Tshokey et al., 2019). In Australia, Shapiro et al. (2015) reported a seroprevalence of 5.1% in pet cats and 9.3% in breeding cats in the state of New South Wales [NSW] (Shapiro, Bosward et al. 2015). Interestingly, no feral cats or shelter cats were seropositive in the same study (Shapiro et al., 2015).

Seroprevalence in dogs in Australia was first reported for the Townsville region of north Queensland, where 21.8% of dogs sampled while attending suburban veterinary practices for routine procedures returned a positive result (Cooper, Hedlefs et al. 2011). A lower seroprevalence was reported more recently for a larger sample of dogs from four diverse subpopulations across various regions of Australia, in which 3.0% of household pet dogs, 2.3% of breeding dogs, 1.9% of shelter dogs and 6.5% of dogs in indigenous communities were seropositive (Shapiro, Norris, Heller, et al., 2016). The discrepancy between these two studies may reflect differences in the studied dog populations and methodology, as the former study was localised to one region and utilised an ELISA optimised with *C. burnetii*-infected and non-infected mice sera (Cooper et al., 2011) while the latter was geographically diverse with more robust laboratory methods utilising immunofluorescence assay [IFA] with a cut-off titre determined from a *C. burnetii*-infected dog (Shapiro, Norris, Heller, et al., 2016).

Most recently in Australia, Ma et al. (2020) investigated seroprevalence and bacterial shedding by pet cats and dogs in very remote and regional communities in western NSW, within a local health district [LHD] reporting high Q fever notification rates in people (> 13 per 100,000 population at the time of the study in 2018). The overall seroprevalence in cats and dogs was 13.1% and 26.1% respectively, utilising IFA previously adapted and validated for each species by Shapiro et al (2016; 2015). Seropositivity differed

greatly between communities, ranging from nil in both species to 45.5% in cats and 55.9% in dogs (Ma et al., 2020). Higher seropositivity was seen within 150 km of Lightning Ridge where an atypical Q fever outbreak occurred in the community in 2015 (Archer et al., 2017), suggesting common exposure of humans and their pets to an environmental source of *C. burnetii* (Ma et al., 2020). Feeding raw kangaroo meat was also associated with an increased risk of seropositivity in cats, a source of exposure previously hypothesised by Shapiro, Norris, Bosward, and Heller (2016) for cattery confined cats. Despite seropositivity, all samples (serum, whole blood, reproductive tissue, reproductive swabs) were negative for *C. burnetii* by PCR, with Ma et al. (2020) concluding pet dogs and cats were unlikely to be an important reservoir of human Q fever infection outside of the peri-parturient period. While parturition appears to be the most significant source of bacterial shedding in dogs and cats, Tozer et al. (2014) did detect *C. burnetii* in dog and cat urine samples by PCR, suggesting some level of risk from other routes of shedding.

The role of horses in Q fever epidemiology is unclear as they have not been identified as the source of infection in human cases to date (Desjardins et al., 2018). However, horses may pose a risk of transmission as *C. burnetii* DNA has been detected by PCR in equine placentas and aborted foetuses in Europe (Leon et al., 2012; Roest et al., 2013), and a pooled detection rate of 11.9% is reported for equine blood and urine sampled in Australia (Tozer et al., 2014). Seropositivity is also described in horses in various countries (Marenzoni et al., 2013) and most recently in horses at a riding stable in Southern France that was located close to cases of Q fever in people (Desjardins et al., 2018).

1.3.3 Wildlife

Vertebrate and invertebrate wildlife may directly transmit *C. burnetii* to humans or play an important role as a reservoir of infection for domestic species that are more commonly associated with human infections. The Three-toed Sloth for example, has been identified as a significant reservoir of human infection in French Guiana, more so than domestic ruminants and pets (Eldin et al., 2017), while wild rabbits have

been associated with Q fever pneumonia in Nova Scotia (Marrie, Williams, Schlech, & Yates, 1986). In the United Kingdom, wild rats exhibit high *C. burnetii* seroprevalence in and around livestock facilities and are considered a source of infection for domestic cats (Webster, Lloyd, & Macdonald, 1995).

Coxiella burnetii has been detected by PCR in blood, faecal, and urine samples of some native animals in Australia (Cooper, Stephens, Ketheesan, & Govan, 2013; Tozer et al., 2014). Kangaroos have been suggested as a reservoir in south-west and central Western Australia [WA], where Banazis, Bestall, Reid, and Fenwick (2010) reported a seroprevalence of 33.5% by ELISA and positive detection by PCR in 12.3% of faecal samples in western grey kangaroos compared to less than 1% seroprevalence and 8.4% positivity for pooled PCR samples (urine and faeces) in cattle and sheep in the same region. Utilising the same methods, Potter, Banazis, Yang, Reid, and Fenwick (2011) reported an overall *C. burnetii* seroprevalence of 24.1% and positive PCR detection in 4.1% of faecal samples throughout mid- to southwestern WA, with results varying between geographic areas within the region in both studies. Cooper, Barnes, Potter, Ketheesan, and Govana (2012) also reported high (20.8%) seroprevalence by ELISA in macropods across Australia, ranging from a low of 7.1% in western Queensland to a high of 30.4% in north Queensland. However, it is not understood whether the detection of *C. burnetii* by PCR in macropod faeces is due to true bacterial shedding from the gastrointestinal tract [GIT] or passive transit through the GIT as a consequence of bacterial ingestion (Banazis et al., 2010). Additionally, the ELISA methodology determined cut-off points in the absence of known negative and positive samples for the species. Banazis et al. (2010) and Potter et al. (2011) utilised pooled high-reacting serum and pooled low-reacting serum samples from the Banazis et al. (2010) study population as positive and negative controls respectively, whilst Cooper et al. (2012) optimised their ELISA with *C. burnetii*-infected and non-infected mice and canine sera, which may have impacted the validity of the results.

Despite positive *C. burnetii* serological and PCR samples from Australian wildlife, causality in human Q fever cases in Australia have not been definitively attributed to wildlife, though some case reports place

a high index of suspicion on macropods as the likely source of infection. Two cases from separate workplace locations were reported in northern NSW, in which both patients directly handled juvenile kangaroos in the weeks prior to illness and otherwise spent more than five hours a day mowing lawns contaminated with kangaroo faeces (Flint et al., 2016). However, a definitive source could not be confirmed in each case, all co-workers at both sites were seronegative for *C. burnetii*, and kangaroo tissue samples and tick specimens collected at one of the workplaces failed to detect *C. burnetii* by PCR (Flint et al., 2016). Severe Q fever resulting in multiorgan failure, disseminated intravascular coagulation and respiratory failure was also described in a patient with frequent occupational contact with deceased macropods in a geographical region reporting up to 40% seroprevalence in kangaroos (Stevenson, Gowardman, Tozer, & Woods, 2015). Similarly, the source of infection could not be confirmed and although contact with deceased kangaroos was considered a potential source of infection, the patient also lived only two kilometres from a paddock with cattle (Stevenson et al., 2015). Further research has been recommended to establish the pathobiology of *C. burnetii* in kangaroos, which is expected to differ from that of placental mammals, to understand bacterial shedding routes and the potential role of macropods and other marsupials in the epidemiology of human Q fever (Banazis et al., 2010).

1.3.4 Ticks

Ticks are another potential reservoir of *C. burnetii*, shedding bacteria in saliva, coxal fluid and faeces, contaminating the skin of the host and the surrounding environment (Eldin et al., 2017). Both hard and soft ticks can harbour the pathogen and transstadial transmission of *C. burnetii* has been experimentally confirmed in *Ixodes ricinus,* with adults shedding bacteria following infection as nymphs (Korner et al., 2020). Bacteria were detectable within *I. ricinus* ticks for at least seven weeks following *in vitro* feeding with a *C. burnetii*-infected blood meal, though excretion via faeces was observed only with blood meals reflective of highly bacteraemic hosts (Korner et al., 2020). While ticks are not considered essential for transmission among vertebrates, they may be an important vector in vertebrate wildlife populations (Eldin

et al., 2017), and some ticks may also contribute to the dispersal of bacteria across geographic regions as they parasitise migratory birds (Tokarevich et al., 2019).

Coxiella burnetii has been detected by PCR in Australian paralysis ticks (*Ixodes holocyclus*), kangaroo ticks (*Amblyomma triguttatum*), and bandicoot ticks (*Haemaphysalis humerosa*) (Bennett et al., 2011; Cooper et al., 2013; Graves et al., 2016; Tozer et al., 2014). While the bacterium is a confirmed Australian tick-transmitted pathogen (Graves & Stenos, 2017), direct transmission to humans via tick bite is rare with only one published report of suspected transmission (Beaman & Hung, 1989). The presence of ticks on pet dogs and cats was not associated with *C. burnetii* seropositivity in very remote and regional communities in western NSW (Ma et al., 2020). However, the transmission of *C. burnetii* between animals and from animals to humans in Australia is not well understood, and ticks may indeed play an important role in Q fever epidemiology as vectors of disease and environmental reservoirs (Cooper et al., 2013; Flint et al., 2016). In particular, *I. holocyclus* and *A. triguttatum* parasitise a wide range of hosts, including wildlife and domestic ruminants, cats, and dogs, and may bite humans (Cooper et al., 2013).

1.4 Routes of human infection

1.4.1 Inhalation

Inhalation is the most common route of infection associated with clinical Q fever in people (Angelakis & Raoult, 2010; Eldin et al., 2017; Tigertt, Benenson, & Gochenour, 1961). Respiratory exposure may occur directly from the source as *C. burnetii* is aerosolized from infected fresh animal tissues, fluids, and excreta, most prominently at and around the time of parturition (Kersh, Fitzpatrick, Self, Priestley, et al., 2013; Welsh, Lennette, Abinanti, & Winn, 1958). Welsh (1958) established the presence of *C. burnetii* in air samples within five to 24 minutes following parturition in sheep, which then persisted for an average of 12 days regardless of placental removal from the birthing area (Welsh et al., 1958). Infected animal tissues can also continue to release bacteria as they desiccate, contributing to the long-term persistence of

bacteria in the environment such that *C. burnetii* has been detected in soil, air and HEPA vacuum samples up to one year following a Q fever outbreak (Kersh, Fitzpatrick, Self, Priestley, et al., 2013). Bacteria may then be dispersed beyond the primary site of contamination within inhalable airborne dust particles by dry and windy environmental conditions (Hogerwerf et al., 2012; O'Connor, Tribe, & Givney, 2015; Tissot-Dupont, Amadei, Nezri, & Raoult, 2004; Tozer et al., 2014). As early as 1948, Q fever outbreaks in Artesia, California, were suspected to be associated with the inhalation of infected dust following arid spells and windstorms (Young, 1948). Since then, windborne spread has been reported in community outbreaks in other countries including France, the Netherlands, the United Kingdom, and Australia (Hackert et al., 2012; Hawker et al., 1998; O'Connor et al., 2015; Tissot-Dupont et al., 2004; Wallensten et al., 2010).

1.4.2 Ingestion

Bacteria may be ingested through the consumption of contaminated unpasteurised dairy products. However, the importance of the ingestion of dairy products as a source of clinical disease is debated (Cerf & Condron, 2006; Eldin, Angelakis, Renvoise, & Raoult, 2013). Several studies have reported an association between the consumption of raw milk and sporadic cases of clinical illness, or with increased seroprevalence (Beck, Bell, Shaw, & Huebner, 1949; Fishbein & Raoult, 1992; Marmion & Stoker, 1958; Signs, Stobierski, & Gandhi). However, seroconversion and clinical illness was not evident when people voluntarily consumed infected raw milk (Krumbiegel & Wisniewski, 1970). Ingestion is therefore considered to present a low, but not negligible risk for infection and disease (Gale, Kelly, Mearns, Duggan, & Snary, 2015).

1.4.3 Human to human transmission

Very rarely, cases of human-to-human transmission of *C. burnetii* are reported. Infected human tissues and blood products present a risk of exposure for medical, laboratory and mortuary staff, and transplant recipients (Osorio, Sarria, Gonzalez-Ruano, Casal, & Garcia, 2003). The pathogen can remain viable and

infectious in donated blood products stored at 1 – 6°C for more than six weeks (Kersh, Priestley, & Massung, 2013), although the likelihood of an infected blood donation in Australia is considered low (Gidding et al., 2019). Q fever following bone marrow transplantation has also been reported (Kanfer et al., 1988). During pregnancy, infected women pose a transmission risk to their unborn child and to hospital staff, as bacteria may be present in the placenta, vaginal secretions and breast milk (Amit, Shinar, Halutz, Atiya-Nasagi, & Giladi, 2014; Raoult & Stein, 1994). Patient to patient transmission has been reported within a maternity ward, which was presumed to have been due to the aerosolisation of vaginally excreted *C. burnetii* (Amit et al., 2014), while medical staff performing an emergency caesarean section on a patient known to have Q fever seroconverted despite having used respiratory protection (Hassidim et al., 2018). Sexual transmission has also been reported due to the presence of bacteria in semen (Milazzo et al., 2001).

1.5 Clinical manifestations

1.5.1 Acute Q fever

1.5.1.1 Clinical Presentation and Management

Clinical signs of acute Q Fever were first described by Derrick in 1937, who documented nine confirmed cases in men aged between 17 and 55 years (Derrick, 1937). The most prominent clinical signs observed were fever of variable length and persistent headache, while a relative bradycardia, sweats, ataxia, conjunctival congestion, photophobia and jaundice were also seen in some patients (Derrick, 1937). Later reports of illness also found fever of variable length, persistent headache, relative bradycardia, malaise and myalgia to be the most consistent findings, while hepatomegaly, splenomegaly, atypical pneumonia, and pancreatitis were also reported among other signs (Kosatsky, 1984; Marrie, 2010; Marrie, Langille, Papukna, & Yates, 1989; Spelman, 1982). Since these early reports of disease, it has become clear that a range of non-specific symptoms may occur following exposure to *C. burnetii*.

Generally, symptomatic Q fever disease is estimated to occur in 20% to 80% of individuals exposed to *C. burnetii*, with symptoms usually apparent after an incubation period of two to three weeks (Million & Raoult, 2015). However, incubation period, symptoms and clinical attack rates vary according to inoculum dose, bacterial strain, and patient factors including age, gender, pregnancy, and comorbidities (Angelakis & Raoult, 2010; Brooke, Kretzschmar, Mutters, & Teunis, 2013; Hackert et al., 2015; Hackert et al., 2012; Million & Raoult, 2015). In symptomatic cases, acute febrile illness with fatigue and headache are most commonly reported, as seen in recent outbreaks in the Netherlands and Australia (Archer et al., 2017; Bond et al., 2016; Limonard et al., 2010; O'Connor et al., 2015). Fever may last more than two weeks and may be accompanied by hepatitis or pneumonia (Dugdale, Chow, Yakirevich, Kojic, & Knoll, 2014; Eldin et al., 2017; Marrie, 2010). In Australia, hepatitis appears to be more common than pneumonia, though patients may exhibit both (Graves & Islam, 2016). Elsewhere, pneumonia is particularly prevalent among Q fever patients in Maritime Canada, while a local *C. burnetii* 'geotype' in French Guiana is responsible for high rates of hospitalisation for Q fever pneumonia accompanied by unusually high antibody titres (Marrie, 2010; Million & Raoult, 2015).

Antiphospholipid antibodies are also a feature of acute *C. burnetii* infection, which may lead to autoimmune disease (Ordi-Ros et al., 1994). New research has established that anti-cardiolipin antibodies can cause acute endocarditis, characterized by non-infectious sterile vegetations of the aortic valve (Million et al., 2016). In rare cases, myocarditis, pericarditis, meningitis, meningoencephalitis, lymphadenitis, bone marrow pathology and cholecystitis have also been reported in acute disease (Eldin et al., 2017).

Doxycycline for 14 days is currently recommended for the treatment of symptomatic acute Q fever in adults, with the exception of pregnant women (Eastwood et al., 2018; Eldin et al., 2017). The USA CDC and Q fever Working Group (2013) recommended doxycycline for 14 days for treatment of acute Q fever in children over the age of eight years, and also in those less than eight years with severe illness or risk

factors for persistent infection (Anderson et al., 2013). Consideration of a shorter course in children less than eight years with uncomplicated illness was recommended due to a risk of dental complications (Anderson et al., 2013). However, Melenotte, Million, and Raoult (2020) recommend doxycycline for 14 days as a suitable treatment regime in all children with acute Q fever, as the risk of dental discoloration or dental enamel hypoplasia, which was extrapolated from safety data for tetracycline, has been shown to be minimal (Gaillard, Briolant, Madamet, & Pradines, 2017). In Australia, it is recommended that children be specifically referred to an infectious disease physician for treatment (Eastwood et al., 2018). If required, alternative treatments include minocycline, clarithromycin, fluoroquinolones, and co-trimoxazole (trimethoprim and sulfamethoxazole) (Eldin et al., 2017).

Where anti-cardiolipin antibodies are detected during acute Q fever, it is recommended that hydroxychloroquine be added and treatment extended to a minimum period of 3 weeks, and then continued until anti-cardiolipin has normalised (Million et al., 2017). It is also recommended that hydroxychloroquine be added for treatment of patients that develop endocarditis during acute Q fever, with treatment extended out to 18 months (Melenotte et al., 2020). Clinical and serological follow-up is recommended for at least six to 12 months, and should anti-phase I immunoglobulin [Ig] G titres >800 persist beyond six months, or where clinical outcome is poor, persistent infection should be considered (Eastwood et al., 2018; Eldin, Melenotte, et al., 2016).

1.5.2 Persistent infection (chronic Q fever)

1.5.2.1 Endocarditis

Endocarditis is the most commonly diagnosed presentation of persistent Q fever (Eldin et al., 2017; Fenollar et al., 2001; Gikas, Kokkini, & Tsioutis, 2010), resulting from *C. burnetii* colonisation of heart valves (Million et al., 2016). Bacteria persist in low numbers within small focal collections of mononuclear cells, inciting fibrosis, calcification, mild inflammation, and vascularisation of the heart valves (Lepidi,

Houpikian, Liang, & Raoult, 2003). Over time, this leads to thickening or remodelling which may be mistaken for non-infectious valvular degenerative damage in the absence of vegetations on echocardiography and where blood culture is negative (Lepidi et al., 2003; Million, Thuny, Richet, & Raoult, 2010). Some patients remain asymptomatic for months or years, during which time the heart valves are progressively damaged until cardiovascular signs occur, including sudden cardiac insufficiency, thromboembolism, or mycotic aneurysm (Eldin et al., 2017; Million & Raoult, 2015). Q fever should therefore be considered a differential diagnosis in all patients with unexplained valvulopathy, especially when accompanied by an inflammatory syndrome or hepatomegaly (Million & Raoult, 2015).

In others, clinical signs may be non-specific, such as relapsing fevers, chills, sweats, weight loss and hepatosplenomegaly (Eldin et al., 2017). Diagnosis of persistent Q fever endocarditis can therefore be challenging, delaying treatment and increasing the risk of relapse and mortality (Lepidi et al., 2003; Million & Raoult, 2015). Risk factors include being male, age above 40 years, increased anticardiolipin antibodies during acute Q fever, and pre-existing valvulopathy including valvular prosthesis, aortic stenosis, mitral insufficiency, mitral valve prolapse or a bicuspid aortic valve (Million & Raoult, 2015). Q fever endocarditis is treated with a combination of doxycycline and hydroxychloroquine for at least 18 – 24 months, and post-treatment serological monitoring should be continued for at least five years (Million et al., 2010).

1.5.2.2 Vascular Infection

Persistent *C. burnetii* vascular infections are established when an existing vascular lesion or graft is primarily colonized following acute infection, or where a mycotic aneurysm occurs secondarily to endocarditis (Botelho-Nevers et al., 2007; Eldin, Melenotte, et al., 2016). Such foci of infection are increasingly detected with improved diagnostic tools and criteria, and increasing awareness of this condition (Eldin et al., 2017). Abdominal and thoracic aortic lesions are most common (Botelho-Nevers et al., 2007; Eldin et al., 2017) and unlike endocarditis, bacteria are present in high numbers (Eldin, Mailhe,

et al., 2016). Histologically, necrotising granulomas within the vascular endothelium are described (Hagenaars et al., 2014).

Life-threatening complications include aorto-duodenal fistula, aorto-caval fistula, and the rupture of a graft or aneurysm, while emboli and osteoarticular infection are also recognised sequalae (Botelho-Nevers et al., 2007; Eldin et al., 2017; Hagenaars et al., 2014). Prior to such complications, infection may remain asymptomatic or present with non-specific signs including fever, weight loss, abdominal pain, and fatigue (Botelho-Nevers et al., 2007). Consequently, vascular infections are underdiagnosed and associated with a poor prognosis and increased mortality (Eldin, Mailhe, et al., 2016). Mortality of 18% is reported at two and a half years follow-up in a cohort of 66 patients (Eldin, Mailhe, et al., 2016) and 25% in 32 patients followed for three years or more (Botelho-Nevers et al., 2007). In addition to antibiotic treatment for 18 – 24 months, surgical removal of infected tissue is required to resolve vascular infection due to the high bacterial load (Botelho-Nevers et al., 2007; Eldin, Mailhe, et al., 2016).

1.5.2.3 Osteoarticular infections

Osteoarticular infections are rare but increasingly reported with improved diagnostics and awareness (Eldin et al., 2017). Lesions are characterised by well-formed granulomas with very few bacteria present (Landais et al., 2007). Pure osteoarticular infections may persist following primary Q fever in otherwise healthy immune-competent patients with no apparent underlying disease (Britton, Macartney, Arbuckle, Little, & Kesson, 2015; Landais et al., 2007), and in patients with prosthetic implants or those who are immunocompromised (Raoult et al., 2000). Persistent multifocal osteomyelitis is most frequently reported in children (Britton et al., 2015), while osteoarticular infections in adults are variable including osteomyelitis, spondylodiscitis, tenosynovitis, arthritis, culture-negative prosthetic joint arthritis, subacromial bursitis, coxitis and sacroiliitis (Eldin et al., 2017; Landais et al., 2007). Spondylodiscitis most often presents concurrently with endocarditis or vascular infection and is often accompanied by psoas abscess (Eldin, Melenotte, et al., 2016). Treatment of adults with doxycycline and hydroxychloroquine for

at least 18 months is recommended, which has been extrapolated from established treatment strategies for endocarditis (Eldin et al., 2017; Landais et al., 2007). Surgical intervention may also be required for resolution (Landais et al., 2007; Million, Bellevegue, et al., 2014).

1.5.2.4 Other foci of persistent infection

Persistent lymphadenitis may occur as an isolated focus of infection or associated with other foci including endocarditis, vascular, or osteoarticular infections (Eldin, Melenotte, et al., 2016). Q fever lymphadenitis has been identified as a risk factor for non-Hodgkin lymphoma (Melenotte et al., 2018) due to the up-regulation of anti-apoptotic processes during *C. burnetii* infection (Melenotte et al., 2019). Other sites of persistent focalized *C. burnetii* infection include bone marrow, pulmonary, pericardial, and gall bladder localisations (Eldin, Melenotte, et al., 2016) and recently a breast implant infection was described (Hassidim et al., 2018). Possible prostatic, thyroid, and laryngeal foci have also been identified with ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography [^{18}F-FDG PET/CT] in association with another confirmed main focus of infection, though the significance and specificity of these additional hypermetabolic foci remain unknown (Eldin, Melenotte, et al., 2016).

1.5.2.5 Antibiotic prophylaxis

Antibiotic prophylaxis with a combination of doxycycline and hydroxychloroquine for at least 12 months can prevent progression of acute Q fever to persistent Q fever endocarditis (Million, Walter, Thuny, Habib, & Raoult, 2013). This prophylactic strategy has been extrapolated to patients with a vascular graft or confirmed aneurysm to reduce the risk of persistent vascular infection (Eldin, Mailhe, et al., 2016). Acute Q fever patients should therefore be screened for risk factors for endocarditis and vascular infection to enable an evaluation of the potential benefit of prolonged antimicrobial therapy. However, a review of 89 Q fever patient records from the period 2005 – 2009 in NSW revealed only six cases in which a complete cardiac examination had been undertaken and 91% of cases had no record of a cardiac history being taken,

prompting efforts to address this deficiency within the medical profession (Hess, Massey, Durrheim, O'Connor, & Graves, 2011).

Q fever experts from the French National Referral Centre for Q Fever, Marseille, recommend routine echocardiography to screen for valvulopathy and serological testing for IgG anticardiolipin in acute Q fever patients, particularly those over 40 years of age (Eldin et al., 2017; Million et al., 2013). Antibiotic prophylaxis for the prevention of endocarditis would then be recommended in patients with valvulopathy or patients over 40 years of age expressing high IgG anticardiolipin (Eldin et al., 2017; Million et al., 2013). Similarly, abdominal computed tomography (CT) or ultrasound to detect abdominal aortic aneurysm has been recommended for acute Q fever patients with known risk factors for aneurysm (over 65 years of age, smoking, or family history of aneurysm). Antibiotic prophylaxis would then be recommended where aneurysm is detected, and in patients with vascular grafts (Eldin, Mailhe, et al., 2016). Currently, there is lack of data to support routine prophylaxis of patients with osteoarticular prostheses or immune compromise (Eldin et al., 2017).

1.5.3 Q fever fatigue syndrome

Q fever fatigue syndrome [QFS] is a recognised sequela of acute Q fever in Australia (Eastwood et al., 2018). Early reports in Australian abattoir workers describe incapacitating fatigue, nausea, headache, sweats, myalgia, arthralgia, alcohol intolerance, and sleep disturbances five to 14 years following acute illness (Marmion, Shannon, Maddocks, Storm, & Penttila, 1996). Q fever fatigue syndrome has since been described internationally and is estimated to occur in approximately 20% of patients (Morroy et al., 2016). However, recognition of this syndrome is controversial as aetiology and diagnostic criteria have not been universally defined and may be varied and multifactorial (Hatchette, Hayes, Merry, Schlech, & Marrie, 2003; Limonard et al., 2016; Morroy et al., 2016). Hypotheses include cytokine dysregulation and immunomodulation due to persistent infection or the persistence of antigen in the absence of viable

bacteria, which may be influenced by host and genetic factors (Harris et al., 2000; Marmion et al., 2005; Morroy et al., 2016; Penttila et al., 1998). Symptoms may also be perpetuated by behavioural and psychogenic factors (Wildman et al., 2002). Regardless of aetiology, the consequences are debilitating for patients and present significant financial cost, as made evident in a large Dutch Q fever outbreak following which severe fatigue and severely impaired general quality of life were reported in 40% of patients four years following acute infection (Limonard et al., 2016).

1.5.4 Q fever in pregnancy

Women infected with *C. burnetii* during pregnancy are less likely to display overt symptoms of Q fever, such as febrile illness (Tissot-Dupont, Vaillant, Rey, & Raoult, 2007). However, adverse pregnancy outcomes including miscarriage, foetal death, malformations, and prematurity have been associated with infection in multiple countries, and are attributed to placentitis and infection of the foetus (Carcopino, Raoult, Bretelle, Boubli, & Stein, 2009; Eldin et al., 2017; Million, Roblot, et al., 2014; Raoult & Stein, 1994). However, obstetrical morbidity may differ between regions with bacterial strain and the ability of health care providers to diagnose Q fever where adverse pregnancy outcomes occur (Angelakis et al., 2013; Million, Roblot, et al., 2014). Foetal loss is more likely when infection occurs during the first trimester, for which anti-phospholipid antibodies may be implicated (Million, Roblot, et al., 2014), while infection later in pregnancy is more likely to result in premature delivery (Carcopino, Raoult, Bretelle, Boubli, & Stein, 2007). The risk of persistent *C. burnetii* infection is also increased in women infected during pregnancy (Carcopino et al., 2009), likely due to altered immune responses favouring bacterial growth and the replicative niche afforded by placental trophoblasts (Amara et al., 2010; Carcopino et al., 2009). *Coxiella burnetii* may then recrudesce during subsequent pregnancies, resulting in recurrent adverse pregnancy outcomes for individuals (Anderson et al., 2013).

Antibiotic treatment for *C. burnetii* during pregnancy has been shown to prevent adverse outcomes (Carcopino et al., 2007; Million, Roblot, et al., 2014), although approaches to diagnosis and management differ between regions. In Australia, there remains uncertainty regarding the consequences of untreated infection during pregnancy and consequently monthly serological monitoring is recommended initially, followed by treatment with trimethoprim and sulfamethoxazole (co-trimoxazole) during pregnancy and doxycycline postpartum where rising antibody titres are confirmed (Gidding & Graves, 2013). Further research into Q fever during pregnancy has been called for in Australia as Q fever remains a potentially under-recognised though treatable cause of adverse pregnancy outcomes, particularly given Q fever is not routinely considered in investigations of stillbirth without a high index of suspicion (Marks & Olenski, 2019).

Elsewhere, the USA CDC and Q fever Working Group (2013) recommend that women diagnosed with acute Q fever during pregnancy receive antibiotic treatment throughout the pregnancy (Anderson et al., 2013). To aid in the appropriate treatment of women, the working group further recommend that all women of child-bearing age who are diagnosed with acute Q fever be pregnancy tested, educated on the risks to the foetus should they become pregnant during treatment and monitoring, and be carefully monitored for recrudescence in any subsequent pregnancies (Anderson et al., 2013). However, as *C. burnetii* infection may remain mild or asymptomatic prior to adverse pregnancy outcomes, researchers in France recommend the broader approach of *C. burnetii* serological screening of all pregnant women in endemic areas and antibiotic treatment where serological titres are indicative of either acute exposure or persistent infection (Million, Roblot, et al., 2014). Across regions, careful serological and clinical monitoring of pregnant Q fever patients for persistent infection is recommended during pregnancy and for at least 24 months post-partum (Anderson et al., 2013; Eldin et al., 2017). Where progression to persistent infection is diagnosed, long term antibiotic management is required and the risk of *C. burnetii* transmission to the child via breastfeeding should be considered (Carcopino et al., 2007).

1.5.5 Q fever in children

Globally, Q fever is rarely reported in children, and this is generally attributed to asymptomatic infection or milder disease being more common in children than in adults (Hackert et al., 2015). Occupational exposure risk is also reduced or negligible in children. However, where community (non-occupational) exposure occurs, lower rates of infection are still seen in children compared to adults (Hackert et al., 2015). In Australia, children aged less than 15 years comprised 2.1% (256/12,164) of notifications nationally from 1991 – 2014 (Sloan-Gardner, Massey, Hutchinson, K.Knope, & Fearnley, 2017) while in NSW, children aged less than 10 years comprised 1.3% (n = 26) of notifications in the state from 2001 – 2010 (Lowbridge, Tobin, Seale, & Ferson, 2012). However, there is increasing concern in Australia regarding Q fever in children following a marked increase in paediatric (<15 years old) Q fever notifications in Queensland from 2000 – 2008 (Tozer, Lambert, Sloots, & Nissen, 2011), and higher than expected seroprevalence (5%) in older children and young adults (10-19 year-olds) in the Hunter-New England region of NSW Wales from 2006 – 2009 (Islam, Ferguson, Givney, & Graves, 2011).

When Q fever does present in children, acute infection is non-specific with clinical manifestations including fever, influenza-like symptoms, respiratory infection, malaise, gastroenteritis-like symptoms, and skin rash (Anderson et al., 2013), which can mimic other childhood infections and result in a lack of suspicion of Q fever (Hackert et al., 2015). In the minority of cases, acute Q fever in children may be severe, with hepatitis, cholecystitis, meningitis, encephalitis, pericarditis, myocarditis, lymphadenitis, rhabdomyolysis, and haemolytic-uremic syndrome reported (Anderson et al., 2013; Eldin et al., 2017; Maltezou et al., 2004). Persistent *C. burnetii* infection in children may be debilitating, with chronic multifocal granulomatous osteomyelitis the most recognised presentation, requiring prolonged antibiotic therapy and often surgical drainage for cure, though long-term follow-up is important to monitor for possible late relapses (Britton et al., 2015; Nourse et al., 2004).

1.6 Immune responses to *Coxiella burnetii* infection

1.6.1 Innate and cell mediated responses

It is accepted that innate immunity and cell mediated immunity [CMI] are most critical for early bacterial control and also later *C. burnetii* clearance during both initial infection and re-challenge (Andoh et al., 2007; Eldin et al., 2017; Zhang et al., 2007). Following the uptake of *C. burnetii* by monocytes, polarisation towards an M1-type response occurs and pro-inflammatory cytokines and receptors are expressed including tumour necrosis factor [TNF], resulting in the control of intracellular *C. burnetii* replication (Benoit, Barbarat, Bernard, Olive, & Mege, 2008). In contrast, *C. burnetii* infection of macrophages leads to their polarisation towards a unique and atypical M2-type response, in which interleukin [IL] IL-6 and IL-10 are upregulated and TNF and TLR2 expression is downregulated, permitting moderate replication of the pathogen within macrophages during acute infection (Benoit et al., 2008).

Subsequent presentation of bacterial antigens to T-lymphocytes induces a T-helper cell type 1 [Th1] response. This is pivotal for achieving pathogen clearance via granuloma formation and an array of microbicidal actions, primarily mediated by interferon gamma [IFN-γ] and TNF, including phagosome maturation and alkalinisation, regulation of nutrients such as iron, and ultimately apoptosis (Andoh et al., 2007; Capo & Mege, 2012; Dellacasagrande, Capo, Raoult, & Mege, 1999; Ghigo et al., 2002; Zhang et al., 2007). Importantly, it is thought that IFN-γ reorients M2 polarised macrophages towards an M1 response to allow for eventual pathogen clearance (Amara, Bechah, & Mege, 2012).

1.6.2 Humoral immune responses

Anti-*C. burnetii* antibodies can usually be detected within two to three weeks of the onset of symptoms (Melenotte et al., 2020). Anti-phase II IgM, IgG and IgA usually appear earlier and reach much higher titres than their anti-phase I counterparts, with anti-phase II IgM and IgA subsiding within months while anti-phase II IgG antibodies may persist for years following infection (Dupuis, Péter, Peacock, Burgdorfer, &

Haller, 1985; Tissot-Dupont & Raoult, 2008). An idealised representation of antibody responses following acute Q fever in humans is presented in **Figure 1.1**. However, antibody responses will vary between individuals and with the bacterial strain (Million & Raoult, 2015).

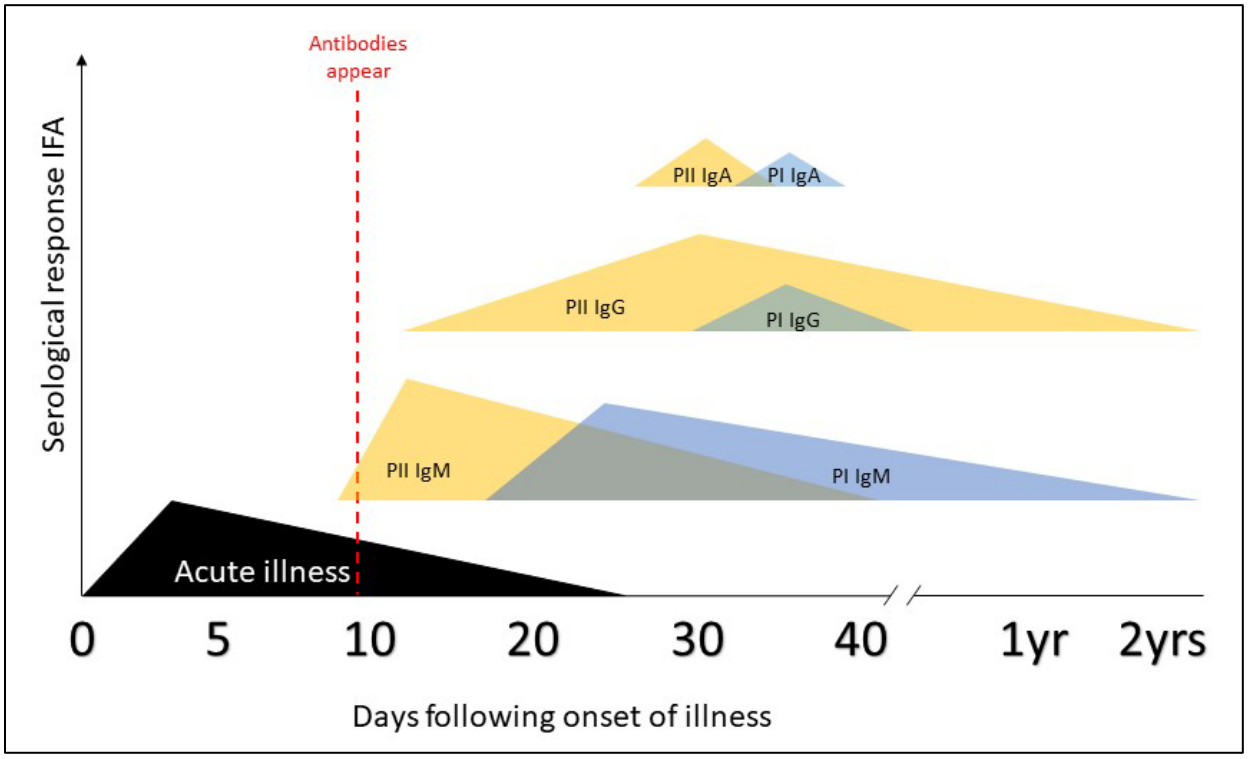

Figure 1.1 Idealised representation of antibody responses following acute Q fever. Diagram based on the clinical and laboratory experience of the Adelaide Q fever Research Group and Infectious Disease Laboratories illustrated by CSL (2009). Results may vary between individuals. Persistent infection is not illustrated.

Despite strong antibody responses to *C. burnetii* infection, the traditional mechanisms of antibody-mediated immunity are not apparent as anti-*C. burnetii* antibodies have not been determined as directly bactericidal, opsonization does not appear to negatively affect intracellular bacterial replication or viability, and pathogen clearance is independent of the complement and Fc receptor [FcR] cellular activation (Shannon, Cockrell, Takahashi, Stahl, & Heinzen, 2009). Desnues et al (2009) demonstrated that opsonization of *C. burnetii* may in fact be favourable for the pathogen, as it resulted in intensive replication within human macrophages *in vitro*. Antibody-mediated immunity is therefore not considered essential

for the clearance of primary *C. burnetii* infection, which is instead dependent on T-cell responses (Andoh et al., 2007).

However, *in vivo* studies in mice suggest that antibody-mediated immunity may be critical in protective immunity, as the passive transfer of immune sera or purified IgG from immunocompetent vaccinated mice to naïve mice afforded significant protection (Zhang et al., 2013; Zhang et al., 2007). In agreement with Shannon et al. (2009), antibody-mediated immunity was independent of complement activation and FcR-mediated effector functions. However, Zhang et al. (2013) hypothesised that in contrast to *in vitro* studies, anti-*C. burnetii* antibodies may indeed neutralize or kill the pathogen *in vivo*. Concurrent T-cell competency remained critical for full antibody-mediated protection and for later control of replication and clearance of the pathogen (Zhang et al., 2013). B-cells may also be important for the avoidance of self-damage to host tissues through their down-regulation of Th1 responses primarily via IL-10 (Andoh et al., 2007).

1.6.3 Autoimmunity

Anticardiolipin antibodies are reported in 47 – 81% of acute Q fever patients (Jansen et al., 2018), and have been associated with fever, thrombosis, thrombocytopenia, haemophagocytic syndrome, hepatitis, cholecystitis, meningitis, acquired non-infectious endocarditis and the progression to persistent infectious endocarditis in patients with pre-existing valvular pathology (Melenotte et al., 2018; Million et al., 2017; Million et al., 2016). Foetal death during infection in early pregnancy may also be a consequence of antiphospholipid syndrome (Million, Roblot, et al., 2014). Camacho, Outschoorn, Tellez, and Sequi (2005) identified that a significant percentage of acute and chronically infected Q fever patients expressed anti-cardiac muscle and anti-smooth muscle antibodies, though their clinical significance was not evaluated.

1.6.4 Persistent infection

Persistent infection is attributed to a failure of the Th1 response to effectively eliminate *C. burnetii*, although the mechanisms behind such failure *in vivo* are yet to be elucidated (Eldin et al., 2017). *In vitro* studies utilising isolated peripheral blood mononuclear cells [PBMCs] from persistent Q fever patients determined that systemically, IFN-γ production and IFN-γ pathway responses appear to remain intact during persistent infection (Schoffelen et al., 2017). The authors postulated that *in vivo* functionality may differ at the local site of infection, particularly as *C. burnetii* exhibits a predilection for specific sites of persistent infection and the bacterial challenge at these sites would be significantly lower than that utilised *in vitro* (Schoffelen et al., 2017).

The cytokine IL-10 is thought to play a key role in sustaining *C. burnetii* infection as it is elevated during persistent infection and in pregnancy, impairing microbicidal activity of host cells and providing positive feedback for further IL-10 production (Eldin et al., 2017). Benoit et al. (2008) described the ability of IL-10 to polarise human monocytes and macrophages towards an M2-type response, in which local bacterial persistence was perpetuated directly by permitting *C. burnetii* replication and indirectly by reducing M1 responses that would otherwise result in IFN-γ production and pathogen clearance (Benoit et al., 2008). Indeed, this immune response appeared to be localised and mediated by specific host-pathogen interactions rather than an intrinsic immune defect, as *in vitro C. burnetii* killing by monocytes was restored following cure (Dellacasagrande, Ghigo, Capo, Raoult, & Mege, 2000). Various other intrinsic and extrinsic factors likely influence immune responses in persistent infection, such as host genetic polymorphisms (Schoffelen et al., 2017), bacterial strain (Sobotta, Hillarius, et al., 2017), and pathological predilection (Amara et al., 2012).

In individuals with persistent *C. burnetii* infection, antibodies against both phase I and phase II antigens are often chronically elevated (Eldin et al., 2017; Healy et al., 2011), with anti-phase I IgG and IgA titres

predominating (Graves & Islam, 2016). As opsonised bacteria are more readily internalised and stimulate the further production of IL-10, antibody persistence may indeed perpetuate persistent infection (Desnues et al., 2009). However, low serological titres have also been described in patients with confirmed foci of persistent infection (Melenotte et al., 2020).

1.7 Diagnosis

1.7.1 Acute Q fever

Upon suspicion of Q fever, it is recommended in Australia to submit blood samples for *C. burnetii* PCR and serology (anti-phase I and phase II IgG and IgM), preferably by IFA within seven days of disease onset, and a convalescent serology specimen collected more than seven days after the initial sample (Eastwood et al., 2018; Gunaratnam et al., 2014). An interval of three to six weeks between initial and convalescent samples is described by the USA CDC Q fever Working Group, which included participation from the Australian Rickettsial Reference Laboratory (Anderson et al., 2013). A diagnosis of acute Q fever may be confirmed by positive *C. burnetii* PCR, or a fourfold rising anti-phase II IgG titre between the initial and convalescent serological samples when tested in parallel (Anderson et al., 2013; Eastwood et al., 2018). Of these, PCR offers the most rapid and useful option for early diagnosis of definitive Q fever when positive (Bae et al., 2019; Graves & Islam, 2016), though it is important to recognise that bacteraemia is transient and a negative PCR does not exclude acute Q fever (Eastwood et al., 2018).

A single serum sample demonstrating elevated anti-phase II IgM or IgG in conjunction with compatible illness is supportive of acute Q fever (Anderson et al., 2013; Eastwood et al., 2018). However, IgM antibodies have a lower specificity than IgG and may exhibit cross-reactivity with other pathogens such as *Legionella* and *Bartonella* (Anderson et al., 2013). Culture of *C. burnetii* may also be utilised to confirm Q fever diagnosis. However, culture is not routinely recommended in Australia as it presents a high risk for laboratory-acquired infection and must be undertaken within biosafety level 3 laboratory conditions

(Eastwood et al., 2018). Where diagnosis of acute Q fever is delayed, illness may be prolonged or fatal (Munckhof et al., 2007).

1.7.2 Persistent Infection

Definitive diagnosis of persistent *C. burnetii* infection is based on the identification of a focus of infection (endocarditis, vascular aneurysm, infection of a vascular aneurysm or prosthesis, chronic hepatitis, osteomyelitis, osteoarthritis) and laboratory confirmation through positive *C. burnetii* PCR, culture, or immunohistochemistry (Anderson et al., 2013). The CDC Q fever Working Group also considers elevated anti-phase I IgG titres ≥800 or ≥1,024 (dependant on laboratory methods) as a major criterion that is sufficient for laboratory confirmation of persistent infection when accompanied by an identified focus of infection, as extrapolated from the modified Duke criteria for infective endocarditis (Anderson et al., 2013). The presence of increased anti-phase I IgA is additionally supportive of persistent infection (Graves & Islam, 2016).

Importantly, diagnosis of persistent *C. burnetii* infection should not be made on serological evidence alone, as serological profiles differ between regions, patients, and with the different clinical entities (Eldin et al., 2017). Laboratory results for a patient may also vary according to the centre in which they are tested (Healy et al., 2011). Indeed, guidance from the French National Referral Centre for Q Fever in Marseille, France, assigns a higher titre (≥6,400) as a major criterion for confirmed *C. burnetii* endocarditis, vascular infections, and prosthetic joint arthritis, while retaining ≥800 for osteoarticular infection (non-prosthetic) and lymphadenitis (Eldin et al., 2017). However, exclusion of persistent infection should not rely on serology alone, as some patients with laboratory confirmed persistent Q fever endocarditis have returned anti-phase I IgG titres <800 (Edouard et al., 2013). In recent years ^{18}F-FDG PET/CT has proven to be an invaluable tool for the detection of persistent *C. burnetii* foci in French Q fever patients (Eldin, Melenotte, et al., 2016).

1.8 Risk factors

1.8.1 Occupation

Occupational exposure associated with the livestock industry is considered the most significant risk for Q fever in Australia; including abattoir and meat workers, farmers, shearers, wool classers, livestock veterinarians, livestock transporters, and professional shooters (Eastwood et al., 2018; Lowbridge et al., 2012; Sloan-Gardner et al., 2017). Occupations involving contact with livestock or their fluids were most commonly reported from 2002 – 2013 in combined notification data for Queensland and NSW, which report the most Q fever notifications nationally (Sloan-Gardner et al., 2017). This is further supported by a recent seroprevalence study in blood donors in these two states, in which *C. burnetii* seropositivity was significantly associated with contact with sheep, cattle and goats, and having worked in an abattoir or assisted at an animal birth (Gidding et al., 2019). Elsewhere, livestock farmers and abattoir workers constituted over 50% of notifications in South Australia [SA] from 2007 – 2017 (Rahaman, Milazzo, Marshall, & Bi, 2019). Prior to the Australian Government funded National Q fever Management Program [NQFMP], which provided free Q fever vaccination for at-risk workers from 2002 – 2006, approximately half of all notified cases were among abattoir workers (NNDSS Annual Report Working Group, 2019). The uptake of this program was most successful in abattoir workers, reaching close to 100% coverage and contributing to a decline in national notification rates by more than 50% (Gidding, Wallace, Lawrence, & McIntyre, 2009). The resultant decline in notifications associated with slaughtering and meat work, saw abattoir and meat workers, butchers and professional shooters account for only 10% of notifications in NSW for the period 2001-2010, while farmers, shearers and graziers predominated (52%) (Lowbridge et al., 2012).

Occupations beyond the immediate livestock industry that are considered to have an increased risk of Q fever include the wider veterinary cohort of veterinarians, veterinary nurses and veterinary students,

agricultural college staff and students, professional dog and cat breeders, wildlife and zoo workers, animal refuge workers, and laboratory workers who handle veterinary specimens or work with *C. burnetii* (Australian Technical Advisory Group on Immunisation, 2018). However, these workers are not well described in published notification or seroprevalence data in Australia to date. Instead, risk is attributed to individual case reports and to investigations identifying non-traditional reservoir host animal species, including cats, dogs and Australian wildlife (Cooper et al., 2011; Cooper et al., 2013; Shapiro, Norris, Heller, et al., 2016; Shapiro et al., 2015; Tozer et al., 2014). Occupational risk also encompasses workers who are not directly exposed to animals or their products, but rather the environments in which they are contained, such as contractors, maintenance workers and groundskeepers (Eastwood et al., 2018). This was evident during an outbreak on an isolated goat farm in regional Victoria from 2012 – 2014, during which administration staff, visiting trade contractors, and the spouse of a farm labourer were infected despite no direct animal exposure (Bond et al., 2016).

While occupation remains the most well recognised risk factor in Australia, 66% (2985/4523) of notifications had no occupation listed in NSW and Queensland notification data for the period 2002 – 2013 and, hence, there is a need for collection of more complete and consistent occupational data in state and national notification databases (Sloan-Gardner et al., 2017). The introduction of the Notifiable Conditions Information Management System in NSW in 2010 has permitted enhanced data collection for the state, with 91.4% of Q fever notifications reporting an occupation in the period 2011 – 2015 (Clutterbuck, Eastwood, Massey, Hope, & Mor, 2018). This enhanced data reported no "high risk occupation" in 47.7% (315/660) of notifications, supporting previous findings that factors beyond occupation are increasingly important, and that the surveillance field 'occupation' alone is no longer considered to adequately capture the risk of exposure (Massey, Irwin, & Durrheim, 2009). Of notifications from NSW and Queensland in the period 2002 – 2013 that stated occupation, 32% reported an occupation of no known risk (Sloan-Gardner et al., 2017). In SA, 15% of notifications from 2007 to 2017 reported no

known occupational risk, with notifications including teachers, childcare and community workers, retirees, and the unemployed among others (Rahaman et al., 2019). During an outbreak in a NSW rural town in 2015, very few cases (21%) reported working in a high-risk occupation (Archer et al., 2017), while an outbreak at an abattoir situated in a peri-urban setting in close proximity to residential properties and a school highlighted the potential for community exposure (Lord et al., 2016). Internationally, a very large community outbreak occurred in the Netherlands from 2007 – 2009, during which over 3500 Q fever notifications were reported (van der Hoek et al., 2010).

1.8.2 Age and sex

Sloan-Gardiner *et al*. (2017) published a review of national Q fever notification data in Australia over the period 1991 – 2014, during which 80% of notifications were in males, and the highest notification rates for both males and females were in the 40 – 59 years age group. In NSW, 75.4% of all notifications from 2005 – 2015 were in males, and the highest number of notifications were in people aged from 45 to 59 years (Clutterbuck et al., 2018). A separate analysis of NSW notification data for the period 2006 – 2012, which assessed risk factors in older adults (> 45 years), identified that Q fever notifications were significantly reduced among those aged 65 years or older, and in women (Karki, Gidding, Newall, McIntyre, & Liu, 2015). Hence, symptomatic Q fever resulting in notification is most prevalent among middle-aged males in Australia as a whole. However, the median age of notified cases has increased nationally over time from 35 years (1991 – 2000) to 47 years (2007 – 2014), as has the proportion of notifications regarding female patients – the latter rising from 15% in 1991 to 25% in 2014 (Sloan-Gardner et al., 2017). In NSW the highest notification rate from 1991 – 2000 was in men aged 20 – 29 years, shifting to men aged 50 – 59 years from 2000 – 2010, while the proportion of notifications in females increased from 16% to 25% in these periods respectively (Lowbridge et al., 2012). These changes have been potentially influenced by improved testing practices, increased participation of females in animal handling occupations, increased awareness among the public and medical professionals leading to improved

detection of non-occupational cases, and the availability of a Q fever vaccine reducing occupational cases (Sloan-Gardner et al., 2017; Tozer et al., 2011), demonstrating the complexities of Q fever risk factors in Australia.

Q fever vaccination has been available in Australia since 1989 and is targeted at workers in high-risk occupations (Seqirus, 2019). As pre-exposed individuals are ineligible for vaccination, it is typically younger adults entering high-risk occupations that benefit from vaccination, contributing to the decreased proportion of young males notified over time. This effect was evident during the NQFMP in which the decline in Q fever notifications was most pronounced in young adult males (Gidding et al., 2009). Since the cessation of the NQFMP, notification numbers have steadily increased (NNDSS Annual Report Working Group, 2019; Sloan-Gardner et al., 2017) and under-vaccination of younger workers entering high-risk workplaces is re-emerging in regions such as SA, where Q fever notifications from 2007 – 2017 were dominated by younger males aged in their twenties and thirties working in abattoirs or with livestock (Rahaman et al., 2019). Hence, there is a close relationship between occupation, age and gender contributing to the diagnosis and subsequent notification of Q fever disease in Australia.

In community (non-occupational) settings, middle-aged and adult male patients are at increased risk of Q fever diagnosis, and adult males more often exhibit clinical Q fever disease of greater severity following natural infection (Textoris, Capo, Raoult, Leone, & Mege, 2010). In a community Q fever outbreak in western NSW during 2014 – 2015, the majority of confirmed cases were aged 40 years or over, and although the proportion of females (43%) was increased compared to historical data for the region (18%), males still predominated (Archer et al., 2017). Adult males and middle-aged persons are similarly considered at increased risk for clinical Q fever disease in other regions including France (Melenotte et al., 2018), the USA (Anderson et al., 2013), and the Netherlands (Roest et al., 2011).

Younger age (< 15 years) also appears protective against clinical Q fever disease (Angelakis & Raoult, 2010) and, in Australia, children are frequently asymptomatic or exhibit only a mild transient acute illness (Armstrong et al., 2019). The lowest Q fever notification rate in Australia over the period 1991 – 2014 was in the 0 – 19 years age group, at 0.7/100,00, with the notification rate increasing to 3.4 and 3.75 per 100,000 in the 20 – 39 and 40 – 59 years groups respectively (Sloan-Gardner et al., 2017). Seroprevalence data similarly report increasing seropositivity with increasing age, reflective of occupational risk in adulthood and increasing lifetime risk of exposure (**Table 1.1**).

Table 1.1 Summary of *C. burnetii* seroprevalence findings by age in in Australia.

Study	***Sampling***	***Seroprevalence findings by age***
Gidding et al. (2019)	Cross sectional study among blood donors in non-metropolitan regions with high Q fever notification rates (Hunter New England in New South Wales and Toowoomba in Queensland) and in metropolitan Sydney and Brisbane in 2014 – 2015.	9% increase in the odds of seropositivity for every five-year increase in age.
Tozer et al. (2011)	Serum bank assembled from non-Q fever related pathology requests collected in south Queensland in 2007 – 2009.	Seroprevalence of 1.3% in 0 – 14 years, 4.6% in the 15 – 39 years group, and >9% in those over 40 years.
Islam et al. (2011).	Residual diagnostic serum samples collected in the Hunter New England region of NSW in 2006 – 2009.	Greatest seroprevalence of 37% in the over 60 years age group compared to only 1% in 0 – 9 years group and 5% in the 10–19 years group.
Parker, Robson, and Bell (2010)	Convenience sample of residual diagnostic laboratory sera from south west Queensland collected in 2001 – 2002, for persons aged under 25 years.	Seroprevalence increased from 2.5% in those aged less than 15 years to 11% in those aged 15 – 24 years.

1.8.3 Rurality

Globally, the association of rurality with *C. burnetii* seroprevalence appears to vary and is influenced by land use, geography, and seasonal conditions (Angelakis & Raoult, 2011; Cikman et al., 2017; Clark & Soares Magalhaes, 2018; Hackert et al., 2012; Tozer et al., 2011). In Australia, Q fever notification rates are greatest in rural areas (Graves & Islam, 2016; Lowbridge et al., 2012; NNDSS Annual Report Working Group, 2019). A prospective cohort study of adults aged 45 years and over in NSW identified the risk of Q fever notification to be lowest in major cities and increased with both rurality and living on farm, with outer regional/remote areas (living on farm) presenting the greatest risk for notification (Karki et al., 2015). Notification data for NSW from 2005 to 2015 identified significantly higher (relative risk 11.39) notification rates in rural areas compared with urban areas; though, substantial variation was observed between local government areas (Clutterbuck et al., 2018).

Similarly, most seroprevalence studies have confirmed increased seropositivity in rural populations compared to metropolitan areas in Australia (Gidding et al., 2019; Islam et al., 2011). Interestingly, one study in south east Queensland utilising residual sera from pathology requests sampled in 2008 – 2009 demonstrated a similar seroprevalence in a major metropolitan area (5.0%) compared to the surrounding rural/remote areas (5.3%), which may reflect a risk of exposure due to the encroachment of housing into areas previously used for agriculture or abattoir sites, environmental conditions such as dust storms, or emerging reservoirs of disease (Tozer et al., 2011). However, number of notifications remained increased in the rural areas during the study period, suggesting rural exposure was more likely to result in clinical disease of greater severity, possibly due to increased inoculation dose, and that greater awareness of Q fever in rural/remote areas resulted in more testing (Tozer et al., 2011). A subsequent study of blood donors sampled in Queensland in 2014 – 2015 reported the more typical distribution with seroprevalence found to be greater in rural Queensland than in metropolitan areas, and differences in methodology were

proposed by the authors of the latter study as possibly accounting for the variable study findings (Gidding et al., 2019).

While the increased risk of Q fever in rural communities is mostly attributed to occupational exposure (Clutterbuck et al., 2018), one fifth of notifications in 2007 in a rural area of NSW reported direct or indirect non-occupational exposure to animals, their tissues and products (Massey et al., 2009). More recently, rurality remained associated with increased seropositivity among Queensland and NSW blood donors reporting rare or no contact with sheep, cattle and goats (Gidding et al., 2019). Risk associated with rurality may therefore be influenced by other factors, such as proximity to livestock industries and animal transport routes given the environmental resilience of *C. burnetii* and the ability for dispersal via wind and animal transport (Gidding et al., 2019; Sloan-Gardner et al., 2017). This was demonstrated in enhanced Q fever notification data for NSW from 2011 – 2015, in which indirect contact with livestock is reported due to being in proximity to livestock, livestock holding areas, or livestock transport trucks, as well as laundering clothing contaminated with livestock faeces (Clutterbuck et al., 2018). The association of Q fever with rurality is echoed in notification data for children for whom occupational exposure is not applicable, with all notifications in children aged less than 10 years in NSW from 2001 – 2010 occurring in regional and rural areas (Lowbridge et al., 2012).

Notifications associated with direct or indirect contact with wildlife or feral animals, which would be more likely in regional and rural areas, also appear to be increasing (Clutterbuck et al., 2018; Massey et al., 2009; Sivabalan, Saboo, Yew, & Norton, 2017). However, notification data for NSW from 2005 to 2015 identified this trend in urban areas also, which may be due to enhanced surveillance from mid-2010 onwards (Clutterbuck et al., 2018). Proposed sources of infection in both rural and urban areas include ticks and aerosolised *C. burnetii* from the excreta of native or feral animals and their ticks, for example kangaroo faeces aerosolized by wind or lawn mowing (Archer et al., 2017; Clutterbuck et al., 2018; Flint et al., 2016; Graves & Islam, 2016).

1.8.4 Environmental influences

Dry and windy weather facilitates the dispersal of *C. burnetii* and, consequently, Q fever notification rates are increased in Australia in areas of low rainfall (Cameron et al., 2017; Lowbridge et al., 2012) and during drought years (Archibald, 2019; Cameron et al., 2017). Interestingly, some coastal regions in Queensland report increased Q fever notifications following periods of high rainfall, which may be due to an increased density of wildlife in the wet periods resulting in an elevated *C. burnetii* burden in the subsequent drier period (Harris, Eales, Squires, Govan, & Norton, 2013; Sivabalan et al., 2017). Similarly, the Northern NSW Local Health District experienced a high notification rate of 10 per 100,000 population, compared to the state average of 2.8 per 100,000, despite high annual rainfall from 2001 – 2010, illustrating the multifactorial nature of the disease (Lowbridge et al., 2012).

Outbreaks around the globe are often associated with dry conditions and winds (Boden, Brasche, Straube, & Bischof, 2014). A South Australian outbreak that occurred in 2004 predominantly affected people who attended a sheep sale yard on a dry day with a moderate wind (18 – 28 km/hr) that resulted in the dispersal of contaminated dust and soil across the sale yard, such that the attendees reported having to wash the dust from their eyes and faces (O'Connor et al., 2015). Similarly, drought, gusty weather and dust storms were reported in the months preceding a community outbreak in Lightning Ridge in western NSW (Archer et al., 2017). Elsewhere, unusual southerly gales of up to 130 km/hr were identified as the probable cause of a large outbreak in Birmingham in the United Kingdom, which dispersed *C. burnetii* from farmland to surrounding urban areas (Hawker et al., 1998). In the Netherlands, exposure-response gradients have been observed in community cases aligning with the wind direction from source farms experiencing abortion storms in goats (Hackert et al., 2012; Schimmer et al., 2010). In the La Crau region of France, increased Q fever incidence is seasonally associated with secondary spring lambing due to strong local winds and decreased rainfall at that time compared to the main lambing drop (Tissot-Dupont et al., 2004).

1.9 Q fever burden in Australia

With over 500 cases notified annually (Australian Government Department of Health, 2017), Q fever is the most commonly reported non-foodborne zoonosis in Australia (Safe Work Australia, 2014). Over the period 1991 – 2014, Queensland reported the highest Q fever notification rate (6.26/100,000) and greatest number of notifications, accounting for 47% (5,730/12,164) of national notifications in Australia (Sloan-Gardner et al., 2017). New South Wales followed with a rate of 3.07/100,000, constituting 40% (4893/12,164) of notifications for the same period, while notification rates and numbers were much smaller in other states and territories in the period; SA 1.06 / 100,00 (n = 391), Victoria 0.70/100,00 (n = 837), WA 0.56/100,00 (n = 269), Northern Territory [NT] 0.56/100,00 (n = 27), Australian Capital Territory [ACT] 0.13/100,00 (n = 14), and Tasmania 0.03/100,00 (n = 3) (Sloan-Gardner et al., 2017). In the 2015 National Notifiable Disease Surveillance System (NNDSS) Annual Report, the highest notification rates remained with Queensland (5.3/100,000) and NSW (3.4/100,000), while there were no notifications for Tasmania and the ACT (NNDSS Annual Report Working Group, 2019).

In the period 2013 – 2017, more than 2,500 cases of Q fever were notified in Australia nationally, representing a significant burden as an estimated 40 – 50% of notified cases require hospitalisation, with mean hospital stays of 4 – 6 days reported (Gidding et al., 2019). Further, structured telephone surveys with confirmed acute Q fever patients in NSW revealed that 93% of patients required time off work or school, ranging from two to 296 days (median 21 days) (Massey et al., 2009). Half of the patients surveyed had not fully recovered within 28 – 93 weeks of illness onset, reporting persisting fatigue, arthralgia, myalgia, fevers, sweats, and endocarditis, while in those that had recovered, the median time to recovery was 12 weeks (range 1 – 35 weeks) (Massey et al., 2009).

The greatest burden of disease is borne by rural communities in Australia, and farmers report significant challenges in managing their businesses after Q fever infection due to prolonged symptoms (Lower et al.,

2017). The Victorian Farmers Federation Livestock Group is cited as estimating a loss of 1,700 weeks in productivity in the livestock industry alone for the state each year due to Q fever (Archibald, 2019). Such losses would be expected to be higher in Queensland and NSW as they report the highest number of Q fever notifications. Worker's compensation claims for Q fever have also been significant in Australia and were estimated to cost $1.3 million annually prior to the NQFMP (Kermode, Yong, Jurley, & Marmion, 2003). In NSW, there were 177 workers compensation claims for Q fever between 2002 and 2012, totalling more than AU$3.5 million (SafeWork-NSW, 2019). In a recent claim settled in Queensland, the plaintiff was awarded AU$1.4 million after contracting Q fever in 2012 whilst employed as a Project Supervisor (Carpenter) upgrading cattle yards and other items at a secondary school farm, which resulted in ongoing disability ("Thomson v State of Queensland & Anor," 2019).

However, the monetary and societal costs are expected to be greater than is currently understood, as notification data is likely to under-represent the true burden of Q fever in Australia (Eastwood et al., 2018; Gidding et al., 2019). Gidding et al. (2019) estimated that 29 – 39% of symptomatic cases in Australia remain undiagnosed, which suggests the true number of cases in the period 2013 – 2017 exceeded 3,500 – 4,000 cases. In the Netherlands, the cost associated with a large Q fever outbreak affecting more than 4,000 people was estimated at 250-600 million Euros over a decade, of which human costs accounted for 85% of this total with loss of quality of life and productivity the greatest burdens (Morroy et al., 2012).

1.10 Q fever vaccination

Uniquely, Australia has a Q fever vaccine (Q-VAX®, Seqirus Pty Ltd., Parkville, Victoria, Australia) for use in humans, which was licensed in March 1989. However, this or any other Q fever vaccine, is not routinely used anywhere else in the world (Armstrong et al., 2019), though Q-VAX® was utilised briefly in the Netherlands in 2011 during a large Q fever outbreak (Isken et al., 2013). Workplace Health and Safety [WH&S] guidelines issued by state governments in Australia regard Q fever vaccination as the highest

order risk control measure for preventing occupational Q fever (SafeWork-NSW, 2019; WorkCover-QLD, 2019). Secondary to this, it is recommended that workers and visitors who are not vaccinated for Q fever and cannot provide evidence of immunity from prior exposure be excluded from visiting at-risk workplaces. Risks should then be further managed through workplace design (e.g. hazard isolation, installation of ventilation and dust suppression systems) and implementing safe work practices including the use of personal protective equipment [PPE] (SafeWork-NSW, 2019; WorkCover-QLD, 2019).

1.10.1 Q-VAX®

1.10.1.1 Formulation

Q-VAX® contains whole cell formalin-inactivated phase I *Coxiella burnetii* Henzerling strain. A minimum of 25µg of antigen is provided in 0.5mL of aqueous solution (sodium chloride, sodium phosphate-monohydrate, sodium phosphate-dihydrate) and thiomersal 0.01% w/v is added as a preservative. Trace amounts of ovalbumin may also be present (Seqirus, 2019).

1.10.1.2 Pre-vaccination testing

Prior to vaccination, patients must undergo pre-vaccination screening to detect pre-existing immunity to *C. burnetii*. This process involves serological testing for *C. burnetii* antibodies, intradermal skin testing with the diluted Q-VAX® Skin Test (Seqirus Pty Ltd., Parkville, Victoria, Australia) and questioning by their vaccine provider on the possibility of previous exposure to *C. burnetii*. The Q-VAX® Skin Test contains the same ingredients as the vaccination. However, it contains only 2.5µg of antigen per 0.5mL of aqueous solution and is further diluted in sodium chloride prior to administration so that the final 0.1ml dose contains 16.7ng of antigen. To perform the skin test, the small dose is injected intra-dermally into the volar surface of the mid-forearm. Seven days after this test dose, the injection site is assessed for a positive reaction, described in the Q-VAX® product information as ‘any induration at the site of the skin test

injection'. Any persons with a positive *C. burnetii* serological result or a positive Q fever skin test should not be vaccinated (Seqirus, 2016).

1.10.1.3 Indications

Q-VAX® is indicated for the immunisation of susceptible adults at identifiable risk of *C. burnetii* infection, including abattoir workers (and those closely associated with the meat industry), farmers, veterinarians, stockyard workers, shearers, animal transporters, others exposed to cattle, sheep or goats or their products, persons culling and processing kangaroos, laboratory personnel handling potentially infected veterinary specimens, and visitors to abattoirs (Seqirus, 2019). Further to this, the Australian Government Immunisation Handbook, which outlines current recommendations for vaccine use in Australia, additionally lists veterinary nurses and veterinary students, professional dog and cat breeders, wildlife and zoo workers who work with high-risk animals, animal refuge workers, and other people exposed to high-risk animals, particularly cattle, camels, sheep, goats and kangaroos, including their products of conception such as placental tissue and birth fluids (Australian Technical Advisory Group on Immunisation, 2018).

1.10.1.4 Safety

Pre-licensure clinical trials for Q-VAX® began in 1981 at two South Australian Abattoirs and over the first two years, 924 abattoir workers were voluntarily vaccinated in an open trial (Marmion et al., 1984). A subset of 464 vaccinees were closely monitored for adverse events following immunisation [AEFI] and very common local reactions included injection site pain (48%) and injection site erythema (33%) lasting one to three days, while injection site swelling was uncommon (0.6%). Transient headache lasting 1 day was a common (9%) systemic reaction, while fever was uncommon (0.2%) (Marmion et al., 1984; Marmion et al., 1990). Follow-up assessments of the injection site were undertaken in 325 vaccinees at 6 – 12 months post-vaccination, with no instances of persistent reaction reported (Marmion et al., 1984).

However, in a follow up questionnaire issued to 869 vaccinated workers, one vaccinee reported a small, mobile lump at the injection site which lasted two months before resolving (Marmion et al., 1990).

Overall, the vaccine was considered low risk and the clinical trials were extended to additional abattoir sites in SA and to workers visiting these sites, with more than 4,000 vaccinated between 1981 and 1988 (Marmion et al., 1990). Throughout this period, AEFI were assessed at SA sites and additionally within a placebo-controlled blinded study in Queensland, in which 200 vaccinees received Q-VAX® and 98 received an influenza vaccine (Flu-Vax; CSL) (Marmion et al., 1990). Local injection site reactions [ISRs] (pain and erythema) remained common, while chronic ISRs were rare. A review of 2,682 vaccinated worker's medical records and questionnaire responses over the period 1981 – 1986 revealed no chronic reactions (Marmion et al., 1990). However, two workers were identified outside of this structured review as having developed sterile injection site abscessation, and a further two workers experienced soft subcutaneous lumps for several months following vaccination which resolved without treatment (Marmion et al., 1990).

Limited post-marketing AEFI data are publicly available via the Australian Government Therapeutic Goods Administration National Database of Adverse Event Notifications [DAEN]. This contains passive surveillance data of adverse events reported for medicinal products in Australia, which is particularly important for monitoring serious AEFI for which expedited reporting is mandatory (Therapeutics Goods Administration, 2000). Serious AEFI are those in which death, life-threatening illness, hospitalisation, persistent or significant disability/incapacity, or a congenital anomaly/birth defect occurs (Bentsi-Enchill et al., 2012). To date, ISRs are the most frequently notified events in this database, followed by headache and pyrexia (Therapeutics Good Administration, 2020), which remains in line with the findings of the initial clinical studies. A limitation of passive databases is under-reporting (Therapeutics Goods Aministration, 2018), as evidenced during Australia's NQFMP (2001 – 2006) where only 86 AEFI (eight of which were serious) were reported despite the administration of close to 50,000 vaccines (Gidding et al., 2009). During

this program, 80% of AEFI were ISRs, with five reports of sterile injection site abscess, while systemic events included fever, headache, arthralgia, myalgia, and allergic reaction (Gidding et al., 2009).

The Q-VAX® product information (Seqirus, 2019) includes the following estimates of incidence for AEFI based on post-marketing data:

- **Very common (>1/10):** Injection site inflammation (e.g. erythema, pain, warmth and swelling)
- **Common (<1/10 and ≥1/100):** Headache, delayed skin reaction presenting up to 6 months after vaccination) at injection site (either vaccination and/or skin test site).
- **Uncommon (<1/100 and ≥1/1,000):** Injection site induration and/or oedema, pyrexia, malaise, fatigue, myalgia, nausea, vomiting, diarrhoea, hyperhidrosis
- **Rare (<1/1,000 and ≥1/10,000):** Injection site abscess formation, granuloma
- **Very Rare (<1/10,000):** Dizziness, arthralgia, lymphadenopathy, chills, chronic fatigue syndrome.

Due to the potential for serious hypersensitivity reactions in people who are already sensitised to *C. burnetii* antigens, administration of Q-VAX® is contraindicated in persons who have a history of Q fever or likely exposure followed by an illness strongly suggestive of Q fever, and in persons who have been previously vaccinated for Q fever (Seqirus, 2019). The Australian Government Immunisation Handbook further states that Q fever vaccination is not recommended for children aged <15 years due to a lack of safety and efficacy data in this age group (Australian Technical Advisory Group on Immunisation, 2018). However, the need for a vaccine for children in Australia has been recognised for many years and preliminary investigations suggest it may be used safely, though prospective studies are indicated (Armstrong et al., 2019).

Further Q-VAX® AEFI data are reported for a community vaccination program in the Netherlands in 2011 during a large community outbreak, which targeted cardiovascular patients due to their high-risk for Q fever complications and included patients who were immune compromised (Isken et al., 2013; Schoffelen,

Wong, et al., 2014). The vaccine was found to be reactogenic but safe; of 970 vaccinees returning an adverse event diary, 80% reported local ISRs and 43% systemic AEFI, including fever in 9% (Schoffelen, Wong, et al., 2014). Two causally related serious adverse events occurred, which were both delayed and persistent ISRs. Compared to the Q-VAX® clinical trial data, the incidence of AEFI was greater in this population, who were older (median 67 years) with an increased proportion of females (40%). This study also identified that females were more likely to experience AEFI and AEFI of greater severity than males, while AEFI were also more frequent in younger age groups (Schoffelen, Wong, et al., 2014). These findings may have implications for Australia's veterinary workforce, which is predominantly female, and for any consideration of the extension of this vaccine to the broader community and children <15 years of age. As the population studied by Schoffelen (2014) was aged with co-morbidities, and the pre-licensure clinical data was collected in a predominantly male profession, there remains a paucity of AEFI data for younger adult females.

1.10.1.5 Efficacy

Absolute vaccine efficacy lasting at least five years was reported for the clinical trial period (Marmion et al., 1990). During the initial trial in SA, 34 cases of Q fever occurred among 1,349 unvaccinated abattoir workers compared to four cases among the 924 vaccinated workers over an 18 month period (Marmion et al., 1984). The limited randomised, blind, placebo-controlled trial in Queensland abattoirs saw none of the 98 Q-VAX® recipients contract Q fever and the trial was discontinued at 15 months after the seventh case of Q fever in the control group, which was considered the ethically acceptable limit to achieve statistical significance (Shapiro et al., 1990). At the conclusion of the trials, eight Q fever cases had been diagnosed in vaccinated workers compared to 97 cases in unvaccinated workers. Importantly, all cases among vaccinated workers occurred < 13 days post-vaccination and were deemed natural infections in which vaccination was given during the incubation period (Marmion et al., 1990). Further to this, a retrospective study of Q fever incidence in workers vaccinated in the period 1985 – 1990 across three of

the SA abattoir trial sites reported only two cases of Q fever in 2,555 vaccinated workers, compared to 55 among 1,365 unvaccinated workers (Ackland, Worswick, & Marmion, 1994). Again, both cases in vaccinated workers followed vaccination during the incubation period of a natural attack (Ackland et al., 1994).

Similarly, during a Q fever outbreak at an abattoir in NSW in 1998, no Q fever cases occurred in workers who had been vaccinated prior to the period of likely exposure, whereas post-exposure vaccination afforded no significant protection (Gilroy et al., 2001). The efficacy of the vaccine was also displayed during the NQFMP in Australia, which saw a decline in national Q fever notifications by over 50% following the vaccination of close to 50,000 workers within abattoirs and farming (Gidding et al., 2009). A meta-analysis of Q-VAX® efficacy data reported the effectiveness at 98% (95% confidence interval 94–99%), which reached 100% after excluding cases diagnosed within 15 days after vaccination (Gefenaite, Munster, van Houdt, & Hak, 2011). A second meta-analysis from O'Neill et. al (2014) also concluded that the vaccine significantly reduces acute clinical Q fever in occupationally exposed individuals, although further studies with improved demographic data were recommended to better understand the protective effect, as were studies specific to other occupations such as veterinary workers (O'Neill et al., 2014).

Most recently, a retrospective cohort study linking Q fever notifications and hospital admissions in Queensland determined a Q fever incidence rate of 5.4 per 100,000 person years follow-up in Q fever vaccinees, drawn from 30 confirmed Q fever cases among 133,819 vaccinees (Woldeyohannes et al., 2020). The incidence among Q fever registry participants listed as non-vaccinated but considered immune at pre-vaccination screening was notably higher at 89.5 per 100,000 person years, reflecting 76 confirmed cases among 18,997 individuals. Whilst the efficacy of Q-VAX® in the vaccinated subjects was determined to be 94.37%, this study raises concerns over the accuracy of pre-vaccination screening tests and false positive screening tests, with further work required to better understand the longevity of immunity afforded by natural exposure (Woldeyohannes et al., 2020).

Whilst highly effective, a review of laboratory confirmed Q fever notifications for the period 1991 – 2014, where vaccination status was recorded, revealed 2% (71/3,218) of patients had been previously vaccinated for Q fever (Sloan-Gardner et al., 2017). In SA, 5% (8/167) of Q fever notifications from 2007 – 2017 reported Q fever vaccination ranging from 83 days to 15 years prior to diagnosis (Rahaman et al., 2019). In Victoria, 2% (13/659) of notifications from 1993 – 2013 occurred in persons vaccinated more than 15 days prior to illness, with a mean onset of 2.5 years (range 18 days – 14.7 years) post-vaccination (Bond, Franklin, Sutton, & Firestone, 2017). However, it is difficult to ascertain true vaccine failures from prolonged incubation periods, with incubation periods of up to 60 days described (O'Connor et al., 2015) and speculation of 93 and 134-day incubation periods in some patients in Australia (Bond et al., 2017). True vaccine failures may reflect an individual's failure to respond to the vaccine antigen or a failure to develop protection against heavy burdens of *C. burnetii* exposure (Gilroy et al., 2001). Waning immunity over time has also been suggested (Rahaman et al., 2019), though immune responses beyond five years post-vaccination are poorly documented.

1.11 Immune response to vaccination

Immunity from vaccination is clinically apparent from approximately 13 days (Marmion et al., 1990), which is supported by the development of cell mediated immune responses within 9 – 15 days post-vaccination (Izzo, Marmion, & Worswick, 1988). T-lymphocyte proliferation predominates the cell mediated response, with post-vaccination reactivity demonstrated to the vaccine strain (Henzerling phase I), the Nine Mile phase II strain, and the Priscilla phase I strain which is associated with endocarditis in Australia (Marmion et al., 1990). Humoral responses follow, with seroconversion evident from two to four weeks post-vaccination (Izzo et al., 1988). Antibodies against both phase I and phase II *C. burnetii* are produced, and during the initial three to four months the IgM antibody class predominates (Marmion et al., 1984; Worswick & Marmion, 1985). Among abattoir workers, anti-phase I IgG later predominated from

20 months post-vaccination, with anti-phase II IgM and IgG less prevalent, while IgA antibodies remained essentially negative post-vaccination (Marmion et al., 1990; Worswick & Marmion, 1985).

During the initial stage of the pre-licensure clinical trials, seroconversion was determined in 54 – 66% of abattoir workers via complement fixation test [CFT] or IFA, though the time points of sampling were not described (Marmion et al., 1984). In a follow-up of 81 of these abattoir workers, 77% were seropositive by CFT, IFA, or a highly sensitive competitive radioimmunoassay [RIA] at 20 – 40 months post-vaccination, reducing to 40% at 41 – 60 months post-vaccination (Izzo et al., 1988). Despite waning antibody with time, 95% of vaccinees exhibited lymphocyte proliferation (lymphocyte stimulation index; LSI) five years post-vaccination utilising PBMCs and *C. burnetii* phase I (Henzerling) and phase II (Nine Mile) antigens (Izzo et al., 1988).

To investigate the longevity of immunity in low-risk populations with no known risk of pre-existing or repeated *C. burnetii* exposure, a small cohort of 32 low-risk workers were also vaccinated during clinical trials (Izzo et al., 1988). Seroconversion by CFT or IFA two to four weeks post-vaccination was 80%, declining to 14% over various time points from five to 96 weeks post-vaccination. Cell mediated responses (LSI) persisted in 86% over various time points from five to 96 weeks post-vaccination, which was considered to support the longevity of vaccine immunity in the absence of a boosting effect from recurrent exposure (Izzo et al., 1988). However, this study was limited in size and duration and there remains a paucity of information on the longevity of immune responses post-vaccination outside of abattoir workers.

1.12 Vaccine uptake

Vaccine uptake in at-risk workers in Australia is not well described, but may be less than ideal given over 500 cases of Q fever are notified annually in Australia (Australian Government Department of Health, 2017). A recent survey in blood donors reported that among participants identified as belonging to groups

recommended for Q fever vaccination, only 10% had been vaccinated (Gidding et al., 2019). Barriers to vaccination include a lack of Q fever knowledge, not being aware of the Q fever vaccine, a perception of not being at risk for Q fever, difficulty in accessing the vaccine, not taking the time to get vaccinated, not being eligible as < 15 years of age, and the vaccination not being provided by employers (Gidding et al., 2019; Massey et al., 2009). Expense is also a barrier as the cost of Q-VAX® is not funded by the Australian Government Pharmaceutical Benefits Scheme and must borne by individuals or employers (Archibald, 2019). The cost of the Q fever vaccination process, including the pre-vaccination screening appointment, skin test and serology, and the secondary appointment for vaccination, varies by provider; a search of advertised prices online ranged from AU$275 to in excess of AU$400 (Easy Street Medical Centre, 2018; Hamilton Medical Centre, 2020; Health Hub Doctors Morayfield, 2019; Pioneer Health, 2020).

The NQFMP confirmed improved vaccine outcomes where these barriers were considered; at-risk groups were targeted, the costs of screening and vaccination were fully funded, over 1000 healthcare workers were trained in screening and vaccination procedures, and community-based vaccination clinics were conducted *en mass* in many areas (Gidding et al., 2009). However, vaccine uptake varied between geographical states and occupational group, demonstrating the multifactorial barriers to vaccine uptake (Gidding et al., 2009). During Phase 1 targeting abattoir workers and sheep shearers, close to 100% uptake was seen in SA and Victoria, while in Queensland and NSW uptake was 75% and 50 – 60% respectively. During phase 2 targeting sheep and cattle farmers and their employees and families, uptake was reduced to only 18% in NSW, 31% in SA, and 43% in Victoria. The time-consuming process of pre-screening and later vaccination was thought to have impacted vaccine uptake (Gidding et al., 2009). Despite low uptake in some cohorts, a significant reduction in Q fever notifications were associated with the program which ran from 2002 – 2006 (Gidding et al., 2009).

Since 2009, national Q fever notifications have been rising (NNDSS Annual Report Working Group, 2019) and vaccine under-coverage in younger workers, particularly abattoir workers and farmers, has been

described following the cessation of the NQFMP (Rahaman et al., 2019). There has been a consistent call from Q fever researchers and Public Health practitioners in Australia for national Government funding to be re-instated for the vaccine in at-risk groups (Archibald, 2019; Lower et al., 2017; Rahaman et al., 2019). Indeed, improving vaccine coverage is expected to be cost effective; in a study now 17 years old, Kermode et al. (2003) demonstrated an incremental cost per life year gained of AU$20,002 and a cost per quality-adjusted life year (QALY) of AU$6,294 were vaccine uptake in meat industry workers to be increased from 65% to 100%. An incremental cost per life year gained of AU$24,950 and a cost per QALY of AU$7,984 was estimated were vaccine uptake to be increased among agricultural workers from zero to 20% (Kermode et al., 2003). Adjusted for inflation of 47.1% over 17 years at an average annual inflation rate of 2.3% (Reserve Bank of Australia, 2021), these costs per QALY are estimated to be AU$9,256 and AU$11,741 in 2020 for each scenario, respectively. In Australia, a medicine with a cost less than $50 000 per QALY gained is more likely to be recommended for funding by the Pharmaceutical Benefits Scheme (Taylor & Jan, 2017) and hence, these data may support arguments for government funding of the Q fever vaccine. However, a thorough health economic evaluation would be required to understand current cost-benefits of vaccination, particularly given the advances in our understanding of Q fever since the Kermode et al. (2003) study was published, including diagnostic methodologies.

1.13 Q fever in Australia's veterinary workforce

Complacency towards zoonoses and infection control is an occupation-wide concern in the veterinary industry within Australia and abroad (Attard et al., 2012; Willemsen et al., 2019). Compared with the human healthcare sector, there is a paucity of studies investigating infection control knowledge and practices in the veterinary sector, and those that have been undertaken confirm a general disregard for the use of infection control practices and poor development of infection control policies and guidelines (Attard et al., 2012; Willemsen et al., 2019). In Australia, the emergence of equine Hendra Virus highlighted the importance of infection control practices and prompted a survey of Australian

veterinarians to investigate zoonotic disease risk perceptions and infection control practices (Dowd, Taylor, Toribio, Hooker, & Dhand, 2013). The survey, undertaken in veterinarians attending a national conference in 2011, revealed that one third of workplaces did not have isolation facilities for animals, one fifth did not have a separate eating area for staff, and the provision and use of PPE was less than adequate. This study also confirmed that veterinarians' perceptions, along with workplace policies and culture, influenced attitudes towards zoonotic disease and increased efforts to encourage infection control practices were recommended (Dowd et al., 2013).

Despite Q fever being the second most commonly reported zoonoses in veterinarians surveyed in 2011 (Dowd et al., 2013), and among the reported zoonoses experienced in veterinary nurses surveyed at a national conference in 2003 (Soest & Fritschi, 2004), limited data exists regarding the knowledge, attitudes, practices, exposure risks, and Q fever vaccination status of Australia's veterinary workforce. Some data regarding exposure risk exists from veterinarians serologically surveyed for zoonotic diseases from 1975 to 1982 and again in 1992 (Giesecke & Barton, 1993). In 1992, 146 veterinarians and 35 non-veterinarians were sampled at the national Australian Veterinary Association [AVA] conference in Adelaide, with the total cohort comprised mostly of veterinarians: mixed practice (29.3%), government administration (9.9%), small animal practice (9.3%), lecturers (8.8%), veterinarians in industry (8.8%), government field officers (5.5%), large animal practice (4.4%), laboratory diagnosticians (4.4%), meat inspectors (3.3%), and students (1.1%). The 35 non-veterinarians included veterinary nurses, animal attendants, scientists, livestock advisors and stock inspectors, meat inspectors, and medical officers. Overall seroprevalence for anti-phase I or phase II antibody was 13.2%, with mixed animal practitioners and government veterinarians comprising most positive results. Clinical Q fever was reported in 4.4% of participants, most of whom were veterinary and non-veterinary meat inspectors (Giesecke & Barton, 1993).

Since 1992, the demographic of the veterinary workforce has evolved to a predominantly female workforce (Australian Veterinary Association, 2015), while Australian universities have introduced mandatory Q fever vaccination for veterinary students (Australian Veterinary Association, 2018). Additionally, advances in veterinary care and changes to agricultural, government, and pet ownership practices will have influenced the way in which veterinary personnel practice, including a significant reduction in the proportion of veterinarians exposed to cattle and mixed animal practice (Australian Veterinary Association, 2015). Hence, *C. burnetii* seroprevalence data from 1992 may no longer be applicable to today's veterinary workforce.

The need for a greater understanding of Q fever in today's Australian veterinary workforce was reinforced by recent atypical Q fever outbreaks occurring in suburban small animal veterinary facilities:

1. A two-year-old fox terrier presented to a veterinary clinic in western Sydney moribund and in shock. The dog underwent an emergency caesarean, which revealed a ruptured uterus and peritonitis, and all pups were deceased. Due to the severity of the patient's condition, a senior veterinary nurse cared for the patient in her home for several weeks after surgery. This nurse was admitted to hospital with Q fever six weeks following the initial surgery, suffering from fever, pancytopenia, pericarditis, pericardial effusion, back pain, rigors, headache, and mild hepatomegaly. Another two nurses who also had contact with the infected dog and deceased puppies experienced flu-like illness, one of which had sought medical attention from a general medical practitioner but remained undiagnosed until testing was performed following the diagnosis of the hospitalised nurse (Gibbons & White, 2014).
2. A breeding queen underwent a caesarean at a small animal veterinary hospital in south west Sydney in 2010. Nine veterinary staff and the queen's owner (a cat breeder) showed serological evidence of acute *C. burnetii* infection. This included staff with no direct contact with the queen

or kittens, and one staff member that was not present at the clinic on the day of the surgery but handled surgical equipment the following day and entered rooms in which the patient had been. Six of the nine veterinary staff were symptomatic with mild to severe flu-like illness. Two of these six required extended hospitalisation; the most severely affected had assisted with surgery and performed mouth to mouth resuscitation on new-born kittens. Of a total of 20 staff at the veterinary clinic, only one veterinarian reported prior Q fever vaccination (Maywood & Boyd, 2011). The queen and a further 26% of cats from the same cattery were seropositive for *C. burnetii* using IFA (Kopecny et al., 2013).

3. Seven cases of Q fever were identified in staff from an animal refuge and an adjacent veterinary clinic in southeast Queensland in 2016. A parturient cat was considered the most likely source of infection, though this was unable to be laboratory confirmed. The cat, which had been caught in a council trap, had prematurely delivered and she, along with her kittens were subsequently euthanised the same day. All seven symptomatic staff were present at the animal refuge facility that day and illness onset ranged over a period of one month, with two patients hospitalised. The primary case assisted with the euthanasia at the animal refuge and attended to the laundry at the veterinary clinic. Staff at the facilities did not routinely use PPE in the handling of animals during euthanasia, even where exposed to parturient products. None of the veterinary clinic staff and only three animal refuge staff had been vaccinated for Q fever (Malo et al., 2018).

These incidents test the general belief that Q fever in Australia is a disease of production animal workers and support the findings of others that the relative importance of non-production species in the Q fever epidemiology of Australia is increasing (Massey, Irwin et al. 2009). These cases also highlight potential under-vaccination of veterinary personnel, particularly veterinary nurses, despite a recommendation for vaccination in Australia's National Immunisation Handbook. This raises serious concerns as Australian law requires employers to show due diligence to ensure the health and safety of themselves and others within

their workplace, including eliminating or minimising WH&S risks as far as is reasonably practicable and providing necessary training and instruction to protect all persons (New South Wales Government, 2015). Yet, we have a report of unvaccinated veterinary personnel performing mouth to mouth resuscitation on new-born kittens (Maywood & Boyd, 2011). However, workplaces cannot provide adequate training and instruction to minimise Q fever risk when there is a paucity of information on Q fever risk specific to the Australian veterinary workforce.

1.14 Scope and aims of thesis

The research in this thesis aims to investigate Q fever disease and vaccination in Australia's veterinary workforce to address the following knowledge gaps identified in this Chapter:

Knowledge Gap	*Aims*	*Rationale*
There is a paucity of studies investigating infection control knowledge and practices in the veterinary sector, and general complacency towards WH&S is reported.	Investigate the knowledge, awareness, and attitudes of Q fever disease and vaccination in Australia's veterinary workers and determine the vaccination status of Australia's veterinary workforce.	Q fever is a vaccine preventable disease. Yet, reports of Q fever among unvaccinated veterinary workers in Australia are emerging. Identifying gaps in knowledge and/or practices will inform intervention strategies to improve WH&S outcomes.
Veterinary workers are not well described in published notification data, while existing seroprevalence data in veterinary workers in Australia pre-dates significant demographic changes in the workforce and Q fever vaccination.	Investigate *C. burnetii* seroprevalence in Australia's veterinary workers and identify demographic and work characteristics associated with seropositivity.	Risk of *C. burnetii* exposure is expected to vary with practice type / animal exposures, with ruminants thought to pose the greatest risk of exposure. However, recent veterinary outbreaks in Australia report exposure from companion animals (cats, dogs), while other studies suggest wildlife may be a source of exposure. Seroprevalence will better inform risk profiles for *C. burnetii* exposure within the veterinary industry.
Immune responses post-Q-VAX® are not well described in occupations outside of abattoir workers, and there is a paucity of data beyond 5-years post-vaccination.	Investigate the longevity of vaccine immunity afforded by Q fever vaccination in Australia's veterinary workers.	Exposure profiles / risk of re-exposure post-vaccination are expected to vary between occupations generally and within the veterinary industry according to animal and environmental exposures. Understanding the longevity of vaccine-induced immunity and the influence of re-exposure will inform WH&S and vaccination policy.
Females and young adults are under-represented in adverse events following immunisation data for Q-VAX®.	Collect further AEFI data for Q-VAX® in younger adults, particularly females.	The majority of veterinary students and workers entering veterinary studies, or the veterinary workforce are young female adults. This study will provide industry-relevant safety data for informing vaccine policy. With increased calls for the vaccination to be extended to children (<15 years), this study will also provide valuable data for any consideration of trialling this vaccine in younger adolescents or children.

This research is intended to provide industry specific insights for the continued improvement of WH&S practices, which comes at a time when the importance of zoonoses and the one health paradigm are increasingly recognised. Aspects of this research may also benefit our understanding of Q fever in Australia generally, and AEFI data are expected to improve the overall safety data available for Q-VAX®.

2 Q Fever Knowledge, Attitudes and Vaccination Status of Australia's Veterinary Workforce

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2.1 Abstract

Q fever, caused by *Coxiella burnetii*, is a serious zoonotic disease in humans with a worldwide distribution. Many species of animals are capable of transmitting *C. burnetii*, and consequently all veterinary workers are at risk for this disease. An effective Q fever vaccine has been readily available and used in Australia for many years in at-risk groups. Little is known about attitudes towards this vaccine and vaccine uptake in veterinary workers. This study aimed to determine the Q fever vaccination status of veterinarians and veterinary nurses in Australia and to assess and compare the knowledge and attitudes towards Q fever disease and vaccination of each cohort. An online cross-sectional survey performed in 2014 targeted all veterinarians and veterinary nurses in Australia. Responses from 890 veterinarians and 852 veterinary nurses were obtained. Binary, ordinal and multinomial logistic regression were used to make comparisons between the two cohorts. The results showed that 74% of veterinarians had sought vaccination compared to only 29% of veterinary nurses. Barriers to vaccination among those not vaccinated did not differ between cohorts, and included a lack of perceived risk, financial expense, time constraints, and difficulty in finding a vaccine provider. Poor knowledge and awareness of Q fever disease and vaccination were additional and notable barriers for the veterinary nursing cohort, suggesting veterinary employers may not be meeting their legal responsibility to educate staff about risks and risk prevention. Further

evaluation is needed to identify the drivers behind seeking and recommending vaccination so that recommendations can be made to improve vaccine uptake.

2.2 Introduction

Q fever is a serious zoonotic disease capable of causing acute and chronic debilitating and life-threatening illness in humans (Angelakis & Raoult, 2010). Following infection by the causative bacterium *Coxiella burnetii*, 20 – 80% of patients become symptomatic in the acute phase, with symptoms most often limited to a flu-like illness (Million & Raoult, 2015). However, 2 – 5% of acute Q fever patients develop severe complications such as hepatitis, atypical pneumonia, myocarditis or meningitis (Angelakis & Raoult, 2010; Kosatsky, 1984; Marrie, 2010; Marrie et al., 1989; Spelman, 1982). Acute infection during pregnancy has been associated with miscarriage, foetal death, premature delivery and low birth weights, with women in their first trimester at greatest risk (Denman & Woods, 2009; Langley, Marrie, LeBlanc, Almudevar, & Raoult, 2003; Raoult, Fenollar, & Stein, 2002). Persistent infection may result in chronic Q fever symptoms, of which endocarditis is most common (Fenollar et al., 2001; Gikas et al., 2010). Women with chronic Q fever may experience recurrent miscarriage or pre-term deliveries (Langley et al., 2003). Chronic Q fever fatigue syndrome [QFS] is a recognised sequelae to acute Q fever, occurring in 10 – 20% of patients (Marmion et al., 2005; Sukocheva et al., 2010; Wildman et al., 2002). Due to the non-specific and variable presentations of both acute and chronic Q fever, diagnosis may be delayed in the absence of a high index of suspicion, prolonging illness and endangering the lives of those affected (Landais et al., 2007; Munckhof et al., 2007).

A wide variety of domestic and wild animal species act as a reservoir for *C. burnetii*, shedding the bacterium in the products of conception, and to a lesser extent in urine, faeces, milk and saliva (Angelakis & Raoult, 2010; Guatteo et al., 2006). Inhalation is the most common route of infection and to date, most human infections have been attributed to cattle, sheep and goats (Angelakis & Raoult, 2010). However,

companion and wild animals may be an under-recognised contributor to *C. burnetii* infection (Abe et al., 2001; Massey et al., 2009; Tozer et al., 2011; Tozer et al., 2014), and outbreaks among veterinary workers have been associated with direct or indirect contact with birth products following cat and dog caesareans in small animal veterinary clinics (Gibbons & White, 2014; Kopecny et al., 2013; Maywood & Boyd, 2011). Studies in Japan, Denmark, the Netherlands and the USA have confirmed a significantly higher seropositivity to *C. burnetii* among veterinarians compared to the general population (Abe et al., 2001; Bosnjak, Hvass, Villumsen, & Nielsen, 2010; de Rooij et al., 2012; Whitney et al., 2009), while Q fever was the second most common zoonosis reported among Australian veterinarians in a recent survey (Dowd et al., 2013).

A whole cell formalin-inactivated Q fever vaccine (Q-VAX®; CSL Biotherapies, Parkville, Vic.) has been available in Australia since 1989 and has a reported efficacy of over 98% (Gefenaite et al., 2011). To date, routine use and licensing has been restricted to Australia, in part due to a perception that the vaccine is "old-fashioned" and concerns regarding adverse events following immunisation (Forland et al., 2010; Marmion, 2007). Any persons who have previously had Q fever or exposure to *C. burnetii* should not receive the vaccination due to an increased risk of adverse events following immunisation (*The Australian Immunisation Handbook*, 2013). Strict pre-vaccination protocols have been successfully implemented in Australia to minimise the risk of adverse events; serology and intradermal skin testing with diluted vaccine to check for evidence of prior exposure. This process requires experienced medical practitioners and may be seen as costly and time consuming (Milazzo, Featherstone, & Hall, 2005).

Currently, the Australian Veterinary Association [AVA] Biosecurity Guidelines and the Australian Immunisation Handbook recommend vaccination of all veterinarians, veterinary students and veterinary nurses (*The Australian Immunisation Handbook*, 2013; Australian Veterinary Association, 2011). The vaccination process is now a course requirement for students enrolled in veterinary and animal science degrees at Australian universities. Outside of this tertiary environment, Q fever vaccination may be

recommended or a compulsory requirement to commence employment for some veterinary and other animal workers. Though, the current vaccination status of the workforce is not known.

The aim of this study was to determine the Q fever vaccination status and compare the knowledge, attitudes and practices of veterinarians and veterinary nurses in Australia regarding Q fever, with the further aim of informing vaccine policy both in Australia and internationally and making recommendations to maximise workplace health and safety [WH&S] for all veterinary personnel.

2.3 Methods

2.3.1 Study design

This cross-sectional study was targeted at all veterinarians and veterinary nurses in Australia over 18 years of age and currently or recently employed in a veterinary workplace. The study was implemented via the Survey Monkey® (Palo Alto, California, USA) platform as an online questionnaire containing 53 questions (13 open, 25 closed and 15 semi-closed) divided across six sections; (1) demographics and veterinary work environment, (2) attitudes towards Q fever illness and vaccination, (3) experience with Q fever disease, (4) experience with Q fever vaccination, (5) knowledge of disease risk, and (6) biosecurity practices (Appendix A). Skip logic was used, and it was not compulsory to answer all questions. Ethics approval was granted by Charles Sturt University School of Animal and Veterinary Sciences Human Ethics Committee (protocol #416/2013/19). A participant information statement was provided to participants and informed consent was sought prior to commencement of the survey.

2.3.2 Recruitment of veterinary nurses

Veterinary nurses were recruited during March and April of 2014. Due to limitations in accessing this unique workforce, which requires no formal registration outside of the state of Western Australia [WA], participants were recruited in a number of ways. A personal email invitation containing a link to the survey was sent on our behalf by the Veterinary Surgeon's Board of WA to all veterinary nurses in this state using

the email address listed with the board. In other states and territories, attempts were made to phone all veterinary clinics to invite veterinary nurses to participate in the survey. The clinic phone lists for New South Wales [NSW] and Tasmania were compiled from practice lists provided by the state's respective veterinary practitioner's boards whilst the remaining state lists were compiled from all clinics listed with the Yellow Pages® phone book. Reminder emails/letters/faxes were sent out two weeks after the first email, letter or fax. The Veterinary Nursing Council of Australia [VNCA] also sent personal emails to members for whom they had an email address recorded.

2.3.3 Recruitment of veterinarians

Initial contact with veterinarians was made through an invitation advertised in the AVA's email newsletter sent to all members on the 11th of April 2014. In May and June of 2014 veterinarians were recruited in a similar fashion to veterinary nurses. Personal email invitations were sent on our behalf by state veterinary surgeons boards where possible. In other states, the contact lists compiled for distributing the survey to clinics for participation of veterinary nurses were revised for the recruitment of veterinarians. Clinics that had previously declined participation of their veterinary nurses were contacted to invite participation from veterinarians and phone calls were also made to check method of contact preference where post or fax had been previously specified. Reminder emails/letters/faxes were sent out 2 weeks after the first. During May 2014, the Veterinary Practitioners Board of NSW also provided details and a link to the survey on their website, which had increased traffic during this month as it coincided with veterinarians' registration renewals.

2.3.4 Data management and analysis

Binary, ordinal and multinomial logistic regression analyses were undertaken to compare the knowledge, attitudes and practices between veterinarian and veterinary nurse cohorts (the exposure) and all models were adjusted for age, gender and state to account for demographic differences of the cohorts. *P*-values

of <0.05 were considered statistically significant and 95% confidence intervals [CI] were calculated. For ordinal outcomes, the assumption of proportionality was evaluated using the Score Test. Where the Score Test was found to be significant (p <0.05), indicating that the assumption was invalid, categories were combined to create binary variables or if appropriate multinomial logistic regression was undertaken.

Agreement with attitudinal statements regarding the importance of, potential harm from, and difficulty in accessing the Q fever vaccination were compared between the two cohorts using binary logistic regression with the positive outcome 'agree'. Q fever knowledge was assessed as self-reported knowledge, with participants asked to rate their level of Q fever knowledge on a scale of one (lowest) to ten (highest), and a Kruskal-Wallis Test undertaken to assess for a statistical difference between the mean rank of each cohort. Perception of vaccine safety, efficacy and expense was compared with ordinal logistic regression. The positive outcome "agree/strongly agree" was modelled over lower levels of agreement in veterinarians compared to veterinary nurses. Self-perceived level of risk (nil, low, moderate, high) of personal exposure to *C. burnetii* was compared between the two cohorts using multinomial logistic regression. Odds ratios were calculated for veterinarians versus veterinary nurses, with logits modelled using 'nil' exposure as the reference category. Respondents were considered to have attempted vaccination if they reported that they had been vaccinated or were positive at pre-vaccination screening. Odds of attempting vaccination and odds of receiving vaccination were compared using separate binary logistic regression models with the positive outcomes 'attempted vaccination' and 'vaccinated', respectively. Multinomial logistic regression was used to determine the odds of vaccination of each cohort across the three most likely vaccination scenarios; (1) actively sought vaccination despite no workplace or study requirement to do so, (2) vaccinated as a requirement of work (3) vaccinated due to a university/ other course requirement.

Practice structures were defined as 'solo' (one veterinarian within the clinic), 'group' (multiple veterinarians within the clinic), 'corporate' (multiple veterinarians with a clinic owned and managed by a

corporate entity), 'university' (clinical, research and/or academia within a university) or 'other'. Practice type by species was determined by the combination of species with which respondents spent >90% of their working hours with. Responses stating "don't know" or "unsure" were excluded from all comparisons. All analyses were performed in SAS® statistical program (2002-2012 SAS Institute Inc; Cary, NC, USA).

2.4 Results

2.4.1 Sampling

A total sample size of 1,742 participants was achieved, comprised of 852 veterinary nurses and 890 veterinarians.

Of the 995 veterinary nurses registered with the WA state veterinary board, 113 were not contactable for this survey due to the absence of a registered email address. The remaining 882 were individually emailed an invitation to participate, although 74 of these emails were subsequently undeliverable. In other states of Australia, phone calls were made to 1,677 clinics inviting participation of veterinary nurses. Of the 1,446 clinics who agreed to receive participation details, 1,286 preferred to receive an email link to the survey (54 of these emails were subsequently undeliverable), while 91 were sent survey details via post and 69 via fax. Personal emails were sent to 917 VNCA members.

Sampling of veterinarians conducted via email invitations sent on our behalf by state veterinary practitioners boards resulted in individual emails sent to 1200 veterinarians in WA and 245 veterinarians in Tasmania. The number of veterinarians registered with these state boards and the number of undeliverable email invitations were not able to be obtained. In the remaining states contacted initially via phone, 1582 clinics agreed to participate with 1458 sent an email link to the survey (23 of these emails were undeliverable), while survey details were sent to 82 clinics via post and 42 clinics via fax. It is not

known how many veterinarians accessed the survey via the AVA's email newsletter (which was viewed by 2537 members), or the NSW veterinary surgeon's board website.

2.4.2 Demographics and veterinary work

Although an accurate response rate was unable to be determined from this survey due to the absence of a single registry by which all veterinarians and veterinary nurses could be contacted, it was estimated that participation by 890 veterinarians and 852 veterinary nurses represented approximately 12% of the estimated 7,400 employed veterinarians and 10% of the estimated 8,600 employed veterinary nurses at the time according to Australian government employment statistics (Australian Bureau of Statistics, 2014a, 2014b). All states and territories were represented and the sample of veterinarians by state reflected similar proportions to veterinary registrations in each state (**Table 2.1**) (Australian Veterinary Association, 2014). The majority of veterinary nurses were female (98%) compared to 63% of veterinarians, and the nursing cohort was, both in range of years and on average, younger and reported fewer years in the veterinary workforce (**Table 2.2**).

Both cohorts mostly worked in small animal practice with a group practice structure predominating (**Table 2.2**). The reported number of staff in veterinary workplaces ranged from one to 299 with a median number of 10 and interquartile range of 10, indicating that most would be categorised as small businesses. Regarding education, most (65%) veterinary nurses had completed Certificate IV level training at a technical tertiary institution although 13% reported no formal education. The veterinarians responding to the survey were mostly graduates of Australian universities (89%), with all seven Australian veterinary schools represented, and 35% of respondents held additional postgraduate qualifications (**Table 2.2**).

Table 2.1 Distribution of veterinarians and veterinary nurses by state; a comparison of respondents to available employment and registration data for Australia's veterinary workforce in 2014.

	Veterinarians			*Veterinary Nurses*	
	Study respondents (n = 890)	**Veterinarians registered by state 2014†**	**ABS data 2014**	**Study Respondents (n=852)**	**ABS data 2014**
State	n (%)	n (%)	%	n (%)	%
Queensland	151 (17%)	2503 (24%)	18.1	187 (22%)	27.3
New South Wales / Australian Capital Territory	292 (33%)	3203 (30%)	42.1	307 (36%)	17.2
Victoria	201 (23%)	2586 (24%)	24.7	163 (19%)	22.9
South Australia	20 (2%)	655 (6%)	7.2	51 (6%)	15.5
Tasmania	48 (5%)	252 (2%)	2.6	8 (<1%)	5.6
Western Australia	156 (18%)	1296 (12%)	4.6	114 (13%)	9.3
Northern Territory	7 (<1%)	134 (1%)	0.6	5 (<1%)	1
Not specified	15 (2%)	-	-	17 (2%)	-

†Registration data reported within the Australian Veterinary Association veterinary workforce survey (2014). ABS; Australian Bureau of Statistics (2014).

Table 2.2 Demographics and veterinary work of participants in study of Q fever knowledge attitudes and practices in 2014 in Australia.

Characteristic	***Veterinarians (n=890) No. (%)*** [†]	***Veterinary Nurses (n=852) No. (%)*** [†]
Gender		
Female	560 (63%)	836 (98%)
Male	321 (36%)	14 (2%)
Not specified	9 (1%)	2 (<1%)
Age		
Range	21-80 years	18-69 years
Mean	40 years	33 years
Median	38 years	31 years
Standard deviation	12 years	10 years
Interquartile Range	19 years	16 years
18-30 years	251 (28%)	403 (48%)
31-40 years	238 (27%)	229 (27%)
41-50 years	202 (23%)	144 (17%)
51+ years	194 (22%)	68 (8%)
Australian State		
Queensland	151 (17%)	187 (22%)
New South Wales / Australian Capital Territory	292 (33%)	307 (36%)
Victoria	201 (23%)	163 (19%)
South Australia	20 (2%)	51 (6%)
Tasmania	48 (5%)	8 (<1%)
Western Australia	156 (18%)	114 (13%)
Northern Territory	7 (<1%)	5 (<1%)
Not specified	15 (2%)	17 (2%)
Years working		
Range	0.2-60 years	0.3-47 years
Mean	16.2 years	10 years
Median	14 years	8 years
Standard deviation	12 years	8 years
Interquartile Range	19 years	10 years
0.2-5 years	192 (22%)	305 (37%)
6-10 years	172 (20%)	222 (27%)
11-20 years	225 (26%)	219 (26%)
21-30 years	157 (18%)	64 (8%)
31+ years	128 (15%)	21 (3%)
Practice Type		
Small animals	512 (58%)	640 (75%)
Farm/mixed animals	297 (33%)	132 (15%)
Equine/other	37 (4%)	17 (2%)

Characteristic	*Veterinarians (n=890) No. (%)* †	*Veterinary Nurses (n=852) No. (%)* †
Not specified	44 (5%)	63 (7%)
Practice Structure		
Corporate‡	32 (4%)	48 (6%)
Group§	575 (65%)	441 (52%)
Solo‖	169 (19%)	256 (30%)
University	31 (3%)	36 (4%)
Other	45 (5%)	19 (2%)
Not specified	38 (4%)	52 (6%)
Highest level of education - veterinary nurses		
Certificate IV	n/a	546 (64%)
Certificate II	n/a	51 (6%)
Diploma/Bachelors/other	n/a	132 (15%)
Nil	n/a	109 (13%)
Not specified	n/a	14 (2%)
Highest level of education - veterinarians		
Undergraduate	571 (65%)	n/a
Grad Certificate/Diploma	111 (13%)	n/a
Masters	50 (6%)	n/a
ANZCVS or equivalent	95 (11%)	n/a
PhD or fellowship	52 (6%)	n/a
Not specified	11 (1%)	n/a
University attended - veterinarians		
The University of Sydney	223 (25%)	n/a
The University of Melbourne	158 (18%)	n/a
Murdoch University	150 (17%)	n/a
The University of Queensland	207 (23%)	n/a
Charles Sturt University	28 (3%)	n/a
James Cook University	21 (2%)	n/a
The University of Adelaide	4 (<1%)	n/a
Other (international)	99 (11%)	n/a

†Unless specified otherwise. Percentages are of total respondents for each parameter. Not all participants responded to all questions. ‡One veterinarian within the clinic §Multiple veterinarians within the clinic ‖Multiple veterinarians within a clinic owned and managed by a corporate entity. ANZCVS; Australian and New Zealand College of Veterinary Scientists.

2.4.3 Attitudes towards vaccination

The majority of both cohorts (97%) agreed that vaccines in general are important in the prevention of disease. Among participants aware of the Q fever vaccine, veterinarians had higher odds (1.82; 95% CI 1.03-3.32; *p* <0.043) of being convinced of the importance of the Q fever vaccine and lower odds (0.52; 95% CI 0.34-0.79; p <0.001) of being concerned that the vaccine may be harmful (**Table 2.3**) compared to veterinary nurses. Close to 40% of each cohort agreed that the vaccine was difficult to access, with veterinarians reporting lower odds (0.74; 95% CI 0.56-0.97; *p* = 0.027) of agreement with this statement (**Table 2.3**).

Table 2.3 Binary logistic regression analysis of attitudes towards the Q fever vaccine among veterinarians and veterinary nurses surveyed in Australia in 2014.

	Agree	***Disagree***	***Total***	***Adjusted Odds Ratio†‡***	***95% CI§***	***P-value‖***
All participants:						
"If a vaccine exists for a certain disease, then vaccination is usually a good way to protect someone against this disease"						
Nurses	727 (97%)	20 (3%)	747	1		
Vets	805 (97%)	22 (3%)	827	1.13	0.55-2.35	0.75
Participants with prior awareness of the Q fever vaccine:						
"I am convinced of the importance of the Q fever vaccine"						
Nurses	443 (93%)	34 (7%)	477	1		
Vets	701 (95%)	35 (5%)	736	1.82	1.03-3.32	0.043
"I worry that the Q fever vaccine will do more harm than good"						
Nurses	71 (15%)	407 (85%)	478	1		
Vets	70 (9%)	667 (91%)	737	0.52	0.34-0.79	<0.001
"It is difficult to get vaccinated for Q fever"						
Nurses	205 (43%)	267 (57%)	472	1		
Vets	287 (39%)	443 (61%)	730	0.74	0.56-0.97	0.027

†Odds of stating "agree", ‡Ratio adjusted for age, gender and state, §Confidence interval; ‖Likelihood ratio Chi-square *p*-value.

2.4.4 Knowledge and perceptions of Q fever vaccination and disease

The majority of both cohorts (98%) agreed (slightly agreed/agreed/strongly agreed) that Q fever is a serious disease with significant health consequences. Self-reported Q fever knowledge on a scale of one (lowest) to ten (highest) was normally distributed among veterinarians (mean =5; median = 5), while that of veterinary nurses was positively skewed (mean = 3.5; median = 3) (**Figure 2.1**). The Kruskal-Wallis Test identified a significant difference (p <0.001) in knowledge between the cohorts. Amongst participants with prior awareness of the Q fever vaccine, there was no significant difference in the perception of vaccine safety between the cohorts. However, veterinarians reported 2.5 times (95% CI 1.6 – 4.1; p <0.001) odds of agreeing that the vaccine is effective and 0.39 times (95% CI 0.29-0.54; p <0.001) odds of agreeing that the vaccine is expensive compared to veterinary nurses (**Table 2.4**). The cost, safety and efficacy of the Q fever vaccine was not known among 43% (342/802), 13% (102/807) and 21% (168/805) of veterinarians and 50% (358/716), 26% (187/722) and 31% (221/722) of veterinary nurses respectively.

Table 2.4 Ordinal logistic regression analysis of the perceptions of the Q fever vaccination among veterinarians and veterinary nurses surveyed in Australia in 2014 who were aware of the Q fever vaccination.

	Strongly disagree/ disagree	***Slightly disagree***	***Slightly agree***	***Agree/ strongly agree***	***Total***	***Adjusted OR†‡***	***95% CI***	***P-value§***
"The Q fever vaccine is safe if appropriately administered"								
Nurses	3 (<1%)	6 (2%)	36(9%)	350 (89%)	395	1		
Vets	2 (<1%)	18 (3%)	61 (9%)	583 (88%)	664	0.975	0.63-1.50	0.909
"The Q fever vaccine is effective in preventing Q fever"								
Nurses	4 (1%)	6(2%)	49(13%)	313(84%)	372	1		
Vets	1 (<1%)	6 (1%)	41 (7%)	573 (92%)	621	2.49	1.55-4.09	0.001
"The Q fever vaccine is too expensive"								
Nurses	86 (31%)	30 (11%)	61 (22%)	97 (35%)	274	1		
Vets	230 (52%)	67 (15%)	71 (16%)	76 (17%)	444	0.39	0.29-0. 54	<0.001

†Odds Ratio: odds of stating "agree/strongly agree" modelled over the lower levels of agreement. Assumption of proportionality met. ‡Adjusted for age, gender and state; Participants stating "Don't know" were excluded from analysis. §Wald Chi-square p-value.

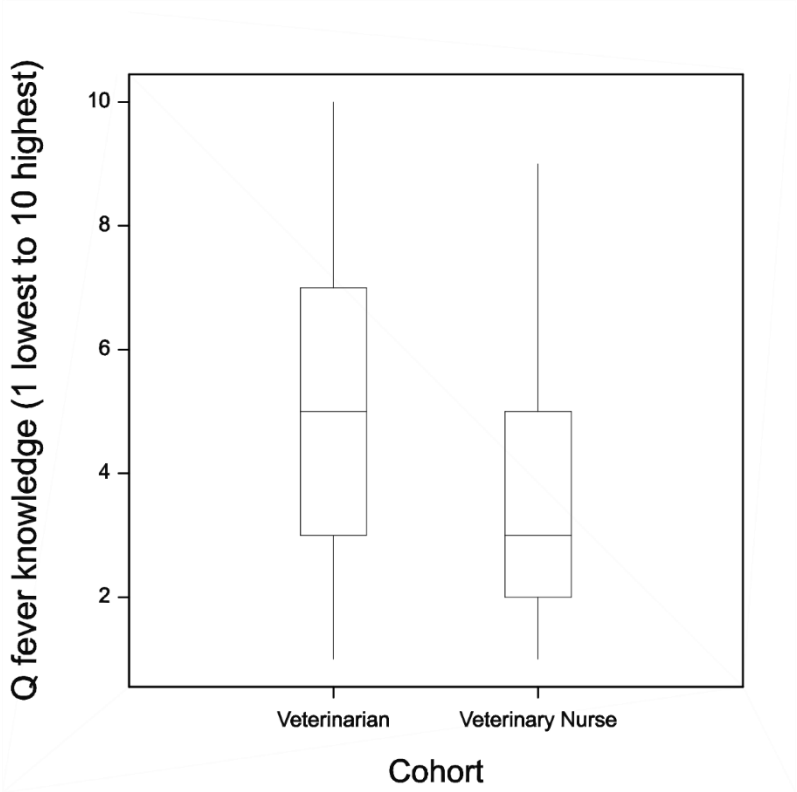

Figure 2.1 Boxplot of self-rated Q fever knowledge among veterinarians and veterinary nurses.

2.4.5 Exposure to *Coxiella burnetii*

Both cohorts considered the risk of exposure to *C. burnetii* to be similar for both veterinarians and veterinary nurses within each practice type (**Figure 2.2**). Ordinal regression analysis of perceived level of exposure to *C. burnetii* did not meet the assumption of proportionality and binary categories were not appropriate. Subsequently multinomial regression was undertaken which, when adjusted for practice type, revealed veterinarians had 12.5 times (95% CI 6.4-25.1; p <0.001) the odds of stating their personal risk as high rather than nil compared to veterinary nurses (**Table 2.5**). Eleven percent (93/850) of veterinarians and 25% (201/793) of veterinary nurses stated they "did not know" their level of exposure and a further 11% (150/793) of veterinary nurses stated 'nil exposure'. Of those veterinary nurses stating 'nil exposure', 91% worked within small animal practices, 7% in large and mixed animal practices, and the remainder in equine or other practices.

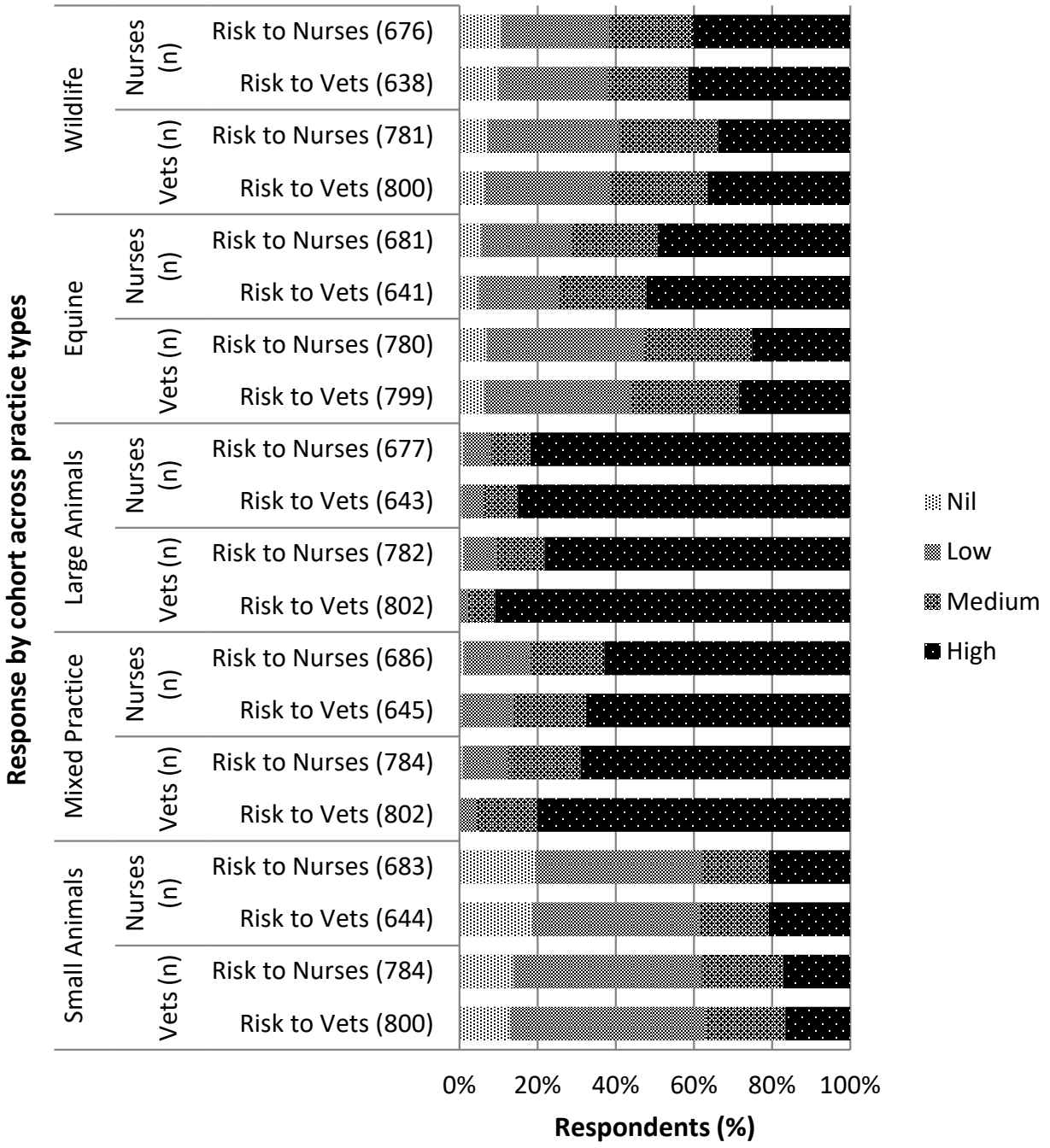

Figure 2.2 Perceived exposure risk of veterinarians and veterinary nurses to *C. burnetii* across different practice types

Table 2.5 Multinomial logistic regression analysis of level of perceived personal exposure to *C. burnetii* among veterinarians versus veterinary nurses surveyed in Australia in 2014.

	Nil	*Low Exposure*				*Moderate Exposure*				*High Exposure*			
	n (%)	n (%)	OR†	95% CI	*P*-value‡	n (%)	OR†	95% CI	*P*-value‡	n (%)	OR†	95% CI	*P*-value‡
Nurses (n=592)	151 (19%)	329 (41%)	1			88 (11%)	1			24 (3%)	1		
Vets (n=757)	33 (4%)	414 (49%)	5.4	3.5-8.7	<0.001	216 (25%)	8.2	4.8-13.9	<0.001	94 (11%)	12.5	6.4-25.1	<0.001

†Adjusted Odds Ratio; logits modelled using "nil exposure" as the reference category and adjusted for age, gender, state and practice type. ‡Wald Chi-square *p*-value. Participants stating "Don't know" were excluded from analysis.

2.4.6 Vaccination status and barriers to vaccination

The majority of veterinarians (587/796; 74%) were either vaccinated (488/587; 61%) or had sought vaccination for Q fever but were unable to be vaccinated due to a positive pre-vaccination screening result (99/587; 12%). This proportion increased to 78% (562/721) among graduates of Australian veterinary schools and decreased to only a third (25/75; 33%) among international graduates (**Table 2.6**). Only 29% (199/688) of veterinary nurses had been vaccinated (162/199; 24%) or had sought vaccination but were unable to be vaccinated due to a positive pre-vaccination screening result (37/162; 5%) (**Table 2.6**). Overall, veterinarians had 13 times (95% CI 9.9-18.1; p <0.001) odds of having attempted vaccination and 10 times (95% CI 7.6 – 12.6; p <0.001) odds of having received the vaccination.

Among those who had attempted vaccination, a positive pre-vaccination screening result was reported by 17% (99/587) of veterinarians and 19% (37/199) of veterinary nurses. Among veterinarians graduating from Australian veterinary schools this percentage fell slightly to 15% (84/562), while 60% (15/25) of international graduates who had attempted vaccination were found to be positive at pre-vaccination screening (**Table 2.6**).

Table 2.6 Q fever vaccination status of veterinarians and veterinary nurses surveyed in Australia in 2014.

	All vets (n=796) **n (%)**	*Vets graduated in Australia (n=721)* **n (%)**	*Vets graduated internationally (n=75)* **n (%)**	*Nurses (n=688)* **n (%)**
Attempted vaccination				
Vaccinated	488 (61%)	478 (66%)	10 (13%)	162 (24%)
Pre-screen positive	99 (12%)	84 (12%)	15 (20%)	37 (5%)
Total attempted	587 (74%)	562 (78%)	25 (33%)	199 (29%)
Not attempted vaccination				
Not aware of the vaccine	57 (7%)	38 (5%)	19 (25%)	205 (30%)
Aware of the vaccine	152 (19%)	121 (17%)	31 (41%)	284 (41%)
Total not attempted	209 (26%)	159 (22%)	50 (67%)	489 (71%)

Veterinarians mostly (81%) received their vaccination as a requirement of a university course, while veterinary nurses were commonly vaccinated as a job requirement (43%). Multinomial regression revealed veterinary nurses had twice the odds (95% CI 1.2-5.0; $p = 0.02$) of having been vaccinated as a job requirement than having actively sought vaccination outside of a job requirement than veterinarians, while veterinarians had 19 times (95% CI 9.1-40.7; $p < 0.001$) odds of having been vaccinated as a university/course requirement than having actively sought vaccination outside of a job requirement than veterinary nurses.

Among respondents who had not attempted Q fever vaccination, 27% (57/209) of veterinarians and 42% (205/489) of veterinary nurses were not aware the vaccine existed (**Table 2.6**). Reasons for non-vaccination among those aware of its existence did not differ significantly between the two cohorts, with the perception that "I will not be seriously affected by Q fever" identified as the most influential reason for not seeking vaccination (**Table 2.7**).

Table 2.7 Proportional odds of the influence of known barriers to vaccination among unvaccinated veterinary nurses versus unvaccinated veterinarians surveyed in Australia in 2014.

	Nil Influence n (%)	*Minor/ Moderate Influence n (%)*	*Major/ sole Influence n (%)*	*Total*	*Adjusted OR†‡*	*95% CI*	*P-value§*
Unable to access a Q fever vaccine provider							
Nurses	194 (71%)	39 (14%)	41 (15%)	274	1		
Vets	98 (66%)	28 (19%)	22 (15%)	148	0.66	0.34-1.28	0.21
Q fever vaccine may not be effective							
Nurses	234 (86%)	35 (13%)	4 (1%)	273	1		
Vets	128 (87%)	17 (12%)	2 (1%)	147	1.65	0.65-4.62	0.305
Unable to afford the financial cost of vaccination							
Nurses	173 (63%)	65 (24%)	36 (13%)	274	1		
Vets	116 (78%)	22 (15%)	10 (7%)	148	1.19	0.60-2.43	0.628
Q fever vaccination may be harmful							
Nurses	211 (77%)	55 (20%)	8 (3%)	274	1		
Vets	109 (74%)	34 (23%)	4 (3%)	147	0.91	0.45-1.91	0.648
Pre-screening and vaccination process is too time consuming							
Nurses	198 (72%)	61 (22%)	15 (5%)	274	1		
Vets	100 (68%)	31 (21%)	15 (10%)	146	0.53	0.26-1.06	0.063
Perception that "I won't be seriously affected by Q fever"							
Nurses	134 (49%)	82 (30%)	60 (22%)	276	1		
Vets	72 (49%)	35 (24%)	41 (28%)	148	1.04	0.58-1.88	0.893

†Odds Ratio: odds of stating "major/sole influence" modelled over the lower levels of influence. Proportionality assumption was met. ‡Adjusted for age, gender and state. §Wald Chi-square *p*-value. Responses from participants who were not aware of the existence of the Q fever vaccine are excluded.

Seven participants who were not vaccinated commented that their medical practitioner had little knowledge of, or had advised against, Q fever vaccination. Such comments included:

"I have seen two GP's…regarding Q fever vaccination… neither one had any real idea of what was involved… one looked it up and made me feel the risks of vaccination were too high."

"My doctor when questioned about the existence of this vaccine did not believe that it existed."

"My doctor was under the impression you only need this vaccine if you are travelling overseas."

2.4.7 Sources of biosecurity information

Clinic protocols and veterinarians within the workplace were identified by participants as the most influential sources of biosecurity information for both veterinarian and veterinary nurse cohorts (**Figure 2.3**).

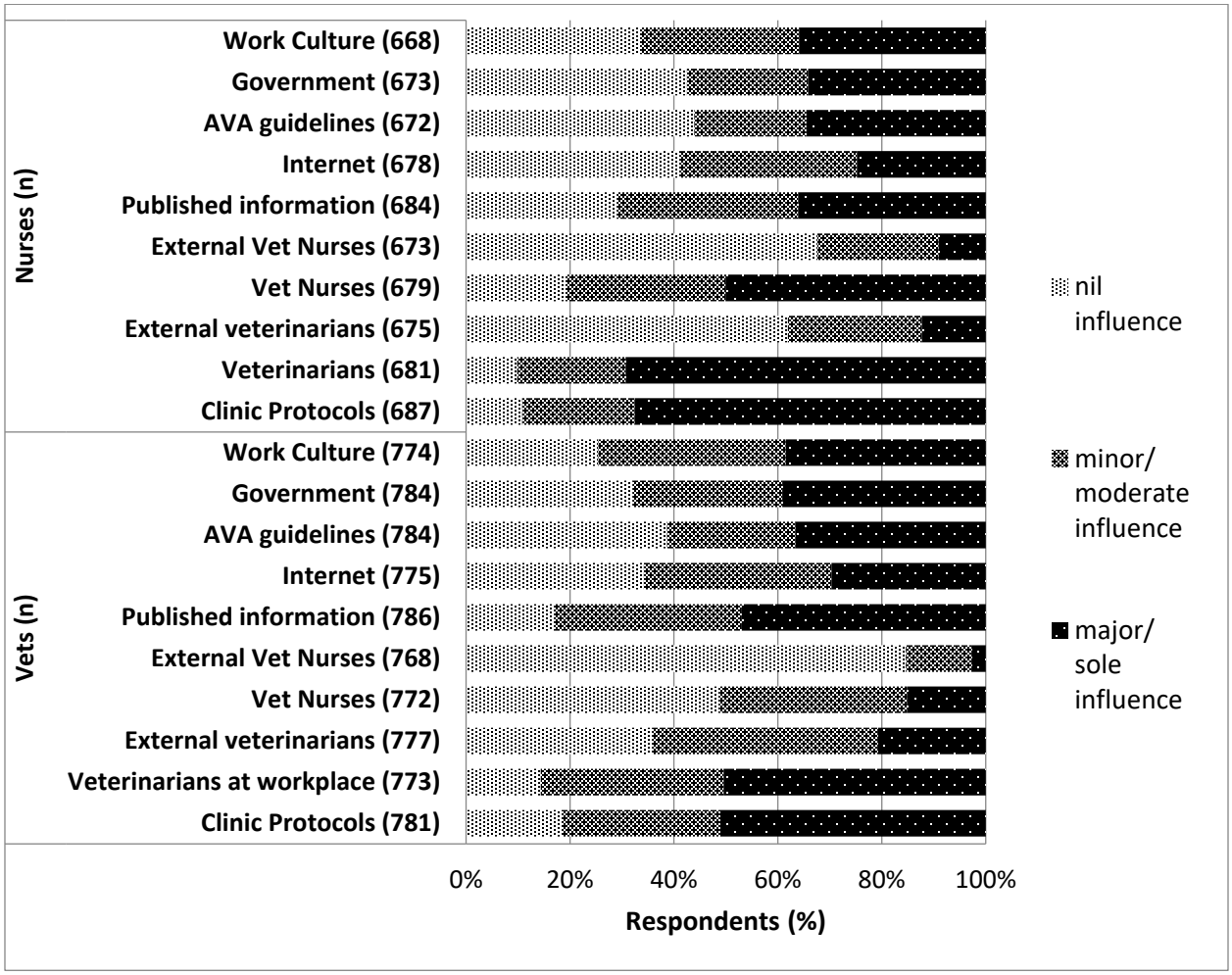

Figure 2.3 The level of influence that different sources of information have regarding work related biosecurity, as reported by veterinarians and veterinary nurses surveyed in 2014.

2.5 Discussion

This study investigated the knowledge, attitudes and practices of Australia's veterinary workforce regarding Q fever disease and vaccination and made comparisons between veterinarians and veterinary nurses. The key finding of this study was a shortfall in Q fever vaccine uptake among the veterinary nursing cohort, with less than a third of participants reporting they had sought vaccination. This was particularly disconcerting as the majority of veterinary nurses participating in the survey were women of child bearing age, who could potentially face an increased risk of chronic disease outcomes if they were to contract Q fever while pregnant (Denman & Woods, 2009). In contrast, the majority of veterinarians had sought vaccination with uptake similar to that observed in abattoir workers during Australia's National Q Fever Management Program [NQFMP] (Gidding et al., 2009) and for other occupational diseases among medical professions (Dinelli et al., 2009). The discrepancy between veterinarians and veterinary nurses seeking vaccination was observed despite the overwhelming majority of both cohorts agreeing that Q fever is a serious disease and that vaccinations are important for prevention of serious diseases. It is important to understand why this gap in vaccine uptake exists to improve Q fever vaccine uptake by veterinary nurses within Australia and to inform the potential introduction of the Q fever vaccine to at-risk cohorts internationally.

Some answers to the question of why veterinary nurses have a lower Q fever vaccination rate were evident from the study and can be supported by theories of health behaviour models. Such models outline the sociodemographic and motivational factors influencing health intentions and subsequent behavioural enaction of intended behaviours, such as vaccine uptake (Armitage & Conner, 2000). In the Health Belief Model, determinants of behaviour are perceived susceptibility, perceived severity, perceived benefits, perceived barriers, health motivation and cues to action (Armitage & Conner, 2000). Hence, the first step towards vaccine uptake as a health behaviour is knowledge of the health risk Q fever poses and awareness of the availability of a vaccine. Veterinary nurses however reported a notable lack of awareness of the

Q fever vaccine and a particularly low level of self-reported Q fever knowledge. Since veterinary nurses identified workplace protocols and veterinarians within their workplace as the two most influential sources of biosecurity information, the shortfall in the two fundamental areas of awareness and knowledge may point to inadequate WH&S protocols and training in the practices in which these veterinary nurses were employed. This is consistent with other studies calling for improved WH&S training within veterinary clinics both in Australia and internationally (Dowd et al., 2013; Jeyaretnam, Jones, & Phillipa, 2000; Lipton, Hopkins, Koehler, & DiGiacomo, 2008; Shirangi, Fritschi, & Holman, 2007; Snow & Rice, 2005; Wright, Jung, Holman, Marano, & McQuiston, 2008).

Once aware of the risks associated with Q fever and the availability of a Q fever vaccination, the formation of an intention to seek vaccination relies on an individual's attitudes, both positive and negative, associated with seeking and receiving the vaccination. Applying the health belief model, determinants of Q fever vaccine uptake will include perceived susceptibility to Q fever disease, perceived severity of Q fever disease, perceived benefits of vaccination and perceived barriers to vaccination (Armitage & Conner, 2000). A lack of perceived susceptibility and severity was evident among non-vaccinates in both cohorts, with the belief that they personally were not at risk of being severely affected by Q fever the most influential reason for not seeking vaccination. This view may reflect a lack of knowledge of the sources of infection and therefore the potential level of exposure for veterinary workers. For example, some workers may not be aware that companion animals are a source of infection, believing that only livestock transmit the pathogen to humans. This is likely in the nursing cohort at least, given that a quarter of veterinary nurses surveyed identified that they did not know their potential risk of exposure to *C. burnetii* and close to one fifth, the majority of whom were engaged in companion animal work, stated nil exposure.

Alternatively, veterinary workers who might identify themselves as being at risk of acquiring Q fever may assume that they have already been exposed to *C. burnetii* due to time in the veterinary industry or

working with higher risk species. Therefore, they may hold the perception that they have pre-existing immunity and subsequently are at lower risk of being severely affected by Q fever and do not require vaccination. Given most people exposed to *C. burnetii* will remain asymptomatic or experience flu-like symptoms, the low level of perceived risk for personally being severely affected by Q fever, reported in unvaccinated workers aware of the vaccine, may reflect an informed risk analysis and their perception of 'severely affected'. Indeed, multi-stage health behaviour models theorise that the variables important to behavioural enaction may vary at different stages of the process of behaviour change (Armitage & Conner, 2000; Weinstein & Sandman, 1992). Further qualitative research, such as focus groups or semi-structured interviews, would assist in our understanding of worker's perceptions of the risk of being severely affected by Q fever.

Along with this lack of perceived vulnerability, perceived costs may be a key barrier to Q fever vaccination (Floyd, Prentice-Dunn, & Rogers, 2000). These costs may include those of time and financial expense, as well as difficulties involved in finding a Q fever vaccination provider and the perceived and reported adverse outcomes following vaccination. The results of this study indicate all these factors were somewhat influential barriers to vaccination for the participants of this study. While these barriers were equally influential for both unvaccinated veterinarians and unvaccinated veterinary nurses, the veterinary nursing cohort (any vaccination status) was significantly more likely to agree that the Q fever vaccine was too expensive. This suggests that expense may be more influential for veterinary nurses, which is not unexpected given their lower wages compared to veterinarians (National Careers Institue, 2021a, 2021b). The perception of being "too expensive" may also reflect cost-benefit analysis, with veterinary nurses perceiving fewer benefits from vaccination than veterinarians.

Further understanding of the reasons behind the gap in vaccination between the two cohorts can be gained from the results of survey questions regarding reasons why participants had sought vaccination. The overwhelming majority of veterinarians surveyed received their Q fever vaccination as a compulsory

requirement of their university studies. This usually occurs during the early years of veterinary or other animal science degrees, often with large numbers of students vaccinated on campus over a short time period or in organised vaccination clinics. Such vaccination programs reduce the cost of vaccination, the difficulty sourcing a trained provider, and the time involved in being vaccinated. In addition, vaccination in this tertiary environment is driven by recommendation from peers and health and safety protocols, along with a desire to avoid both negative health and learning outcomes. Such adherence to subjective norms has been shown to be an integral component of health models, such as the theory of planned behaviour (Armitage & Connor, 2001). The Australian NQFMP is further evidence of the success of vaccination *en mass*. The Australian government program funded the costs of screening and vaccination of more than 50,000 abattoir workers, sheep shearers, farm workers and their families. Workplace or community mass vaccination clinics were held, resulting in a program uptake ranging from 50 to 100% in the initial phase (Gidding et al., 2009).

However, the opportunity for *en mass* vaccination of veterinary nurses is limited, as veterinary clinics are mostly small businesses as opposed to universities, abattoirs, and farming communities with substantially larger cohorts requiring vaccination. Additionally, veterinary nursing in Australia is typically taught in the workplace with supplementary or formal training via technical tertiary institutions. A Certificate IV was the most commonly reported highest level of education by veterinary nurses, for which concurrent employment or placement in a veterinary clinic for practical learning is required (Australian Industry and Skills Commitee, 2020; TAFE NSW, 2021; TAFE Queensland, 2021). Hence, the risk of exposure to *C. burnetii* occurs within the workplace rather than the educational institution and therefore, vaccination is not typically mandatory for veterinary nursing education, though it may be recommended (TAFE Queensland, 2021). Hence, veterinary nurses do not typically have access to vaccination programs via their educational institution and instead, they may need to seek out vaccination independently or rely on individual workplace vaccination programs which may or may not be available in their workplace. In the

former group, the barriers to vaccination outlined above become much more influential. Although, there is anecdotal evidence that an increasing number of veterinary clinics are now approaching Q fever vaccination as their responsibility.

This study also raised concerns regarding a lack of appropriate advice on Q fever vaccination from general medical practitioners in Australia, which builds on the reported challenges general practitioners face regarding the diagnosis and management of Q fever (Gunaratnam et al., 2014). In some instances, it is reported that Q fever vaccination of apparently at-risk individuals was actively discouraged. Other studies have recommended greater collaboration between medical practitioners and veterinarians regarding zoonoses and risk mitigation in particular, as general medical practitioners tend not to be comfortable with advising on the role of animals in the transmission of zoonotic agents and associated risk factors (Grant & Olsen, 1999; Speare et al., 2015). Hence, further research is recommended to investigate Q fever knowledge and attitudes towards Q fever vaccination in medical practitioners.

Accessing the Australian veterinary workforce for the purpose of this study proved difficult due to the absence of a governing central body through which the workforce could be contacted. Despite state registration requirements for veterinarians, access via state veterinary boards was limited as was contacting individuals via the AVA. Veterinary nurses were only contactable by the WA state veterinary board or the VNCA. It is expected that a greater number of responses may be achieved for both cohorts with uniform access to individuals, rather than businesses, as demonstrated by the national AVA workforce survey of veterinarians distributed annually by all state boards to individuals for online completion. The AVA workforce survey has achieved response rates varying from 15% to 29% of registered veterinarians. In comparison, this survey received responses from 8.4% of veterinarians registered in 2014. However, it is not directly comparable since not all registered veterinarians were contactable in the current study due to the inability to access them via state boards, as was the case for the AVA surveys. Other veterinary workforce studies in Australia have relied upon data collection from professional

conferences; however, these typically achieve smaller sample sizes and greater selection bias among participants (Dowd et al., 2013; Soest & Fritschi, 2004). A novel approach to data collection was required for this study to maximise the number of responses and reduce selection bias in order to improve sample representativeness. Although this resulted in a non-uniform approach across all states and the inability to accurately assess response rate, the numbers achieved were remarkable and the sampling across states was considered representative of the workforce (Table 2.1). In addition, the distribution of age and gender were comparable to those reported in AVA national workforce surveys and Australian government statistics (Australian Bureau of Statistics, 2014a; Australian Veterinary Association, 2012, 2013, 2014). The proportion engaged in each practice type was also similar to those reported in the AVA national workforce surveys (Australian Veterinary Association, 2012, 2013, 2014).

Currently, there is little information available on the demographics of the Australian population of veterinary nurses beyond Australian government employment statistics. These statistics report 99% of Australian veterinary nurses are female which was reflected in the results of this survey (Australian Bureau of Statistics, 2014b). However, the veterinary nurse respondents in this study were older and more highly educated than those reflected in government statistics, and the states of WA and NSW may be over-represented in this study, while South Australia and Queensland appear to be under-represented (**Table 2.7**) (Australian Bureau of Statistics, 2014b). Coverage bias associated with web-based surveys is expected to be minimal due to the option of alternative methods for survey participation and the context of contact via the workplace and professional associations (Cook, Heath, & Thompson, 2000).

The results of this study most likely indicate a 'best-case' scenario for this workforce for a number of reasons. Firstly, a selection bias may have occurred towards participation from those familiar with the subject and/or with a higher level of concern about Q fever (Fan & Yan, 2010). Secondly, the veterinary nursing participants in this study had a higher level of formal qualifications than their profession as a whole. As a result, the knowledge and awareness of Q fever could be expected to be lower in the greater

population of veterinary workers, particularly veterinary nurses. Additionally, self-reported levels of knowledge tend to be over-claimed (Hülür, Wilhelm, & Schipolowski, 2011). In surveys, there is also a risk that responses may be inaccurate, particularly due to a tendency towards socially desired responses (Trelle, 2002). To reduce the impact of such bias, respondents were given the option to state "Don't know" or opt out of responding to questions. Bias may also result from careless reading/answering, particularly in professional/busy cohorts participating in a long questionnaire, or from misunderstanding of questions (Trelle, 2002).

2.6 Conclusion

The low levels of Q fever knowledge and uptake of the Q fever vaccine in veterinary nurses suggests inadequate WH&S information and training, in relation to Q fever, as is required by Australian law. Other barriers to vaccination included financial expense, time, and difficulty in finding a Q fever vaccine provider; these were influential on veterinarians also. Veterinarians were mostly vaccinated in mass vaccination clinics during tertiary education, reducing the impact of these barriers, and indicating the potential for workplace vaccination clinics to improve the vaccination status of veterinary nurses. This study highlights the need for additional studies to identify the drivers behind seeking and recommending vaccination so that further recommendations on improving Q fever vaccine uptake by Australian veterinary workers can be made. Evaluation of the knowledge and attitudes towards this vaccine among the medical profession may also be warranted. Until then, some veterinary workers remain unnecessarily at risk and unaware of the dangers posed to them by *C. burnetii* while veterinarians and clinics may be failing to provide adequate duty of care.

3 Willingness of Veterinarians in Australia to Recommend Q fever Vaccination in Veterinary Personnel

The content of this chapter is published:

Sellens E, Norris JM, Dhand NK, Heller J, Hayes L, Gidding HF, Willaby H, Wood N, Bosward KL. Willingness of veterinarians in Australia to recommend Q fever vaccination in veterinary personnel: Implications for workplace health and safety compliance. *PLoS One*. 2018 Jun 1;13(6):e0198421. doi: 10.1371/journal.pone.0198421.

3.1 Abstract

Q fever vaccine uptake among veterinary nurses in Australia is low, suggesting poor recommendation of the vaccine among veterinary personnel. This study aimed to determine the willingness of veterinarians to recommend Q fever vaccination to veterinary personnel and to identify factors influencing Q fever vaccine uptake by veterinary nurses in Australia. An online cross-sectional survey targeted veterinarians and veterinary nurses in Australia in 2014. Responses were analysed using multivariable logistic regression. Factors significantly (p <0.05) associated with a willingness to recommend the vaccination, expressed by 35% (95% CI 31 – 38%) of veterinarians (n = 828), were (1) being very concerned for colleagues regarding *Coxiella burnetii* (OR 4.73), (2) disagreeing the vaccine is harmful (OR 3.80), (3) high Q fever knowledge (OR 2.27), (4) working within small animal practice (OR 1.67), (5) disagreeing the vaccine is expensive (OR 1.55), and (6) age, with veterinarians under 39 years most likely to recommend vaccination. Of the veterinary nursing cohort who reported a known Q fever vaccination status (n = 688), 29% (95% CI 26 – 33%) had sought vaccination. This was significantly (p <0.05) associated with (1) agreeing the vaccine is important (OR 8.34), (2) moderate/high Q fever knowledge (OR 5.51), (3) working in Queensland (OR 4.00), (4) working within livestock/mixed animal practice (OR 3.24), (5) disagreeing the

vaccine is expensive (OR 1.86), (6) strong reliance on work culture for biosecurity information (OR 2.5), (7) perceiving personal exposure to *Coxiella burnetii* to be at least low/moderate (OR 2.14), and (8) both agreeing the vaccine is safe and working within a corporate practice structure (OR 4.28). The study identified the need for greater compliance with workplace health and safety laws in the veterinary industry and calls for employers to establish strict workplace vaccination policy. Further education of veterinary personnel is required to raise awareness of the potential for occupational exposure to *C. burnetii* in workers across all practice types.

3.2 Introduction

Q fever is a vaccine-preventable zoonotic disease caused by the bacterium *Coxiella burnetii,* which is distributed worldwide, with the exception of New Zealand and French Polynesia (Million & Raoult, 2015). Although acute illness is usually limited to non-specific flu-like symptoms, up to five percent of patients experience severe illness requiring hospitalisation and one percent of acute clinical cases are fatal (Angelakis & Raoult, 2010; Kosatsky, 1984; Marrie, 2010; Marrie et al., 1989; Raoult, Marrie, & Mege, 2005; Spelman, 1982). Patients with cardiovascular lesions, immunosuppression or pregnancy are predisposed to persistent focalized *C. burnetii* infections, such as endocarditis, vascular infections, and bone and joint infections (Million & Raoult, 2015). Infection during pregnancy may lead to adverse pregnancy outcomes including miscarriage (Million & Raoult, 2015; Raoult et al., 2005). Diagnosis is often delayed in the absence of suspicion; highlighting the importance of prevention in at-risk cohorts (Healy et al., 2011; van der Hoek et al., 2011).

Farmed cattle, sheep and goats are most commonly implicated in human Q fever (Angelakis & Raoult, 2010). However, human outbreaks associated with dogs and cats are well described (Gibbons & White, 2014; Komiya, Sadamasu, Toriniwa, et al., 2003; Kopecny et al., 2013; Kosatsky, 1984) and *C. burnetii* has been found within a wide range of host species (Angelakis & Raoult, 2010; Tozer et al., 2014). People

directly exposed to animals are considered at greatest risk of exposure to *C. burnetii*, and Q fever is subsequently a workplace health and safety [WH&S] concern for farmers, meat processors, and veterinarians globally (Angelakis & Raoult, 2011).

Q fever is associated with considerable costs to individuals, businesses, and public health systems, including medical expenses, lost work hours and compensation claims. A large outbreak which occurred in the Netherlands from 2007 – 2011 is estimated to have cost over 300 million Euros, with delayed expenses associated with chronic fatigue syndrome an ongoing prominent burden (Asseldonk, Prins, & Bergevoet, 2013). In Scotland, an outbreak among abattoir workers in 2006 highlighted the burden and complexities of managing outbreaks and the long term follow up of affected workers (Wilson et al., 2010). Within Australia, Q fever is the most commonly reported notifiable zoonotic disease, excluding food-borne pathogens (Safe Work Australia, 2014). Here, the cost of compensation alone is estimated to exceed $1.3 million Australian dollars annually (Kermode et al., 2003). However, this is likely to be underestimated, as many cases remain un-diagnosed where there is a lack of suspicion (Kermode et al., 2003).

While personal protective equipment offers some protection during risky procedures, human vaccination is the most effective measure for preventing Q fever and related societal costs. A whole-cell Q fever vaccine (Q-VAX®; Seqiris, Parkville, Vic.) has been available in Australia since 1989 and is recommended for workers with high occupational risk; including veterinary workers (*The Australian Immunisation Handbook*, 2013). Targeted Q fever vaccination of people within high-risk occupations was shown to significantly reduce the burden of Q fever in Australia and proved to be cost-effective (Gidding et al., 2009; Kermode et al., 2003). Uptake of the Q fever vaccine among at-risk workers in Australia is variable. Uptake by abattoir workers and sheep shearers targeted in a nation-wide government funded vaccine program ranged from 50 – 100% across different Australian states (Gidding et al., 2009). Uptake by veterinarians, who since the early 1990s have been mostly vaccinated *en mass* upon commencing university studies, is

estimated at 74% (Sellens et al., 2016). In contrast, a best-case estimate of 29% for uptake among veterinary nurses was reported (Sellens et al., 2016), with the low uptake attributed to a lack of awareness of the Q fever vaccine, a lack of knowledge regarding Q fever disease, and an increase in the influence of barriers to vaccination where vocational vaccine programs or protocols are not routine (Sellens et al., 2016). The poor vaccine uptake among veterinary nurses may also be due to inadequate WH&S protocols in veterinary clinics and veterinarians not recommending Q fever vaccination, as these two sources of biosecurity information are the major influential sources for this cohort (Sellens et al., 2016). This raises serious concerns regarding WH&S within the Australian veterinary industry.

This study aimed to determine the willingness of veterinarians in Australia to recommend Q fever vaccination to other veterinary personnel and to identify significant factors influencing the uptake of the Q fever vaccine by veterinary nurses in Australia. The results of this study have the potential to inform WH&S and Q fever vaccination protocols within the Australian veterinary industry and provide valuable knowledge for the implementation of Q fever vaccination internationally.

3.3 Methods

3.3.1 Study design and recruitment

An online cross-sectional survey targeting all actively working veterinarians and veterinary nurses in Australia was undertaken from March to June of 2014 via the Survey Monkey® platform. The details of this study and recruitment have been described previously (Sellens et al., 2016). Briefly, the questionnaire contained 53 questions pertaining to (1) demographics and veterinary work environment, (2) attitudes towards Q fever illness and vaccination, (3) experience with Q fever disease, (4) experience with Q fever vaccination, (5) knowledge of disease risk, and (6) biosecurity practices.

Veterinary nurses in Australia are not required to be formally registered with state veterinary boards, with the exception of those in Western Australia (WA). Veterinarians, however, are required to maintain

registration with the veterinary board of the state in which they practice. A personal email invitation to participate in this survey was sent on our behalf to veterinary nurses in WA, and veterinarians in WA and Tasmania, from their respective state veterinary boards. Elsewhere, contact via state veterinary boards was not possible and participants were primarily recruited via their workplace. Researchers attempted to phone all veterinary clinics in Australia to invite participation and a follow up invitation was sent via email, or fax or post where preferred, to consenting clinics to be shared with staff. Additionally, the survey was advertised by (1) the Australian Veterinary Association [AVA] in an e-newsletter distributed to members on the 11th of April 2014, (2) the Veterinary Practitioners Board of New South Wales on their website during May 2014, and (3) the Veterinary Nursing Council of Australia [VNCA] as a personal email invitation to members.

The study was performed in accordance with the Declaration of Helsinki. A participant information statement outlining the risks and benefits of the study was provided to participants upon invitation, and again in the first pages of the survey. Consent to participate was confirmed through commencement of the survey. Ethics approval was granted by Charles Sturt University School of Animal and Veterinary Sciences Human Ethics Committee (protocol #416/2013/19).

3.3.2 Data management and analysis

3.3.2.1 Outcome variables

Two outcome variables were drawn from the questionnaire data. A dichotomous outcome variable was created for veterinarians from responses to the survey question "*Thinking about vaccination for Q fever across each occupation group within each practice type, what would be your recommendations for Q fever vaccination?*" Veterinarians who indicated that they slightly, moderately or strongly recommended vaccination for veterinarians, veterinary nurses and kennel hands/animal handlers across all veterinary practice types were considered 'willing to recommend vaccination'.

A second dichotomous outcome variable reflecting vaccination status was assessed for the veterinary nursing cohort. Those nurses who were vaccinated or had attempted vaccination but were unable to receive the vaccine due to a positive pre-vaccination screening result, were considered to have 'sought vaccination' which was the positive outcome variable. Veterinary nurses who stated 'unsure' for their vaccine status were excluded from the statistical analysis.

3.3.2.2 Explanatory variables

For the outcome variables 'willing to recommend Q fever vaccination' and 'sought vaccination', 38 and 33 explanatory variables were assessed, respectively. Age, gender, state, practice type, practice structure, and education were considered potential confounders.

The continuous variables 'age', 'years working' and 'self-rated Q fever knowledge' were categorized into ordinal variables for each cohort determined by their distribution into quantiles. Practice type was defined by the proportion of hours respondents spent per week with each species: (a) 'small animal' where >90% of work hours were spent with cats, dogs, pocket pets, wildlife or birds; (b) 'equine' where >90% of work hours were spent with horses; (c) 'livestock' where >90% of work hours were spent with cattle, sheep or goats; (d) 'other' where >90% of work hours were spent with zoo, fish, or other species, and the remainder were classified as (e) 'mixed' animal practice. Due to the low number of veterinary nurses working in some practice types, a dichotomous variable was created; 'livestock/mixed animal practice' reflecting exposure to farm animals which are most commonly implicated as sources of Q fever in Australia, and 'small/equine/wildlife/other' reflecting exposure to species that are less commonly implicated in Q fever cases in Australia (Gunaratnam et al., 2014; Karki et al., 2015). Participant's practice structure was determined by the practice environment in which most weekly hours were spent. Hours were stated for (a) solo practice (single vet), (b) group/multi-vet practice, (c) corporate practice (group practices owned by a large corporation), (d) government, (e) industry, (f) laboratory, (g) university, (h) abattoir, and (i) other. Due to the small number of participants working outside of solo and group practices and the

similarities in general management styles of practice structures (c) through to (i), the latter were combined as one practice structure labelled "corporate/other".

3.3.2.3 Statistical analysis

Initially, contingency tables and univariable associations between explanatory and outcome variables were determined, assisted by UniLogistic SAS macro (Dhand, 2010). Variables exhibiting some association (p <0.25) were then considered for multivariable logistic analysis, excluding those for which >10% of responses were missing. Collinearity was assessed using the Spearman rank correlation coefficient and Chi-square test of significance. Where two variables were found to be collinear (coefficient >0.7; p <0.05) one of the pair of collinear variables was excluded from multivariable analysis. Multivariable model building, aided by MultiLogistic SAS macro (Dhand, 2009), was undertaken via forward stepwise selection retaining variables with a p-value <0.05 in the final model.

All significant variables within the model and potential confounders were tested for interaction prior to assessment of confounding. Interaction terms were retained in the model where significant (p <0.05), and potential confounders were forced into the model if they caused >20% change in the coefficients of variables already in the model. The Likelihood-ratio test was used to determine the significance of the full models and Hosmer-Lemeshow goodness of fit tests were performed on final models.

3.4 Results

3.4.1 Sampling

Eligible responses from 1,742 participants were received: 890 veterinarians and 852 veterinary nurses. This resulted from telephone contact with 1,677 clinics, of which 1,446 and 1,582 consented to receive the survey for participation of veterinary nurses and veterinarians respectively. Additionally, personal invitation emails sent to 882 veterinary nurses and 1200 veterinarians registered with the WA state

veterinary board, 245 veterinarians registered with the veterinary board of Tasmania, and 917 veterinary nurse members of the VNCA.

It was not possible to calculate a response rate for the survey as the number of veterinarians and veterinary nurses that viewed an invitation to participate is not known. Referring to government employment statistics however, the number of responses represented 12% of the estimated 7,400 employed veterinarians and 10% of the estimated 8,600 employed veterinary nurses in Australia at the time (Australian Bureau of Statistics, 2014a, 2014b). Further results of sampling, including the characteristics and demographics of the study sample have been previously described in detail (Sellens et al., 2016).

3.4.2 Willingness to recommend Q fever vaccination

Of the 890 veterinarians who participated, 828 responses were complete for the variable 'willing to recommend vaccination'. Of these, 287 (35%; 95% confidence interval [CI] 31-38%) were considered willing to recommend Q fever vaccination. Generally, a greater proportion of veterinarians were willing to recommend Q fever vaccination to workers in livestock and mixed animal practices than other practice types (**Figure 3.1**).

Of the 38 explanatory variables assessed, 11 were excluded due to >10% missing data. The first reflected self-rated biosecurity knowledge and the low response rate is attributed to respondents visually missing the question during survey completion. The further 10 excluded variables pertained to sources of biosecurity information, and survey fatigue is likely responsible for the poor responses as all were drawn from the final question of the survey. Of the remaining 27 variables, 19 exhibited some univariable association (p <0.25) with the outcome (**Table S3-1**). Four were excluded due to significant correlation with other variables and the remaining 15 variables were tested in the multivariable analysis.

Multivariable modelling identified six variables significantly associated with the outcome 'willing to recommend vaccination'; (1) level of concern that colleagues may be exposed to *C. burnetii*, (2) concern that the Q fever vaccine will do more harm than good, (3) age, (4) self-rated Q fever knowledge, (5) gender, and (6) perception of vaccine expense. No significant interaction terms were identified. Practice type was found to confound the association between the outcome and three of the significant variables; (1) level of concern that colleagues may be exposed to *C. burnetii* and (2) self-rated Q fever knowledge and (3) gender. The inclusion of practice type rendered gender non-significant; however, gender was retained in the model as it confounded the association of age with the outcome. The Hosmer and Lemeshow Goodness-of-Fit test was not significant (p = 0.59) demonstrating there was no reason to believe the model was not a good representation of the data.

The final multivariable model included 772 complete case responses of which 270 (35%) were willing to recommend the Q fever vaccination. Veterinarians willing to recommend Q fever vaccination were more likely to; (1) report higher levels of concern that colleagues may be exposed to *C. burnetii*, (2) disagree that the Q fever vaccine will do more harm than good, (3) work in small animal practice, (4) self-report higher levels of Q fever knowledge, (5) be under 39 years of age, and (6) disagree that Q fever vaccination is expensive (**Table 3.1**).

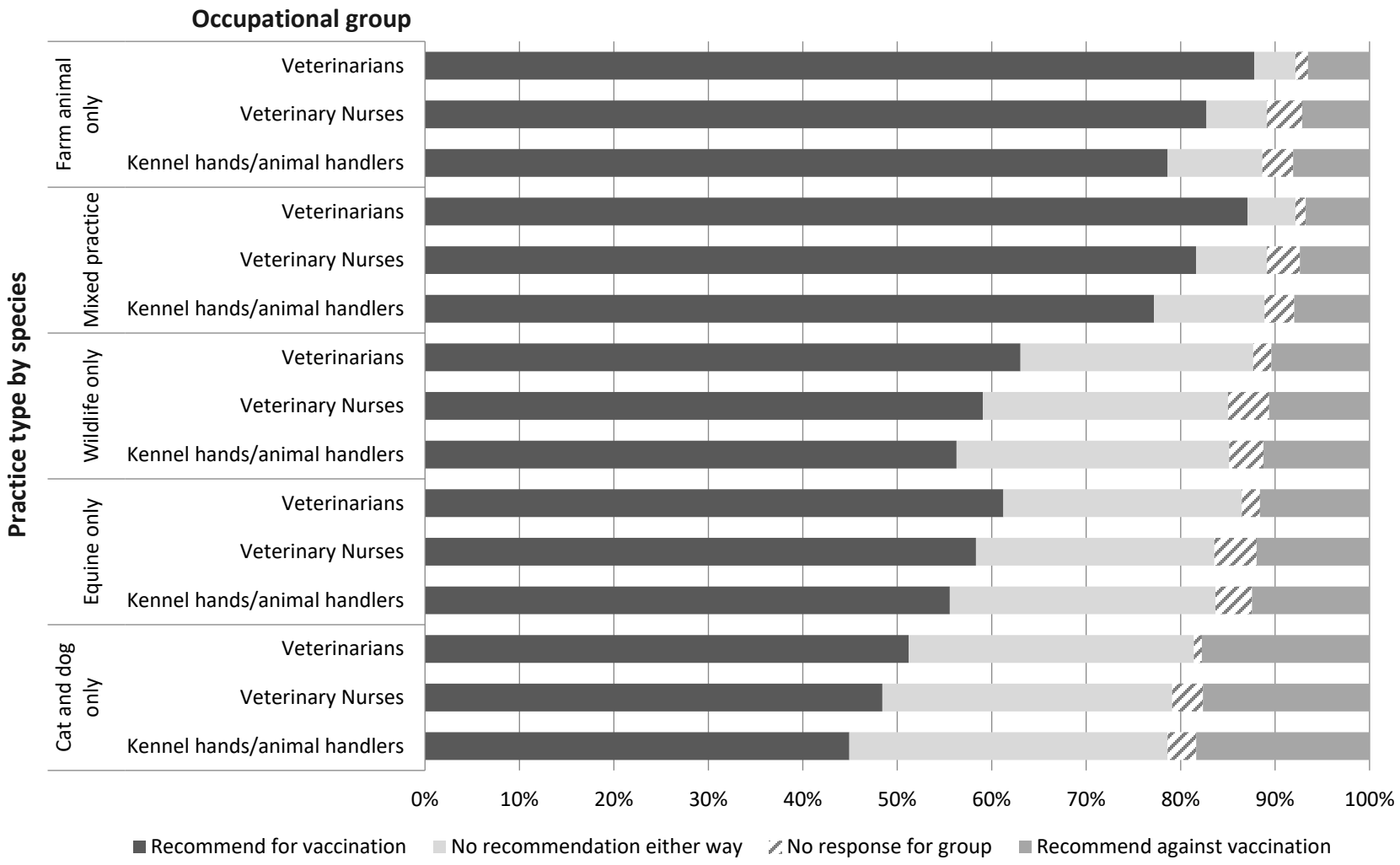

Figure 3.1 Responses from veterinarians (n = 828) surveyed in Australia in 2014 reflecting their recommendation for Q fever vaccination in veterinary personnel. The percentage of veterinarians recommending for vaccination, against vaccination, and with no recommendation either way for each occupational group (veterinarians, veterinary nurses, kennel hands/ animal handlers) within each practice type queried is shown. Not all respondents provided a response for recommendation for all groups.

Table 3.1 Final multivariable model parameter estimates and odds ratios of factors associated with a willingness to recommend Q fever vaccination among veterinarians surveyed in Australia in 2014.

Variable	*b*	*SE(b)*	*Odds Ratio*	*95% LCL*	*95% UCL*	*P-value*[†]
Intercept	-3.61	0.5	.	.	.	<0.001
Concern that colleagues may be exposed to C. burnetii						<0.001
Nil concern	.	.	Ref	.	.	
Slight concern	0.89	0.23	2.43	1.54	3.87	
Moderate concern	1.5	0.26	4.47	2.71	7.46	
Very concerned	1.53	0.32	4.62	2.47	8.74	
"I worry that the Q fever vaccine will do more harm than good"						<0.001
Agree	.	.	Ref	.	.	
Disagree	1.4	0.38	4.05	2.02	9.07	
Practice type in which most hours have been spent throughout career						0.008
Mixed/ Large animal	.	.	Ref	.	.	
Small animal	0.51	0.19	1.67	1.16	2.42	
Other	0.68	0.44	1.97	0.82	4.59	
Self-rated Q fever knowledge						0.019
≤3/10	.	.	Ref	.	.	
4-5/10	0.2	0.24	1.22	0.76	1.95	
6-7/10	0.6	0.24	1.82	1.14	2.92	
≥8/10	0.79	0.29	2.2	1.24	3.92	
Age						0.019
≤30 years	.	.	Ref	.	.	
31-38 years	-0.12	0.22	0.89	0.58	1.38	
39-48 years	-0.69	0.24	0.5	0.31	0.8	
≥49 years	-0.48	0.25	0.62	0.37	1.01	
"The Q fever vaccination is too expensive"						0.039
Agree	.	.	Ref	.	.	
Disagree	0.44	0.19	1.55	1.07	2.27	
Don't know	0.03	0.24	1.03	0.64	1.65	

Model adjusted for gender. b; regression coefficient. SE; standard error. OR; profile-likelihood odds ratio. LCL; 95% lower confident limit. UCL; 95% upper confidence limit. Ref; Reference category.
[†]Likelihood ratio *p*-value.

3.4.3 Factors influencing vaccine uptake by veterinary nurses

Of the 852 veterinary nurse respondents, 729 entered a response for vaccination status. Those stating 'unsure' (n=41) were excluded from further analysis. Of the 688 remaining veterinary nurses, 199 (29%; 95% CI 26-33%) had sought vaccination for Q fever (Sellens et al., 2016). Of 33 explanatory variables assessed, one was excluded due to >10% missing data; self-rated knowledge of biosecurity. This was attributed to respondents visually missing the question during survey completion. Twenty-five of the remaining 32 variables exhibited some univariable association (p <0.25) with the outcome (**Table S3-2**). Four were excluded due to significant correlation with other variables and the remaining 22 variables were included in multivariable analysis.

The final multivariable model included 573 complete case responses of which 166 (29%) had sought vaccination. Nine main effects were identified (**Table 3.2**). Interaction was found between perception of Q fever vaccine safety and practice structure in which most hours were spent (**Table 3.2**). Age and gender were forced into the model as significant confounders. Age confounded the association between perception of Q fever vaccine safety and the outcome, while gender confounded the association of practice structure with the outcome. The Hosmer and Lemeshow Goodness-of-Fit test was not significant (p = 0.23) demonstrating there was no reason to believe the model was not a good representation of the data.

Veterinary nurses who had sought vaccination were more likely to; (1) be convinced of the importance of the Q fever vaccination, (2) self-report higher levels of Q fever knowledge, (3) work in Queensland, (4) work with animal species more commonly associated with Q fever, (5) disagree that the vaccine is expensive, (6) rely mostly or solely on workplace culture as a source of biosecurity information, (7) report greater likelihood of exposure to *Coxiella burnetii*, and (8) both agree that Q fever vaccination is safe and work in corporate/other practice structures.

Table 3.2 Final multivariable model parameter estimates and odds ratios of factors significantly associated with Q fever vaccination status of veterinary nurses in Australia in 2014.

Variable	*b*	*SE(b)*	*Odds Ratio*	*95% LCL*	*95% UCL*	*P-value*[†]
Intercept	-4.09	0.98	.	.	.	**0.009**
"I am convinced of the importance of the Q fever vaccination"						**0.001**
Disagree	.	.	Ref	.	.	
Agree	2.12	0.8	8.34	2.16	56.35	
Self-rated Q fever knowledge						**<0.001**
1/10	.	.	Ref	.	.	
2-3/10	0.58	0.44	1.78	0.78	4.36	
4-5/10	1.17	0.45	3.21	1.37	8.05	
6+/10	1.71	0.47	5.51	2.28	14.29	
State						**<0.001**
WA / NT	.	.	Ref	.	.	
SA/Tasmania/Victoria	0.43	0.5	1.54	0.59	4.24	
NSW / ACT	0.22	0.47	1.24	0.51	3.25	
Queensland	1.39	0.49	4	1.59	10.81	
Practice type						**0.001**
Small/equine/wildlife/other	.	.	Ref	.	.	
Large / mixed	1.18	0.3	3.24	1.8	5.93	
"The Q fever vaccination is too expensive"						**<0.001**
Agree	.	.	Ref	.	.	
Disagree	0.62	0.31	1.86	1.01	3.45	
Don't know	-0.55	0.29	0.58	0.33	1.02	
Reliance on work culture for biosecurity information						**0.022**
Nil	.	.	Ref	.	.	
Minor/moderate	0.33	0.4	1.39	0.65	3.12	
Major/sole	0.92	0.41	2.5	1.15	5.69	
Perceived average personal level of exposure to Coxiella burnetii throughout career						**0.028**
Nil/very low	.	.	Ref	.	.	
Low/moderate	0.76	0.27	2.14	1.26	3.66	
High/very high	1.07	0.65	2.91	0.83	10.85	
Don't know	0.16	0.37	1.17	0.56	2.41	

Variable		*b*	*SE(b)*	*Odds Ratio*	*95% LCL*	*95% UCL*	*P-value*[†]
		******Interaction Term******					
"The Q fever vaccination is safe if appropriately administered"	*Practice structure in which most hours are spent*						**0.032**
Disagree / don't know	***Solo***	.	.	Ref			
	Group	.	.	0.15	0.02	0.87	
	Corporate / other	.	.	0.36	0.04	2.33	
Agree	***Solo***	.	.	Ref			
	Group	.	.	1.14	0.66	1.99	
	Corporate / other	.	.	4.28	1.87	10.15	

Positive outcome = 'sought vaccination'. Model adjusted for age and gender. b; regression coefficient. SE; standard error. OR; profile-likelihood odds ratio. LR; likelihood ratio test. LCL; 95% lower confidence limit. UCL; 95% upper confidence limit. Ref; reference category. NSW; New South Wales. ACT; Australian Capital Territory. SA; South Australia. WA; Western Australia. NT; Northern Territory.

[†]Likelihood ratio *p*-value.

3.4.4 Responsibility for health and safety in the workplace

Responses to questions pertaining to responsibility for WH&S were missing for 12% and 20% of the veterinarian and veterinary nursing cohorts, respectively. This was likely due to survey fatigue, being the final questions in the survey. Hence, these variables were excluded from multivariable analysis. However, these results are presented separately (**Figure 3.2**), as they are considered pertinent to the discussion of other findings.

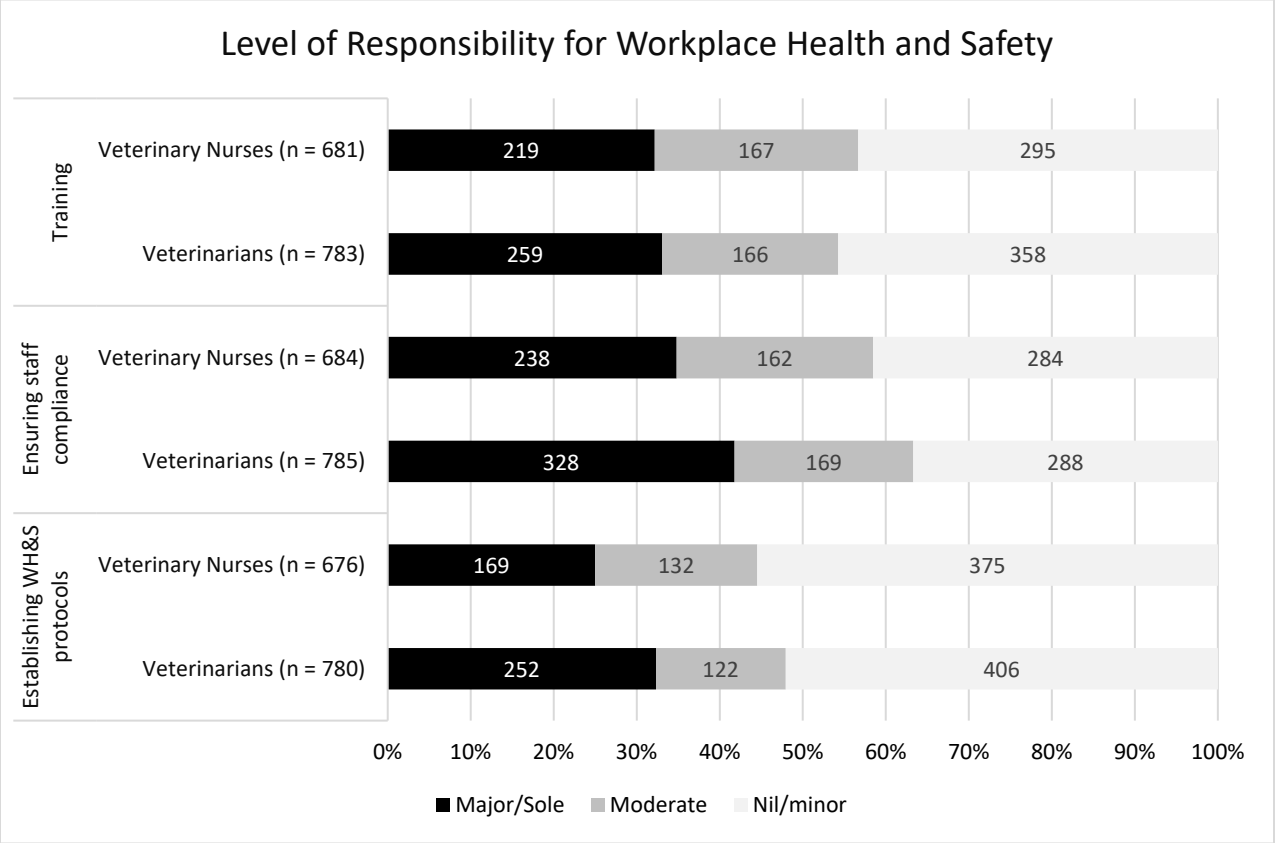

Figure 3.2 Level of responsibility for workplace health and safety reported by veterinarians and veterinary nurses surveyed in Australia in 2014.

3.5 Discussion

According to Safe Work Australia's model WH&S laws, which form the basis of WH&S laws implemented by Australian states and territories, person's conducting business must identify reasonably foreseeable hazards that could give rise to risks to health and safety, and must eliminate or minimise those risks so far as is reasonably practicable (Safe Work Australia, 2019). The model Work Health and Safety Regulations 2011 detail a hierarchical approach to risk mitigation, which prioritises the substitution of a risk with something that gives rise to lesser risk, followed by isolating the hazard from any person exposed to it, and then implementing engineering controls. If a risk remains despite these actions, administrative controls and finally the provision of personal protective equipment is required (Safe Work Australia, 2021). Duty holders must also provide any information, training, instruction or supervision that is necessary to protect all persons from risk to their health and safety arising from work (Safe Work Australia, 2019). The model laws also place a duty on workers to take reasonable care for his or her own health and safety, take reasonable care that his or her acts or omissions do not adversely affect the health and safety of other persons, and comply with any reasonable instruction, policy or procedure that is given by the person conducting the business relating to health or safety at the workplace (Safe Work Australia, 2019). Failure to comply with such laws implemented by state and territory governments can result in legal action against business owners or employees, and hefty workers' compensation claims (New South Wales Government, 2015).

Regarding Q fever, the first WH&S priority should be the implementation of a pre-screening and vaccination program as part of WH&S protocols (SafeWork-NSW, 2019). Close to 50% of veterinarians responding to questions regarding their level of WH&S responsibility indicated moderate or major/sole responsibility for establishing WH&S protocols, and greater than 60% indicated moderate or major/sole responsibility for ensuring staff compliance with WH&S protocols. However, this study identified that only 35% of veterinarians surveyed in 2014 demonstrated some level of willingness to recommend the Q fever

vaccination to veterinary personnel across all practice types. This may reflect a lack of consideration for Q fever vaccination in WH&S protocols in Australian veterinary clinics, which requires further investigation.

Small animal veterinarians and veterinarians reporting high Q fever knowledge scores were most likely to recommend Q fever vaccination in veterinary personnel across all practice types. The finding that veterinarians associated with livestock and mixed animal practice were less likely to recommend vaccination across all practice types reflects a lack of knowledge of the relevance of Q fever across all veterinary practice types and to all employed veterinary personnel. As Q fever is most often associated with ruminants in Australia and many places worldwide (Angelakis & Raoult, 2011; Eastwood et al., 2018; Lowbridge et al., 2012), veterinarians working with these high-risk species and veterinarians with low Q fever knowledge may not identify that other species pose a threat of Q fever. This is further supported by the finding of decreased vaccine uptake in veterinary nurses working with species not traditionally associated with Q fever.

With cases of Q fever being reported among small animal workers both within Australia and abroad (Gibbons & White, 2014; Komiya, Sadamasu, Toriniwa, et al., 2003; Kopecny et al., 2013), it is essential that veterinary personnel are educated that all animal species, and particularly periparturient animals, pose a potential threat of Q fever. Improving knowledge of the risk of exposure to *C. burnetii* for all veterinary personnel should improve both vaccination recommendation and uptake. This is further supported by the findings that increased concern for colleagues regarding exposure to *C. burnetii* was associated with recommendation by veterinarians, and veterinary nurses reporting higher Q fever knowledge and those perceiving at least a low/moderate level of exposure to *C. burnetii* were more likely to take up Q fever vaccination.

Age was found to be a significant factor for recommendation of Q fever vaccination, with veterinarians aged less than 39 years more likely to recommend the vaccination. This may reflect improvements over time in the teaching of public health at a tertiary level, vaccine availability from 1989 onwards, and the implementation of strict Q fever vaccination protocols within Australian veterinary schools since that time (Charles Sturt University, 2021; James Cook University, 2020; Murdoch University, 2018; University of Adelaide, 2021; University of Melbourne, 2021; University of Queensland, 2020; University of Sydney, 2021).

Concern that the Q fever vaccine may be harmful significantly decreased the odds of veterinarians recommending the vaccine. Although the vaccine results in local injection site reactions in up to 80% of vaccinees (Marmion et al., 1990; Schoffelen, Wong, et al., 2014; Seqirus, 2016), serious adverse events are extremely rare due to strict pre-vaccination protocols including serological and intradermal skin testing to screen for pre-existing immunity (Marmion, 2007; Schoffelen, Wong, et al., 2014; Seqirus, 2016). Both the skin test and vaccine have been proven very safe (Schoffelen, Wong, et al., 2014) and efficacy is reported to be greater than 97% (Gefenaite et al., 2011; Marmion, 2007). The notion that this vaccine is likely to be harmful when administered appropriately is unfounded and overcoming this perception through education and awareness should help to improve vaccine recommendation.

Although the pre-vaccination screening process markedly improves safety (Marmion, 2007), it does result in a more complicated, time consuming and expensive vaccination process. This study identified that the perception that the Q fever vaccine was expensive was a significant factor associated with reduced vaccine recommendation by veterinarians and reduced vaccine uptake among veterinary nurses. The perception of vaccine expense in both cohorts may reflect a lack of perceived benefit from vaccination, as the most influential reason for veterinary workers not seeking vaccination was the perception that they will not be seriously affected by Q fever (Sellens et al., 2016). Education of veterinary workers to improve Q fever knowledge and promote the benefits of vaccination, both for individual health and for compliance with

WH&S laws, may improve the perception of vaccine expense in both cohorts. Additionally, the cost of the vaccination process could potentially be decreased through group vaccination and vaccine subsidies from the government or employers; strategies which have proven to increase vaccine uptake and reduce disease burden among other at-risk cohorts (Gidding et al., 2009). Indeed, the AVA has recommended that governments subsidise Q fever vaccination for all veterinary industry workers and students and the addition of the vaccine to the Pharmaceutical Benefits Scheme (Australian Veterinary Association, 2018).

Veterinary nurses working in the state of Queensland reported significantly higher odds of vaccination than other Australian states. This is not surprising given that it was Queensland in which the first case of Q fever was described in 1935 (Derrick, 1937), and that the state has the highest annual Q fever notification rate in Australia (Safe Work Australia, 2014). However, Q fever is present in all states of Australia (Australian Government Department of Health) and efforts to improve vaccine uptake are required nationally. Current national AVA guidance on Q fever states:

> *"Veterinary practices must have a Q fever risk management protocol in place for all staff, clients and visitors to the practice, to ensure their protection. This requirement applies to both livestock and companion animal practice... Evidence of vaccination or previous Q fever exposure should be assessed at the time of employment, and the associated risks discussed during the induction process" (Australian Veterinary Association, 2018).*

Numerous other national and state-based sources of information similarly discuss Q fever risk reduction, prioritising vaccination and emphasising the onus on employers to protect workers, including the national AVA Guidelines for Veterinary Personal Biosecurity (Australian veterinary Association, 2017), state government health and WH&S departments (NSW Ministry of Health, 2019; SA Health, 2020; Work Safe Victoria, 2015; WorkCover-QLD, 2019; WorkSafe Tasmania, 2020), and the Australian Q fever Register

(Australian Meat Processor Corporation, 2021). However, the results of this study suggest this information may not be reaching veterinary employers and workers, or that it is either not be being implemented in WH&S protocols or that WH&S protocols are not being followed in practice. Veterinary and veterinary nursing conferences and continuing education providers could offer education on Q fever and provide tools to help veterinary managers implement Q fever vaccination protocols and programs within clinics. Consideration of compulsory national registration for veterinary nurses would improve access to this occupational group for the purposes of providing accurate WH&S information.

Veterinary nurses who reported workplace culture as a major influence regarding biosecurity practices were more likely to have sought Q fever vaccination. This finding implies that veterinary workplaces in which organisational culture places a high level of importance on safety foster positive attitudes towards Q fever vaccination. This is further supported by the positive association with having sought vaccination and employment within corporate practices and other organizations including abattoirs, government facilities and universities. These employers tend to have formal business-like structures with clear WH&S policies and procedures underpinning workplace safety culture; attributes that have been shown to positively impact the uptake of occupational vaccines among other healthcare workers (Bish, Yardley, Nicoll, & Michie, 2011; Isaacson, Roemheld-Hamm, Crosson, Dicicco-Bloom, & Winston, 2009; Yassi, Lockhart, Buxton, & McDonald, 2010). Unfortunately, WH&S attitudes and practices are typically inadequate in the majority of Australian veterinary practices (Dowd et al., 2013) and the findings of this study provide further evidence that a change in WH&S culture is required within the industry as a whole. Indeed, a wholistic approach to improving WH&S culture and overall wellbeing in veterinary workers could be effective in improving Q fever vaccination uptake, rather than isolated messages or protocols regarding only Q fever. This effect was observed in Canadian health workers, who reported influenza vaccination campaigns as "hypocritical" when they were not so actively encouraged in other areas of health and well-being, such as hand-washing and healthy eating (Yassi et al., 2010).

Limitations of this study relating to accessing this unique workforce, response rates, sample representativeness, and selection bias have been discussed in detail previously (Sellens et al., 2016). Briefly, access to the veterinary workforce proved difficult, particularly the veterinary nursing cohort who are not subject to strict registration requirements. A novel approach was required to ensure contact with as many veterinary workers as possible; however, this led to an unknown denominator for calculation of response rates. Alternative methods, such as data collection at professional conferences, would have allowed for accurate response rates but resulted in much smaller sample sizes and greater selection bias. Given the limitations, the sample size achieved in this study was exceptional and the cohort of veterinarians was considered representative of the population when compared to government employment and Australian Veterinary Association statistics (Australian Bureau of Statistics, 2014a; Australian Veterinary Association, 2014).

Selection bias towards participation from those familiar with Q fever vaccination may have contributed to these results representing a 'best-case' scenario for vaccine recommendation and uptake. For the veterinary nursing cohort, the exclusion of veterinary nurses stating 'unsure' for their Q fever vaccination status would have compounded this bias, as the authors propose that these participants were most likely not vaccinated as this vaccine is relatively 'memorable' given the complexity of vaccination and frequency of local adverse reactions. However, exclusion of this small proportion of respondents is not likely to have affected the outcome of regression analysis. Importantly, to be considered willing to recommend vaccination, veterinarians in this study had to at least slightly recommend the vaccination to veterinarians, veterinary nurses and kennel hands across all practice types queried. Ideally, veterinarians should be strongly recommending the Q fever vaccination to all veterinary personnel. Additionally, the veterinary nursing cohort responding to the questionnaire was likely to be older and more highly educated than the veterinary nursing population as a whole (Australian Bureau of Statistics, 2014b; Sellens et al., 2016),

indicating that a bias towards participation by those more familiar with the topic of Q fever may exist. As such, the proportion of veterinarians willing to recommend vaccination and veterinary nurses taking up vaccination represent a best-case scenario for this workforce, with the need for improvement within the industry probably being even more pressing than highlighted by these study results.

To date, routine use of Q-VAX® has been limited to occupational cohorts within Australia. The vaccine was successfully implemented for a short time in the Netherlands, where vaccination was targeted towards patients pre-disposed to Q fever complications; mostly elderly people with immunosuppressive, cardiac, or vascular disease (Isken et al., 2013; Schoffelen, Herremans, et al., 2013; Schoffelen, Wong, et al., 2014). As the outbreak subsided, the vaccination program was discontinued and ongoing prevention of human Q fever instead focussed on vaccination of livestock to limit *C. burnetii* shedding (Asseldonk et al., 2013). Barriers to the routine use of Q-VAX® in the Netherlands and elsewhere focus largely on the complexities and expense of the pre-vaccination screening process and the reactogenicity of the vaccine (Gidding et al., 2009; Ruiz & Wolfe, 2014; Schoffelen, Wong, et al., 2014); barriers also highlighted in this study. However, targeted use of Q-VAX® for occupationally at-risk cohorts has proven to be cost-effective in the Australian setting (Kermode et al., 2003). Should the current vaccine be considered for similar occupationally targeted use outside of Australia, the results of this study should be considered.

3.6 Conclusion

This study suggests a lack of compliance with WH&S laws regarding employer and worker responsibilities to reduce or eliminate the threat of hazards within the workplace, including those posed by infectious diseases such as Q fever. Improved compliance could be achieved through education and awareness campaigns that highlight the potential for occupational exposure to *C. burnetii* across all practice types, and the importance, safety, and benefits of the Q fever vaccine. Veterinary employers should aim to establish workplace protocols that facilitate the vaccination process and reduce the cost or improve the

perception of cost-benefit of this vaccine, particularly where financial subsidies are not provided. More broadly, improving overall WH&S practices and culture within Australia's veterinary industry is called for, which should protect individuals and practices not only from the potential costs of Q fever, but also other zoonoses and workplace hazards. The recommendations from this study are applicable for Q fever awareness programs generally and could inform any planned introduction of the Q fever vaccine within at risk populations internationally.

3.7 Supplementary materials

Table S3-1 Contingency table and univariable association of considered explanatory variables against the outcome variable "willing to recommend Q fever vaccination" for veterinarians surveyed in Australia in 2014.

Explanatory Variable	*Willing (n)*	*Not Willing (n)*	*Total (n)*	*P-value[t]*
"I am convinced of the importance of the Q fever vaccine"				
Disagree	5	47	816	**<0.001**
Agree	280	484		
"I worry that the Q fever vaccine will do more harm than good"				
Agree	12	83	817	**<0.001**
Disagree	275	447		
Level of concern of personal exposure to the bacteria causing Q fever				
Not concerned	70	247	814	**<0.001**
Slightly concerned	95	151		
Moderately concerned	79	96		
Very concerned	38	38		
Level of concern that colleagues could be exposed to the bacteria causing Q fever				
Not concerned	44	207	810	**<0.001**
Slightly concerned	93	172		
Moderately concerned	95	105		
Very concerned	50	44		
Level of concern that family could be exposed to the bacteria causing Q fever				
Not concerned	99	301	811	**<0.001**
Slightly concerned	101	146		
Moderately concerned	50	58		
Very concerned	32	24		
"The Q fever vaccine is safe if appropriately administered"				
Agree	261	421	803	**<0.001**
Disagree	2	20		
Don't know	16	83		
"The Q fever vaccine is too expensive"				
Agree	56	97	798	**<0.001**
Disagree	135	172		
Don't know	87	251		

Explanatory Variable	*Willing (n)*	*Not Willing (n)*	*Total (n)*	*P-value[t]*
"The Q fever vaccine is effective in preventing Q fever"				
Agree	244	385	801	**<0.001**
Disagree	2	5		
Don't know	32	133		
Vaccination status				
Vaccinated	236	350	813	**<0.001**
Not vaccinated	44	164		
Unsure	2	17		
Self-rated Q fever knowledge score from 1 (lowest) to 10 (highest)				
1-3	54	198	813	**<0.001**
4-5	69	146		
6-7	103	128		
8+	56	59		
Perceived average personal level of exposure to Coxiella burnetii throughout career				
Don't know	18	69	828	<0.001
Nil/very low	64	162		
Low/moderate	160	263		
High/very high	45	47		
Year of graduation from veterinary degree				
Prior to 1990	60	148	825	**0.001**
1990 - 1999	57	150		
2000 - 2008	81	132		
2009 onwards	87	110		
Personally knowing someone who has been diagnosed with Q fever				
No	145	337	807	**0.001**
Yes	135	190		
Age				
18-30	96	130	825	**0.005**
31-38	72	122		
39-49	56	148		
50+	61	140		

Explanatory Variable	*Willing (n)*	*Not Willing (n)*	*Total (n)*	*P-value[t]*
Years in total engaged in veterinary employment working directly with animals				
0-6	99	138	828	**0.008**
7-14	85	146		
15-25	52	135		
26+	51	122		
State of current workplace				
NSW/ACT	107	171	818	**0.047**
Qld	57	83		
SA/Tas/Vic	72	176		
WA/NT	49	103		
Gender				
Male	94	207	821	**0.136**
Female	189	331		
Practice structure in which most hours are spent working				
Solo	47	118	812	**0.166**
Group	197	349		
Corporate/Other	32	69		
"Q fever is a serious disease"				
Agree	272	499	804	**0.225**
Disagree	4	15		
Don't know	3	11		
Role within practice				
Practice owner	88	196	827	**0.264**
Veterinary associate	166	287		
Other	33	57		
"If a vaccine exists for a certain disease, then vaccination is usually a good way to protect someone against this disease"				
Disagree	5	16	822	**0.265**
Agree	282	519		
"It is difficult to get vaccinated for Q fever"				
Agree	109	218	804	**0.328**
Disagree	175	302		

Explanatory Variable	*Willing (n)*	*Not Willing (n)*	*Total (n)*	*P-value*[t]
University attended for veterinary school				
University of Sydney	81	134	828	**0.369**
University of Melbourne	44	105		
Murdoch University	44	99		
University of Queensland	71	125		
Other	47	78		
Highest level of tertiary education attained				
Nil	194	342	828	**0.396**
Grad Certificate, Diploma, Masters	47	107		
ANZCVS or equivalent, PhD, or fellowship	46	92		
Number of staff employed by workplace				
1-7	85	178	781	**0.502**
8-11	68	120		
12-18	51	102		
19+	69	108		
Personally having had Q fever disease				
No	248	474	814	**0.622**
Yes	34	58		
Practice type in which most hours have been spent throughout career				
Mixed/Large animal	106	185	823	**0.697**
Small animal	168	329		
Other	11	24		

NSW; New South Wales. ACT; Australian Capital Territory. SA; South Australia. WA; Western Australia. NT; Northern Territory.
[t]Likelihood ratio chi-square *p*-value.

Table S3-2 Contingency table and univariable association of all considered explanatory variables against the outcome variable "sought Q fever vaccination" for veterinary nurses surveyed in Australia in 2014.

Explanatory Variable	*Vaccinated (n)*	*Not Vaccinated (n)*	*Total (n)*	*P-value*[‡]
Self-rated Q fever knowledge score from 1 (lowest) to 10 (highest)				
1	11	134	684	<0.001
2-3	47	190		
4-5	65	107		
6+	74	56		
Perceived average personal level of exposure to Coxiella burnetii throughout career				
Nil/very low	64	240	686	<0.001
Low/moderate	100	103		
High/very high	15	8		
Don't know	18	138		
"The Q fever vaccine is safe if appropriately administered"				
Agree	184	305	677	<0.001
Disagree/Don't know	12	176		
"The Q fever vaccine is too expensive"				
Agree	71	133	672	<0.001
Disagree	73	62		
Don't know	52	281		
"The Q fever vaccine is effective in preventing Q fever"				
Agree	166	288	678	<0.001
Disagree/Don't know	30	194		
Personally knowing someone who has been diagnosed with Q fever				
No	105	381	683	<0.001
Yes	91	106		
Practice type in which most hours have been spent throughout career				
Small/equine/other	134	430		<0.001
Large/mixed practice (traditionally associated with Q fever)	61	57		
State of current workplace				
NSW/ACT	68	186	680	<0.001
Qld	77	82		
SA/Tas/Vic	36	143		
WA/NT	17	71		

Explanatory Variable	*Vaccinated (n)*	*Not Vaccinated (n)*	*Total (n)*	*P-value*[‡]
"I am convinced of the importance of the Q fever vaccine"				
Disagree	4	72	673	<0.001
Agree	193	404		
"Q fever is a serious disease"				
Agree	194	425	682	<0.001
Disagree/Don't know	3	60		
Level of concern that colleagues could be exposed to the bacteria causing Q fever				
Not concerned	50	215	684	<0.001
Slightly concerned	58	146		
Moderately concerned	58	86		
Very concerned	31	40		
Practice structure in which most hours are spent working				
Solo	53	163	666	<0.001
Group	92	272		
Corporate/Other	43	43		
Level of concern of personal exposure to the bacteria causing Q fever				
Not concerned	65	226	684	0.001
Slightly concerned	56	146		
Moderately concerned	50	83		
Very concerned	26	32		
"It is difficult to get vaccinated for Q fever"				
Agree	67	221	662	0.001
Disagree	130	244		
Level of influence of workplace health & safety culture regarding information about work related biosecurity				
Major/sole	82	143	630	0.003
Minor/moderate	83	239		
Nil	16	67		
"I worry that the Q fever vaccine will do more harm than good"				
Disagree	7	12	684	0.006
Agree	191	474		
Level of concern that family could be exposed to the bacteria causing Q fever				
Not concerned	88	265	684	0.008
Slightly concerned	47	126		
Moderately concerned	40	60		

Explanatory Variable	*Vaccinated (n)*	*Not Vaccinated (n)*	*Total (n)*	*P-value*[†]
Very concerned	23	35		
Level of influence of workplace protocols regarding information about work related biosecurity				
Major/sole	129	306	648	0.062
Minor/moderate	54	131		
Nil	3	25		
Level of influence of AVA guidelines regarding information about work related biosecurity				
Major/sole	75	148	634	0.085
Minor/moderate	71	186		
Nil	36	118		
Level of influence of vet nurses external to clinic regarding information about work related biosecurity				
Major/sole	14	44	633	0.136
Minor/moderate	113	243		
Nil	54	165		
Level of influence of government sources regarding information about work related biosecurity				
Major/sole	72	147	635	0.220
Minor/moderate	77	208		
Nil	33	98		
Highest level of education attained relating to veterinary nursing				
Nil	35	59	687	0.236
Certificate III	13	30		
Certificate IV	116	320		
Diploma/Bachelors/Other	34	80		
Level of influence of the vet nurses within clinic regarding information about work related biosecurity				
Major/sole	101	223	640	0.237
Minor/moderate	68	196		
Nil	12	40		
Gender				
Female	193	482	686	0.240
Male	5	6		
Level of influence of veterinarians external to clinic regarding information about work related biosecurity				
Major/sole	21	58	636	0.244
Minor/moderate	115	257		
Nil	45	140		

Explanatory Variable	*Vaccinated (n)*	*Not Vaccinated (n)*	*Total (n)*	*P-value*[ł]
Number of staff employed by workplace				
<6	42	137	670	0.271
6-9	58	127		
10-15	42	110		
16+	49	105		
Years in total engaged in veterinary employment working directly with animals				
<5	49	131	681	0.426
5-8	58	113		
9-15	51	132		
16+	39	108		
"If a vaccine exists for a certain disease, then vaccination is usually a good way to protect someone against this disease"				
Disagree	7	12	684	0.452
Agree	191	474		
Level of influence of the veterinarians within clinic regarding information about work related biosecurity				
Major/sole	133	313	642	0.665
Minor/moderate	47	128		
Nil	5	16		
Age				
18-26	56	142	680	0.777
27-32	49	108		
33-42	44	120		
43+	50	111		
Level of influence of the internet regarding information about work related biosecurity				
Major/sole	46	116	639	0.796
Minor/moderate	114	274		
Nil	23	66		
Level of influence of personal research through veterinary journals, textbooks, and websites etc. regarding information about work related biosecurity				
Major/sole	66	170	644	0.924
Minor/moderate	107	256		
Nil	13	32		

NSW; New South Wales. ACT; Australian Capital Territory. SA; South Australia. WA; Western Australia. NT; Northern Territory.

[ł]Likelihood ratio chi-square *p*-value.

4 *Coxiella burnetii* Seroprevalence in Unvaccinated Veterinary Workers in Australia

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4.1 Abstract

Q fever (caused by *Coxiella burnetii*) is a serious zoonotic disease that occurs almost world-wide. Occupational contact with animals increases the risk of exposure and Q fever vaccination is recommended for veterinary workers in Australia. This cross-sectional study aimed to investigate *C. burnetii* seroprevalence among unvaccinated veterinary workers in Australia, and determine factors associated with a positive serological result. During 2014 and 2015, participants recruited at veterinary conferences and workplace vaccination clinics completed a questionnaire and provided a blood sample for *C. burnetii* serology. Participants were predominantly veterinarians (77%), but veterinary support staff, animal scientists, and administration workers also participated. Blood samples (n = 192) were analysed by an immunofluorescence assay and considered positive where the phase I or phase II IgG titre was ≥1/50. Seroprevalence was 19% (36/192; 95% CI 14 – 25%). A positive serological result was significantly associated with (1) working in outer regional/remote areas (odds ratio [OR] 6.2; 95% CI 1.9 – 20.8; reference = major cities; p = 0.009) and (2) having spent more than 50% of total career working with ruminants (OR 4.8; 95% CI 1.7 – 13.5; reference = <15% of career; p = 0.025). These findings confirm an increased risk of exposure to *C. burnetii* compared to the general population, providing new evidence to support Q fever vaccination of veterinary workers in Australia.

4.2 Background

Q fever is a disease of people caused by the zoonotic pathogen *Coxiella burnetii*, a small obligate intracellular Gram negative bacterium (Angelakis & Raoult, 2010). Except for New Zealand and French Polynesia, *C. burnetii* has been described worldwide; occurring as sporadic cases, small clusters, or large outbreaks such as occurred in the Netherlands in 2007 – 2010 (Million & Raoult, 2015; Roest et al., 2011). A range of non-specific symptoms may occur following exposure to *C. burnetii*, which vary according to the route of transmission, inoculum dose, geographical region, and patient factors (Angelakis & Raoult, 2010). Infection may be asymptomatic, or present as a self-limiting flu-like illness which often remain undiagnosed (Million & Raoult, 2015). However, some Q fever patients experience severe symptoms including but not limited to pneumonia, hepatitis, obstetric complications including foetal death, and persistent endocardial, vascular and osteoarticular infections (Angelakis & Raoult, 2010; Carcopino et al., 2007; Million & Raoult, 2015, 2017). Post Q fever fatigue syndrome [QFS] is experienced by up to 20% of Q fever patients and may persist for years with debilitating consequences (Morroy et al., 2016).

Many production, companion and wild animal species harbour *C. burnetii*, shedding bacteria into the environment in placental tissues, faeces, urine, and milk (Angelakis & Raoult, 2011; Tozer et al., 2014). Large numbers of bacteria are also shed in the faeces of ticks, an important reservoir for the pathogen (Angelakis & Raoult, 2010). Cattle, sheep and goats have been implicated as the source of *C. burnetii* in most human Q fever outbreaks globally; however, other species including cats and dogs have been associated with disease in people (Angelakis & Raoult, 2010; Komiya, Sadamasu, Toriniwa, et al., 2003; Kopecny et al., 2013; Marrie, Durant, Williams, Mintz, & Waag, 1988). Environments may remain contaminated with viable *C. burnetii* for many months, with bacterial spread occurring via wind and animal transport (Kersh, Fitzpatrick, Self, Priestley, et al., 2013; Nusinovici, Frossling, Widgren, Beaudeau, & Lindberg, 2015; Tissot-Dupont et al., 2004; Tozer et al., 2014; Wallensten et al., 2010). Inhalation is the

primary route of infection for people, while transmission via ingestion, transcutaneous, transfusion and sex have also been reported (Angelakis & Raoult, 2010).

Since 1989, a Q fever vaccine for people (Q-VAX®, Seqiris Pty. Ltd., Parkville, Victoria, Australia) has been licensed for use in Australia, where it is recommended for those with an occupational risk of exposure to *C. burnetii*, including abattoir workers, farmers and veterinary personnel (*The Australian Immunisation Handbook*, 2013), Veterinary students at all Australian veterinary schools are routinely vaccinated prior to, or on commencement of their veterinary studies. As a result, approximately 74% of all veterinarians in Australia have sought vaccination for Q fever, with 61% receiving the vaccination and 12% unable to be vaccinated due to evidence of prior exposure to *C. burnetii* (Sellens et al., 2016). Veterinary nurses in Australia report lower vaccine uptake, with only29% having sought vaccination (24% vaccinated; 5% showing evidence of prior exposure). This is due to a variety of reasons, including a perception that they are not at risk of exposure to *C. burnetii* (Sellens et al., 2016). Medical practitioners in Australia have also reportedly advised veterinary workers against Q fever vaccination on occasion due to a perception that the workers were not at risk of Q fever (Sellens et al., 2016).

Seroprevalence studies can be utilised to estimate exposure as antibodies against phase II *C. burnetii* are usually highly persistent following natural infection, with immunoglobulin [Ig] G persisting longer than other antibody classes (Teunis et al., 2013). Seroprevalence for *C. burnetii* has been reported for veterinary populations in other countries but not yet in Australia. Most commonly, immunofluorescence assays [IFA] for phase I and phase II IgG and IgM have been utilised, with these studies reporting a *C. burnetii* seroprevalence of 65.1% in Dutch livestock veterinarians (n = 189) (Van den Brom et al., 2013), 58.3% in livestock veterinarians in Belgium (n = 82) (Pozzo et al., 2017), 18.7% in veterinary students in the Netherlands (n = 674) (de Rooij et al., 2012), and 13.5% in veterinarians in Japan (n = 267) (Abe et al., 2001). Enzyme-linked immunosorbent assays [ELISA] for phase I and II IgG were utilised in studies reporting a *C. burnetii* seroprevalence of 22.2% and 38.2% in veterinarians in the USA (n = 508) and

Germany (n = 424) respectively (Bernard et al., 2012; Whitney et al., 2009). Complement Fixation Test for phase II IgG was utilised to study veterinary students in Spain where *C. burnetii* seroprevalence was reportedly 11.0% (Valencia, Rodriguez, Puñet, & Giral, 2000). Risk factors for seropositivity were similar between these studies, with recurrent factors including increasing age or years of veterinary work or study, increasing contact with livestock, and rural location.

This study aimed to investigate the *C. burnetii* seroprevalence among veterinary workers in Australia. These data are essential for gaining an understanding of the risk of exposure to *C. burnetii* associated with veterinary work in Australia. The findings will assist veterinary workers and medical practitioners in making informed decisions regarding the prevention of Q fever, particularly with regards to Q fever vaccination.

4.3 Methods

4.3.1 Study design and recruitment

This study was a cross-sectional survey of the veterinary workforce in Australia. Participation required the provision of a blood sample and completion of a paper questionnaire. Participation was voluntary, and individuals could elect to receive a copy of their Q fever serological result. Veterinary workers from Australia who were over the age of 18 years were eligible to participate. Both vaccinated and unvaccinated workers were recruited, but only unvaccinated workers were included in this seroprevalence study.

Veterinary workers were opportunistically recruited during the Australian Veterinary Association [AVA] national conference in Perth in 2014, the AVA NSW divisional conference in Goulburn in 2014, and the AVA Pan Pacific Veterinary Conference in Brisbane in 2015. Attendees at these conferences, who were mostly veterinarians, were free to approach the study booth during conference hours. Additional participants, including research, clinical, and administrative staff, were recruited via Q fever vaccination clinics for staff within the veterinary departments at the University of Sydney Camperdown and Camden

campuses in 2015. Participants from the vaccination clinics were enrolled by research staff and sampled at the time of pre-vaccination screening appointments.

4.3.2 Questionnaire

Participants were required to complete a questionnaire at the time of blood sampling, which contained 15 questions regarding demographics, history of workplace animal exposures, Q fever disease, and Q fever vaccination (Appendix B). Surveys were labelled with the participant's unique lab identification number only.

4.3.3 Laboratory methods

4.3.3.1 Blood samples

Approximately 10ml of venous blood was taken from participants, refrigerated, and couriered as soon as practically possible to the Australian Rickettsial Reference Laboratory [ARRL], Victoria, Australia, for *C. burnetii* serology. Where it was not possible to courier samples in a timely manner, they were centrifuged, and serum stored frozen at -20°C until transport. Each sample was labelled using a unique lab identification number, and no patient details were made available to the ARRL. Survey responses were manually entered into a Microsoft® Access® database (Microsoft Corporation, Washington, USA).

4.3.3.2 Serology

The IFA was conducted using a National Association of Testing Authorities accredited in-house indirect IFA (accreditation No. 14342). The IFA utilised fluorescein-labelled goat anti-human IgM, IgG, IgA and a mixture of all three anti-isotypes combined (Kirkegaard & Perry Laboratories, MD, USA), separately for phase I and phase II bacterial antigens (a total of eight tests per serum dilution). Phase I (cat # 1227) and phase II (cat # 1123) *Coxiella burnetii* antigens (Serion-Virion, Germany) were obtained from DKSH Australia. These semi-purified bacterial antigens were fixed onto micro-wells on glass slides. The slides were then treated with patient sera, then the four different fluorescein-labelled goat anti-human

immunoglobulins; one to each well to detect antibodies against *C. burnetii*. Positive and negative control sera were run on every slide.

Patient samples were initially screened at a 1/25 dilution and a 1/400 dilution; the latter to detect any prozone phenomenon. When positive on screening immunofluorescence, samples were titrated out further to a 1/3200 dilution or to a definitive endpoint where appropriate. The highest dilution of patient serum showing immunofluorescence equivalent to the positive control was designated as the endpoint of the titration and recorded as the patient's antibody titre.

4.3.3.3 Interpretation

Participants returning an anti-phase II or anti-phase I IgG titre ≥1/50 were considered positive. Serological profiles of participants returning a positive result were further classified as past or relatively recent exposure, as per the criteria outlined in **Box 4-1**, adapted from Healy et al. (2011). A category for 'chronic infection' was not included in the interpretation, due to the complexities of diagnosing persistent infection. Instead, the serological profiles were additionally assigned as 'possible increased risk for persistent infection (endocarditis / vascular infection)' where phase I IgG was ≥1/800, and phase I IgA was ≥1/50. These serological criteria were derived from criteria proposed by Raoult (2012), based on the modified "Duke" criteria, and modified by Graves at the Australian Rickettsial Reference Laboratory. Importantly, these criteria do not imply a diagnosis of chronic (or focal, persistent) Q fever, but may prompt further investigation for a focus of persistent infection in a clinical scenario, particularly where other risk factors for persistent infection exist (Million & Raoult, 2015; Raoult, 2012).

Box 4-1. *Coxiella burnetii* serology classification criteria applied to veterinary workers participating in a seroprevalence study in Australia, 2014-2015. Criteria were adapted from Healy et al. (2011).

Category	Criteria
Serological Result	
Positive	Phase II or phase I IgG titre ≥1/50
Equivocal	Phase II IgG and/or phase I IgG = 1/25
Negative	Phase II IgG and phase I IgG <1/25
Serological classification of seropositive workers	
Relatively recent exposure	Phase I and/or phase II IgG ≥1/50 and phase II IgM ≥1/50
Past exposure	Phase I and/or phase II IgG ≥1/50 and phase II IgM <1/50

4.3.4 Statistical analysis

4.3.4.1 Variables

The primary outcome of interest was whether the worker was *C. burnetii* seropositive or seronegative, with equivocal (titre of 1/25) serological results considered negative for this outcome. The secondary outcome of interest was whether the serological profile of the worker reflected relatively recent exposure to *C. burnetii* (**Box 4-1**). These outcomes were dichotomous.

Explanatory variables were drawn from the questionnaire. Categorical responses were grouped according to biologically or demographically sensible categories. Continuous explanatory variables were categorised if the association between the variable and the outcome was not linear on the log odds scale. A category for missing data was included for variables with any incomplete responses. This category was only included in statistical analysis where it represented five percent or more of the total responses.

Explanatory variables included gender, age, job description, total years working with animals, total hours per week currently working with animals, percentage of career working with small animals (dogs, cats, pocket pets), percentage of career working with ruminants (cattle, sheep, goats), percentage of career working with horses, percentage of career working with other species, percentage of career not working directly with animals, currently working in a private practice, currently working in a laboratory, currently working in government / industry (excluding abattoirs), currently working in an "other" type of organisation.

Workplace postcode (four-digit mail delivery number) was utilised to generate two additional variables: (1) Australian geographical state, and (2) Australian Statistical Geography Standard [ASGS] Remoteness Area. The ASGS was developed by the Australian Bureau of Statistics [ABS] and divides Australia into broad geographic regions that share common characteristics of remoteness for statistical purposes (Australian Bureau of Statistics, 2018). Study postcodes were matched to remoteness areas utilising the ABS July 2016 remoteness structure. Where a postcode spanned more than one remoteness category, the category allocated was the one for which the majority of that postcode was assigned.

4.3.4.2 Modelling

Univariable logistic regression analyses were undertaken to identify the unadjusted association between explanatory variables and each of the two outcome variables. Variables with a *p*-value of < 0.25 in the univariate analyses were considered in the multivariable logistic regression modelling procedures, which were undertaken manually via forward and backward stepwise selection. Where a strong correlation (Spearman rank correlation coefficient > 0.7; $p < 0.05$) was identified between variables, the variable with the least significant association with the outcome was excluded from multivariable modelling. Gender and age were included in multivariable modelling as confounders *a priori* due to previous Australian studies reporting increased seroprevalence with male gender and increasing age (Gidding et al., 2019; Islam et al., 2011; Tozer et al., 2011). These were also included to account for bias in the cohort due to the

exclusion of previously vaccinated workers that were proportionately likelier to be female and younger. Biologically plausible interactions identified *a priori* exhibiting some (p <0.25) univariable association with the outcome were tested and retained if they caused more than a 20% change in the beta values of significant variables in the model. The significance of the full model was determined with the Likelihood-ratio test, and a Hosmer-Lemeshow goodness of fit test was performed on the final model to determine if the model was a good fit for the data.

4.3.5 Ethics approval

Primary ethics approval was granted through the University of Sydney Human Research Ethics Committee (#2014/245), and secondary approval through the Charles Sturt University Human Research Ethics Committee (#2015/289). The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

4.4 Results

4.4.1 Responses

The serological results of 192 veterinary workers who had not been previously vaccinated for Q fever were included in this study. The response rate for the vaccination clinics at the University of Sydney was 92%, reflecting participation from 19/22 and 37/39 clinic attendees at the Camden and Camperdown campuses, respectively. At the AVA national conference in Perth (2014), 189/730 delegates were sampled of whom 74 were not vaccinated for Q fever. At the AVA NSW divisional conference in Goulburn (2014) 48/81 delegates were sampled, of whom 14 were not vaccinated for Q fever. The AVA Pan Pacific Conference in Brisbane (2015) was attended by 1110 delegates of whom 124 were from New Zealand and ineligible. Of the remaining 986 delegates, 95 were sampled of whom 48 were not vaccinated for Q fever.

An overall response rate could not be determined for this study due to sampling at conferences, where considerable overlap of attendees is expected, and the exact number of eligible workers is not known.

4.4.2 Demographics

Of the 192 unvaccinated veterinary workers, 77% were veterinarians (**Table S4-1**). The median age of participants was 50 years (range 18 – 75; IQR 20), and females constituted 53% of the study cohort. All Australian geographical states and territories were represented. However, the state category of NSW/ACT was overrepresented at 57% (NSW 53%; ACT 4%), compared to available national veterinary workforce data for 2014 (NSW 28%; ACT 2%; combined 30%) (Australian Veterinary Association, 2014). Participants mostly worked in major cities (64%), followed by inner regional areas (22%) and outer regional/remote areas (9%), which was similar to the distribution of the general Australian population for these remoteness categories (70%, 28% and 11% respectively) according to census data (National Rural Health Alliance, 2015). Further information on the demographic and work characteristics of the studied cohort are reported as supplementary data (**Table S4-1**).

4.4.3 Serology

Thirty-six (19%; 95% confidence interval [CI] 14-25%) of the 192 veterinary workers were *C. burnetii* seropositive. Of the positive serological profiles, 53% (19/36) were classed as past exposure and 47% (17/36) were classed as relatively recent (**Table 4.1**). Three (8%) of the seropositive workers returned a serological profile consistent with a possible increased risk for persistent infection.

Table 4.1 Summary of serological results and serological profiles of veterinary workers participating in a *Coxiella burnetii* seroprevalence study, Australia, 2014 – 2015.

	n	*%*	*95% Lower Confidence Limit*	*95% Upper Confidence Limit*
Serological Result[†]				
Positive	36	18.8%	13.9%	24.9%
Negative	151	78.6%	72.3%	83.9%
Equivocal	5	2.6%	1.1%	6.0%
Column Total	192	100.0%	.	.
Serological profile of seropositive workers				
Relatively recent exposure	17	47.2%	32.0%	63.0%
Past exposure	19	52.8%	37.0%	68.0%
Column Total	36	100.0%	.	.

[†]Refer to **Box 4-1** for criteria used to classify serological profile.

4.4.4 Previous Q fever diagnosis

Four participants reported having been medically diagnosed with Q fever, confirmed with laboratory testing (**Table 4.2**). Three of these patients were veterinarians with varied animal exposures, all having worked in the veterinary industry for 35 years or more. The fourth was an administration worker within a small animal veterinary clinic in a major city, who had worked in the industry for only 6 years and reported no direct occupational animal handling in that time. All four Q fever patients returned a positive serological profile, including two who were diagnosed over 30 years ago (**Table 4.2**). The administration worker, who was most recently diagnosed, exhibited a serological profile of possible increased risk of persistent infection (**Table 4.2**).

Table 4.2 Characteristics and serological results of veterinary workers participating in a *Coxiella burnetii* seroprevalence study, Australia, 2014 – 2015, who reported being medically diagnosed with Q fever during their career.

Role	***Years Working***	***Career animal exposure***	***Workplace remoteness***[†]	***Year of diagnosis***	***P1 IgA titre***	***P1 IgM titre***	***P1 IgG titre***	***P2 IgA titre***	***P2 IgM titre***	***P2 IgG titre***
Admin	6	No animal handling (100%);	Major City	2014	1/100	1/50	1/1600	1/200	1/100	1/1600
Vet	40	Small animals (89.5%); Ruminants (7.5%); Horses (1%); Other (2%)	Inner Regional	1980	1/25	<1/25	1/400	<1/25	1/100	<1/25
Vet	43	Small animals (16%); Ruminants (65%); Horses (16%); Other (3%)	Major City	1982	<1/25	<1/25	1/100	<1/25	<1/25	<1/25
Vet	35	Small animals (25%); Ruminants (45%); Horses (30%); Other (0%)	Outer Regional	2002	<1/25	<1/25	1/800	<1/25	<1/25	1/1600

[†]According to the Australian Statistical Geography Standard (Australian Bureau of Statistics, 2018). Admin; administration worker. Vet; veterinarian. P1; Phase I *C. burnetii*. P2; Phase II *C. burnetii.*

4.4.5 Variables associated with a positive *Coxiella burnetii* serological result

The univariable association between each variable and a positive serological result is shown in the supplementary data (**Table S4-1**). Two variables were identified as having a significant univariable association with a positive *C. burnetii* serological result; (1) workplace remoteness classification (p = 0.004), and (2) percent of career spent working with ruminants (sheep, cattle, goats) (p = 0.002) (**Table S4-1**). Seroprevalence among veterinary workers currently working in outer regional / remote areas was 53% (9/17; 95% CI 31-74%), compared to 13% (16/123; 95% CI 8-20%) among metropolitan workers and 21% (9/43; 95% CI 11-35%) among inner regional workers. Seroprevalence among workers who had spent more than 50% of their total career working with ruminants was 38% (12/32; 95% CI 21-54%), while seroprevalence among workers who had spent 15% or less of their total career working with ruminants was 11% (13/118; 95% CI 7-18%) (**Table S4-1**).

Five variables exhibited some association (p <0.25) with a positive serological result (**Table S4-1**). Number of years working in the veterinary industry was excluded from multivariable modelling due to a strong correlation (spearman rank coefficient 0.81; p <0.001) with age. No significant interactions were identified and no confounders, with the exception of age and gender, were retained in the final model.

In the final model there were two significant variables: (1) workplace remoteness area classification (p = 0.025) and (2) percent of total career spent working with ruminants (sheep, cattle, goats) (p = 0.009) (**Table 4.3**). Veterinary workers who returned a positive *C. burnetii* serological result were most likely to be currently working within outer regional / remote areas of Australia (odds ratio [OR] 6.2; 95% CI 1.9–20.8; reference category = major cities), and most likely to have spent more than 50% of their total career working with ruminants (OR 4.8; 95% CI 1.7 – 13.5; reference category = 15% or less) (**Table 4.3**). The final model was significant (p = 0.003), and the Hosmer and Lemeshow Goodness-of-Fit test was not significant (p = 0.439), suggesting the model was a good representation of the data.

Table 4.3 Final multivariable model for factors significantly associated with a positive *Coxiella burnetii* serological result among Australian veterinary workers sampled from 2014 – 2015.

Parameters	*Seroprevalence % (n)*	*Odds ratio*	*95% LCL*	*95% UCL*	*P-value*[†]
% of career working with ruminants (cattle, sheep, goats)					0.009
15% or less	11% (13/118)	ref	.	.	
>15% up to 50%	26% (11/42)	2.5	1.0	6.6	
More than 50%	38% (12/32)	4.8	1.7	13.5	
Workplace remoteness[‡]					0.025
Major Cities of Australia	13% (16/123)	ref	.	.	
Inner Regional Australia	21% (9/43)	1.7	0.6	4.5	
Outer Regional / Remote Australia	53% (9/17)	6.2	1.9	20.8	
Missing / not classified	22% (2/9)	2.3	0.3	11.4	

n = 192; Model adjusted for age and gender. [†]Wald Chi Square *p*-value. LCL; lower confidence limit. UCL; upper confidence limit. [‡]Remoteness determined from postcode according to the Australian Statistical Geography Standard (Australian Bureau of Statistics, 2018).

4.4.6 Variables associated with relatively recent exposure

Seventeen workers were classified as having a serological profile suggestive of a relatively recent exposure. Univariable analysis revealed one variable with a significant association with this outcome: job description (p = 0.025). Veterinary support workers (nurses, kennel hands, farm hands) were more likely to have been recently exposed than veterinarians and animal scientists (OR 5.1; 95% CI 1.4 – 16.4), comprising 29% (5/17) of the recently exposed cohort compared to only 9% of the total cohort. However, all these support workers were sampled within workplace vaccination clinics across two veterinary sites, and this clustering may have biased the result. Other workers who were positive for this outcome were unrelated by workplace.

Age category was not significant for relatively recent exposure (p = 0.283). However, the median age of the recently exposed group (40 years; IQR 16 years) was significantly younger (p = 0.0106) than the median age of the remainder of the cohort (51 years; IQR 21 years), as assessed using the Wilcoxon Rank Sum

Scores method and Kruskal-Wallis Test for significance. Again, this result may be influenced by the clustering of veterinary support staff previously reported, as the cohort of support staff studied were younger (median 35 years; IQR 18) than the general cohort.

Although geographical state was not found to be significant (p = 0.393), some states exhibited high odds ratios for recent exposure. Recently exposed workers were more likely to work in WA/NT (OR 5.8; 95% CI 0.7 – 122.7) or NSW/ACT (OR 4.1; 95% CI 0.8 – 75.8) compared to the reference category of SA/Tasmania/Victoria. These odds remained high (WA/NT OR 5.9, 95% CI 0.6 – 131.8; NSW/ACT OR 3.2, 95% CI 0.5 – 62.2) after adjusting for job description, to account for over-representation of nurses from NSW/ACT, and for age, gender and rurality. Recently exposed workers were also more likely to be working within outer regional/remote areas (OR 2.4; 95% CI 0.5 – 9.1) but again this was not significant (p = 0.605). None of the workers currently working in government (n=15), laboratories (n=10), or "other" organisations (n=30) returned a profile suggestive of recent exposure, indicating these workplaces may be lower risk for *C. burnetii* exposure. A larger sample size of workers is required to investigate these associations further.

4.5 Discussion

This study assessed *C. burnetii* seroprevalence among veterinary workers in Australia who have not been vaccinated for Q fever. Overall seroprevalence for the cohort studied was 19%. A positive serological result was associated with increasing exposure to ruminants and working in regional or remote areas. These associations are consistent with similar studies in veterinary populations around the globe (Abe et al., 2001; Bernard et al., 2012; Chang et al., 2010; de Rooij et al., 2012; Pozzo et al., 2017; Valencia et al., 2000; Van den Brom et al., 2013; Whitney et al., 2009), and the overall seroprevalence was similar to that of veterinarians in the USA (22.2%) (Whitney et al., 2009) and veterinary students in the Netherlands (18.7%) (de Rooij et al., 2012). These findings confirm that veterinary workers in Australia have an

increased risk of exposure to *C. burnetii* compared to general populations, with previous studies reporting an overall seroprevalence of two to seven percent for general populations in Queensland and New South Wales (Gidding et al., 2019; Islam et al., 2011; Tozer et al., 2011). The results additionally suggest that there may be a considerable proportion of unvaccinated veterinary workers that may be eligible for, and could benefit from, Q fever vaccination.

Due to antibody decline over time and heterogeneity of individual antibody responses, the 19% seroprevalence reported represents the minimum level of *C. burnetii* exposure in this study cohort. Additional analysis of cell mediated immune response, via intradermal skin test or interferon-gamma production by T-lymphocytes following in-vitro stimulation with *C. burnetii* antigen, would likely identify additional workers as positive for previous *C. burnetii* exposure (Schoffelen, Joosten, et al., 2013). Unfortunately, such testing was beyond the scope of this study. This does not impact the validity of comparisons with other studies, as this limitation is universal to seroprevalence studies.

Increasing career exposure to ruminants (cattle, sheep and goats) was significantly associated with a positive *C. burnetii* serological result, confirming that ruminants pose a high risk of exposure to *C. burnetii* among veterinary workers in Australia. Similarly, veterinarians in the USA who worked with cattle were found to have an increased risk for *C. burnetii* exposure (OR 2.8) (Whitney et al., 2009), as did veterinarians working with cattle (OR 2.8) or sheep (OR 2.1) in Bavaria (Bernard et al., 2012). In southern Belgium, seroprevalence among veterinarians having contact with livestock was 58.5% compared to 6.3% among veterinarians working only with companion animals (Pozzo et al., 2017).

Veterinary workers within remote or outer regional locations were significantly more likely to be seropositive for *C. burnetii*. This association remained significant after adjusting for ruminant contact in multivariable modelling. Rural location was found to be a significant factor for *C. burnetii* exposure in veterinarians in the Netherlands (OR 6.6) (Van den Brom et al., 2013), while studies in Taiwan and Belgium

identified rurality to be significant during univariable, but not multivariable, analysis (Chang et al., 2010; Pozzo et al., 2017). Globally, the association of rurality with *C. burnetii* seroprevalence appears to vary, and is influenced by land use, geography, and seasonal conditions (Angelakis & Raoult, 2011; Cikman et al., 2017; Clark & Soares Magalhaes, 2018; Hackert et al., 2012; Tozer et al., 2011). In Australia, rural populations of NSW and Queensland report increased *C. burnetii* seroprevalence and Q fever disease notifications (Gidding et al., 2019; Islam et al., 2011; Lowbridge et al., 2012; Tozer et al., 2011). Rural location may increase exposure to *C. burnetii* due to the proximity of farms, livestock facilities, and animal transport routes (Clark & Soares Magalhaes, 2018; O'Connor et al., 2015). Geographical dispersal of *C. burnetii* may also be more pronounced where there is higher densities of wild and domesticated animal species and greater inter-species interaction; a concept that remains largely understudied (Clark & Soares Magalhaes, 2018).

This study also confirms that veterinary workers who were predominantly exposed to non-ruminant species and those in metropolitan areas remain at higher risk of *C. burnetii* exposure, reporting 11% and 13% seroprevalence respectively, compared to general populations in Australia which report a seroprevalence of two to five percent in metropolitan areas (Gidding et al., 2019; Tozer et al., 2011). This increased risk is supported by reports of Q fever outbreaks associated with cat and dog births (Gibbons & White, 2014; Kopecny et al., 2013; Malo et al., 2018), Q fever disease among cat breeders (Shapiro, Norris, Bosward, et al., 2016), and the detection of *C. burnetii* in a large variety of domestic and wild animal species in Australia (Cooper et al., 2012; Shapiro, Norris, Heller, et al., 2016; Shapiro et al., 2015; Tozer et al., 2014). While reports of disease associated with non-ruminant species and within metropolitan areas is low, the consequence of clinical Q fever disease for those affected may be severe.

Although symptomatic disease rates differ with demographics, geographic region, exposure setting (endemic versus outbreak), and with bacterial dose and strain (Brooke et al., 2013; Hackert et al., 2015; Hackert et al., 2012; Million & Raoult, 2015),. symptomatic Q fever disease is generally estimated to occur

in 20% to 80% of individuals exposed to *C. burnetii* (Million & Raoult, 2015). In this study, only four (11%) of the 36 seropositive workers reported having been medically diagnosed with Q fever, which may reflect under diagnosis of clinical Q fever disease in the studied cohort. Two workers remained seropositive more than 30 years after Q fever diagnosis, suggesting recurrent exposure or the long-term persistence of antibodies against *C. burnetii* following infection. Antibody profiles suggestive of possible persistent (chronic) infection were identified in three (8%) of the workers, which was similar to that reported for veterinarians in Belgium (12%) (Pozzo et al., 2017). These serological profiles appear to be more common in veterinarians following Q fever diagnosis compared to patients with no occupational risk of exposure, and may be due to persistent or recurrent exposure rather than pathological disease (Wielders et al., 2015).

Few individuals in this study exhibited a serological profile suggestive of relatively recent exposure. Among those that did, veterinary support staff were over-represented. However, these support staff were clustered within two related but geographically separated workplaces, possibly reflecting a workplace outbreak. Additionally, an administration worker from an urban small animal clinic, who reported no direct occupational animal exposure, had been recently diagnosed with Q fever. These findings highlight the risk of exposure of support and administration staff, who remain largely unvaccinated for Q fever in Australia. Efforts should be made by all veterinary employers and veterinary workers in Australia to ensure that Q fever vaccination is recommended and available to veterinary support staff (Sellens et al., 2016; Sellens, Norris, et al., 2018).

Current employment within government, laboratories or “other” organisations (excluding abattoirs) was possibly protective against relatively recent *C. burnetii* exposure. Compared to private practice, these roles may require less contact with animals, explaining the possible reduced risk for recent *C. burnetii* exposure. However, working in these organisations was not protective for a positive serological result generally. This may be because employment within such organisation is either highly competitive or more

attractive to experienced workers looking for a change from clinical work, resulting in these workers having usually spent many years working intensively with animals prior to securing such positions.

The participants in this study were not aware of their *C. burnetii* serostatus at the time of sampling, and there were no incentives offered for participation. Subsequently, these results are likely to be valid for the sample. However, they may not be generalizable to all veterinary workers, particularly veterinary support staff who were under-represented and mostly clustered within two related workplaces. Additional seroprevalence studies in Australia should aim to gather further data from veterinary support workers, who report a low level of knowledge regarding Q fever and remain largely unvaccinated (Sellens et al., 2016).

4.6 Conclusion

This study contributes valuable information for employers and employees within the veterinary industry. The findings confirm that veterinary workers have an increased risk of exposure to *C. burnetii*, supporting the Australian Government recommendation for Q fever vaccination of all veterinarians, veterinary students, and veterinary nurses. This recommendation should be extended to cover broader veterinary support staff, such as kennel hands, farm hands and administration workers, particularly in regional and remote areas and clinics working with ruminants. This study also highlights that four out of five unvaccinated veterinarians are potentially eligible for Q fever vaccination despite many years working with animals. These findings will assist medical practitioners and veterinary workers in making informed decisions regarding the prevention of Q fever, particularly with regards to Q fever vaccination.

4.7 Supplementary materials

Table S4-1 Demographics and work characteristics of the whole cohort, and the seropositive and seronegative cohorts, of veterinary workers participating in a *Coxiella burnetii* seroprevalence study, Australia, 2014 – 2015.

Variable	*Whole Cohort*		*Seropositive Workers*		*Seronegative Workers*		*P-value*[‡]
	n	*%*	*n*	*%*	*n*	*%*	
Gender							**0.474**
Female	101	53%	17	47%	84	54%	
Male	91	47%	19	53%	72	46%	
Column Total	192	100%	36	100%	156	100%	
Age Categories (years)							**0.795**
18-30	22	11%	4	11%	18	12%	
31-45 years	48	25%	10	28%	38	24%	
45-60 years	84	44%	17	47%	67	43%	
>60 years	38	20%	5	14%	33	21%	
Column Total	192	100%	36	100%	156	100%	
State							**0.626**
Queensland	22	11%	4	11%	18	12%	
NSW / ACT	109	57%	19	53%	90	58%	
WA / NT	20	10%	6	17%	14	9%	
SA / Victoria / Tasmania	34	18%	6	17%	28	18%	
Not stated[†]	7	4%	1	3%	6	4%	
Column Total	192	100%	36	100%	156	100%	
Workplace Remoteness Category[§]							**0.004**
Major City	123	64%	16	44%	107	69%	
Inner Regional	43	22%	9	25%	34	22%	
Outer Regional / Remote	17	9%	9	25%	8	5%	
Not stated / unclassified	9	5%	2	6%	7	4%	
Column Total	192	100%	36	100%	156	100%	
Current Job Role							**0.413**
Veterinarian	147	77%	28	78%	119	76%	
Animal Scientist	11	6%	1	3%	10	6%	
Veterinary nurse / farm hand / kennel hand	18	9%	5	14%	13	8%	
Administration	15	8%	1	3%	14	9%	

Variable	*Whole Cohort*		*Seropositive Workers*		*Seronegative Workers*		*P-value‡*
	n	*%*	*n*	*%*	*n*	*%*	
Not stated†	1	1%	1	3%	0	0%	
Column Total	192	100%	36	100%	156	100%	
Total years working with animals (years)							**0.974**
0 – 10 years	45	23%	8	22%	37	24%	
11 – 20 years	42	22%	7	19%	35	22%	
21 – 30 years	46	24%	9	25%	37	24%	
> 30 years	55	29%	11	31%	44	28%	
Not stated†	4	2%	1	3%	3	2%	
Column Total	192	100%	36	100%	156	100%	
Total hours per week currently working directly with animals (hours)							**0.888**
Nil	23	12%	5	14%	18	12%	
Up to 15 hours	56	29%	9	25%	47	30%	
>15 up to 38 hours	51	27%	9	25%	42	27%	
More than 38 hours	62	32%	13	36%	49	31%	
Column Total	192	100%	36	100%	156	100%	
Percent of career working with small animals (dogs, cats, pocket pets)							**0.081**
15% or less	46	24%	8	22%	38	24%	
>15% up to 50%	38	20%	12	33%	26	17%	
More than 50%	108	56%	16	44%	92	59%	
Column Total	192	100%	36	100%	156	100%	
Percent of career working ruminants (cattle, sheep, goats)							**0.002**
15% or less	118	61%	13	36%	105	67%	
>15% up to 50%	42	22%	11	31%	31	20%	
More than 50%	32	17%	12	33%	20	13%	
Column Total	192	1	36	100%	156	100%	
Percent of career working horses							**0.275**
15% or less	161	84%	28	78%	133	85%	
>15%	31	16%	8	22%	23	15%	
Column Total	192	100%	36	100%	156	100%	
Percent of career working other species							**0.186**
15% or less	175	91%	35	97%	140	90%	
>15%	17	9%	1	3%	16	10%	
Column Total	192	100%	36	100%	156	100%	

Variable	*Whole Cohort*		*Seropositive Workers*		*Seronegative Workers*		*P-value*[‡]
	n	*%*	*n*	*%*	*n*	*%*	
Percent of career not working directly with animals							**0.849**
Less than 85%	180	94%	34	94%	146	94%	
85% or more	12	6%	2	6%	10	6%	
Column Total	192	100%	36	100%	156	100%	
Currently working with animals in private practice							**0.616**
Yes	121	63%	24	67%	97	62%	
No	71	37%	12	33%	59	38%	
Column Total	192	100%	36	100%	156	100%	
Currently working with animals within a laboratory							**0.477**
Yes	10	5%	1	3%	9	6%	
No	182	95%	35	97%	147	94%	
Column Total	192	100%	36	100%	156	100%	
Currently working with animals within a government agency							**0.239**
Yes	15	8%	1	3%	14	9%	
No	177	92%	35	97%	142	91%	
Column Total	192	100%	36	100%	156	100%	
Currently working with animals within an industry role							**0.477**
Yes	10	5%	1	3%	9	6%	
No	182	95%	35	97%	147	94%	
Column Total	192	100%	36	100%	156	100%	
Currently working with animals within an "other" type of organisation							**0.485**
Yes	30	16%	7	19%	23	15%	
No	162	84%	29	81%	133	85%	
Column Total	192	100%	36	100%	156	100%	

[†]Not stated category excluded from statistical analysis. [‡]Wald Chi Square *p*-value reflects the significance of the univariable association of each variable with a positive *C. burnetii* serology. [§]Workplace remoteness determined from postcodes according to the Australian Statistical Geography Standard (Australian Bureau of Statistics)

5 Immune Responses to *Coxiella burnetii* in Veterinary Workers Previously Vaccinated for Q fever

5.1 Abstract

Repeat doses of Australia's whole cell Q fever vaccine (Q-VAX®, Seqiris Pty. Ltd) are contraindicated due to a risk of adverse events following immunisation in those with pre-existing exposure. However, our current understanding of long-term immune responses to the vaccine relies heavily on data from clinical trials in abattoir workers. Due to the very high risk of re-exposure in these workers, it remains unclear if vaccine-induced immunity was influenced by natural re-exposure and if the results translate to other occupations and population demographics. This study investigated long-term immune responses in veterinary workers following Q fever vaccination through convenience sampling in 2014 and 2015 at veterinary professional conferences and at two veterinary schools within Australia. Anti-phase I and anti-phase II *Coxiella burnetii* antibody titres were determined by IFA for IgM, IgG and IgA antibody classes (n = 203). Cell mediated immunity [CMI] was measured in a sub-set (n = 55) using a commercial interferon gamma release assay [IGRA], modified for stimulation of peripheral blood mononuclear cells rather than fresh whole blood. Time since vaccination ranged from one to 25 years. Overall seroprevalence was 15% (31/203), with no participants returning a seropositive result more than 18 years post-vaccination. Of the seropositive participants, 84% (26/31) had been vaccinated within 10 years of sampling. In multivariable modelling, time since vaccination was the only significant explanatory variable for a positive serological result (p = 0.025), with seropositive participants more likely to have been vaccinated up to five years (OR 4.8; 95% CI 1.6 – 16.8) or six to 10 years (OR 3.3; 1.2 – 10.8) prior to sampling (reference > 10 years). Anti-phase II IgG and IgM were most prevalent, identified in 19 (9%) and 18 (9%) of the 203 participants, while anti-phase I IgG was detected in only nine (4%). There was little evidence to support a boosting effect to

serological titres from natural re-exposure. Seroprevalence in the IGRA subset was 7% (4/55); three of the seropositive participants were IGRA negative and the fourth returned a borderline IGRA result. Of the total 55 IGRA participants, three (5%) returned positive and two (4%) returned borderline results, all of whom were vaccinated within six to 10 years of sampling. The results of this study suggest that previously described ongoing measurable immune responses following Q fever vaccination in abattoir workers may not translate to other occupational cohorts due to the likely effect of enhanced antigenic boosting from the abattoir environment. However, the comparatively reduced measurable outcomes of this study may not correlate with waning immunity, as no participants reported having had Q fever. As CMI is considered critical for clearing *C. burnetii* infection, further studies are recommended to investigate CMI responses in vaccinated veterinary workers and other at-risk cohorts in Australia.

5.2 Introduction

The zoonotic bacterial pathogen *Coxiella burnetii* exhibits smooth-to-rough variation of the lipopolysaccharide [LPS] in the cell wall, with two distinct phases recognised (Amano & Williams, 1984). Virulent phase I bacteria are 'smooth' with full-length LPS, while avirulent phase II bacteria are 'rough' with truncated LPS (Hackstadt et al., 1985; Vishwanath & Hackstadt, 1988). Phase I bacteria exhibit several strategies to evade host immune responses and enable survival and multiplication within phagosomes of monocyte-macrophages (Aderem & Underhill, 1999; Capo et al., 1999; Eldin et al., 2017; Shannon et al., 2005; Vishwanath & Hackstadt, 1988). Phase II bacteria lack these strategies and are phagocytosed and killed via lysosomal activation or complement mediated pathways in serum (Baca et al., 1981; Baca & Paretsky, 1983; Capo et al., 1999; Vishwanath & Hackstadt, 1988). Consequently, phase II bacteria are not isolated from naturally or experimentally infected immunocompetent hosts, occurring only with repeated passage through embryonated eggs or within cell culture (Baca & Paretsky, 1983; Vishwanath & Hackstadt, 1988).

Inactivated whole-cell phase I *C. burnetii* vaccines provide immunity against clinical Q fever, with efficacy attributed in part to the immune response to phase I LPS (Zhang et al., 2007). Australia has a licensed whole-cell formalin-inactivated Q fever vaccine (Q-VAX®, Seqiris Pty. Ltd., Parkville, Victoria, Australia), prepared with phase I *C. burnetii* Henzerling strain (Seqirus, 2019). The vaccine is currently licensed for use in persons 15 years and over and is recommended for occupational groups exposed to animals and their products, including veterinary workers (Australian Technical Advisory Group on Immunisation, 2018; Seqirus, 2019). Despite the duration of protective immunity following vaccination being unknown, repeat doses of Q-VAX® are contraindicated due to a risk of adverse events following immunisation in those who have had prior exposure to *C. burnetii* (Seqirus, 2019). Potential vaccinees must undergo strict pre-vaccination testing which includes *C. burnetii* serology and a skin test in which a very small dose of the diluted vaccine is injected intradermally to allow subjective assessment for a cell mediated response (Seqirus, 2019). Based on the incidence of Q fever in vaccinated subjects during clinical trials, immunity is expected to last beyond five years (Seqirus, 2019) and clinical efficacy has since been estimated as 94% or greater based on clinical trials and reviews of Australian Q fever notification databases for possible vaccine failures (Bond et al., 2017; Gefenaite et al., 2011; Marmion, 2007; Woldeyohannes et al., 2020; Zhang & Samuel, 2004).

Data regarding the longevity of measurable vaccine-induced immune responses rely heavily on pre-licensure clinical trials undertaken from 1981 – 1988 in abattoir workers, one of the highest risk groups for Q fever in Australia (Marmion et al., 1990). Immune responses were investigated up to 60 months (five years) post-vaccination; seropositivity was evident in 77% at 20 – 40 months post-vaccination, reducing to 40% at 41 – 60 months post-vaccination, when the results of a complement fixation test [CFT], immunofluorescence assay [IFA], and a highly sensitive competitive radioimmunoassay [RIA] were all considered (Izzo et al., 1988). At 60 months, cell mediated immunity [CMI] was evident in 95% of vaccinees, demonstrated as lymphocyte proliferation (lymphocyte stimulation index; LSI) following

stimulation of peripheral blood mononuclear cells [PBMCs] with *C. burnetii* phase I (Henzerling) and phase II (Nine Mile) antigens (Izzo et al., 1988). A more recent study measured circulating antibody with IFA and CMI by interferon gamma (IFN-γ) release assay [IGRA] in 11 Q-VAX® recipients vaccinated within seven years of sampling, and all were positive on at least one of the two assays, though the demographic of the studied cohort was not described (Kersh, Fitzpatrick, Self, Biggerstaff, et al., 2013). The combination of IFA, the "gold standard" for *C. burnetii* serology (Bizzini et al., 2015), and IGRA was considered to provide the most sensitive indicator of immunity post-vaccination (Kersh, Fitzpatrick, Self, Biggerstaff, et al., 2013).

It remains unclear if the longevity of vaccine-induced immune responses, which are primarily attributed to studies in predominantly male abattoir workers, translates to other occupations. Repeated *C. burnetii* exposure in very high-risk workplaces such as abattoirs, may provide a boosting effect to prolong the apparent duration of vaccine-induced immunity. Indeed, a recent study in Australian blood bank donors returned a *C. burnetii* seroprevalence of only 10% in participants previously vaccinated for Q fever (Gidding et al., 2019), and Q fever among previously vaccinated persons has been recently described in Australia, with disease onset between five- and 15-years post-vaccination (Bond et al., 2017; Rahaman et al., 2019). Further studies with improved demographic data have been recommended to better understand the protective effect, including occupation-specific studies such as in veterinary workers (O'Neill et al., 2014).

It is important to recognise that in Australian veterinary workers the risk for *C. burnetii* exposure differs between individuals within the profession according to animal exposures and location rurality (Sellens et al., 2020). Exposure risk is also likely to differ over the duration of an individual's career. As females predominate in the workforce, pregnancy and absence to care for children may result in considerable time periods of reduced or nil animal exposure, particularly those exposures considered higher risk for *C. burnetii*. Hence, individuals are expected to experience vastly different opportunities for boosting from natural exposure, and some may be at risk of Q fever should vaccine induced immunity wane in the

absence of natural re-exposure. Therefore, this study aimed to describe immune responses to *C. burnetii* in veterinary workers previously vaccinated for Q fever, and to investigate the association of these responses with time since vaccination and demographic variables including type of animal exposures.

5.3 Methods

5.3.1 Sample population

Convenience sampling of Australian veterinary workers over the age of 18 years was undertaken at veterinary conferences in 2014 (AVA national conference, Perth; AVA NSW divisional conference, Goulburn) and 2015 (AVA Pan Pacific Veterinary Conference, Brisbane), and at veterinary schools within the University of Sydney Camperdown and Camden campuses, and Charles Sturt University Wagga Wagga campus. Although vaccinated and unvaccinated workers were recruited, only samples obtained from vaccinated participants were included in this study. Results for unvaccinated participants have been published previously (Sellens et al., 2020). Veterinary workers included veterinarians, veterinary students, veterinary nurses, animal scientists, and administrative staff at veterinary facilities.

5.3.2 Sample collection

Participation required the provision of a 10mL venous blood sample for serological analysis, and the completion of a paper questionnaire. Serum samples were refrigerated and couriered as soon as practically possible to the Australian Rickettsial Reference Laboratory (ARRL), Victoria, Australia, for *C. burnetii* serology. A sub-set of participants (n = 55) at the 2014 National AVA conference in Perth provided an additional 10 – 15 mL of venous blood collected into heparinised tubes for analysis of CMI. These were collected into pre-prepared 50mL plain tubes containing 400µL heparin and 19.6mL of supplemented RPMI Medium 1640 + L-Glutamine (supRPMI) (Invitrogen, MA, USA), which were stored at 4°C and warmed to room temperature prior to blood collection. The CMI samples were couriered to a nearby laboratory for processing within 8 hours.

The questionnaire (Appendix B) contained 15 questions relating to demographics, history of workplace animal exposures, and history of Q fever disease and vaccination. Surveys were de-identified, labelled only with the unique laboratory identification number, and data was manually entered into a Microsoft® Access® database (Microsoft Corporation, WA, USA).

5.3.3 Laboratory methods and interpretation

5.3.3.1 Serology

Serology was undertaken at ARRL utilising an in-house indirect IFA as previously described (Sellens et al., 2020). Briefly, semi-purified phase I and phase II bacterial antigens (Serion-Virion, Germany) were fixed onto micro-wells on glass slides. The slides were treated with patient sera, followed by four different fluorescein-labelled goat anti-human immunoglobulins (IgM, IgG, IgA and a mixture of all three anti-isotypes combined; Kirkegaard & Perry Laboratories, MD, USA) to detect antibodies against *C. burnetii*. These immunoglobulins were applied separately, to total eight tests per patient per serum dilution. Positive and negative control sera were run on every slide. Initial screening of sera was undertaken at a 1/25 dilution and a 1/400 dilution, the latter to detect any prozone phenomenon. Positive samples were then titrated out further to a 1/3200 dilution or to a definitive endpoint. The patient's antibody titre was recorded as the highest dilution showing immunofluorescence similar to the positive control. A cut-off value of 1/50 was considered positive for anti-phase I and anti-phase II IgM, IgG, and IgA antibody titres. Inconclusive titre results (= 1/25) were considered negative for data analysis.

5.3.3.2 Cell mediated immunity

A commercial *C. burnetii* IGRA kit (Q-detect™, Innatoss Laboratories BV, Oss, Netherlands) was utilised in this study. The enzyme-linked immunosorbent assay [ELISA] measures the IFN-γ production of immune cells following *in vitro* stimulation with heat-killed *C. burnetii* (Q-detect™ antigen Cb2009-02629). Sensitivity is reported as 93% and specificity as at least 90% when the kit is used as per the manufacturer's

instructions (Innatoss Laboratories BV, 2015). Due to logistical constraints, the method for this study was modified in consultation with the manufacturer, with stimulation of PBMCs undertaken rather than fresh whole blood.

Within eight hours of sampling, PBMCs were harvested and prepared for frozen storage. The samples were halved, and each half (~7.5 mL) layered over 15 mL of warmed Lymphoprep™ (Nycomed, Switzerland) and immediately centrifuged (500 g, 30 minutes, 18°C). The PBMC layer from each half was extracted and combined, washed with supRPMI, centrifuged (500*g*, 10 minutes, 18°C) and the supernatant discarded. Cells were then washed in 2% Heat Inactivated Filter Sterilised Foetal Calf Serum (HI-FCS; JRH Biosciences, KS, USA) in supRPMI, centrifuged (500*g*, 7 minutes, 18°C), and the supernatant discarded. The cells were re-suspended in 2% HI-FCS in supRPMI and checked for viability using trypan blue exclusion. The cells were stored in 1mL aliquots at a target concentration of 7.5 – 15 x10^6 cells per mL in 2% HI-FCS in supRPMI + 15% DMSO in HI-FCS (1:1), and frozen immediately for liquid nitrogen storage.

Prior to stimulation, cryovials were thawed in a warm water bath (37°C) and warmed R10 (RPMI 1640 [GIBCO ThermoFisher, Australia] with 10% HI-FCS [Corning Mediatech, Australia], 2% Penicillin-Streptomycin [Sigma, Australia] and 1% L- glutamax [GIBCO, ThermoFisher Australia]) slowly added. The contents were transferred to conical tubes and the cells were again twice washed in R10 and centrifuged (300 g, 12 minutes, Beckman Coulter Allegra X22R), resuspended in R10, and checked for viability using trypan blue exclusion. Cells were incubated overnight at 37.5°C and 5% CO_2 in R10 suspension totalling 10 mL, and the viability then rechecked.

Stimulation of PBMCs with *C. burnetii* antigen was undertaken for 24 hours at 37°C in accordance with the Q detect ELISA kit manufacturer's instructions for using 96-wells plates (Innatoss Laboratories BV, 2015), with 300µL of the cellular suspension added to each well rather than 180µL of whole blood. For

each participant, two test samples were incubated with Q-detect™ antigen (Cb2009-02629), two with positive (phytohaemagglutinin) and two with negative (RPMI) controls to total six sample wells per participant. Following stimulation, 200µL of suspension from each well was removed and stored frozen for later use in the ELISA component of the kit.

The ELISA was performed as per the manufacturer's instructions to determine the IFN-γ production in each sample, with each ELISA plate also including a standard curve (Innatoss Laboratories BV, 2015). An automated plate washer (Tecan, Austria) was used for each washing step and the optical density of each well was determined with a microplate spectrophotometer (SpectraMax 340PC, Molecular Devices, CA, USA) at 450nm within 30 minutes of stopping the reaction. The results were analysed and interpreted in consultation with the IGRA manufacturer (Innatoss Laboratories BV, Netherlands) to determine the IFN-γ concentration and relative response [RR] for each sample. The *C. burnetii*-induced IFN-γ production was calculated as the IFN-γ concentration in the test sample minus that of the negative control sample. The RR for each test sample was also determined in relation to the positive and negative controls. The results were interpreted according to the Q detect ELISA kit manufacturer's criteria (**Box 5-1**).

Box 5-1 Q-detect™ interpretation criteria for IFN-γ production (Innatoss Laboratories BV, 2015).

Result	**IFN-γ production & Relative Response (RR) criteria**
Positive	[IFN-γ >16 pg/mL and RR >0.60] **or** [IFN-γ >64 pg/mL and RR>0.40]
Borderline	16 pg/mL < IFN-γ < 64 pg/mL and RR >0.60
Negative	IFN-γ < 16 pg/mL or RR < 0.40
Inconclusive	Negative control IFN-γ > 40 pg/mL or positive control IFN-γ < 40 pg/mL or RR <0.1

5.3.4 Statistical Analysis

5.3.4.1 Variables

The primary outcome of interest was the proportion of positive results for serology and IGRA, with individual participant's results dichotomised as either positive or negative. The primary explanatory variable of interest was time since vaccination. Other explanatory variables included gender, current age, age at the time of vaccination, geographical state, geographical remoteness, job description, total years working with animals, total hours per week currently working with animals, percentage of career working with small animals (dogs, cats, pocket pets), ruminants (cattle, sheep, goats), horses, and other species, percentage of career not working directly with animals, currently working in private practice, currently working within laboratory/government/industry, and currently working within an 'other' type of organisation (excluding abattoirs).

Workplace postcode was used to determine geographical state and remoteness category. The latter was assigned according to the Australian Bureau of Statistics July 2016 data for Australian Statistical Geography Standard [ASGS] Remoteness Structure, which divides Australia into regions that share common characteristics of remoteness (Australian Bureau of Statistics, 2018). Categorical responses for explanatory variables were grouped according to biologically or demographically related categories. Collinearity was assessed between continuous explanatory variables by the Pearson correlation coefficient where both variables were normally distributed, or the Spearman rank correlation coefficient where the assumption of normality was not met. Associations between each continuous variable and the outcome on a log odds scale were also assessed, and variables categorised for modelling where this association was not linear. A category for missing data was included for variables where responses were incomplete, and the missing category was included in statistical analysis where it comprised five percent or more of the total responses.

5.3.4.2 Modelling

The univariable association between the primary outcome variable and explanatory variables were assessed with logistic regression modelling using the SAS© statistical program (SAS Institute Inc., Cary, NC, USA). The positive outcome in modelling procedures was a positive test result. Multivariate modelling via backwards selection was undertaken, retaining variables with a *p*-value <0.05 as significant in the final model. Gender and age at vaccination were considered confounders *a priori* for multivariable modelling, as these are known to influence immune responses at the time of vaccination (Klein, Marriott, & Fish, 2015; Sellens, Bosward, et al., 2018). Other plausible confounders considered in the modelling were rurality and exposure to ruminants, as these were associated with seropositivity in unvaccinated veterinary workers in Australia (Sellens et al., 2020). Significant variables and potential confounders were tested for interaction, and interaction terms were retained in the model where significant (p <0.05). Confounders were forced into the model if they caused >20% change in the coefficients of variables already in the model. The Likelihood-ratio test was used to determine the significance of the full model and a Hosmer-Lemeshow goodness of fit tests was performed on the final model.

5.3.5 Ethics Statement

Primary ethics approval was granted through the University of Sydney Human Research Ethics Committee (#2014/245), and secondary approval through the Charles Sturt University Human Research Ethics Committee (#2015/289).

5.4 Results

5.4.1 Demographic characteristics

Q fever vaccination was reported by 212 participants, none of whom reported being subsequently diagnosed with Q fever. Five participants reported a vaccination date prior to Q-VAX® licensing in 1989 and four could not recall a date of vaccination. Data for these nine participants were excluded from

analysis. In the remaining 203 participants, time since vaccination ranged from one to 25 years (median 8; interquartile range [IQR] 9). The majority (171; 84%) were veterinarians, and the remainder were veterinary students (17; 8%), animal scientists (12; 6%), and veterinary nursing and administration staff (3; 1%) (**Table S 5-1**). Females predominated (70%). Age ranged from 20 – 75 years (median 31; IQR 17). The median age of females (30 years; IQR 14) was younger than that of males (38 years; IQR 22; Kruskal-Wallis Test $p = 0.020$). All states and territories were represented, with NSW over-represented (50%) for the veterinary workforce (Australian Veterinary Association, 2014) (**Table S 5-1**). Participants worked mostly in major cities (43%) or inner regional (38%) areas, while 14% were from outer regional or remote areas of Australia.

The median number of years working in the veterinary industry was seven (range 0 – 53; IQR 13). The median time spent working with animals per week was 38 hours (range 1 – 80; IQR 32) and 93% of participants had spent their entire career time working directly with animals. Most (61%) had spent the majority (>50%) of their career working with small animals (dogs, cats, pocket pets), 13% with ruminants (cattle, sheep, goats), while only 5% and 1% reported working the majority of their career with horses or other species, respectively (**Table S 5-1**). Only 5% reported no animal contact for more than 15% of their career. Currently working within private practice was reported by 71% of participants, within a laboratory, industry, or government organisation was reported by 17%, and 13% reported currently working within another (unspecified) type of organisation. No participants reported current employment within an abattoir.

Continuous variables age, years working, and years since vaccination were not normally distributed, and their correlation was assessed using Spearman's rank correlation coefficient. A strong positive correlation was identified between age and years working in the veterinary industry ($r_s = 0.65$; $p < 0.001$), and a moderate positive correlation was identified between age and years since vaccination ($r_s = 0.57$; $p < 0.001$), and between years working in the veterinary industry and years since vaccination ($r_s = 0.52$; $p < 0.001$).

5.4.2 *Coxiella burnetii* serology

5.4.2.1 Seroprevalence

Coxiella burnetii seroprevalence in the 203 participants was 15% (31/203; 95% confidence interval [CI] 11 – 21%). Positive anti-phase II IgG and IgM titres were most prevalent (**Table 5.1**), identified in 19 (9%) and 18 (9%) of the 203 participants. Anti-phase I IgG ≥50 was detected in nine (4%) participants, of whom five expressed higher anti-phase I IgG titres than the total anti-phase II titres (**Table 5.1**). No participants returned positive titres for anti-phase I IgM, or to either phase IgA.

5.4.2.2 Time since vaccination and seropositivity

Time since vaccination ranged from one to 25 years and no participants returned a positive serological result beyond 18 years post-vaccination. A linear relationship was not evident between inverse antibody titres and time since vaccination (**Figure 5.1**). Most seropositive vaccinees (26/31; 84%) had been vaccinated within 10 years of sampling. The median time since vaccination was significantly different (Kruskal-Wallis Test p = 0.004) between the seropositive cohort (7 years; IQR 5) and the seronegative cohort (9.5 years; IQR 11). However, the association between years since vaccination as a continuous variable and the likelihood (log odds) of a positive serological result was not linear. Therefore, years since vaccination was categorised for further analysis as 'up to five years' (n = 49), 'six to 10 years' (n = 76), and 'more than 10 years' (n = 78). Categorised time since vaccination was inversely associated with seropositivity in the univariate analysis (p = 0.030).

Table 5.1 *Coxiella burnetii* serological profiles in seropositive veterinary workers previously vaccinated for Q fever and sampled in Australia from 2014 to 2015.

Participant ID	*Years since vaccination*	*Anti-phase II C. burnetii antibody*				*Anti-phase I C. burnetii antibody*			
		IgA	**IgM**	**IgG**	**Total**	**IgA**	**IgM**	**IgG**	**Total**
Predominantly anti-phase II response									
399	1	<25	<25	100	**100**	<25	<25	50	**50**
400	2	<25	50	<25	**50**	<25	<25	<25	**<25**
130	3	<25	<25	100	**100**	<25	<25	25	**25**
146	4	<25	400	<25	**400**	<25	<25	<25	**<25**
166	4	<25	100	50	**100**	<25	<25	<25	**<25**
405	4	<25	100	<25	**100**	<25	<25	<25	**<25**
132	5	<25	100	800	**800**	<25	<25	100	**100**
149	5	<25	50	400	**400**	<25	<25	<25	**<25**
271	5	<25	200	<25	**200**	<25	<25	<25	**<25**
80	6	<25	<25	200	**200**	<25	<25	25	**25**
127	6	<25	<25	100	**100**	<25	<25	25	**25**
178	6	<25	50	100	**100**	<25	<25	25	**25**
74	7	<25	<25	100	**100**	<25	<25	50	**50**
175	7	<25	<25	50	**50**	<25	<25	<25	**<25**
199	7	<25	50	1600	**1600**	<25	<25	<25	**<25**
255	7	<25	50	<25	**50**	<25	<25	<25	**<25**
408	7	<25	100	200	**200**	<25	<25	<25	**<25**
163	8	<25	50	200	**200**	<25	<25	<25	**<25**
335	8	<25	50	<25	**50**	<25	<25	<25	**<25**
137	9	<25	50	<25	**50**	<25	<25	<25	**<25**
156	9	25	25	800	**800**	<25	<25	400	**400**
273	10	<25	200	800	**800**	<25	<25	<25	**<25**
172	11	<25	50	50	**50**	<25	<25	<25	**<25**
173	11	<25	100	<25	**100**	<25	<25	<25	**<25**
439	11	<25	50	<25	**50**	<25	<25	<25	**<25**
385	18	<25	<25	50	**50**	<25	<25	<25	**<25**
Predominantly anti-phase I response									
339	1	<25	<25	25	**25**	<25	<25	800	**800**
117	4	<25	<25	50	**50**	<25	<25	100	**100**
13	7	<25	<25	<25	**<25**	<25	<25	50	**50**
105	10	<25	<25	<25	**<25**	<25	<25	100	**100**
284	13	25	<25	50	**50**	<25	<25	800	**800**

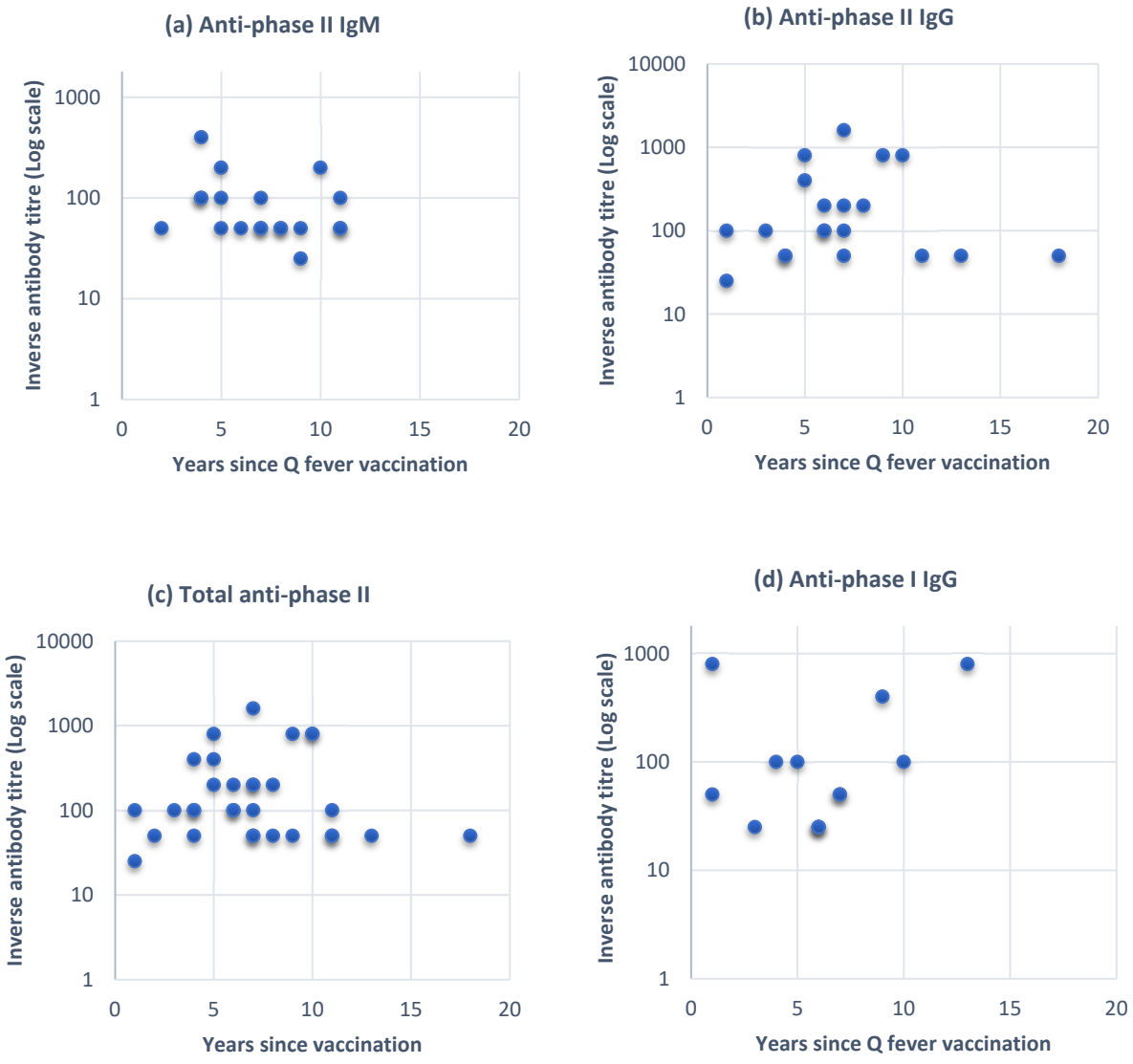

Figure 5.1 Inverse antibody titre versus the number of years post-Q fever vaccination in *C. burnetii* seropositive veterinary workers previously vaccinated for Q fever and sampled in Australia from 2014 to 2015. Titres are shown on a $\log_{10}$ scale for (a) anti-phase II *C. burnetii* IgM, (b) anti-phase II *C. burnetii* IgG, (c) total anti-phase II, and (d) anti-phase I *C. burnetii* IgG.

5.4.2.3 Age at time of vaccination and seropositivity

Age at the time of vaccination ranged from 13 to 65 years in the 203 participants, with a median of 21 years (IQR 7). Three participants reported vaccination before the age at which Q-VAX® is approved for use (15 years), with their vaccination administered by a private general practitioner (n = 2) or organised through their secondary school (n = 1). The median age at the time of vaccination in the seropositive participants was 19 years (range 17 – 60; IQR 8), which did not significantly differ (Kruskal-Wallis Test p = 0.190) from that of the seronegative participants in whom the median age at vaccination was 21 years (range 13 – 65; IQR 7.5). Age at time of vaccination was categorised as adolescent (< 20), adult (20-40 years), or middle aged and above (> 40 years). Seroprevalence among those vaccinated before 20 years of age was 21%, and this age category reported increased odds of seropositivity (OR 1.9; 95% CI 0.56 – 8.74; reference age > 40 years). However, this univariable association was non-significant (p = 0.192) (**Table S 5-1**).

5.4.2.4 Association of other demographic variables with seropositivity

The seropositive group was comprised of a greater proportion of females (77%) than the seronegative group (69%), though there was no significant association between gender and a positive serological result (p = 0.327; **Table S 5-1**). The age of the seropositive group (median 28 years; IQR 7 years) was younger (Kruskal-Wallis Test p = 0.004) than that of the seronegative group (median 34 years; IQR 18.5). Age was categorised for statistical analysis, as its association with the likelihood (log odds) of a positive serological result was not linear. Categorised age was significantly associated with seropositivity (p = 0.023), and seropositive participants were most likely aged 20 – 30 years (OR 3.2; 95% CI 1.1 – 11.5; reference >45 years; **Table S 5-1**).

Similarly, seropositivity declined as years working in the veterinary industry increased, which approached significance (p = 0.051). Some association (p <0.2) was identified with the variable currently working with animals within a laboratory, industry, or government organisation (excludes abattoirs) (p = 0.094).

Seroprevalence was lower among those that were working in such organisations (2/35; 6%) compared to those not (18%), and the likelihood of a positive serological result was greater among those not working in such organisations (OR 3.34; 95% CI 1.0 – 22.6). No statistical association was apparent for other variables and a positive outcome (**Table S 5-1**).

5.4.2.5 Multivariable modelling

The primary explanatory variable for multivariable modelling was time since vaccination, and gender and age at vaccination were considered confounders *a priori*. Age and years working were excluded from multivariable modelling due to their correlation with years since vaccination. All other variables were considered in the multivariable modelling procedure, but none were retained as significant. Time since vaccination remained the only significant explanatory variable. No interaction was observed, and rurality and ruminant exposure were not found to confound the association between the explanatory variable and the outcome. The full model was significant (p = 0.023) and the Hosmer and Lemeshow Test for goodness of fit was non-significant (p = 0.643). Seropositive participants were more likely to have been vaccinated up to five years (OR 4.8; 95% CI 1.6 – 16.8) or six to 10 years (OR 3.3; 1.2 – 10.8) prior to sampling, compared to more than 10 years (p = 0.025) (**Table 5.2**).

Table 5.2 Association between time since vaccination and *C. burnetii* seroprevalence in veterinary workers previously vaccinated for Q fever and sampled in Australia from 2014 to 2015. The model was adjusted for age at vaccination and gender.

Years since vaccination	***Seroprevalence***		***Adjusted Odds Ratio***	***95% Confidence interval***		***P-value***
	n	**%**		**Lower limit**	**Upper limit**	
Up to 5 years	11	22%	4.82	1.58	16.8	0.025
6-10 years	15	20%	3.31	1.18	10.83	
> 10 years	5	6%	*Reference*	-	-	

5.4.3 Interferon gamma release assay

Of the 203 participants included in the analysis, 55 had IFN-γ production measured as an indicator of cell mediated immunity. Time since vaccination ranged from one to 24 years (median 10; IQR 14). This subset more closely reflected the distribution by state of registered veterinarians in Australia (Australian Veterinary Association, 2014), with improved representation of participants from Queensland (9/55; 16%), Victoria (12/55; 22%) and South Australia (8/55; 15%), and a reduced proportion from NSW (18/55; 33%) than in the whole cohort. The sample was comprised of veterinarians (n = 50) and veterinary students (n = 5). The demographic and work history of this subset were otherwise representative of the total sample, though seroprevalence was lower at 7% (4/55; 95% CI 2 – 17 %).

Three (5%) of the 55 IGRA participants returned a positive and two (4%) a borderline result. These five participants were female veterinarians, aged 24 to 29 years, and vaccinated whilst at university within six to 10 years of sampling. The proportion of positive or borderline IGRA results within 10 years of vaccination was 16% (5/32). There was generally poor agreement between serological and IGRA results; of the five borderline or positive IGRA participants, three were seronegative, one returned an equivocal serological result, and only one returned a positive serological result with a phase I IgG titre of 50 (**Table 5.3**). Overall, 15% (8/55; 95% CI 7 – 27%) of this subset exhibited either a positive serological result or a positive or borderline IGRA result. Due to the small number of positive results, statistical modelling was not undertaken for the IGRA results.

Table 5.3 Markers of immunity to *C. burnetii* in 55 veterinary workers previously vaccinated for Q fever and sampled in Australia in 2014 for both serology and cell mediated immunity.

Serology (IFA)	**Cell mediated immunity (Q-detect™ IGRA)**			
	Positive	***Borderline***	***Negative***	***Total***
Positive	0	1	3	**4**
Equivocal	1	0	1	**2**
Negative	2	1	46	**49**
Total	**3**	**2**	**50**	**55**

5.5 Discussion

This is the first study to report immune responses following Q fever vaccination in veterinary workers. Overall, 15% of participants exhibited antibodies to *C. burnetii* within 25 years of Q fever vaccination. Gidding et al. (2019) similarly reported a 10% seroprevalence in blood donors reporting previous Q fever vaccination. Seropositivity in the current study was associated with time since vaccination and a higher seroprevalence was observed within five years (22%) and from five to 10 years (20%) post-vaccination, while there were no positive results beyond 18 years post-vaccination. In previously studied abattoir workers, overall seroprevalence was higher at 55 – 65% from 20 – 60 months post-vaccination (Marmion et al., 1990). Although Marmion et al. (1990) combined the results of IFA, CFT and RIA, positive IFA results were reported in at least 45%. This suggests the higher prevalence in abattoir workers may have been influenced by other factors including re-exposure. However, seroprevalence can be difficult to compare between studies due to differences in methodology (Healy et al., 2011).

In addition to overall seroprevalence, the prevalence of anti-phase I IgG antibodies was higher among abattoir workers than in the current study. In abattoir workers, anti-phase I IgG predominated with a prevalence of 45% in workers sampled between 20 – 60 months post-vaccination, followed by anti-phase II IgM (19%), anti-phase II IgG (13%), and anti-phase I IgM (8%) (Marmion et al., 1990). In the current study in veterinary workers, anti-phase I IgG antibodies were observed in only 4%, whereas anti-phase II IgG and anti-phase II IgM were most prevalent at 9% and 8% respectively. This difference may support recurrent exposure as a contributing factor to the higher seroprevalence observed in abattoir workers, as unvaccinated individuals with pre-existing natural immunity demonstrate a predominantly anti-phase I response upon skin testing or vaccination, effectively a boosting response (Schoffelen, Herremans, et al., 2014; Worswick & Marmion, 1985). In unvaccinated individuals, anti-phase I antibodies are also typically very low following acute *C. burnetii* infection, with anti-phase II IgG titres reaching much higher levels and persisting for longer (Dupuis et al., 1985; Tissot-Dupont & Raoult, 2008). Predominant and persistent anti-

phase I titres in unvaccinated persons are seen with persistent infection, where they are often markedly elevated (≥1/800) (Healy et al., 2011), or in apparently healthy individuals with increased risk of recurrent occupational *C. burnetii* exposure, as demonstrated in unvaccinated veterinary workers in Australia (Sellens et al., 2020) and Belgium (Pozzo et al., 2017).

In the present study, anti-phase I IgG predominated in only six participants. Two of the six, returned a notably high anti-phase I IgG titre (1/800). One was a veterinary student sampled one-year post-vaccination who did not demonstrate a positive titre in any other antibody class. If phase I IgG is indeed related to a boosting effect, it is possible that this individual was vaccinated despite pre-existing immunity, or that vaccine-induced antibodies were only short-lived and subsequently boosted from natural exposure. Indeed, veterinary students in Australia are exposed to a variety of animal species including ruminants. The second participant reporting a high anti-phase I IgG titre (1/800) had been sampled 13 years post-vaccination and reported 50% of their career was spent with dairy cattle and 10% with beef cattle. Boosting from re-exposure is certainly possible, as exposure to ruminants is a known risk factor for *C. burnetii* exposure in Australian veterinarians (Sellens et al., 2020). A further four participants demonstrating primarily anti-phase I IgG expressed titres of 1/50 or 1/100 and had spent most of their career with cats and dogs, species that have occasionally been associated with *C. burnetii* exposure in Australia (Gibbons & White, 2014; Kopecny et al., 2013). However, without further studies to investigate the effect of re-exposure in previously vaccinated persons that are seronegative, the interpretation of serological profiles remains unclear.

No further evidence of serological boosting from natural exposure was identified in the study cohort. Unlike Australia's unvaccinated veterinary workers (Sellens et al., 2020) and the general population (Gidding et al., 2019; Islam et al., 2011), seropositivity in this cohort was not associated with rurality or increasing exposure to ruminants. The decline in seroprevalence after 10 years further supports a lack of evidence of serological boosting in the data, as the risk of *C. burnetii* exposure is not expected to

significantly decline with increasing time in the veterinary profession (Sellens et al., 2020). While increasing age was associated with a decline in seropositivity, this effect was most likely due to correlation with time since vaccination. Significant immune senescence is seen in the aged (> 65 years old) (Farber, Yudanin, & Restifo, 2014; Simon, Hollander, & McMichael, 2015), whereas most participants in this study were much younger both at the time of vaccination and at the time of sampling, and no significant association was found between age at vaccination and the likelihood of a seropositive result.

Therefore, most antibodies detected in this study cohort are expected to be primarily vaccine induced. The absence of a linear decline in titres over time, also reported by Kersh, Fitzpatrick, Self, Biggerstaff, et al. (2013) in a small sample of Q-VAX® recipients, may be due to heterogeneity in vaccine-induced antibody responses. The low overall seroprevalence suggests that serological responses in vaccinated veterinary workers both initially and upon re-exposure to *C. burnetii* may be only short-lived in the majority. Similarly, in a cohort of unvaccinated persons with pre-existing immunity identified by pre-vaccination screening (serology and skin test), serological boosting from the Q-VAX® Skin Test was found to be waning at 12 months post-skin test (Schoffelen, Herremans, et al., 2014). This was most evident in those seronegative prior to the skin test, with only 43% seropositive at 6-months and 23% at 12 months post-skin test (Schoffelen, Herremans, et al., 2014). However, the Schoffelen (2014) study was conducted in in a community setting in older patients with comorbidities and may not translate to younger cohorts in occupational settings or to boosting responses in vaccinated persons versus those naturally exposed.

Despite the low seroprevalence in the current study, none of the 203 vaccinated participants reported having had Q fever disease. In contrast, a cohort of 192 unvaccinated veterinary workers concurrently sampled returned four reports of laboratory confirmed Q fever (Sellens et al., 2020). The vaccine appears to have afforded long-term protection in this study cohort despite waning antibody over time. While there is uncertainty over the role of antibodies in *C. burnetii* immunity, mice studies have demonstrated that IgM and IgG antibodies appear to contribute to protective immunity following vaccination (Zhang et al.,

2013), and that B-cells may be important for the avoidance of self-damage to host tissues following infection (Andoh et al., 2007). Therefore, waning antibody in veterinary workers may have implications for vaccine efficacy. However, it is accepted that innate immunity and cell mediated immunity (CMI) are most critical for *C. burnetii* control and clearance during both initial exposure and re-challenge (Andoh et al., 2007; Eldin et al., 2017; Zhang et al., 2007). Therefore, in the absence of measurable antibody, cell mediated immune memory may provide a robust indication of immunity.

Interferon gamma responses in this study were lower than expected, as previous studies have reported positive IFN-γ responses in 60 – 81 % of Q fever vaccinees (Izzo & Marmion, 1993; Kersh, Fitzpatrick, Self, Biggerstaff, et al., 2013; Schoffelen, Herremans, et al., 2013). The reduced IFN-γ responses may be due to a variety of factors. Firstly, these results may be reflective of this population for the given sampling method, with a best-case scenario of 16% reporting a positive or borderline IGRA response when sampled at random within 10 years post-vaccination. While memory T-cell populations may persist for decades following some vaccinations such as smallpox, individual circulating memory T-cells have shorter half-lives (1 – 12 months) (De Boer & Perelson, 2013) and their maintenance may rely on signalling from repeated antigen exposure or from cross-reactivity to self-antigens or to other environmental or commensal organisms (Farber et al., 2014). Therefore, in the absence of recurrent exposure, *C. burnetii* specific memory T-cells may diminish within the peripheral circulation following vaccination. T-cell-mediated memory responses with high protective capacity may also be compartmentalised in tissue sites, as most memory T-cells within the body reside in tissues, including lymphoid tissues, intestine, lung and skin (Farber et al., 2014). Therefore, the failure to detect immune memory in PBMCs may not reflect an absence of immune memory in Q fever vaccinees, particularly as the route of vaccine exposure is not typical of the route of natural infection. Due to the lower-than-expected prevalence, a larger sample size is required to investigate further, and a study design in which vaccinees are repeatedly tested over time could provide more reliable information on individual IFN-γ responses post-vaccination. Our

understanding of long-term immune memory post-vaccination could also be enhanced with further studies in which vaccinees returning a negative IGRA result are re-exposed with Q-VAX® Skin Test and the IGRA repeated.

Secondly, modifications to sampling methodology may have impacted the performance of IGRA. While the use of PBMCs in IGRAs is common, the results may not be comparable to fresh whole blood. This is because PBMCs are maintained within culture media lacking granulocytes, platelets, cytokines, growth factors, and other factors important for cell viability and immune responses (Hartmann, Emnéus, Wolff, & Jungersen, 2016). Indeed, Izzo *et al.* (1993) reported poor IFN-γ responses in Q fever vaccinees following stimulation of PBMCs with phase I antigen. Only 17% returned a positive result, which was offset by the addition of interleukin-2 to the cell medium (Izzo & Marmion, 1993). Contrary to this, stimulation with anti-phase II antigen saw 73% positive for IFN-γ response in the same cohort, demonstrating that phase I *C. burnetii* antigen may have a down-regulatory action on IFN-γ formation by isolated PBMCs stimulated *in vitro* in Q fever vaccinees (Izzo & Marmion, 1993). Therefore, the use of a phase I antigen with PBMCs in this study may have contributed to poor IGRA results. While other studies reporting strong IFN-γ responses in Q fever vaccinees have utilised phase I antigen, these have used fresh whole blood samples rather than PBMCs (Kersh, Fitzpatrick, Self, Biggerstaff, et al., 2013; Schoffelen, Herremans, et al., 2013). However, strong IFN-γ responses have been reported following phase I *C. burnetii* stimulation of PBMCs from chronic Q fever patients, though stimulation times of 48 hours (double that of this study) were utilised (Schoffelen et al., 2017) and responses utilising fresh whole blood in Q fever patients were greater than those seen in vaccinees (Schoffelen, Herremans, et al., 2013). While freezing and thawing may also have potentially affected cell responses, previously frozen PBMCs were successfully utilised by Schoffelen et al. (2017) and positive control responses were adequate in the current study.

Finally, the commercial IGRA used in this study has been optimised for the detection of both active and latent *C. burnetii* infection to support the diagnosis of Q fever disease or Q-fever related chronic fatigue

syndrome, and to detect prior natural exposure to support further medical decisions including the decision to vaccinate (Innatoss Laboratories BV, 2015). The assay may therefore lack sensitivity in detecting responses in individuals exposed via vaccination rather than natural exposure, particularly long-term responses in the absence of recent *C. burnetii* re-exposure. Indeed, Q fever patients demonstrate higher IFN-γ responses than is initially seen with Q fever vaccination (Schoffelen, Herremans, et al., 2013), while IFN-γ responses in mice following *C. burnetii* challenge were significantly higher in naive compared to vaccinated individuals (Zhang et al., 2007). Indeed, the use of the Q-detect™ kit with fresh whole blood sampled from a smaller cohort of Q fever vaccinees sampled at an Australian scientific conference yielded mostly (5/7) borderline results and two positive results, whereas a previously infected participant returned a strong positive (Graves et al., 2018). However, this was a preliminary study, and a larger cohort is required to validate the performance of Q-detect™ in vaccinated individuals. The IGRA was also developed within a specific geographic location, the Dutch village of Herpen, following the Dutch Q fever outbreak and utilises heat-killed bacteria originating from the Dutch *Coxiella* strain as the antigen (Cb2629) (Innatoss Laboratories BV, 2015). This Dutch strain may not perform as well in the IGRA in individuals with pre-existing immunity from exposure to the vaccine strain (Henzerling) or local strains in Australia.

Overall, the results of the IGRA cannot be interpreted without further understanding of the use of the Q-detect™ kit for detecting immunity in Q-VAX® vaccinees, for use with PBMCS, and for use in the Australian setting. Due to the very high cost of the commercial IGRA, further investigations were not feasible in this cohort study. The use of this IGRA in Australia for its intended purpose, as an aid in diagnosis of Q fever or detection of prior natural exposure, may be similarly hindered by expense and logistics. The stimulation of a large number of fresh whole blood samples is required within 12 hours of sampling, which may not be feasible outside of an outbreak or mass screening setting.

5.6 Conclusions

Despite the low prevalence of positive serological and IGRA results, Q fever disease was not reported in any vaccinees. Importantly, the serological findings of this study suggest that our current understanding of the longevity of vaccine immunity, which is based on studies in predominantly male abattoir workers, may not translate to other at-risk professions including veterinary workers. This could have implications for waning immunity in individuals where recurrent exposure is less likely than in abattoir workers. As re-vaccination is currently contraindicated, further studies are recommended to understand the longevity of vaccine immunity, particularly cell mediate responses, in veterinary workers.

5.7 Supplementary materials

Table S 5-1 Univariable association between explanatory variables and a positive *C. burnetii* serological result in a sample of veterinary workers previously vaccinated for Q fever and sampled in Australia in 2014 and 2015.

	Whole Cohort		Seroprevalence				95% Confidence Interval	
Variable	**n**	**%**	**n**	**%**	**Chi Square p-value‡**	**Odds Ratio**	**Lower Limit**	**Upper Limit**
Categorised Years since Q fever vaccination					0.030			
Up to 5 years	49	24%	11	22%		4.23	1.43	14.24
6-10 years	76	37%	15	20%		3.59	1.31	11.55
> 10 years	78	38%	5	6%		*Reference*		
Total	203	100%	31	15%				
Gender					0.327			
Female	142	70%	24	17%		1.57	0.67	4.14
Male	61	30%	7	11%		*Reference*		
Column Total	203	100%	31	15%				
Age Category (years)					0.023			
20-30	94	46%	22	23%		3.21	1.13	11.53
31-45 years	63	31%	5	8%		0.91	0.23	3.85
>45 years	46	23%	4	9%		*Reference*		
Column Total	203	100%	31	15%				
Age at Vaccination (years)					0.192			
Adolescence (< 20)	75	37%	16	21%		1.90	0.56	8.74
Younger adult (20-40 years)	104	51%	12	12%		0.91	0.26	4.26
Older adult (> 40 years)	24	12%	3	13%		*Reference*		
Column Total	203	100%	31	15%				

Variable	Whole Cohort		Seroprevalence		Chi Square p-value[‡]	Odds Ratio	95% Confidence Interval	
	n	%	n	%			Lower Limit	Upper Limit
State					0.336			
Queensland	25	12%	1	4%		*Reference*		
NSW/ACT	106	52%	16	15%		4.27	0.81	78.88
WA/NT	19	9%	3	16%		4.5	0.52	95.49
Tasmania/SA/Victoria	46	23%	10	22%		6.67	1.16	126.34
Not stated[†]	7	3%	1	14%		-	-	-
Column Total	203	100%	31	15%				
Workplace Location Remoteness Category[§]					0.517			
Major City	87	43%	16	18%		2.93	0.76	19.33
Inner Regional	77	38%	12	16%		2.40	0.60	16.11
Outer Regional / Remote	28	14%	2	7%		*Reference*		
Not stated / unclassified	11	5%	1	9%		1.30	0.06	15.10
Column Total	203	100%	31	15%				
Current Job Role					0.888			
Veterinarian	171	84%	27	16%		1.22	0.31	8.07
Veterinary Student	17	8%	2	12%		0.87	0.09	8.07
Other (animal scientist = 12; vet nurse = 2; administration = 1)	15	7%	2	13%		*Reference*		
Column Total	203	100%	31	15%				
Years Working in the Veterinary Industry					0.051			
Up to 5	86	42%	20	23%		4.39	1.18	28.65
6-10	47	23%	7	15%		2.54	0.56	17.87
11-20	37	18%	2	5%		0.83	0.10	7.25

Variable	Whole Cohort		Seroprevalence		Chi Square p-value‡	Odds Ratio	95% Confidence Interval	
	n	%	n	%			Lower Limit	Upper Limit
>20	31	15%	2	6%		*Reference*		
Not stated†	2	1%	0	0%				
Column Total	203	100%	31	15%				
Total hours per week currently working directly with animals (hours)					0.714			
Up to 15 hours	69	34%	9	13%		*Reference*		
>15 up to 38 hours	40	20%	6	15%		1.18	0.37	3.55
More than 38 hours	90	44%	16	18%		1.44	0.61	3.62
Not stated†	4	2%	0	0%				
Column Total	203	100%	31	15%				
Percent of career working with small animals (dogs, cats, pocket pets)					0.510			
15% or less	34	17%	3	9%		*Reference*		
>15% up to 50%	45	22%	8	18%		2.23	0.59	10.87
More than 50%	123	61%	20	16%		2.01	0.63	8.91
Not stated†	1	0%	0	0%				
Column Total	203	100%	31	15%				
Percent of career working ruminants (cattle, sheep, goats)					0.790			
15% or less	122	60%	20	16%		1.57	0.49	7.04
>15% up to 50%	53	26%	8	15%		1.42	0.37	6.95
More than 50%	27	13%	3	11%		*Reference*		
Not stated†	1	0%	0	0%				
Column Total	203	100%	31	15%				

Variable	Whole Cohort n	Whole Cohort %	Seroprevalence n	Seroprevalence %	Chi Square p-value‡	Odds Ratio	95% Confidence Interval Lower Limit	95% Confidence Interval Upper Limit
Percent of career working horses					0.305			
15% or less	168	83%	23	14%		*Reference*		
>15% up to 50%	23	11%	6	26%		2.23	0.74	6.00
>50%	11	5%	2	18%		1.40	0.21	5.88
Not stated†	1	0%	0	0%				
Column Total	203	100%	31	15%				
Percent of career working with other species					0.560			
15% or less	191	94%	30	16%		*Reference*		
>15%	11	5%	1	9%		1.86	0.34	34.82
Not stated†	1	0%	0	0%				
Column Total	203	100%	31	15%				
Percent of career spent not working with animals					0.630			
15% or less	191	94%	30	16%		1.86	0.34	34.82
>15%	11	5%	1	9%		*Reference*		
Not stated†	1	0%	0	0%				
Column Total	203	100%	31	15%				
Currently working with animals in private practice					0.495			
Yes	144	71%	24	17%		1.37	0.58	3.64
No	55	27%	7	13%		*Reference*		
Not stated†	4	2%	0	0%				
Column Total	203	100%	31	15%				

Variable	Whole Cohort n	Whole Cohort %	Seroprevalence n	Seroprevalence %	Chi Square p-value‡	Odds Ratio	95% Confidence Interval Lower Limit	95% Confidence Interval Upper Limit
Currently working with animals within laboratory, industry, or government (excludes abattoirs)					0.094			
Yes	35	17%	2	6%		*Reference*		
No	164	81%	29	18%		3.34	1.00	22.61
Not stated†	4	2%	0	0%				
Column Total	203	100%	31	15%				
Currently working with animals within an "other" type of organisation (unspecified)					0.799			
Yes	23	11%	4	17%		*Reference*		
No	176	87%	27	15%		1.162	0.32	3.39
Not stated†	4	2%	0	0%				
Column Total	203	100%	31	15%				

†Not stated category excluded from statistical analysis. ‡Wald Chi Square *p*-value reflects the significance of the univariable association of each variable with a positive *C. burnetii* serology. §Workplace remoteness determined from postcodes according to the Australian Statistical Geography Standard (Australian Bureau of Statistics).

6 Frequency of Adverse Events Following Q Fever Immunisation in Young Adults

The content of this chapter is published:

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6.1 Abstract

Q fever is a zoonosis of concern in many countries. Vaccination is the most effective means of prevention, and since 1989, Australia has had a licensed Q fever vaccine, Q-VAX®. This vaccine was also used in the Netherlands in 2011 following the largest recorded Q fever outbreak globally. There is a paucity of available data regarding adverse events following immunisation [AEFI] for young adult females. Such data are important for informing future vaccination recommendations both within Australia and internationally. This study collected Q fever vaccine (Q-VAX®) AEFI data in veterinary and animal science students at Australian universities. Students were enrolled at the time of vaccination and were emailed a link to an online AEFI survey one week later. Of the 60% (499/827) that responded, 85% were female and the median age was 18 years. Local injection site reactions [ISRs] occurred in 98% (95%; CI 96–99%) of respondents, of which 30% (95% CI 24–32%) were severe. Systemic AEFI occurred in 60% (95%; CI 55–64%) of respondents within the seven days following immunisation. Medical attention was sought by 19/499 (3.8%) respondents, of whom one sought treatment at a hospital emergency department. Females were more likely than males to experience any local ISR (odds ratio [OR] 9.3; 95% CI 2.5–33.8; $p < 0.001$), ISRs of greater severity (OR 2.5; 95% CI 1.5–4.2; $p < 0.001$), and any systemic AEFI (OR 1.9; 95% CI 1.1–3.1; $p = 0.016$). These safety data suggest that a high frequency of adverse events following immunisation

should be expected in young adults, particularly females. However, the consequences of Q fever disease are potentially far more debilitating.

6.2 Introduction

Q fever is a zoonotic disease in humans causing significant concern for public health in many countries (Angelakis & Raoult, 2011; Million & Raoult, 2015). The causative bacterium, *Coxiella burnetii*, is harboured by many domestic and wildlife species and shed into the environment in the placental tissues, urine, milk, and faeces of infected animals (Angelakis & Raoult, 2010; Guatteo et al., 2006; Raoult et al., 2005). Transmission to humans is primarily via inhalation of the highly infective and environmentally resilient spore-like phase of the bacterium, which is easily spread over long distances by wind (Hogerwerf et al., 2012; Kersh, Fitzpatrick, Self, Priestley, et al., 2013). While cattle, sheep, and goats are most commonly implicated in human infections, other species including domestic cats and dogs may also pose a risk (Angelakis & Raoult, 2010; Gibbons & White, 2014; Komiya, Sadamasu, Toriniwa, et al., 2003; Kosatsky, 1984; Shapiro et al., 2015; Tozer et al., 2014).

Acute Q fever is symptomatic in 20 – 80% of cases, with non-specific clinical signs varying by country, age, and gender (Million & Raoult, 2015). While a flu-like illness is most common, severe symptoms including atypical pneumonia, hepatitis, and myocarditis have been described, and up to 1.5% of acute cases are fatal (Angelakis & Raoult, 2011; Million & Raoult, 2015). Patients with co-morbidities are predisposed to persistent focalised *C. burnetii* infection, of which endocarditis in patients with pre-existing heart valve lesions is most commonly reported (Angelakis & Raoult, 2011; Million & Raoult, 2015). Infections during pregnancy may be associated with adverse pregnancy outcomes, such as miscarriage and pre-term birth, and predispose the patient to persistent infection (Denman & Woods, 2009; Langley et al., 2003; Million & Raoult, 2015; Raoult et al., 2002). Post Q fever fatigue, a debilitating syndrome presenting as protracted fatigue and often arthralgia and myalgia, occurs in up to 20% of all Q fever cases (Morroy et al., 2016;

Sukocheva et al., 2010; Wildman et al., 2002). Due to the variable and non-specific presentation of Q fever syndromes, diagnosis and treatment may be delayed or missed in the absence of suspicion and the treatment of persistent infections can be particularly complicated, emphasising the importance of prevention (Angelakis & Raoult, 2011).

A whole-cell formalin inactivated Q fever vaccine (Q-VAX®; Seqirus, Parkville, Victoria, Australia) has been licensed for use in Australia since 1989. With a reported efficacy of greater than 97% and very few vaccine failures described, vaccination offers the most effective measure for the prevention of Q fever (Bond et al., 2017; Gefenaite et al., 2011; Marmion, 2007). In Australia, vaccination is currently recommended for people with a high occupational risk of Q fever, including abattoir workers, farmers, and veterinary personnel (Australian Technical Advisory Group on Immunisation, 2018). Vaccine uptake is high among occupations where vaccination is mandated, including abattoir workers who are vaccinated prior to commencing their employment, and veterinarians who are vaccinated early in their university study (Gidding et al., 2009; Sellens et al., 2016). However, uptake is variable among farmers and low among veterinary nurses (Gidding et al., 2009; Sellens et al., 2016); in the latter, the perception that the vaccine is safe was associated with increased likelihood of vaccination (Sellens, Norris, et al., 2018). Despite the availability of an effective vaccine, over 500 cases of Q fever are notified annually in Australia (Australian Government Department of Health, 2017), and the true burden of disease is likely much greater as many cases may remain undiagnosed; particularly in groups not considered occupationally at-risk.

Internationally, Q-VAX® was used in the Netherlands in 2011 following a large Q fever outbreak. The outbreak occurred from 2007 – 2009, in which time over 3500 cases of Q fever were notified (van der Hoek et al., 2010). In contrast to the occupationally based use of the vaccine in Australia, a community-based vaccination program was initiated in which patients at high-risk for Q fever complications were identified by general practitioners and referred for Q fever vaccination. Vaccine compliance was high and a total of 1368 patients were vaccinated for Q fever in the Netherlands in 2011 (Isken et al., 2013).

In the future, there may be a shift towards community-based vaccination within some regions of Australia, as seroprevalence data raise concerns regarding community-based exposure to *C. burnetii*. In Queensland, *C. burnetii* seroprevalence in metropolitan populations (5%) is similar to that of rural/remote populations (5.3%) (Tozer et al., 2011). In New South Wales (NSW), the Hunter-New England region has an overall seroprevalence of 7%, with some local areas reaching 22%. In this region, seroprevalence among 10 – 19-year-olds was found to be higher than expected at 5% (Islam et al., 2011). Localised outbreaks in Australia also suggest a community-based approach may be required, with cases occurring in people with no history of high-risk exposure activities (Archer et al., 2017; Bond et al., 2016), and an outbreak at an abattoir in south-western Sydney, New South Wales, raising concerns due to its close proximity to residential properties and a school (Lord et al., 2016).

Sufficient and available vaccination safety data are required to support an increase in vaccine uptake among occupational cohorts where vaccination is not mandated, and to support any future recommendations for a change from an occupationally based to a community-wide vaccination program within Australia. Pre-licensure adverse events following immunisation [AEFI] data for Q-VAX® were collected from 1981 – 1988 during trials in Australian abattoirs where vaccinees were predominantly male (mean age 29 years) (Ackland et al., 1994; Marmion et al., 1984; Marmion et al., 1990). More recently, AEFI data were collected during the large-scale vaccination program in the Netherlands (Schoffelen, Wong, et al., 2014). Although this study included a greater proportion of females (40%) than the Australian studies, vaccinees were older (median 67 years) and suffered significant co-morbidities (Schoffelen, Wong, et al., 2014).

Additional data are available from the Australian Government Therapeutic Goods Administration National Database of Adverse Event Notifications [DAEN], a passive surveillance system. This system provides important safety data, particularly regarding serious AEFI for which reporting is mandatory. Serious AEFI are defined as such where death, life-threatening illness, hospitalisation, persistent or significant

disability/incapacity, or a congenital anomaly/birth defect occurs (Bentsi-Enchill et al., 2012). Generally though, the surveillance system underestimates overall AEFI (Therapeutics Goods Aministration, 2018), as demonstrated during Australia's National Q fever Management Program [NQFMP] where only 86 AEFI (eight of which were serious) were reported during the program (2001 – 2004) despite the administration of close to 50,000 vaccines (Gidding et al., 2009). It is also not possible to determine the AEFI reporting rate from the DAEN, as the number of Q-VAX® doses administered over time is not reliably recorded.

Consequently, there is a paucity of detailed AEFI data available for young adults, particularly females, and a complete lack of data for paediatric populations (<15 years old). This study aims to provide safety data for Q-VAX® in young adults in a format that is more descriptive than previously reported, with a focus on young adult females in particular. These data will provide useful information for both medical practitioners and young adult vaccinees, and for any future consideration of vaccine trials in paediatric populations.

6.3 Materials and methods

6.3.1 Subjects

University students enrolled in animal science or veterinary science courses in Australia are routinely vaccinated against Q fever in their first year of study, following routine pre-vaccination testing procedures. University students are mostly young adults (<25 years old) and in Australia over 75% of the veterinary science cohort are women. Students from three universities in Australia were enrolled in the study at the time of their Q fever vaccination: (1) veterinary science students at the University of Sydney in 2013 and 2014, (2) veterinary science, animal science, equine science, and veterinary technology students at Charles Sturt University in 2014 and 2015, and (3) veterinary science and veterinary technology students from the University of Queensland in 2014 and 2015. Students who were negative on pre-vaccination screening and subsequently received the Q fever vaccination were invited to participate in the study.

Primary ethics approval was granted by the University of Sydney human research ethics committee (Protocol #15012; Project # 2012/1686). Secondary approvals were granted by Charles Sturt University School of Animal and Veterinary Sciences ethics in human research committee (protocol #2015/003), and the University of Queensland institutional human research ethics committee (approval #2014000328).

6.3.2 Q-VAX® and Q-VAX® Skin Test

Q-VAX® and Q-VAX® Skin Test contain whole cell formalin-inactivated phase I *Coxiella burnetii* Henzerling strain. The Q-VAX® vaccine contains a minimum of 25µg of antigen in 0.5mL of aqueous solution, which is administered subcutaneously in the upper arm (Seqirus, 2016). The Q-VAX® Skin Test contains 2.5µg antigen per 0.5mL of aqueous solution and is further diluted in sodium chloride prior to administration. The final 0.1mL dose contains 16.7ng of antigen and is delivered intra-dermally into the volar surface of the mid-forearm as part of the pre-vaccination screening process (Seqirus, 2016).

In addition to antigen, Q-VAX® and Q-VAX® Skin Test contain sodium chloride, sodium phosphate-monohydrate, and sodium phosphate-dihydrate. Thiomersal 0.01% w/v is added as a preservative (Seqirus, 2016). Product information is included as Appendix C.

6.3.3 Pre-vaccination testing

Prior to vaccination, as part of the recommended protocol prescribed by the vaccine manufacturer, participants were questioned by a medical practitioner regarding the possibility of previous exposure to *C. burnetii*, and underwent serological and intra-dermal skin testing to assess for pre-existing sensitisation to *C. burnetii* antigens resulting from prior natural exposure or vaccination. Blood was collected and the intra-dermal skin test injection (Q-VAX® Skin Test) given on the same day. The skin test reaction was subjectively assessed by a medical practitioner, experienced in reading Q VAX® Skin Test results, seven days post-injection. Blood samples were sent to commercial labs for serological profiling: (1) University of Sydney samples to Douglass Hanley Moir (Macquarie Park, NSW, Australia) utilising an indirect

immunofluorescence assay [IFA], (2) University of Queensland samples to the Queensland Medical Laboratory (Murarrie, QLD, Australlia) utilising an enzyme-linked immunosorbent assay [ELISA], and (3) Charles Sturt University samples to Symbion Laverty Pathology (Macquarie Park, NSW, Australia) utilising an ELISA. Vaccination was administered if both the serology and skin test were negative, and no history of probable prior exposure was identified.

6.3.4 Data collection and survey design

Eligible participants were enrolled by university medical or research staff immediately following their vaccination. Consent forms were signed in which participants provided a contact email address. One week after vaccination, vaccinees enrolled in the study were emailed a link to participate in an online survey administered via the Survey Monkey® platform. The survey contained two closed and seven semi-closed questions pertaining to local and systemic AEFI (Appendix D). Within this survey, participants were asked if they had experienced each local reaction, the size of each reaction, and at what time point following vaccination the local reactions had occurred. For systemic events, participants were asked if they had experienced each event within the seven days following vaccination. Gender was also asked as a closed question in the survey, while age and vaccination location were recorded at enrolment and later matched with questionnaire responses.

6.3.5 Statistical analysis

Descriptive statistics were generated for demographic and adverse events data to assess the frequency and severity of AEFI reported. The frequency of AEFI reported was compared between females and males using generalised linear mixed modelling (PROC GLIMMIX procedure) in the SAS© statistical program (SAS Institute Inc., Cary, NC, USA). Outcome variables for the frequency of AEFI were binary, reflecting whether each AEFI was reported to have occurred or not. Gender was tested as a fixed effect for each outcome. A *p*-value of <0.05 was considered statistically significant. The odds ratios and their 95% confidence limits

were presented. The year of vaccination and the location of vaccination were included as random effects to account for clustering.

Local injection site reactions [ISRs] were further graded for severity based on the level of pain reported and measurement of the area of erythema and/or swelling. The criteria for grading local ISRs, outlined in **Box 6-1** were the same as those used by Schoffelen, Wong, et al. (2014) for Q-VAX®. The overall grade for ISRs assigned for each vaccinee corresponded with the highest grade reported for any of the individual local ISRs. The ordinal outcome variable "ISR grade" was comprised of three categories: grade 1 (mild), grade 2 (moderate), and grade 3-4 (severe). This outcome was compared between females and males, also using generalised linear mixed modelling with year and location of vaccination included as random effects.

Box 6-1 Criteria for grading the severity of local injection site reactions (ISRs) following Q fever vaccination. Vaccinees were assigned an overall ISR grade corresponding to the highest reported grade across the three symptoms.

Grade	***Severity***	***Description of Pain***	***Area of Erythema or Swelling***
1	Mild	Pain to the touch, no obstruction of use	<2.5cm
2	Moderate	Pain on movement, some interference with normal activity	2.5 to <7.5cm
3	Severe	Considerable pain in rest, obstruction of use	7.5 to <15 cm
4	Extensive	-	15cm or greater

6.4 Results

6.4.1 Demographics

A total of 839 vaccinees were enrolled at the time of vaccination and provided consent and contact details for participation in the online survey. Of these, 12 were not contactable due to incorrect email addresses. Survey responses were received from 499 vaccinees across the three locations from 2013 – 2016, resulting in a response rate of 60% (499/827). The majority (85%) of respondents were female, and the median age was 18 years (interquartile range 2 years). Age distribution was similar for both females and males and the demographics of respondents were much the same across the three university locations (**Table 6.1**).

Table 6.1 Demographic information of Q fever vaccinees participating in the adverse events following immunisation study across the three study locations.

Variable	***University of Sydney***	***University of Queensland***	***Charles Sturt University***	***Total Vaccinees***
Gender				
Females n (%)	194 (85.5)	106 (80.9)	124 (87.9)	424 (85.0)
Males n (%)	33 (14.5)	25 (19.1)	16 (11.3)	74 (14.8)
Unspecified n (%)	0 (0)	0 (0)	1 (<1)	1 (<1)
Column Total	227 (100)	131 (100)	141 (100)	499 (100)
Age				
Range (years)	17-43	17-29	17-38	17-43
Mean (years)	20.3	19.0	19.7	19.8
Median (years)	19.0	18.0	19.0	18.0
Interquartile Range	3.0	3.0	2.0	2.0
Year of vaccination				
2013 n (%)	143 (63.0)	0 (0)	0 (0)	143 (28.7)
2014 n (%)	75 (33.0)	21 (16.0)	19 (13.5)	115 (23.0)
2015 n (%)	9 (4.0)	110 (84.0)	55 (39.0)	174 (34.9)
2016 n (%)	0 (0)	0 (0)	67 (47.5)	67 (13.4)
Column Total	227 (100)	131 (100)	141 (100)	499 (100)

6.4.2 Injection site reactions

Local ISRs were reported by 98% (95% CI 96-99%) of respondents. Injection site pain occurred in 95% (95% CI 92-96%), while erythema and swelling were less common, each reported by 58% (95% CI 54-62%) of respondents (**Table 6.2**). Pronounced ISRs (grades 3-4) occurred in 30% (130/473; 95% CI 24-32%) of respondents (**Figure 6.1**). The majority (76%; 366/481) of ISRs appeared within 24 hours of vaccination, 23.5% (113/481) between days 2-5, and less than one percent (2/481) occurred more than five days post-vaccination. Females were significantly more likely to report local ISRs (**Table 6.2**) and ISRs of a higher grade (**Figure 6.1**) than males.

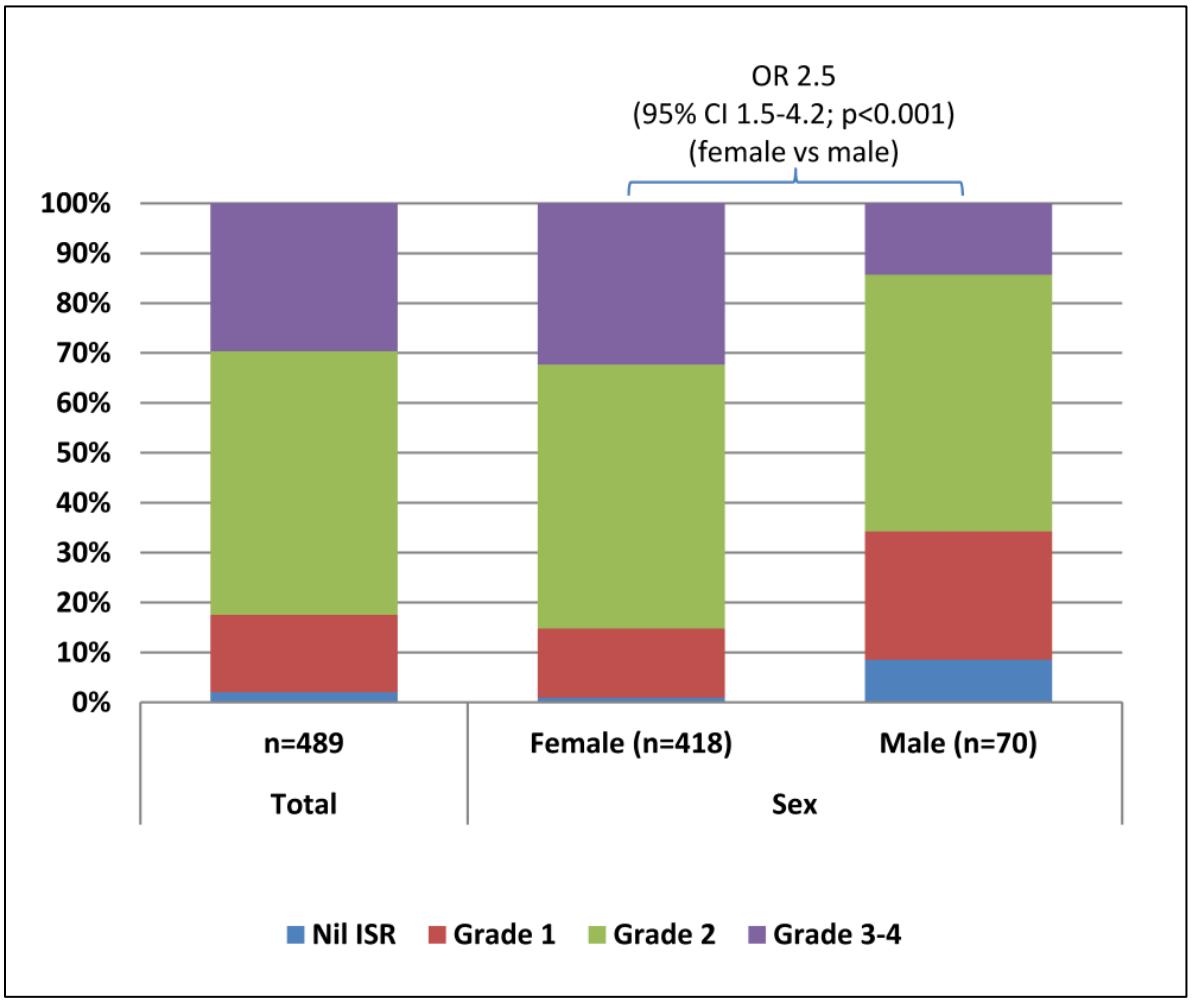

Figure 6.1 Proportion of total respondents and of females and males assigned to each grade of injection site reaction (ISR) following Q fever immunisation. Females were more likely to report severe ISRs.

Table 6.2 Local injection site reactions (ISRs) reported by respondents following Q fever vaccination. The odds ratio for reporting "yes" for each ISR is shown for females versus males.

	All respondents	***Females***	***Males***
Any Local Injection Site Reaction			
Yes n (%)	489 (98.0)	420 (99.1)	68 (91.9)
No n (%)	10 (2.0)	4 (<1)	6 (8.1)
Column Total n (%)	499 (100)	424 (100)	74 (100)
OR (95% CI; p-value)	-	9.3 (2.5-33.8; <0.001)	ref
Injection Site Pain			
Yes n (%)	473 (94.8)	411 (96.9)	61 (82.4)
No n (%)	26 (5.2)	13 (3.1)	13 (17.6)
Column Total n (%)	499 (100)	424 (100)	74 (100)
OR (95% CI; p-value)	-	6.7 (3.0-15.2; <0.001)	ref
Injection Site Swelling			
Yes n (%)	289 (57.9)	257 (60.6)	32 (43.2)
No n (%)	208 (41.7)	165 (38.9)	42 (56.8)
Not Specified n (%)	2 (<1)	2 (0.5)	0 (0)
Column Total n (%)	499 (100)	424 (100)	74 (100)
OR (95% CI; p-value)	-	2.5 (1.5-4.3; <0.001)	ref
Injection Site Erythema			
Yes n (%)	289 (57.9)	257 (60.6)	31 (41.9)
No n (%)	207 (41.5)	165 (38.9)	42 (56.8)
Not Specified n (%)	3 (<1)	2 (< 1)	1 (1.4)
Column Total n (%)	499 (100)	424 (100)	74 (100)
OR (95% CI; p-value)	-	3.3 (1.9-5.7; <0.001)	ref

OR; Odds Ratio. CI; Confidence interval. Ref; reference category for odds ratio. Odds ratio adjusted for year and location of vaccination.

6.4.3 Systemic adverse events

Systemic AEFI occurred in 60% (95% CI 55-64%) of respondents within the seven days following immunisation. Headache (44%; 95% CI 40-48%) and lethargy (43%; 95% CI 38–47%) were most commonly reported. Joint pain was experienced by 25% (95%; CI 21-29%) and fever was reported by 17.2% (95%; CI 14-21%) of respondents (**Table 6.3**). Females were significantly more likely to report experiencing any systemic vaccine reaction, and more specifically, lethargy (**Table 6.3**).

Table 6.3 Systemic adverse events experienced by Q fever vaccinees. The odds ratio for reporting "yes" for each adverse event is shown for females versus males.

	All Respondents	***Females***	***Males***
Any Systemic AEFI			
Yes n (%)	297 (59.5)	263 (62.0)	34 (45.9)
No n (%)	202 (40.5)	161 (38.0)	40 (54.1)
Column Total n (%)	499 (100)	424 (100)	74 (100)
OR (95% CI; p-value)	-	1.9 (1.1-3.1; 0.016)	ref
Fever			
Yes n (%)	86 (17.2)	76 (17.9)	10 (13.5)
No n (%)	409 (82.0)	344 (81.1)	64 (86.5)
Not Specified n (%)	4 (<1)	4 (<1)	0 (0)
Column Total n (%)	499 (100)	424 (100)	74 (100)
OR (95% CI; p-value)	-	1.4 (0.7-2.8; 0.384)	ref
Headache			
Yes n (%)	219 (43.9)	194 (45.8)	25 (33.8)
No n (%)	276 (55.3)	226 (53.3)	49 (66.2)
Not Specified n (%)	4 (<1)	4 (<1)	0 (0)
Column Total n (%)	499 (100)	424 (100)	74 (100)
OR (95% CI; p-value)	-	1.6 (1.0-2.8; 0.058)	ref
Lethargy			
Yes n (%)	213 (42.7)	190 (44.8)	23 (31.1)
No n (%)	283 (56.7)	233 (55.0)	49 (66.2)
Not Specified n (%)	3 (<1)	1 (<1)	2 (2.7)
Column Total n (%)	499 (100)	424 (100)	74 (100)
OR (95% CI; p-value)	-	1.7 (1.0-3.0; 0.048)	ref
Joint Pain			
Yes n (%)	123 (24.6)	112 (26.4)	11 (14.9)
No n (%)	374 (74.9)	311 (73.3)	62 (83.8)
Not Specified n (%)	2 (<1)	1 (<1)	1 (1.4)
Column Total n (%)	499 (100)	424 (100)	74 (100)
OR (95% CI; p-value)	-	2.0 (1.0-3.9; 0.055)	ref

OR; Odds Ratio. CI; Confidence interval. Ref; reference category for odds ratio. Odds ratio adjusted for year and location of vaccination.

6.4.4 Medical attention following vaccination

Medical attention for AEFI was sought from a health provider by 19/499 (3.8%) respondents. Of these, nine sought attention for both local ISRs and systemic AEFI, six sought attention for only local ISRs, and four sought attention for only systemic AEFI. These vaccinees sought help from general practitioners (n = 7), university health services (n = 7), and pharmacists (n = 3). One sought help from a doctor within their family, and one (0.2%) presented to an emergency department experiencing a pronounced (grade 4) injection site reaction and all four systemic events. Data on the outcome of this last patient were not available through the survey.

6.5 Discussion

This study successfully recruited a large number of young adults to provide detailed data for adverse events following Q fever immunisation with Q-VAX®. Further supporting data are provided regarding the safety of this vaccine in this age bracket, with an emphasis on young adult females who have been under-represented in previous reports. The overall proportion of respondents in this study that experienced local and/or systemic adverse events exceeded pre-licensure clinical trial data reported for Q-VAX® (**Table 6.4**). Schoffelen, Wong, et al. (2014) similarly reported a higher proportion of AEFI following Q-VAX® (**Table 6.4**). These increased AEFI may be explained by demographic, social and educational factors, and methodology.

Table 6.4 Summary of the proportion of overall respondents experiencing acute adverse events following Q fever vaccination in the current study and other available published data for Q-VAX®.

	Independent Data		***Clinical Trial Data***	***Registration Holder Surveillance Data***
	Current Study	**Schoffelen, Wong, et al. (2014)**	**Marmion et al. (1990)**	**Seqirus (2016)**
Study population	Veterinary students; median age 18 years, predominantly female	Persons in community with high risk of Q fever due to comorbidities; median age 67 years	Abattoir workers; median age 29 years, predominantly male	Not applicable
Number of vaccinees for which AEFI results are reported	499	970	464	Not applicable
Any local or systemic AEFI	98%	82%	*	*
Any Local ISR	98%	80%	*	*
Injection Site Pain	95%	*	48%	≥10%
Injection Site Swelling	58%	*	*	≥10%
Injection Site Erythema	58%	*	33%	≥10%
Any Systemic AEFI	60%	43%	*	*
Headache	44%	*	9%	<10% and ≥1%
Lethargy	43%	*	*	<1% and ≥0.1%
Joint Pain	25%	*	*	<0.01%
Fever	17%	9%	0.2%	<1% and ≥0.1%

* Data not published. ISR; Injection site reaction; AEFI; adverse event following immunisation.

Both this study and Schoffelen, Wong, et al. (2014) identified that a greater proportion of females reported local and systemic AEFI, and AEFI of a higher severity, following Q fever vaccination compared to males. Gidding et al. (2009) also identified a 2:1 ratio of females to males in passive adverse event notifications to the DAEN surveillance system during Australia's NQMP from 2001 – 2004. These findings provide evidence that females are more likely to experience AEFI than males and explains why the overall proportion of vaccinees experiencing AEFI in this cohort of predominantly female vaccinees was higher than pre-licensure clinical trials, where the majority of vaccinees were male. Females also experienced more severe AEFI symptoms in these studies, with the exception of Gidding et al. (2009) where males reported more severe symptoms. The latter finding was drawn from passive surveillance and may reflect a decreased propensity for males to report less severe AEFI than females.

Gender based differences in vaccine reactogenicity are reported for many vaccines with predominantly females reporting increased AEFI (Beyer, Palache, Kerstens, & Masurel, 1996; Cook, 2014). The cause of this difference is multifactorial and hypotheses include (1) elevated humoral and cell-mediated immune responses in females in response to vaccination, (2) increased perception of pain in females, (3) increased likelihood of subcutaneous rather than intramuscular deposition of vaccines in females due to a thicker subcutaneous layer than males, and (4) the social expectations of males to be stoic and pain tolerant (Beyer et al., 1996; Cook, 2014; Fink & Klein, 2015; Klein, Jedlicka, & Pekosz, 2010).

The immune response to *C. burnetii* is strongly influenced by gender, with the majority of genes modulated following infection being sex-dependent (Textoris et al., 2010). Cell mediated immunity [CMI] plays an essential role in the control of early *C. burnetii* infection (Andoh et al., 2007). Compared to males, females exhibit stronger CMI responses and have reduced bacterial numbers following *C. burnetii* infection (Leone et al., 2002). However, CMI is associated with more severe ISRs following immunisation (Cook, 2014; Klein et al., 2010), which explains why females exhibited increased ISRs of greater severity following Q fever vaccination. While the proportion experiencing severe ISRs (30%) may have been

exaggerated due to self-reporting from recall, the result is comparable to that observed by Schoffelen, Wong, et al. (2014) who reported these reactions in 20% of all respondents from a cohort comprised of older patients with significant comorbidities, including immunosuppression. Gender differences in immune response also contribute to males more often exhibiting clinical Q fever disease of greater severity following natural infection (Textoris et al., 2010).

Age also influences immune responses, with younger age appearing protective against clinical Q fever disease following *C. burnetii* infection (Angelakis & Raoult, 2010). This can be explained by a decline in T cell function with increasing age (Moro-García, Alonso-Arias, & López-Larrea, 2013). As T cells are essential for *C. burnetii* clearance (Andoh et al., 2007), a more robust response in younger people provides immune protection from disease, but may also increase the likelihood and severity of AEFI following vaccination. Schoffelen, Wong, et al. (2014) reported increased AEFI in the younger (<50 years) non-immune suppressed cohort of vaccinees; exceeding 90% for females in this category. The cohort in this current study consisted of young adults, and age may have contributed to the increased AEFI reported. However, the effect of gender appears to be more important as pre-clinical vaccine trials also included younger workers.

Educational and social factors may have contributed to the increase in AEFI reported here compared to pre-licensure clinical trials. The vaccinees in this study were university students with an interest in science and medicine. They were likely more educated than the vaccinees in the pre-licensure clinical trials, most of whom were abattoir workers (Marmion et al., 1990), and may have been more highly motivated to observe and report even minor adverse events, contributing to the increase in AEFI reported here. Indeed, medical students participating in vaccine studies have similarly demonstrated increased reporting of AEFI (Beyer et al., 1996). Additionally, young adults are more likely to experience health anxiety, which can lead to exaggeration of symptoms (Gerolimatos & Edelstein, 2012). This may have contributed to the number of respondents seeking medical attention for their vaccine reactions. Young adults demonstrated

increased healthcare seeking behaviours for AEFI following the tetanus and diphtheria-toxoid vaccine (Jackson et al., 2009), and such behaviour may have been further exaggerated for this cohort if they had recently moved away from home and support networks to commence their studies.

The main limitation of this study was the response rate of 60%, which may have favoured participation from vaccinees that experienced an AEFI, and from females generally as they are known to be more likely to participate in surveys (Cook, 2014; Fink & Klein, 2015). Indeed, Schoffelen, Wong, et al. (2014) reported a response rate of 71% (74% for females; 68% for males) and identified that 80% of the respondents had experienced local AEFI, compared to only 31% of vaccinees who did not respond but were questioned at a later follow-up. Thus, the overall frequency of AEFI may be inflated by an over-representation of symptomatic respondents and under-representation of males generally, who would have been more likely to be asymptomatic. However, the difference between females and males may be even more pronounced than that reported due to under-representation of males. As the gender of vaccinees was not recorded at the time of enrolment in this study, and AEFI data for non-responders is not available, it is not possible to assess the extent of gender bias in these results. Some bias is expected, as 85% of respondents were female compared to 79% of veterinary students commencing their studies between 2013 and 2016 in Australian universities (Department of Education Skills and Employment, 2013 - 2016). Consequently, these results represent a worst-case scenario for the cohort studied. The best-case scenario reflects a frequency of 59% (489/827) for injection site reactions and 36% (297/827) for systemic reaction. This assumes all non-responders were asymptomatic, which is unlikely, and still results in a higher reported frequency of AEFI than pre-licensure trials.

Information collected from vaccinees was limited to one week following vaccination. A serious AEFI will not have been captured in this study if it occurred more than one week following vaccination or resulted in the vaccinee being too ill to participate in the survey. One vaccinee did report seeking medical attention at a hospital emergency department, which may have been classified as serious if hospitalisation was

required; however, the outcome of this patient was not captured in this survey. A search of the DAEN revealed 33 case reports of adverse events, none of which recorded death as an outcome following administration of Q-VAX® from January 2013–June 2016; the time frame in which this study was undertaken (Therapeutic Goods Administration, 2017). This study also lacked a control cohort to assess background rates of the symptoms of interest, which may have been coincidental to vaccination due to other causes (World Health Organization, 2018). Given the anatomical relationship of injection site reactions, this limitation is most applicable to systemic events reported.

6.6 Conclusions

These data contribute useful information on the safety profile of Q-VAX® in young adults, with an emphasis on females who have been under-represented in previous studies and for whom detailed AEFI data has not been specifically reported. Q-VAX® was found to be reactogenic among respondents, and a high frequency of vaccine reactions should be expected in young adults, particularly females. However, AEFI were mostly non-severe and few vaccinees sought medical attention. Ideally, a less reactogenic but equally effective Q fever vaccination is needed. Until such a vaccine is available, the high likelihood of experiencing transient non-severe adverse events following Q fever immunisation should not deter people from seeking vaccination, as the consequences of Q fever disease are potentially far more debilitating. These results are important for policymakers and healthcare providers as they provide further safety data on young adults and females and would be useful if a trial of this vaccine in younger adolescents and children was to be considered in the future.

7 Conclusions and Future Directions

This research provides critical new insights into *C. burnetii* exposure and Q fever vaccination in Australia's veterinary workforce:

	Contribution	***Conclusions***	***Limitations***	***Future directions***
Chapter 2	First study to investigate knowledge and attitudes regarding Q fever disease and vaccination, and to quantify Q fever vaccine uptake in veterinary workers in Australia.	There is a need for increased Q fever knowledge in veterinary workers. There is a lack of awareness of the Q fever vaccine and shortfall in vaccine uptake in veterinary nurses.	Survey responses may be inaccurate. Difficulty accessing the veterinary nursing cohort; sample of these workers was older and more highly educated than those reflected in government statistics.	Investigation into the prevalence and adequacy of WH&S protocols, with regards to Q fever, in Australian veterinary clinics. Follow-up studies to measure changes knowledge and attitudes and vaccine uptake over time where strategies are implemented for improvement.
Chapter 3	First study to investigate factors associated with Q fever vaccine uptake in veterinary nurses and recommendation by veterinarians in Australia.	Improvements in WH&S compliance and culture in Australian veterinary practices could increase Q fever vaccine awareness and uptake.		
Chapter 4	Reports current *C. burnetii* seroprevalence and exposure risk factors in veterinary workers in Australia.	Rural locations present an increased risk for *C. burnetii* exposure in veterinary workers, independent of exposure to ruminants. Most unvaccinated workers are potentially eligible for Q fever vaccination despite many years working with animals. Recommendation for the Q fever vaccine should be broadened to include veterinary support staff generally, encompassing administration, kennel hands, volunteers, etc.	Findings may not be generalizable to all veterinary workers. Veterinary support staff were under-represented and mostly clustered within two workplaces.	Further seroprevalence studies in a larger sample of veterinary support workers.

	Contribution	*Conclusions*	*Limitations*	*Future directions*
Chapter 5	First study to report immune responses following Q fever vaccination in veterinary workers, and the largest study to report seroprevalence and serological profiles in vaccinated persons beyond 5-years post-vaccination.	Despite a low prevalence of positive serological and IGRA results, and little evidence of serological boosting from re-exposure, Q fever disease was not reported in any vaccinated veterinary workers. Current understanding of the longevity of Q fever vaccine immunity, which is based on studies in predominantly male abattoir workers, may not translate to other at-risk professions.	Year of Q fever vaccination was self-reported. IGRA results could not be interpreted with confidence.	Further studies are recommended to understand the longevity of vaccine-induced immune responses in veterinary workers, particularly cell mediate responses, and immune responses observed with re-exposure to *C. burnetii* in vaccinated persons.
Chapter 6	Bridges a gap in knowledge of adverse events following immunisation (AEFI) for Q-VAX® in young adult females.	A higher frequency of AEFI, and AEFI of greater severity should be expected in females than in males. The risk-benefit profile remained positive.	AEFI were self-reported. Limited to one week following vaccination. Serious AEFI may not have been captured. Lack of control cohort.	Investigation of AEFI in younger adolescents. Development of a less reactogenic vaccine.

7.1 Complacency towards workplace health and safety

Chapters Two and Three investigated the knowledge and attitudes of Australian veterinary workers regarding Q fever disease and vaccination, quantified Q fever vaccine uptake, and established positive influences and barriers to vaccination, with veterinarians and veterinary nurses investigated as unique cohorts. These chapters provide further evidence of complacency towards WH&S in the veterinary industry in Australia, as previously described by Attard et al. (2012). A low level of vaccine uptake was identified in Australia's veterinary nurses (29%) and in veterinarians who had graduated from international veterinary schools (33%), with key barriers including a lack of awareness of the Q fever vaccine and a perception that they would not be seriously affected by Q fever. This was in stark contrast

to uptake in veterinarians graduating from Australian veterinary schools (78%), which was primarily driven by the improved vaccine outcomes with mandatory vaccination programs in Australian veterinary schools. The Australian government funded NQFMP is another example of a successful vaccination program in at-risk cohorts, which targeted abattoir workers and farmers and their families from 2002 – 2006 (Gidding et al., 2009). Such programs provide a clear WH&S protocol and *status quo* among peers in support of vaccination, whilst reducing barriers including expense, time, and access to a provider. In the absence of such vaccination programs, the vaccination of veterinary nurses, support staff and international graduate veterinarians is reliant on their individual experiences with education and employment awareness of the existence of the Q fever vaccine, and their ability to seek out and afford vaccination.

A significant contribution of this work was the identification of the importance of workplace culture as a driver of vaccine uptake. Whilst most veterinary workers reported veterinarians and workplace protocols as the most influential sources of biosecurity information, workplace culture was the only source of biosecurity information significantly associated with vaccine uptake. This not only highlights the importance of WH&S culture, but also supports the conclusions of Attard et al. (2012) that there is an absence of standard protocols for infection control and little emphasis on measures to reduce the prevalence of zoonotic disease transmission in Australian veterinary clinics. However, Chapter 3 identified corporate practices are a likely exception which, alongside government agencies and universities, were positively associated with Q fever vaccine uptake.

Whilst industry recommendations are available for WH&S protocols, including the Australian Veterinary Association Guidelines for Veterinary Personal Biosecurity, compliance of individuals with recommended infection control protocols is generally poor in the veterinary industry and only marginally increased with multimodal educational campaigns (Dowd et al., 2013; Willemsen et al., 2019). Veterinary support staff may also prove difficult to access for health promotion in the absence of mandatory registration and where there is low subscription to industry groups, such as the Veterinary Nurses Council of Australia.

Hence, it is vital that individual veterinary practices implement strict Q fever vaccine policies to comply with WH&S legislation, such as those at Australia's veterinary schools where Q fever vaccination is mandated unless pre-existing immunity is demonstrated, or medical exemption is granted (Charles Sturt University, 2021; James Cook University, 2020; Murdoch University, 2018; University of Adelaide, 2021; University of Melbourne, 2021; University of Queensland, 2020; University of Sydney, 2021). Of interest, the University of Sydney has recently transitioned away from providing an organised Q fever vaccination program and veterinary students are now required to arrange their own vaccination at least two weeks prior to commencement of studies (University of Sydney, 2021). This change may lead by example for the industry, as it highlights the absolute importance of the vaccine prior to animal contact and sets the expectation for individuals to take responsibility for their own health. If veterinary clinics followed this approach with staff, they may similarly achieve their desired vaccine outcomes at the local level. Indeed, this would be similar to the Australian government's successful "no jab no pay" approach to childhood immunisations (Hull, Beard, Hendry, Dey, & Macartney, 2020), as monetary outcomes (employment) would depend on appropriate vaccination status.

Strong leadership from industry organisations, such as the AVA and state veterinary boards, and support from state government work safety departments regarding Q fever vaccine policy should continue. However, further actions are clearly required to achieve compliance. As recommended by the Australian Veterinary Association (2018), the addition of the current Q fever vaccine to the Pharmaceuticals Benefits Scheme needs to be considered, which may reduce the complexity and costs of mandating vaccination, particularly in small businesses. Increased application of compliance and enforcement tools by state work safety departments may be required, such as improvement or infringement notices, enforceable undertakings, and civil or criminal prosecutions (Safe Work Australia, 2011). Finally, the Australian Government's recent announcement of a boost to funding for the development of a new Q fever vaccine holds promise for the future, as it aims to reduce barriers to vaccination by negating the need for pre-

vaccination testing; this should result in an efficacious user-friendly vaccine that is more widely available, less expensive, and which requires less time commitment from vaccinees and providers (Gunders & Phillips, 2020).

7.2 *Coxiella burnetii* exposure in today's veterinary workforce

Previously, Giesecke and Barton (1993) identified mixed animal practice as a significant risk factor for *C. burnetii* exposure in veterinary workers. However, from 1970 onwards the veterinary workforce has seen significantly fewer veterinarians working with cattle and in mixed animal practice over time, with a growth in demand for small animal practices (Australian Veterinary Association, 2015). Despite this trend, *C. burnetii* seroprevalence has not declined, with the current research identifying a seroprevalence of 19% in unvaccinated workers, increased from 13.2% in 1992 (Giesecke & Barton, 1993). Whilst ruminant species remained the greatest risk for exposure via direct occupational contact in the current research, the findings add to the increasing body of evidence that other species, and indirect or non-occupational animal exposures may be an increasing source of *C. burnetii* exposure in Australia (Archer et al., 2017; Bond et al., 2016; Gibbons & White, 2014; Kopecny et al., 2013; Malo et al., 2018; Massey et al., 2009; Sloan-Gardner et al., 2017). This is further supported by the association identified between rurality and *C. burnetii* exposure independent of time spent working directly with ruminant species. A limitation of this study was the absence of questioning on potential for non-occupational or indirect exposure to *C. burnetii*, which should be considered in future research.

Those returning a serological profile of recent *C. burnetii* exposure were younger, with veterinary support workers over-represented; highlighting the importance of vaccination of all employees prior to commencing work in veterinary facilities and complementing earlier recommendations for strict workplace vaccination policies. However, as most unvaccinated workers were seronegative at the time of sampling and therefore potentially eligible for Q fever vaccination, increasing time spent working within

the veterinary industry should not preclude seeking vaccination. Importantly, broadening of the Australian Immunisation Handbook recommendation should be considered to list veterinary workers, veterinary students and volunteers in veterinary facilities generally, rather than current specific recommendations for "veterinarians, veterinary nurses and veterinary students" (Australian Technical Advisory Group on Immunisation, 2018). This would encompass workers and volunteers in support roles, such as kennel hands and administration workers that are not clearly identified in the current recommendations, which could facilitate improved compliance with WH&S regulations in veterinary businesses. Though, a larger sample of support workers than was achieved in this study may be considered necessary to inform such a recommendation.

Contradictory to the observed seroprevalence, the proportion of participants reporting a Q fever diagnosis was reduced from 4.4% in 1992, which were reported predominantly in meat inspectors (Giesecke & Barton, 1993), to 1.5% in unvaccinated veterinary workers participating in the current seroprevalence study, despite 7.1% of the former cohort having been vaccinated for Q fever. This may suggest that a shift towards *C. burnetii* exposure via non-ruminant species, indirect contact or non-occupational exposure may be associated with decreased risk for, or severity of clinical Q fever. However, this current study identified Q fever disease in an administrative worker with no occupational contact with animals, and outbreaks associated with cats and a dog report serious clinical consequences (Gibbons & White, 2014; Kopecny et al., 2013; Malo et al., 2018). Hence, this finding may instead reflect under-diagnosis of Q fever due to a lower suspicion in workers not directly involved with ruminant species, which may be a symptom of deficiencies in the diagnosis and management of Q fever by medical practitioners in Australia (Hess et al., 2011; Lindsay, Rohailla, & Miyakis, 2018).

Lindsay et al. (2018) identified gaps in knowledge of the epidemiology and diagnosis of acute Q fever among medical clinicians practicing in the Northern NSW Local Health District. In the cohort (n = 45), who were mostly hospital based; 37% were not aware of the Q fever vaccine and more than half were not

aware of the potential for long-term complications of Q fever. Hess et al. (2011) reported cardiac history being taken in less than 10% of Q fever patients in NSW in 2005 – 2006, and fewer having had a complete cardiac exam performed. These findings are alarming, given the severe impact persistent infection and Q fever fatigue syndrome have on the lives of those affected; post-infection debility was self-reported in 37.5% of Australian veterinary workers with a diagnosis of Q fever in the Giesecke and Barton (1993) study. Collectively, these studies suggest a need for further education of medical professionals in Australia in the prevention, diagnosis and management of Q fever.

Increased seroprevalence coupled with under-vaccination of the predominantly female veterinary nursing cohort also raises concerns for Q fever and pregnancy in this workforce. A survey of Australian female veterinarians graduating from 1960 – 2000 identified an increased risk of preterm delivery, spontaneous abortion and birth defects compared to the general population. Exposure to anaesthetic gases, radiation, pesticides, cytotoxic drugs, increased working hours, and large animal practice were identified as risk factors (Shirangi, Bower, Holman, Preen, & Bruce, 2014; Shirangi, Fritschi, & Holman, 2008, 2009; Shirangi, Fritschi, Holman, & Bower, 2009). However, these studies did not investigate zoonotic pathogens as risk factors nor consider Q fever vaccination status. Similarly, adverse health outcomes associated with pregnancy or other debility and fatigue were not considered in the current research. Given the underdiagnosis of Q fever and lack of routine consideration for *C. burnetii* in pregnancy complications in Australia (Marks & Olenski, 2019), this may reflect a missed opportunity for the investigation of an association between *C. burnetii* exposure and obstetrical complications in the Australian setting. Elsewhere, studies of pregnancy outcomes in veterinary workers report mixed findings (Meisner, Vora, Fuller, Phipps, & Rabinowitz, 2018), and the need for a greater understanding of reproductive health hazards in veterinary workers, including zoonoses, and with improved representation of veterinary support staff, is increasingly recognised (Scheftel, Elchos, Rubin, & Decker, 2017). This is supported by research from Denmark in which a review of national notification data for the period 2007 – 2011

identified 19 pregnancies in 12 women with positive or equivocal *C. burnetii* serology. All were farmers or cattle veterinarians, four experienced obstetrical complications across nine pregnancies of whom three were veterinarians (Nielsen et al., 2014).

7.3 Post-vaccination immune responses

Chapter Five demonstrated that vaccine-induced antibody responses were short-lived in the majority of the veterinary cohort, with little evidence of serological boosting from re-exposure at the time point of sampling. The serological profiles described in the current study aid in the interpretation of serological profiles previously observed in pre-licensure clinical trials (Marmion et al., 1990); the higher seroprevalence reported in abattoir workers in clinical trials is likely attributed to re-current *C. burnetii* exposure, confirming that the findings of the clinical trials may not translate to other occupational cohorts and the wider community. While no vaccinated workers reported having had Q fever, concern for waning immunity following Q fever vaccination was recently raised by Rahaman et al. (2019). Collectively, these findings question the current understanding of the longevity of immunity from Q-VAX® in the absence of re-exposure, which will have implications for vaccine protocols given repeat vaccination is currently contraindicated (Australian Technical Advisory Group on Immunisation, 2018).

Concerns for the vaccine process were also raised by Woldeyohannes et al. (2020) following Q fever notifications in persons previously deemed immune by the pre-vaccination screening process. This may have occurred if the natural immunity detected at pre-screen had subsequently waned and re-challenge resulted in clinical illness. Alternatively, it may reflect a failure of the pre-screening methodology, as the results of the skin test are subjective, discordant results between laboratories for serology by IFA is reported (Graves et al., 2018; Healy et al., 2011), cross-reactions with *Legionella* and *Bartonella* have been described with serology (La Scola & Raoult, 1996; Musso & Raoult, 1997), and it is unknown whether *Coxiella*-like bacteria (CLB) cross-reactions occur in *C. burnetii* serological tests in Australia (Oskam et al.,

2018). This is concerning with respect to the research presented in Chapter Two, as 12% (99/587) of veterinarians and 5% (37/162) of veterinary nurses reported being unable to be vaccinated due to a positive pre-vaccination screening result, and these workers may be unknowingly susceptible to Q fever.

Quantitative assessment of cell-mediated immune responses will be critical to further understanding the longevity of post-vaccination immunity in cohorts with varied opportunity for re-exposure, and for informing decisions to vaccinate. Whilst this study utilised the commercially available Q-detect™ IGRA developed in the Netherlands, the results were disappointing and unreliable after modifying the stimulation method for PBMCs rather than fresh whole blood. However, a preliminary study utilising Q-detect™ in a cohort of attendees at an Australian scientific conference demonstrated positive results in two of seven previously vaccinated persons, borderline results in the remaining five vaccinated persons, and a strong positive result in one participant with prior Q fever infection (Graves et al., 2018). Hence, Q-detect™ or other IGRA should not be discounted for use in future studies investigating cell mediated immunity post-vaccination. However, the methods should be validated for use post-vaccination versus use in the detection of natural exposure.

Future studies could repeat the sampling approach described in Chapter Five or undertake a longitudinal cohort study, though the latter would require an extended period of time. Should such studies similarly report a low prevalence of positive results for IGRA, re-challenge via the Q-VAX® Skin-test, which is considered safe in immune individuals, may aid in determining the extent of immune memory if vaccinees exhibit exaggerated responses compared to a control cohort. Should future studies or other data establish waning immunity over time, vaccination protocols may need to evolve to include the routine re-evaluation of immunity utilising a combination of serology and IGRA in individuals either vaccinated or previously deemed immune from prior exposure. While re-vaccination with Q-VAX® is not currently recommended, repeat vaccination may require consideration following individual assessment of immune status, perhaps

with reduced doses such as that administered in the skin test or to people returning indeterminate pre-vaccination screening results (Australian Technical Advisory Group on Immunisation, 2018).

7.4 Adverse events following immunisation [AEFI]

Chapter Six provided further AEFI data for Q-VAX®, with greater representation of younger adults and females than in previous studies. The study identified that a higher frequency of suspected adverse events, and adverse events of greater severity can be expected in females than in males following Q-VAX®. However, reported AEFI were non-serious, and the risk-benefit remains positive as the consequences of Q fever disease are potentially far more debilitating. These findings provide further data to support the extension of the existing vaccine more broadly within at-risk communities in regional and rural areas.

In response to Q fever notifications in children (Tozer et al., 2011) and a push from farmers for Q fever vaccination in children (Becker, 2017), these AEFI data have bridged a gap in knowledge to permit investigations into the use of Q-VAX® in children under 15 years of age. A small clinical trial for the assessment of the safety and immunogenicity of Q-VAX® in children aged 10 to 15 years was implemented through the Children's Hospital at Westmead (Australian New Zealand Clinical Trials Registry, 2017) and building on this trial, an Australian government Medical Research Future Fund grant was awarded in June 2020 to the University of Sydney for further investigation of Q fever vaccination in rural adolescents (Hunt, 2020). These ongoing studies should also benefit young adolescents that begin agricultural studies at secondary school, and those undertaking paid work, work-experience, or volunteering in animal industries.

7.5 Dissemination of research

The research presented in this thesis is a noteworthy example of collaboration between the veterinary and medical professions in a time when a One Health approach is increasingly important. The findings have been published as four research articles in high impact, peer reviewed journals (Sellens et al., 2020;

Sellens, Bosward, et al., 2018; Sellens et al., 2016; Sellens, Norris, et al., 2018), with at least one further publication expected. Communication of this research directly to Australia's veterinary profession has occurred through oral presentations at National and Pan-Pacific AVA conferences (see Contributions to Conference Proceedings), a Webinar hosted via the AVA Public Health Interest Group, and a short piece in the AVA national newsletter prompting veterinarians not to forget their nurses regarding Q fever awareness and prevention. Further dissemination to both the veterinary and medical professions has been achieved through poster presentations at domestic and international conferences, including those hosted by the Australian Infectious Disease Society, The Marie Bashir Institute for Infectious Diseases and Biosecurity, the International Society for Veterinary Epidemiology and Economics, and the International Association for Ecology and Health (see Contributions to Conference Proceedings). The presence of our research team at veterinary conferences to undertake blood sampling and survey the knowledge attitudes and perceptions of veterinarians, has also raised awareness of Q fever and invited conversations from attendees. Across all formats, we have been met with enthusiasm from our audience to learn more and act where possible. It is hoped that this enthusiasm continues, and that these research findings are embraced by key stakeholders in the veterinary industry in particular. Above all else, the findings should contribute to improving vaccine uptake in the wider profession, as prevention is better than a cure, and for some Q fever patients a cure may never come.

8 References

Abe, T., Yamaki, K., Hayakawa, T., Fukuda, H., Ito, Y., Kume, H., . . . Hirai, K. (2001). A seroepidemiological study of the risks of Q fever infection in Japanese veterinarians. *European Journal of Epidemiology, 17*(11), 1029-1032.

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.

Aderem, A., & Underhill, D. M. (1999). Mechanisms of phagocytosis in Macrophages. *Annual Review of Immunology, 17*, 593-623.

Amano, K., & Williams, J. C. (1984). Chemical and Immunological Characterization of Lipopolysaccharides from Phase I and Phase II *Coxiella burnetii*. *Journal of Bacteriology, 160*(3), 994-1002. doi:10.1128/JB.160.3.994-1002.1984

Amara, A. B., Bechah, Y., & Mege, J. L. (2012). Immune response and *Coxiella burnetii* invasion. *Advances in Experimental Medicine and Biology, 984*, 287-298. doi:10.1007/978-94-007-4315-1_15

Amara, A. B., Ghigo, E., Priol, Y. L., Le´polard, C., Salcedo, S. P., Lemichez, E., . . . Mege, J.-L. (2010). *Coxiella burnetii*, the agent of Q fever, replicates within trophoblasts and induces a unique transcriptional response. *PloS One, 5*(12), e15315. doi:10.1371/journal.pone.0015315

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. doi:10.1093/cid/ciu151

Anderson, A., Bijlmer, H., Fournier, P. E., Graves, S., Hartzell, J., Kersh, G. J., . . . Sexton, D. J. (2013). Diagnosis and management of Q fever--United States, 2013: recommendations from CDC and the Q Fever Working Group. *MMWR: Recommendations and Reports, 62*(RR-03), 1-30. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23535757

Andoh, M., Zhang, G., Russell-Lodrigue, K. E., Shive, H. R., Weeks, B. R., & Samuel, J. E. (2007). T cells are essential for bacterial clearance, and gamma interferon, tumor necrosis factor alpha, and B cells are crucial for disease development in *Coxiella burnetii* infection in mice. *Infection and Immunity, 75*(7), 3245-3255. doi:10.1128/IAI.01767-06

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. doi:10.3201/eid2202.150106

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

Angelakis, E., & Raoult, D. (2010). Q Fever. *Veterinary Microbiology, 140*(3-4), 297-309. doi:10.1016/j.vetmic.2009.07.016

Angelakis, E., & Raoult, D. (2011). Emergence of Q Fever. *Iranian Journal of Public Health, 40*(3), 1-18.

Archer, B. N., Hallahan, C., Stanley, P., Seward, K., Lesjak, M., Hope, K., & Brow, 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.

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

Armitage, C. J., & Conner, M. (2000). Social cognition models and health behaviour: A structured review. *Psychology & Health, 15*(2), 173-189. doi:10.1080/08870440008400299

Armitage, C. J., & Connor, M. (2001). Efficacy of the Theory of Planned Behaviour: A meta-analytic review. *British Journal of Social Psychology, 40*, 471-499.

Armstrong, M., Francis, J., Robson, J., Graves, S., Mills, D., Ferguson, J., & Nourse, C. (2019). Q fever vaccination of children in Australia: Limited experience to date. *Journal of Paediatrics and Child Health*. doi:10.1111/jpc.14364

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

Arricau-Bouvery, N., Souriau, A., Bodier, C., Dufour, P., Rousset, E., & Rodolakis, A. (2005). Effect of vaccination with phase I and phase II *Coxiella burnetii* vaccines in pregnant goats. *Vaccine, 23*(35), 4392-4402. doi:10.1016/j.vaccine.2005.04.010

Asseldonk, M. A. P. M. v., Prins, J., & Bergevoet, R. H. M. (2013). Economic assessment of Q fever in the Netherlands. *Preventive Veterinary Medicine, 112*(1-2), 27-34. doi:10.1016/j.prevetmed.2013.06.002

Attard, K., Burrows, E., Kotiranta-Harris, K., Hedlefs, R., Ketheesan, N., & Govan, B. (2012). Veterinary infection control in Australia: is there control? *Australian Veterinary Journal, 90*(11), 438-441. doi:10.1111/j.1751-0813.2012.00971.x

Australian Bureau of Statistics. (1st June 2017). Australian Statistical Geography Standard (ASGS). Retrieved from http://www.abs.gov.au/websitedbs/D3310114.nsf/home/Australian+Statistical+Geography+Standard+(ASGS)

Australian Bureau of Statistics. (2014a). ABS Labour Force Survey: Veterinarian. Retrieved from http://docs.employment.gov.au/system/files/doc/other/234711veterinarianaus_0.pdf

Australian Bureau of Statistics. (2014b). ABS Labour Force Survey: Veterinary Nurses. Retrieved from http://joboutlook.gov.au/occupation.aspx?search=alpha&tab=stats&cluster=&code=3613

Australian Bureau of Statistics. (2018, 16 March 2018). Remoteness Structure, July 2016. *Australian Statistical Geography Standard (ASGS): Volume 5.* Retrieved from http://www.abs.gov.au/AUSSTATS/abs@.nsf/Lookup/1270.0.55.005Main+Features1July%202016?OpenDocument

Australian Government Department of Health. Australian National Notifiable Diseases Surveillance System. Retrieved from http://www9.health.gov.au/cda/source/rpt_2_sel.cfm

Australian Government Department of Health. (2017). Number of notifications of Q fever, Australia, in the period of 1991 to 2016 and year-to-date notifications for 2017. *National Notifiable Diseases Surveillance System.* Retrieved from http://www9.health.gov.au/cda/source/rpt_3_sel.cfm

Australian Government Department of Health. (2019, 24th April 2019). Australian national notifiable diseases by disease type. *National Notifiable Diseases Surveillance System (NNDSS).* Retrieved from https://www1.health.gov.au/internet/main/publishing.nsf/Content/cda-surveil-nndss-casedefs-distype.htm

The Australian Immunisation Handbook. (2013). (10th ed.). Canberra, Australia: Australian Government Department of Health and Ageing.

Australian Industry and Skills Commitee. (2020, 05 November 2020). Veterinary Nursing. Retrieved from https://nationalindustryinsights.aisc.net.au/industries/animal-care-and-management/veterinary-nursing

Australian Meat Processor Corporation. (2021). Australian Q fever Register. Retrieved from https://www.qfever.org/

Australian New Zealand Clinical Trials Registry. (2017). Trial Review: ACTRN12617000375358. Retrieved from https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=372443. (ACTRN12617000375358). from Australian New Zealand Clinical Trials Registry https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=372443

Australian Technical Advisory Group on Immunisation. (2018). *Australian Immunisation Handbook*. Canberra: Australian Government Department of Health Retrieved from https://immunisationhandbook.health.gov.au/

Australian Veterinary Association. (2011). Guidelines for Veterinary Personal Biosecurity. In: Australian Veterinary Association Limited.

Australian Veterinary Association. (2012). *Australian veterinary workforce survey 2012*. Australian Veterinary Association Limited

Australian Veterinary Association. (2013). *Australian veterinary workforce survey 2013*. Australian Veterinary Association Limited

Australian Veterinary Association. (2014). *Australian veterinary workforce survey 2014*. Australian Veterinary Association Limited

Australian Veterinary Association. (2015). *The Australian Veterinary Association Report on Projection Modelling for the Veterinarian Workforce*. The Australian Veterinary Association Limited

Australian veterinary Association. (2017). Guidelines for Veterinary Personal Biosecurity. In (Vol. 3rd Edition). St. Leonards, NSW: Australian veterinary Association.

Australian Veterinary Association. (2018). Q Fever protection in veterinary practice. In (Vol. 07 Dec 2018). St. Leonards, NSW: Australian Veterinary Association.

Baca, O. G., Akporiaye, E. T., Aragon, A. S., Martinez, I. L., Robles, M. V., & Warner, N. L. (1981). Fate of Phase I and Phase II *Coxiella burnetii* in Several Macrophage-Like Tumor Cell Lines. *Infection and Immunity, 33*(1), 258-266. doi:10.1128/IAI.33.1.258-266.1981

Baca, O. G., & Paretsky, D. (1983). Q Fever and *Coxiella burnetii*: a Model for Host-Parasite Interactions. *Microbiological Reviews, 47*(2), 127-149.

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

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*(2-4), 337-345. doi:10.1016/j.vetmic.2009.12.002

Beaman, M. H., & Hung, J. (1989). Pericarditis associated with tick-borne Q fever. *Australian and New Zealand Journal of Medicine, 19*(3), 254-256. doi:10.1111/j.1445-5994.1989.tb00258.x

Bechah, Y., Verneau, J., Ben Amara, A., Barry, A. O., Lepolard, C., Achard, V., . . . Mege, J. L. (2014). Persistence of *Coxiella burnetii*, the agent of Q fever, in murine adipose tissue. *PloS One, 9*(5), e97503. doi:10.1371/journal.pone.0097503

Beck, M. D., Bell, J. A., Shaw, E. W., & Huebner, R. J. (1949). Q Fever Studies in Southern California: II. An Epidemiological Study of 300 Cases. *Public Health Reports (1896-1970), 64*(2), 41-56. doi:10.2307/4586820

Becker, J. (2017, 20th February). Farmers push for Q fever vaccination to include children as drug study launches. *ABC Rural*. Retrieved from https://www.abc.net.au/news/rural/2017-02-20/farmers-push-for-q-fever-vaccination-to-include-children/8286348

Bennett, M. D., Woolford, L., Banazis, M. J., O'Hara, A. J., Warren, K. S., Nicholls, P. K., . . . 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. doi:10.1007/s10393-011-0729-3

Benoit, M., Barbarat, B., Bernard, A., Olive, D., & Mege, J. L. (2008). *Coxiella burnetii*, the agent of Q fever, stimulates an atypical M2 activation program in human macrophages. *European Journal of Immunology, 38*(4), 1065-1070. doi:10.1002/eji.200738067

Bentsi-Enchill, A., Bahri, P., Blum, M., Heininger, U., Matos dos Santos, E., Sjölin-Forsberg, G., & Eds. (2012). *Definition and Application of Terms for Vaccine Pharmacovigilance*. Retrieved from

Geneva: https://cioms.ch/publications/product/definitions-and-applications-of-terms-for-vaccine-pharmacovigilance/

Bernard, H., Brockmann, S. O., Kleinkauf, N., Klinc, C., Wagner-Wiening, C., Stark, K., & Jansen, A. (2012). High Seroprevalence of *Coxiella burnetii* Antibodies in Veterinarians Associated with Cattle Obstetrics, Bavaria, 2009. *Vector-borne and Zoonotic Diseases, 12*(7), 552-557. doi:doi.org/10.1089/vbz.2011.0879

Berri, M., Souriau, A., Crosby, M., & Rodolakis, A. (2002). Shedding of *Coxiella burnetii* in ewes in two pregnancies following an episode of *Coxiella* abortion in a sheep flock. *Veterinary Microbiology, 85*(1), 55-60. doi:10.1016/S0378-1135(01)00480-1

Beyer, W. E., Palache, A. M., Kerstens, R., & Masurel, N. (1996). Gender differences in local and systemic reactions to inactivated influenza vaccine, established by a meta-analysis of fourteen independent studies. *European Journal of Clinical Microbiology and Infectious Disease, 15*(1), 65-70.

Bish, A., Yardley, L., Nicoll, A., & Michie, S. (2011). Factors associated with uptake of vaccination against pandemic influenza: A systematic review. *Vaccine, 29*(38), 6472–6484. doi:10.1016/j.vaccine.2011.06.107

Bizzini, A., Peter, O., Baud, D., Edouard, S., Meylan, P., & Greub, G. (2015). Evaluation of a new serological test for the detection of anti-*Coxiella* and anti-*Rickettsia* antibodies. *Microbes and Infection, 17*(11-12), 811-816. doi:10.1016/j.micinf.2015.09.015

Boden, K., Brasche, S., Straube, E., & Bischof, W. (2014). Specific risk factors for contracting Q fever: lessons from the outbreak Jena. *International Journal of Hygiene and Environmental Health, 217*(1), 110-115. doi:10.1016/j.ijheh.2013.04.004

Bond, K., Vincent, G., Wilks, C., Franklin, L., Sutton, B., Stenos, J., . . . Firestone, S. (2016). One Health approach to controlling a Q fever outbreak on an Australian goat farm. *Epidemiology and Infection, 144*(6), 1129-1141. doi:10.1017/S0950268815002368

Bond, K. A., Franklin, L. J., Sutton, B., & Firestone, S. M. (2017). Q-Vax Q fever vaccine failures, Victoria, Australia 1994–2013. *Vaccine, 18*(35(51)), 7084-7087. doi:10.1016/j.vaccine.2017.10.088

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. doi:10.1016/s0378-1135(98)00247-8

Bosnjak, E., Hvass, A. M. S. W., Villumsen, S., & Nielsen, H. (2010). Emerging evidence for Q fever in humans in Denmark: role of contact with dairy cattle. *Clinical Microbiology and Infection, 16*, 1285–1288.

Botelho-Nevers, E., Fournier, P. E., Richet, H., Fenollar, F., Lepidi, H., Foucault, C., . . . Raoult, D. (2007). *Coxiella burnetii* infection of aortic aneurysms or vascular grafts: report of 30 new cases and evaluation of outcome. *European Journal of Clinical Microbiology and Infectious Diseases, 26*(9), 635-640. doi:10.1007/s10096-007-0357-6

Britton, P. N., Macartney, K., Arbuckle, S., Little, D., & Kesson, A. (2015). A Rare Case of Q Fever Osteomyelitis in a Child From Regional Australia. *Journal of the Pediatric Infectious Diseases Society, 4*(3), e28-31. doi:10.1093/jpids/piu095

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

Buhariwalla, F., Cann, B., & Marrie, T. J. (1996). A Dog-Related Outbreak of Q Fever. *Clinical Infectious Diseases, 23*(4), 753-755. doi:10.1093/clinids/23.4.753

Burnet, F. M., & Freeman, M. (1937). Experimental studies on the virus of Q fever. *Medical Journal of Australia, 2*, 299-302.

Burton, P. R., Stueckemann, J., Welsh, R. M., & Paretsky, D. (1978). Some Ultrastructural Effects of Persistent Infections by the Rickettsia *Coxiella burnetii* in Mouse L Cells and Green Monkey Kidney (Vero) Cells. *Infection and Immunity, 21*(2), 556-566. doi:10.1128/IAI.21.2.556-566.1978

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. doi:10.1016/j.jfms.2006.11.006

Camacho, M. T., Outschoorn, I., Tellez, A., & Sequi, J. (2005). Autoantibody profiles in the sera of patients with Q fever: characterization of antigens by immunofluorescence, immunoblot and sequence analysis. *Journal of Autoimmune Diseases, 2*, 10. doi:10.1186/1740-2557-2-10

Cameron, B., Bierl, C., Davenport, T., Vollmer-Conna, U., Hickie, I., Wakefield, D., & Lloyd, A. (2017). Drought and Q fever: the association between trends in the incidence of infection and rainfall in rural Australia. In J. C. Caetano simõe, S. F. Anastácio, & G. J. da silva (Eds.), *The Principles and Practice of Q Fever: The One Health Paradigm.* (pp. 301–315.). New York: Nova science publishers.

Capo, C., Lindberg, F. P., Meconi, S., Zaffran, Y., Tardei, G., Brown, E. J., . . . Mege, J. (1999). Subversion of Monocyte Functions by *Coxiella burnetii*: Impairment of the Cross-Talk Between a_Vb_3 integrin and CR3. *Journal of Immunology, 163*(11), 6078-6085.

Capo, C., & Mege, J. L. (2012). Role of innate and adaptive immunity in the control of Q fever. *Advances in Experimental Medicine and Biology, 984*, 273-286. doi:10.1007/978-94-007-4315-1_14

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. doi: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. doi:10.1111/j.1749-6632.2009.04519.x

Cerf, O., & Condron, R. (2006). *Coxiella burnetii* and milk pasteurization: an early application of the precautionary principle? *Epidemiology and Infection, 134*(5), 946-951. doi:10.1017/S0950268806005978

Chang, C. C., Lin, P. S., Hou, M. Y., Lin, C. C., Hung, M. N., Wu, T. M., . . . Lin, L. J. (2010). Identification of risk factors of *Coxiella burnetii* (Q fever) infection in veterinary-associated populations in southern Taiwan. *Zoonoses and Public Health, 57*(7-8), e95-101. doi:10.1111/j.1863-2378.2009.01290.x

Charles Sturt University. (2021). School of Animal & Veterinary Sciences: Current Students. Retrieved from https://science.csu.edu.au/schools/animal-vet/current/current-students

Cikman, A., Aydin, M., Gulhan, B., Karakecili, F., Ozcicek, A., Kesik, O. A., . . . Gültepe, B. (2017). The seroprevalence of *Coxiella burnetii* in Erzincan, Turkey: Identification of the risk factors and their relationship with geographical features. *Journal of Vector Borne Diseases, 54*(2), 157-163.

Clark, N. J., & Soares Magalhaes, R. J. (2018). Airborne geographical dispersal of Q fever from livestock holdings to human communities: a systematic review and critical appraisal of evidence. *BMC Infectious Diseases, 18*(1), 218. doi:10.1186/s12879-018-3135-4

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*. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/30626297

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. doi:10.1128/JB.186.21.7344–7352.2004

Cook, C., Heath, F., & Thompson, R. L. (2000). A meta-analysis of response rates in web- or internet-based surveys. *Educational and Psychological Measurement, 60*(6), 821-836.

Cook, I. F. (2014). Sex differences in injection site reactions with human vaccines. *Human Vaccines, 5*(7), 441-449. doi:10.4161/hv.8476

Cooper, A., Barnes, T., Potter, A., Ketheesan, N., & Govana, B. (2012). Determination of *Coxiella burnetii* seroprevalence in macropods in Australia. *Veterinary Microbiology, 155*(2-4), 317–323. doi:10.1016/j.vetmic.2011.08.023

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. doi: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. doi:10.1089/vbz.2011.0853

Cox, H. R. (1939). Studies of a filter-passing infectious agent isolated from ticks V. Further attempts to cultivate in cell-free media suggested classification 1. *Public Health Reports (1896-1970), 54*(40), 1822-1827.

CSL. (2009). A guide to Q fever and Q fever vaccination. In. Parkville, Victoria, Australia: CSL Biotherapies.

Davis, G. E. a. C., H.R. (1938). A filter-passing infectious agent isolated from ticks. I. Isolation from *Dermacentor andersoni,* reactions in animals, and filtration experiments. *Public Health Reports (1896-1970), 53*, 2259–2261.

De Boer, R. J., & Perelson, A. S. (2013). Quantifying T lymphocyte turnover. *Journal of Theoretical Biology, 327*, 45-87. doi:10.1016/j.jtbi.2012.12.025

de Rooij, M. M., Schimmer, B., Versteeg, B., Schneeberger, P., Berends, B. R., Heederik, D., . . . Wouters, I. M. (2012). Risk factors of *Coxiella burnetii* (Q fever) seropositivity in veterinary medicine students. *PloS One, 7*(2), e32108. doi:10.1371/journal.pone.0032108

Dejan, L., Tamara, I., Katarina, N., Dragan, B., & Sonja, O. (2019). Seroprevalences of *Rickettsia conorii*, *Ehrlichia canis* and *Coxiella burnetii* in Dogs from Montenegro. *Acta Parasitologica, 64*(4), 769-778. doi:10.2478/s11686-019-00098-w

Dellacasagrande, J., Capo, C., Raoult, D., & Mege, J.-L. (1999). Survival in Monocytes: The Role of Cell IFN-γ-Mediated Control of *Coxiella burnetii*. *Journal of Immunology, 162*(4), 2259-2265.

Dellacasagrande, J., Ghigo, E., Capo, C., Raoult, D., & Mege, J. L. (2000). *Coxiella burnetii* survives in monocytes from patients with Q fever endocarditis: involvement of tumor necrosis factor. *Infection and Immunity, 68*(1), 160-164. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10603382

Denman, J., & Woods, M. (2009). Acute Q fever in pregnancy: report and literature review. *Internal Medicine Journal, 39*(7), 479-481. doi:10.1111/j.1445-5994.2009.01933.x

Department of Education Skills and Employment. (2013 - 2016). *Special Courses*. Canberra, Australia: Australian Government Retrieved from https://docs.education.gov.au/documents/2013-special-courses

Derrick, E. H. (1937). "Q" fever, a new fever entity: clinical features, diagnosis and laboratory investigation. *Medical Journal of Australia, 2*, 281-299.

Desjardins, I., Joulié, A., Pradier, S., Lecollinet, S., Beck, C., Vial, L., . . . Leblond, A. (2018). Seroprevalence of horses to *Coxiella burnetii* in an Q fever endemic area. *Veterinary Microbiology, 215*, 49. doi:10.1016/j.vetmic.2017.11.012

Desnues, B., Imbert, G., Raoult, D., Mege, J.-L., & Ghigo, E. (2009). Role of specific antibodies in *Coxiella burnetii* infection of macrophages. *Clinical Microbiology and Infection, 15*, 161-162. doi:10.1111/j.1469-0691.2008.02208.x

Dhand, N. K. (2009). SAS Macros for Statistical Modelling. Retrieved from http://sydney.edu.au/vetscience/biostat/macros/multi_about.shtml

Dhand, N. K. (2010). UniLogistic: a SAS Macro for descriptive and univariable logistic regression analyses. *Journal of Statistical Software, 35*, 1–15.

Dinelli, M. I., Moreira, T., Paulino, E. R., da Rocha, M. C., Graciani, F. B., & de Moraes-Pinto, M. I. (2009). Immune status and risk perception of acquisition of vaccine preventable diseases among health care workers. *American Journal of Infection Control, 37*(10), 858-860. doi:10.1016/j.ajic.2009.04.283

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. doi:10.1016/j.prevetmed.2013.04.002

Dugdale, C., Chow, B., Yakirevich, E., Kojic, E., & Knoll, B. (2014). Prolonged pyrexia and hepatitis: Q fever. *American Journal of Medicine, 127*(10), 928-930. doi:10.1016/j.amjmed.2014.06.003

Dupuis, G., Péter, O., Peacock, M., Burgdorfer, W., & Haller, E. (1985). Immunoglobulin responses in acute Q fever. *Journal of Clinical Microbiology, 22*(4), 484. doi:10.1128/JCM.22.4.484-487.1985

Duron, O., Noë, V., McCoy, K. D., Bonazzi, M., Sidi-Boumedine, K., Morel, O., . . . 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 Pathogen, 11*(5), e1004892. doi:10.1371/journal.ppat.1004892

Eastwood, K., Graves, S. R., Massey, P. D., Bosward, K., van den Berg, D., & Hutchinson, P. (2018). Q fever: A rural disease with potential urban consequences. *Australian Journal of General Practice, 47*(3), 5555. doi:10.31128/AFP-08-17-4299

Easy Street Medical Centre. (2018). Q Fever Vaccination. Retrieved from https://easystreetmedical.com.au/portfolio-items/q-fever-vaccination/

Edouard, S., Million, M., Lepidi, H., Rolain, J. M., Fournier, P. E., La Scola, B., . . . Raoult, D. (2013). Persistence of DNA in a cured patient and positive culture in cases with low antibody levels bring into question diagnosis of Q fever endocarditis. *Journal of Clinical Microbiology, 51*(9), 3012-3017. doi:10.1128/JCM.00812-13

Eldin, C., Angelakis, E., Renvoise, 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. doi:10.4269/ajtmh.12-0212

Eldin, C., Mailhe, M., Lions, C., Carrieri, P., Safi, H., Brouqui, P., & Raoult, D. (2016). Treatment and Prophylactic Strategy for *Coxiella burnetii* Infection of Aneurysms and Vascular Grafts: A Retrospective Cohort Study. *Medicine (Baltimore), 95*(12), e2810. doi:10.1097/MD.0000000000002810

Eldin, C., Mélenotte, C., Mediannikov, O., Ghigo, E., Million, M., Edouard, S., . . . Raoult, D. (2017). From Q Fever to *Coxiella burnetii* Infection: a Paradigm Change. *Clinical Microbiology Reviews, 30*(1), 115-190. doi:10.1128/CMR.00045-16

Eldin, C., Melenotte, C., Million, M., Cammilleri, S., Sotto, A., Elsendoorn, A., . . . Raoult, D. (2016). 18F-FDG PET/CT as a central tool in the shift from chronic Q fever to *Coxiella burnetii* persistent focalized infection: A consecutive case series. *Medicine (Baltimore), 95*(34), e4287. doi:10.1097/MD.0000000000004287

Fan, W., & Yan, Z. (2010). Factors affecting response rates of the web survey: A systematic review. *Computers in Human Behavior, 26*(2), 132-139. doi:10.1016/j.chb.2009.10.015

Farber, D. L., Yudanin, N. A., & Restifo, N. P. (2014). Human memory T cells: generation, compartmentalization and homeostasis. *Nature Reviews: Immunology, 14*(1), 24-35. doi:10.1038/nri3567

Fenollar, F., Fournier, P.-E., Carrieri, M. P., Raoult, D., Habib, G., & Messana, T. (2001). Risks Factors and Prevention of Q Fever Endocarditis. *Clinical Infectious Diseases, 33*(1), 312-316.

Fink, A. L., & Klein, S. L. (2015). Sex and Gender Impact Immune Responses to Vaccines Among the Elderly. *Physiology, 30*(6), 408–416. doi:10.1152/physiol.00035.2015

Fishbein, D. B., & Raoult, D. (1992). A cluster of *Coxiella burnetii* infections associated with exposure to vaccinated goats and their unpasteurized dairy products. *American Journal of Tropical Medicine and Hygiene, 47*(1), 35-40. doi:10.4269/ajtmh.1992.47.35

Flint, J., Dalton, C. B., Merritt, T. D., Graves, S., Ferguson, J. K., Osbourn, M., . . . Durrheim, D. N. (2016). Q fever and contact with kangaroos in New South Wales. *Communicable Diseases Intelligence Quarterly Report, 40*(2), E202-203. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27522129

Floyd, D. L., Prentice-Dunn, S., & Rogers, R. W. (2000). A Meta-Analysis of Research on Protection Motivation Theory. *Journal of Applied Social Psychology, 30*(2), 407-429.

Forland, F., Jansen, A., Gomes, H. d. C., Nøkleby, H., Escriva, A.-B., Coulombier, D., & Giesecke, J. (2010). *Risk Assessment on Q Fever*. Retrieved from Stockholm:

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. doi:10.1177/1098612X15584693

Gaillard, T., Briolant, S., Madamet, M., & Pradines, B. (2017). The end of a dogma: the safety of doxycycline use in young children for malaria treatment. *Malaria Journal, 16*(1), 148. doi:10.1186/s12936-017-1797-9

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*, 1083-1095. doi:10.1111/jam.12778

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. doi:10.1016/j.vaccine.2010.11.008

Gerolimatos, L. A., & Edelstein, B. A. (2012). Predictors of health anxiety among older and young adults. *International Psychogeriatrics, 24*(12), 1998-2008. doi:10.1017/S1041610212001329

Ghigo, E., Capo, C., Tung, C.-H., Raoult, D., Gorvel, J.-P., & Mege, J.-L. (2002). *Coxiella burnetii* Survival in THP-1 Monocytes Involves the Impairment of Phagosome Maturation: IFN-γ Mediates its Restoration and Bacterial Killing. *Journal of Immunology, 169*(8), 4488-4495. doi:10.4049/jimmunol.169.8.4488.

Gibbons, G. C., & White, P. J. (2014). *Q fever in a veterinary hospital; an unusual epidemiology*. Paper presented at the Zoonoses 2014, Brisbane, Australia.

Gidding, H. F., Faddy, H. M., Durrheim, D. N., Graves, S., Nguyen, C., Hutchinson, P., . . . Wood, N. (2019). Q fever seroprevalence among metropolitan and non-metropolitan blood donors in New South Wales and Queensland. *Medical Journal of Australia, 210*(7), 309-315. doi:10.5694/mja2.13004

Gidding, H. F., & Graves, S. (2013). Could it be Q fever? *Medical Journal of Australia, 198*(1), 9-10. doi:10.5694/mja12.11461

Gidding, H. F., Wallace, C., Lawrence, G. L., & McIntyre, P. B. (2009). Australia's national Q fever vaccination program. *Vaccine, 27*(14), 2037-2041. doi:10.1016/j.vaccine.2009.02.007

Giesecke, P. R., & Barton, M. D. (1993). *The AVA/Curtin Serological Survey of Veterinarians in Australia for Core Zoonotic Infections.* Paper presented at the Australian Veterinarians in Public Health and Australian Veterinarians in Industry, Gold Coast Scientific Program, Coolangatta, Queensland, Australia.

Gikas, A., Kokkini, S., & Tsioutis, C. (2010). Q fever: clinical manifestations and treatment. *Expert Review of Anti-Infective Therapy, 8*(5), 529-539. doi:10.1586/eri.10.29

Gilroy, N., Formica, N., Beers, M., Egan, A., Conaty, S., & Marmion, B. (2001). Abattoir-associated Q fever: a Q fever outbreak during a Q fever vaccination program. *Australian and New Zealand Journal of Public Health, 25*(4), 362-367. doi:10.1111/j.1467-842x.2001.tb00595.x

Gimenez, D. F. (1964). Staining Rickettsiae in Yolk Sac Cultures. *Stain Technology, 39*(135-40). doi:10.3109/10520296409061219

Gorvel, L., Textoris, J., Banchereau, R., Amara, A. B., Tantibhedhyangkul, W., Bargen, K. v., . . . Mege, J. L. (2015). Intracellular Bacteria Interfere with Dendritic Cell Functions: Role of the Type I Interferon Pathway. *PloS One, 9*(6), e99420. doi:10.1371/journal.pone.0099420

Grant, S., & Olsen, C. W. (1999). Preventing zoonotic diseases in immunocompromised persons: the role of physicians and veterinarians. *Emerging Infectious Diseases, 5*(1), 159-163. doi:10.3201/eid0501.990121

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), 55-59. doi: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). doi:10.3390/tropicalmed1010004

Graves, S. R., Ross, P., Nguyen, C., Jeppesen, M., Stenos, J., & Robson, J. (2018). *A preliminary comparison of five assays for detecting past exposure to Coxiella burnetii for use prior to human Q Fever vaccination*. Paper presented at the Australian Society for Microbiology Annual Scientific Meeting, Brisbane, Australia.

Graves, S. R., & Stenos, J. (2017). Tick-borne infectious diseases in Australia. *Medical Journal of Australia, 206*(7), 320-324. doi:10.5694/mja17.00090

Guatteo, R., Beaudeau, F., Berri, M., Rodolakis, A., Joly, A., & Seegers, H. (2006). Shedding routes of *Coxiella burnetii* in dairy cows: implications for detection and control. *Veterinary Research, 37*(6), 827-833. doi:10.1051/vetres:2006038

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. doi:10.1016/j.vetmic.2010.10.007

Guimard, T., Amrane, S., Prudent, E., 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. doi:10.4269/ajtmh.17-0251

Gunaratnam, P., Massey, P., Eastwood, K., Durrhein, D., Graves, S., Coote, D., & Fisher, L. (2014). Diagnosis and management of zoonoses – a tool for general practice. *Australian Family Physician, 43*(3), 124-128.

Gunders, J., & Phillips, A. (2020, 6th August 2020). Q fever vaccine funding boosted as the hunt for a better solution continues. *ABC News*. Retrieved from https://www.abc.net.au/news/rural/2020-08-06/q-fever-research-funding-boosted-in-search-for-better-vaccine/12526106

Hackert, V. H., Dukers-Muijrers, N. H., van Loo, I. H., Wegdam-Blans, M., Somers, C., & Hoebe, C. J. (2015). *Coxiella burnetii* Infection Is Lower in Children than in Adults After Community Exposure: Overlooked Cause of Infrequent Q Fever Reporting in the Young. *The Pediatric Infectious Disease Journal, 34*(12), 1283-1288. doi:10.1097/INF.0000000000000871

Hackert, V. H., van der Hoek, W., Dukers-Muijrers, N., de Bruin, A., Al Dahouk, S., Neubauer, H., . . . Hoebe, C. J. (2012). Q fever: single-point source outbreak with high attack rates and massive numbers of undetected infections across an entire region. *Clinical Infectious Diseases, 55*(12), 1591-1599. doi:10.1093/cid/cis734

Hackstadt, T., Peacock, M. G., Hitchcock, P. J., & Cole, R. L. (1985). Lipopolysaccharide Variation in *Coxiella burnetii*: Intrastrain Heterogeneity in Structure and Antigenicity. *Infection and Immunity, 48*(2), 359-365. doi:10.1128/IAI.48.2.359-365.1985

Hagenaars, J. C., Koning, O. H., van den Haak, R. F., Verhoeven, B. A., Renders, N. H., Hermans, M. H., . . . van Suylen, R. J. (2014). Histological characteristics of the abdominal aortic wall in patients with

vascular chronic Q fever. *International Journal of Experimental Pathology, 95*(4), 282-289. doi:10.1111/iep.12086

Hamilton Medical Centre. (2020). Q FEVER VACCINE. Retrieved from http://hamiltonmedicalcentre.com.au/q-fever-vaccine

Handley, J., Paretsky, D. and Stueckemann, J. (1967). Electron Microscopic Observations of *Coxiella burnetii* in the Guinea Pig. *Journal of Bacteriology, 94*(1), 263-267. doi:10.1128/JB.94.1.263-267.1967

Harris, P., Eales, K. M., Squires, R., Govan, B., & Norton, R. (2013). Acute Q fever in northern Queensland: variation in incidence related to rainfall and geographical location. *Epidemiology and Infection, 141*(5), 1034-1038. doi:10.1017/S0950268812001495

Harris, R. J., Storm, P. A., Lloyd, A., Arens, M., & Marmion, B. P. (2000). Long-term persistence of *Coxiella burnetii* in the host after primary Q fever. *Epidemiology and Infection, 124*(3), 543–549. doi:10.1017/s0950268899003763

Hartmann, S. B., Emnéus, J., Wolff, A., & Jungersen, G. (2016). Revisiting the IFN-γ release assay: Whole blood or PBMC cultures? — And other factors of influence. *Journal of Immunological Methods, 434*(July 2016), 24-31. doi:10.1016/j.jim.2016.04.003

Hassidim, A., Elinav, H., Michael-Gayego, A., Benenson, S., Yaalomy, S., Meir, K., . . . Tzur, T. (2018). Breast Implant Q Fever as a Source of In-Hospital Transmission. *Clinical Infectious Diseases, 66*(5), 793–795. doi:10.1093/cid/cix912

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. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12825734

Havas, K. A., & Burkman, K. (2011). A Comparison of the Serological Evidence of *Coxiella burnetii* Exposure Between Military Working Dogs and Feral Canines in Iraq. *Military Medicine, 176*(10), 1101-1103. doi:10.7205/milmed-d-11-00025

Hawker, J. I., Ayres, J. G., Blair, I., Evans, M. R., Smith, D. L., Smith, E. G., . . . 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.

Health Hub Doctors Morayfield. (2019, 25th November 2019). Q Fever Testing and Vaccine. Retrieved from https://www.healthhubdoctorsmorayfield.com.au/services/q-fever-testing-and-vaccine/

Healy, B., van Woerden, H., Raoult, D., Graves, S., Pitman, J., Lloyd, G., . . . 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. doi:10.1093/cid/cir132

Heinzen, R. A., Hackstadt, T., & Samuel, J. E. (1999). Developmental biology of *Coxiella burnetii*. *Trends in Microbiology, 7*(4), 149-154. doi:10.1016/s0966-842x(99)01475-4

Hess, I. M., Massey, P. D., Durrheim, D. N., O'Connor, S., & Graves, S. R. (2011). Preventing Q fever endocarditis: a review of cardiac assessment in hospitalised Q fever patients. *Rural Remote Health, 11*(4), 1763. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22115319

Higgins, D., & Marrie, T. J. (1990). Seroepidemiology of Q fever among cats in New Brunswick and Prince Edward Island. *Annals of the New York Academy of Sciences, 590*(27), 1-4. doi:10.1111/j.1749-6632.1990.tb42231.x

Hogerwerf, L., Borlee, F., Still, K., Heederik, D., van Rotterdam, B., de Bruin, A., . . . Wouters, I. M. (2012). Detection of *Coxiella burnetii* DNA in inhalable airborne dust samples from goat farms after mandatory culling. *Applied and Environmental Microbiology, 78*(15), 5410-5412. doi:10.1128/AEM.00677-12

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. doi:10.1128/iai.70.8.4747-4749.2002

Hull, B. P., Beard, F. H., Hendry, A. J., Dey, A., & Macartney, K. (2020). "No jab, no pay": catch-up vaccination activity during its first two years. *Medical Journal of Australia, 213*(8), 364-369. doi:10.5694/mja2.50780

Hülür, G., Wilhelm, O., & Schipolowski, S. (2011). Prediction of self-reported knowledge with over-claiming, fluid and crystallized intelligence and typical intellectual engagement. *Learning and Individual Differences, 21*, 742-746. doi:10.1016/j.lindif.2011.09.006

Hunt, G. (2020). $35.9 million boost for rare cancers, rare diseases and unmet medical needs [Press release]. Retrieved from https://www.health.gov.au/ministers/the-hon-greg-hunt-mp/media/359-million-boost-for-rare-cancers-rare-diseases-and-unmet-medical-needs

Hussain, S. K., Broederdorf, L. J., Sharma, U. M., & Voth, D. E. (2010). Host Kinase Activity is Required for *Coxiella burnetii* Parasitophorous Vacuole Formation. *Frontiers in Microbiology, 1*, 137. doi:10.3389/fmicb.2010.00137

Innatoss Laboratories BV. (2015). User Manual Q-detect. In. The Netherlands: Innatoss Laboratories BV.

Isaacson, N., Roemheld-Hamm, B., Crosson, J. C., Dicicco-Bloom, B., & Winston, C. A. (2009). Organizational Culture Influences Health Care Workers' Influenza Immunization Behavior. *Family Medicine, 41*(3), 202-207. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19259843

Isken, L. D., Kraaij-Dirkzwager, M., Bondt, P. E. V.-d., Rümke, H. C., Wijkmans, C., Opstelten, W., & Timen, A. (2013). Implementation of a Q fever vaccination program for high-risk patients in the Netherlands. *Vaccine, 31*(23), 2617–2622. doi:10.1016/j.vaccine.2013.03.062

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. doi:10.4269/ajtmh.2011.10-0268

Izzo, A. A., & Marmion, B. P. (1993). Variation in interferon-gamma responses to *Coxiella burnetii* antigens with lymphocytes from vaccinated or naturally infected subjects. *Clinical and Experimental Immunology, 94*(3), 507-515. doi:10.1111/j.1365-2249.1993.tb08226.x

Izzo, A. A., Marmion, B. P., & Worswick, D. A. (1988). Markers of Cell-Mediated Immunity after Vaccination with an Inactivated, Whole-Cell Q Fever Vaccine. *Journal of Infectious Diseases, 157*(4), 781-799. doi:10.1093/infdis/157.4.781

Jackson, L. A., Yu, O., Belongia, E. A., Hambidge, S. J., Nelson, J., Baxter, R., . . . Iskander, J. (2009). Frequency of medically attended adverse events following tetanus and diphtheria toxoid vaccine in adolescents and young adults: a Vaccine Safety Datalink study. *BMC Infectious Diseases, 9*, 165. doi:10.1186/1471-2334-9-165

James Cook University. (2020). WHS-PRO-023 Infection Control Procedure. In. Townsville, QLD, Australia: James Cook University.

Jansen, A. F. M., Raijmakers, R. P. H., Keijmel, S. P., van Der Molen, R. G., Vervoort, G. M., van Der Meer, J. W. M., . . . Bleeker-Rovers, C. P. (2018). Autoimmunity and B-cell dyscrasia in acute and chronic Q fever: A review of the literature. *European Journal of Internal Medicine, 54*, 6-12. doi:10.1016/j.ejim.2018.06.007

Jerrels, T. R., Hinrichs, D. J., & Mallavia, C. P. (1974). Cell envelope analysis of *Coxiella burneti* phase I and phase II. *Canadian Journal of Microbiology, 20*, 1465-1470.

Jeyaretnam, J., Jones, H., & Phillipa, M. (2000). Disease and injury among veterinarians. *Australian Veterinary Journal, 78*(9), 625-619.

Kanfer, E., Farrag, N., Price, C., MacDonald, D., Coleman, J., & Barrett, A. J. (1988). Q fever following bone marrow transplantation. *Bone Marrow Transplantation, 3*(2), 165-166. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3048481

Karki, S., Gidding, H. F., Newall, A. T., McIntyre, P. B., & Liu, B. C. (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), 438. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/26654610

Kermode, M., Yong, K., Jurley, S., & Marmion, B. P. (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. doi: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 Vaccine Immunology, 20*(2), 129-133. doi:10.1128/CVI.00613-12

Kersh, G. J., Fitzpatrick, K. A., Self, J. S., Priestley, R. A., Kelly, A. J., Lash, R. R., . . . Anderson, A. D. (2013). Presence and persistence of *Coxiella burnetii* in the environments of goat farms associated with a Q fever outbreak. *Applied and Environmental Microbiology, 79*(5), 1697-1703. doi:10.1128/AEM.03472-12

Kersh, G. J., Priestley, R., & Massung, R. F. (2013). Stability of *Coxiella burnetii* in stored human blood. *Transfusion, 53*(7), 1493-1496. doi:10.1111/j.1537-2995.2012.03912.x

Khavkin, T., & Tabibzadeh, S. S. (1988). Histologic, Immunofluorescence, and Electron Microscopic Study of Infectious Process in Mouse Lung after Intranasal Challenge with *Coxiella burnetii*. *Infection and Immunity, 56*(7), 1792-1799. doi:10.1128/IAI.56.7.1792-1799.1988

Klein, S. L., Jedlicka, A., & Pekosz, A. (2010). The Xs and Y of immune responses to viral vaccines. *Lancet Infectious Diseases, 10*(338-349). doi:10.1016/S1473-3099(10)70049-9

Klein, S. L., Marriott, I., & Fish, E. N. (2015). Sex-based differences in immune function and responses to vaccination. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 1*(1), 9-15. doi:10.1093/trstmh/tru167

Komiya, T., Sadamasu, K., Kang, M.-i., 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. doi:10.1292/jvms.65.1047

Komiya, T., Sadamasu, K., Toriniwa, H., Kato, K., Arashima, Y., Fukushi, H., . . . 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. doi:10.1007/s10156-003-0237-7

Kopecny, L., Bosward, K. L., Shapiro, A., & 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. doi:10.1177/1098612X13487360

Korner, S., Makert, G. R., Mertens-Scholz, K., Henning, K., Pfeffer, M., Starke, A., . . . Ulbert, S. (2020). Uptake and fecal excretion of *Coxiella burnetii* by *Ixodes ricinus* and *Dermacentor marginatus* ticks. *Parasites and Vectors, 13*(1), 75. doi:10.1186/s13071-020-3956-z

Kosatsky, T. (1984). Household outbreak of Q fever pneumonia related to parturient cat. *The Lancet Infectious Diseases, 2*(8417-8418), 1447-1449. doi:10.1016/s0140-6736(84)91633-7

Krumbiegel, E. R., & Wisniewski, J. H. (1970). Consumption of Infected Raw Milk by Human Volunteers. *Archives of Environmental Health: An International Journal, 21*(1), 63-65. doi:10.1080/00039896.1970.10667193

La Scola, B., & Raoult, D. (1996). Serological cross-reactions between *Bartonella quintana*, *Bartonella henselae*, and *Coxiella burnetii*. *Journal of Clinical Microbiology, 34*(9), 2270-2274. doi:10.1128/JCM.34.9.2270-2274.1996

Landais, C., Fenollar, F., Constantin, A., Cazorla, C., Guilyardi, C., Lepidi, H., . . . Raoult, D. (2007). Q fever osteoarticular infection: four new cases and a review of the literature. *European Journal of Clinical Microbiology and Infectious Diseases, 26*(5), 341-347. doi:10.1007/s10096-007-0285-5

Langley, J. M., Marrie, T. J., LeBlanc, J. C., Almudevar, A., & Raoult, D. (2003). *Coxiella burnetii* seropositivity in parturient women is associated with adverse pregnancy outcomes. *American Journal of Obstetrics and Gynecology, 189*(1), 228-232. doi:10.0167/mob.2003.448

Larson, C. L., Martinez, E., Beare, P. A., Jeffrey, B., Heinzen, R. A., & Bonazzi, M. (2016). Right on Q: genetics begin to unravel *Coxiella burnetii* host cell interactions. *Future Microbiology, 11*(7), 919–939. doi:10.2217/fmb-2016-0044

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. doi:10.1016/j.prevetmed.2011.11.001

Leone, M., Honstettre, A., Lepidi, H., Capo, C., Bayard, F., Raoult, D., & Mege, J.-L. (2002). Effect of Sex on *Coxiella burnetii* Infection: Protective Role of 17β-Estradiol. *The Journal of Infectious Diseases, 189*(2), 339-345. doi:10.1086/380798

Lepidi, H., Houpikian, P., Liang, Z., & Raoult, D. (2003). Cardiac valves in patients with Q fever endocarditis: microbiological, molecular, and histologic studies. *Journal of Infectious Diseases, 187*(7), 1097-1106. doi:10.1086/368219

Limonard, G. J., Nabuurs-Franssen, M. H., Weers-Pothoff, G., Wijkmans, C., Besselink, R., Horrevorts, A. M., . . . Groot, C. A. (2010). One-year follow-up of patients of the ongoing Dutch Q fever outbreak: clinical, serological and echocardiographic findings. *Infection, 38*(6), 471-477. doi:10.1007/s15010-010-0052-x

Limonard, G. J., Peters, J. B., Besselink, R., Groot, C. A., Dekhuijzen, P. N., Vercoulen, J. H., & Nabuurs-Franssen, M. H. (2016). Persistence of impaired health status of Q fever patients 4 years after the first Dutch outbreak. *Epidemiology and Infection, 144*(6), 1142-1147. doi:10.1017/S0950268815002216

Lindsay, P. J., Rohailla, S., & Miyakis, S. (2018). Q Fever in Rural Australia: Education Versus Vaccination. *Vector Borne and Zoonotic Diseases, 18*(11), 632-634. doi:10.1089/vbz.2018.2307

Lipton, B. A., Hopkins, S. G., Koehler, J. E., & DiGiacomo, R. F. (2008). A survey of veterinarian involvement in zoonotic disease prevention practices. *Journal of the American Veterinary Medical Association, 233*(8), 1242-1249.

Lord, H., Fletcher-Lartey, S., Weerasinghe, G., Chandra, M., Egana, N., Schembrib, 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). doi: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. *NSW Public Health Bulletin, 23*(1-2), 31-35. doi:10.1071/NB11037

Lower, T., Corben, P., Massey, P., Depczynski, J., Brown, T., Stanley, P., . . . Durrheim, D. (2017). Farmers' knowledge of Q fever and prevention approaches in New South Wales. *Australian Journal of Rural Health, 25*(5), 306-310. doi:10.1111/ajr.12346

Ma, G. C., Norris, J. M., Mathews, K. O., Chandra, S., Slapeta, 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. doi:10.1016/j.actatropica.2020.105416

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. doi:10.1111/1753-6405.12784

Maltezou, H. C., Constantopoulou, I., Constantina, K., Vlahou, V., Georgakopoulos, D., Kafetzis, D. A., & Raoult, D. (2004). Q Fever in Children in Greece. *American Journal of Tropical Medicine and Hygiene, 70*(5), 540-544.

Marenzoni, M. L., Stefanetti, V., Papa, P., Proietti, P. C., Bietta, A., Coletti, M., . . . c, K. H. (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. doi:10.1016/j.vetmic.2013.09.027

Marks, S., & Olenski, M. (2019). Q Fever in the First Trimester: A Case Report from Northern Rural New South Wales. *Tropical Medicine and Infectious Disease, 4*(2). doi:10.3390/tropicalmed4020090

Marmion, B. P. (2007). Q fever: the long journey to control by vaccination. *Medical Journal of Australia, 186*(4), 164-166.

Marmion, B. P., Kyrkou, M., Worswick, D. A., Esterman, A., Ormsbee, R. A., Wright, J., . . . Feery, B. (1984). Vaccine Prophylaxis of Abattoir-Associated Q Fever. *The Lancet, 324*(8417-8418), 1411-1414. doi:10.1016/S0140-6736(84)91617-9

Marmion, B. P., Ormsbee, R. A., Kyrkou, M., Wright, J., Worswick, D. A., Izzo, A. A., . . . Shapiro, R. A. (1990). Vaccine prophylaxis of abattoir-associated Q fever: eight years' experience in Australian abattoirs. *Epidemiology and Infection, 104*(275-287).

Marmion, B. P., Shannon, M., Maddocks, I., Storm, P., & Penttila, I. (1996). Protracted debility and fatigue after acute Q fever. *Lancet, 347*(9006), 977-978. doi:10.1016/s0140-6736(96)91469-5

Marmion, B. P., & Stoker, M. G. P. (1958). The Epidemiology Of Q Fever In Great Britain: An Analysis Of The Findings And Some Conclusions. *The British Medical Journal, 2*(5100), 809-816. doi:10.1136/bmj.2.5100.809

Marmion, B. P., Storm, P. A., Ayres, J. G., Semendric, L., Mathews, L., Winslow, W., . . . Harris, R. J. (2005). Long-term persistence of *Coxiella burnetii* after acute primary Q fever. *Quarterly Journal of Medicine, 98*(1), 7-20. doi:10.1093/qjmed/hci009

Marrie, T., Williams, J., Schlech, W., & Yates, L. (1986). Q fever pneumonia associated with exposure to wild rabbits. *The Lancet, 327*(8478), 427-429. doi:10.1016/S0140-6736(86)92380-9

Marrie, T. J. (2010). Q fever pneumonia. *Infectious Disease Clinics of North America, 24*(1), 27-41. doi:10.1016/j.idc.2009.10.004

Marrie, T. J., Durant, H., Williams, J. C., Mintz, E., & Waag, D. M. (1988). Exposure to Parturient Cats: A Risk Factor for Acquisition of Q Fever in Maritime Canada. *Journal of Infectious Diseases, 158*(1), 101-108.

Marrie, T. J., Langille, D., Papukna, V., & Yates, L. (1989). Truckin' pneumonia - an outbreak of Q fever in a truck repair plant probably due to aerosols from clothing contaminated by contact with newborn kittens. *Epidemiology and Infection, 102*(1), 119-127.

Massey, P. D., Irwin, M., & Durrheim, D. N. (2009). Enhanced Q fever risk exposure surveillance may permit better informed vaccination policy. *Communicable Diseases Intelligence, 33*(1), 41-45. Retrieved from http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3301i.htm

Matthewman, L., Bryson, N., Kelly, P., Rycroft, A., Hayter, D., & Raoult, D. (1997). Exposure of cats in southern Africa to *Coxiella burnetii*, the agent of Q fever. *European Journal of Epidemiology, 13*(4), 477–479. doi:10.1023/a:1007317032647

Maurin, M., & Raoult, D. (1999). Q fever. *Clinical Microbiology Reviews, 12*(4), 518-553. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC88923/

Maywood, P., & Boyd, R. (2011, 30 June-2 July 2011). *Q fever in a small animal hospital.* Paper presented at the Australian College of Veterinary Scientists College Science Week Scientific Meeting: Epidemiology Chapter and Aquatic Animal Health Chapter Proceedings, Gold Coast, Australia.

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. doi:10.1128/JB.147.3.1063-1076.1981

Mege, J., Maurin, M., Capo, C., & Raoult, D. (1997). *Coxiella burnetii:* the 'query' fever bacterium. A model of immune subversion by a strictly intracellular microorganism. *FEMS Microbiology Reviews, 19*(4), 209-217. doi:10.1111/j.1574-6976.1997.tb00298.x

Meisner, J., Vora, M. V., Fuller, M. S., Phipps, A. I., & Rabinowitz, P. M. (2018). Maternal veterinary occupation and adverse birth outcomes in Washington State, 1992-2014: a population-based

retrospective cohort study. *Occupational and Environmental Medicine, 75*(5), 359-368. doi:10.1136/oemed-2017-104817

Melenotte, C., Mezouar, S., Ben Amara, A., Benatti, S., Chiaroni, J., Devaux, C., . . . Raoult, D. (2019). A transcriptional signature associated with non-Hodgkin lymphoma in the blood of patients with Q fever. *PloS One, 14*(6), e0217542. doi:10.1371/journal.pone.0217542

Melenotte, C., Million, M., & Raoult, D. (2020). New insights in *Coxiella burnetii* infection: diagnosis and therapeutic update. *Expert Review of Anti-Infective Therapy, 18*(1), 75-86. doi:10.1080/14787210.2020.1699055

Melenotte, C., Protopopescu, C., Million, M., Edouard, S., Carrieri, M. P., Eldin, C., . . . Raoult, D. (2018). Clinical Features and Complications of *Coxiella burnetii* Infections From the French National Reference Center for Q Fever. *JAMA Network Open, 1*(4), e181580. doi:10.1001/jamanetworkopen.2018.1580

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.

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. doi:10.1086/321878

Million, M., Bardin, N., Bessis, S., Nouiakh, N., Douliery, C., Edouard, S., . . . Raoult, D. (2017). Thrombosis and antiphospholipid antibody syndrome during acute Q fever: A cross-sectional study. *Medicine (Baltimore), 96*(29), e7578. doi:10.1097/MD.0000000000007578

Million, M., Bellevegue, L., Labussiere, A. S., Dekel, M., Ferry, T., Deroche, P., . . . Raoult, D. (2014). Culture-negative prosthetic joint arthritis related to *Coxiella burnetii*. *American Journal of Medicine, 127*(8), 786 e787-786 e710. doi:10.1016/j.amjmed.2014.03.013

Million, M., & Raoult, D. (2015). Recent advances in the study of Q fever epidemiology, diagnosis and management. *Journal of Infection, 71*, S2-S9. doi:10.1016/j.jinf.2015.04.024

Million, M., & Raoult, D. (2017). No such thing as chronic Q fever. *Emerging Infectious Diseases, 23*(5), 856. doi:10.3201/eid2305.151159

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. doi:10.1093/cid/ciu259

Million, M., Thuny, F., Bardin, N., Angelakis, E., Edouard, S., Bessis, S., . . . Raoult, D. (2016). Antiphospholipid Antibody Syndrome With Valvular Vegetations in Acute Q Fever. *Clinical Infectious Diseases, 62*(5), 537-544. doi:10.1093/cid/civ956

Million, M., Thuny, F., Richet, H., & Raoult, D. (2010). Long-term outcome of Q fever endocarditis: a 26-year personal survey. *Lancet Infectious Diseases, 10*(8), 527-535. doi:10.1016/S1473-3099(10)70135-3

Million, M., Walter, G., Thuny, F., Habib, G., & Raoult, D. (2013). Evolution from acute Q fever to endocarditis is associated with underlying valvulopathy and age and can be prevented by prolonged antibiotic treatment. *Clinical Infectious Diseases, 57*(6), 836-844. doi:10.1093/cid/cit419

Moro-García, M. A., Alonso-Arias, R., & López-Larrea, C. (2013). When aging reaches CD4+ T-cells: phenotypic and functional changes. *Frontiers in Immunology, 4*(107). doi:10.3389/fimmu.2013.00107

Morroy, G., Keijmel, S. P., Delsing, C. E., Bleijenberg, G., Langendam, M., Timen, A., & Bleeker-Rovers, C. P. (2016). Fatigue following Acute Q-Fever: A Systematic Literature Review. *PloS One, 11*(5), e0155884. doi:10.1371/journal.pone.0155884

Morroy, G., Prins, J., Bergevoet, R., Schneeberger, P., Bor, H. H. J., Hoek, W. v. d., . . . Polder, J. J. (2012). Of goats and humans; the societal costs of the Dutch Q fever saga. *International Journal of Infectious Diseases, 16S*(2012), e158–e316. doi:10.1016/j.ijid.2012.05.914

Munckhof, W. J., Runnegar, N., Gray, T. J., Taylor, C., Palmer, C., & Holley, A. (2007). Two rare severe and fulminant presentations of Q fever in patients with minimal risk factors for this disease. *Internal Medicine Journal, 37*(11), 775-778. doi:10.1111/j.1445-5994.2007.01489.x

Murdoch University. (2018). Immunisation Policy. In. Murdoch, WA, Australia: Murdoch University.

Musso, D., & Raoult, D. (1997). Serological cross-reactions between *Coxiella burnetii* and *Legionella micdadei*. *Clinical and Diagnostic Laboratory Immunology, 4*(2), 208-212.

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. doi:10.1292/jvms.60.251

National Careers Institue. (2021a). Your career - Veterinarian. Retrieved from https://yourcareer.gov.au/careers/2347/veterinarian

National Careers Institue. (2021b). Your Career - Veterinary Nurse. Retrieved from https://yourcareer.gov.au/careers/3613/veterinary-nurse

National Center for Emerging and Zoonotic Infectious Diseases. (2018, 4th April 2018). Bioterrorism Agents/Diseases. *Emergency Preparedness and Response.* Retrieved from https://emergency.cdc.gov/agent/agentlist-category.asp

National Rural Health Alliance. (2015). The little book of rural health numbers: Demography. Retrieved from http://ruralhealth.org.au/book/demography

New South Wales Government. (2015, 1st September 2015). Work Health and Safety Act 2011 No 10. Retrieved from http://www.legislation.nsw.gov.au/maintop/view/inforce/act+10+2011+cd+0+N

Nielsen, S. Y., Molbak, 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. doi:10.3201/eid2006.130584

NNDSS Annual Report Working Group. (2019). Australia's notifiable disease status, 2015: Annual report of the National Notifiable Diseases Surveillance System. *Communicable Diseases Intelligence, 43*(2019). doi:10.33321/cdi.2019.43.6

Nourse, C., Allworth, A., Jones, A., Horvath, R., McCormack, J., Bartlett, J., . . . Robson, J. M. (2004). Three cases of Q fever osteomyelitis in children and a review of the literature. *Clinical Infectious Diseases, 39*(7), e61-66. doi:10.1086/424014

NSW Ministry of Health. (2019). *Q fever and veterinary staff*. NSW Ministry of Health Retrieved from https://www.health.nsw.gov.au/Infectious/factsheets/Pages/q-fever-veterinary-staff.aspx

Nusinovici, S., Frossling, J., Widgren, S., Beaudeau, F., & Lindberg, A. (2015). Q fever infection in dairy cattle herds: increased risk with high wind speed and low precipitation. *Epidemiology and Infection, 143*(15), 3316-3326. doi:10.1017/S0950268814003926

O'Neill, T. J., Sargeant, J. M., & Poljak, Z. (2014). The effectiveness of *Coxiella burnetii* vaccines in occupationally exposed populations: a systematic review and meta-analysis. *Zoonoses and Public Health, 61*(2), 81-96. doi:10.1111/zph.12054

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. doi:10.1017/S0950268814001083

Ordi-Ros, J., Selva-O'Callaghan, A., Monegal-Ferran, F., Monasterio-Aspiri, Y., Juste-Sanchez, C., & Vilardell-Tarres, M. (1994). Prevalence, significance, and specificity of antibodies to phospholipids in Q fever. *Clinical Infectious Diseases, 18*(2), 213-218. doi:10.1093/clinids/18.2.213

Oskam, C., Owens, J., Codello, A., Gofton, A., & Greay, T. (2018). Rethinking *Coxiella* infections in Australia. *Microbiology Australia, 39*(4), 223-225. doi:10.1071/MA18069

Osorio, S., Sarria, C., Gonzalez-Ruano, P., Casal, E. C., & Garcia, A. (2003). Nosocomial transmission of Q fever. *Journal of Hospital Infection, 54*(2), 162-163. doi:10.1016/s0195-6701(03)00111-7

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. doi:10.1111/j.1753-6405.2010.00478.x

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, 91*(8), 549-560. doi:10.1093/qjmed/91.8.549

Pinsky, R. L., Fishbein, D. B., Greene, C. R., & Gensheimer, K. F. (1991). An Outbreak of Cat-Associated Q Fever in the United States. *Journal of Infectious Disease, 164*(1), 202-204. doi:10.1093/infdis/164.1.202

Pioneer Health. (2020). Are You At Risk Of Q Fever? Retrieved from https://pioneerhealth.com.au/Services/Further-Services/Q-Fever

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. doi:10.7589/0090-3558-47.4.821

Pozzo, F. D., Martinelle, L., Leonard, P., Renaville, B., Renaville, R., Thys, C., . . . Saegerman, C. (2017). Q Fever Serological Survey and Associated Risk Factors in Veterinarians, Southern Belgium, 2013. *Transboundary and Emerging Diseases, 64*(3), 959–966. doi:10.1111/tbed.12465

Rahaman, M. R., Milazzo, A., Marshall, H., & Bi, P. (2019). Spatial, temporal, and occupational risks of Q fever infection in South Australia, 2007-2017. *Journal of Infection and Public Health, 13*(4), 544-551. doi:10.1016/j.jiph.2019.10.002

Raoult, D. (2012). Chronic Q fever: Expert opinion versus literature analysis and consensus. *Journal of Infection, 65*(2), 102-108. doi:10.1016/j.jinf.2012.04.006

Raoult, D., Fenollar, F., & Stein, A. (2002). Q fever during pregnancy: diagnosis, treatment, and follow-up. *Archives of Internal Medicine, 162*(6), 701-704. doi:10.1001/archinte.162.6.701

Raoult, D., Marrie, T. J., & Mege, J. L. (2005). Natural history and pathophysiology of Q fever. *The Lancet Infectious Diseases, 5*(4), 219-226. doi:10.1016/s1473-3099(05)70052-9

Raoult, D., & Stein, A. (1994). Q fever during pregnancy--a risk for women, fetuses, and obstetricians. *New England Journal of Medicine, 330*(5), 371. doi:10.1056/nejm199402033300518

Raoult, D., Tissot-Dupont, H., Foucault, C., Gouvernet, J., Fournier, P. E., Bernit, E., . . . Weiller, P. J. (2000). Q fever 1985-1998. Clinical and epidemiologic features of 1,383 infections. *Medicine (Baltimore), 79*(2), 109-123. doi:10.1097/00005792-200003000-00005

Reserve Bank of Australia. (2021). Inflation Calculator. Retrieved from https://www.rba.gov.au/calculator/annualDecimal.html

Rodolakis, A., Berri, M., He´chard, C., Caudron, C., Souriau, A., Bodier, C. C., . . . Arricau-Bouvery, N. (2007). Comparison of *Coxiella burnetii* Shedding in Milk of Dairy Bovine, Caprine, and Ovine Herds. *Journal of Dairy Science, 90*(12), 5352–5360. doi:10.3168/jds.2006-815

Roest, H.-J., Gelderen, B. v., Dinkla, A., Frangoulidis, D., Zijderveld, F. v., Rebel, J., & Keulen, L. v. (2012). Q Fever in Pregnant Goats: Pathogenesis and Excretion of *Coxiella burnetii*. *PloS One, 7*(11), e48949. doi:10.1371/journal.pone.0048949

Roest, H., van Solt, C., Tilburg, J., Klaassen, C., Hovius, E., Roest, F., . . . van Zijderveld, F. (2013). Search for possible additional reservoirs for human Q fever, The Netherlands. *Emerging Infectious Diseases, 19*(5), 834-835. doi:10.3201/eid1905.121489

Roest, H. I. J., Tilburg, J. J. H. C., Van Der Hoek, W., Vellema, P., Van Zijderveld, F. G., Klaassen, C. H. W., & Raoult, D. (2011). The Q fever epidemic in The Netherlands: history, onset, response and reflection. *Epidemiology and Infection, 139*(1), 1-12. doi:10.1017/S0950268810002268

Ruiz, S., & Wolfe, D. N. (2014). Vaccination against Q fever for biodefense and public health indications. *Frontiers in Microbiology, 5*(726), 1-7. doi:10.3389/fmicb.2014.00726

SA Health. (2020, 08 November 2020). Q fever - including symptoms, treatment and prevention. Retrieved from https://www.sahealth.sa.gov.au/wps/wcm/connect/public+content/sa+health+internet/conditions/infectious+diseases/q+fever/q+fever+including+symptoms+treatment+and+prevention

Safe Work Australia. (2011). *National Compliance and Enforcement Policy*. Safe Work Australia,

Safe Work Australia. (2014). Occupational Disease Indicators Report 2014. In. Canberra, ACT, Australia: Safe Work Australia.

Safe Work Australia. (2019). *Model Work Health and Safety Act 2010*. Canberra: Parliamentary Counsel's Committee

Safe Work Australia. (2021). *Work Health and Safety Regulations 2011*. Canberra: Parliamentary Counsel's Committee

SafeWork-NSW. (2019, 18th July 2019). Q fever. *Safe Work.* Retrieved from https://www.safework.nsw.gov.au/hazards-a-z/diseases/q-fever

Sanchez, J., Souriau, A., Buendia, A. J., Arricau-Bouvery, N., Martinez, C. M., Salinas, J., . . . 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. doi:10.1016/j.jcpa.2006.06.003

Sandoz, K. M., Popham, D. L., Beare, P. A., Sturdevant, D. E., Hansen, B., Nair, V., & Heinzen, R. A. (2016). Transcriptional Profiling of *Coxiella burnetii* Reveals Extensive Cell Wall Remodeling in the Small Cell Variant Developmental Form. *PloS One, 11*(2), e0149957. doi:10.1371/journal.pone.0149957

Sandoz, K. M., Sturdevant, D. E., Hansen, B., & Heinzen, R. A. (2014). Developmental transitions of *Coxiella burnetii* grown in axenic media. *Journal of Microbiological Methods, 96*, 104–110. doi:10.1016/j.mimet.2013.11.010

Scheftel, J. M., Elchos, B. L., Rubin, C. S., & Decker, J. A. (2017). Review of hazards to female reproductive health in veterinary practice. *Journal of the American Veterinary Medical Association, 250*(8), 862-872. doi:10.2460/javma.250.8.862

Schimmer, B., Ter Schegget, R., Wegdam, M., Zuchner, L., de Bruin, A., Schneeberger, P. M., . . . van der Hoek, W. (2010). The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. *BMC Infectious Diseases, 10*, 69. doi:10.1186/1471-2334-10-69

Schoffelen, T., Herremans, T., Sprong, T., Nabuurs-Franssen, M., van der Meer, J. W., Joosten, L. A., . . . van Deuren, M. (2014). Immunogenicity of the Q fever skin test. *Journal of Infection, 69*(2), 161-164. doi:10.1016/j.jinf.2014.03.008

Schoffelen, T., Herremans, T., Sprong, T., Nabuurs-Franssen, M., Wever, P. C., Joosten, L. A., . . . van Deuren, M. (2013). Limited humoral and cellular responses to Q fever vaccination in older adults with risk factors for chronic Q fever. *Journal of Infection, 67*(6), 565-573. doi:10.1016/j.jinf.2013.08.008

Schoffelen, T., Joosten, L. A., Herremans, T., de Haan, A. F., Ammerdorffer, A., Rumke, H. C., . . . van Deuren, M. (2013). Specific interferon gamma detection for the diagnosis of previous Q fever. *Clinical Infectious Diseases, 56*(12), 1742-1751. doi:10.1093/cid/cit129

Schoffelen, T., Textoris, J., Bleeker-Rovers, C. P., Ben Amara, A., van der Meer, J. W., Netea, M. G., . . . van de Vosse, E. (2017). Intact interferon-gamma response against *Coxiella burnetii* by peripheral blood mononuclear cells in chronic Q fever. *Clinical Microbiology and Infection, 23*(3), 209 e209-209 e215. doi:10.1016/j.cmi.2016.11.008

Schoffelen, T., Wong, A., Rümke, H. C., Netea, M. G., Timen, A., Deuren, M. v., & Bondt, P. E. V.-d. (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*, 6622–6630. doi:10.1016/j.vaccine.2014.09.061

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. doi:10.1111/zph.12658

Sellens, E., Bosward, K. L., Willis, S., Heller, J., Cobbold, R., Comeau, J. L., . . . Wood, N. (2018). Frequency of Adverse Events Following Q Fever Immunisation in Young Adults. *Vaccines, 6*(4), 83. doi:10.3390/vaccines6040083

Sellens, E., Norris, J. M., Dhand, N. K., Heller, J., Hayes, L., Gidding, H. F., . . . Bosward, K. L. (2016). Q Fever Knowledge, Attitudes and Vaccination Status of Australia's Veterinary Workforce in 2014. *PloS One, 11*(1), e0146819. doi:10.1371/journal.pone.0146819

Sellens, E., Norris, J. M., Dhand, N. K., Heller, J., Hayes, L., Gidding, H. F., . . . Bosward, K. L. (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. doi:10.1371/journal.pone.0198421

Seo, M. G., Lee, S. H., VanBik, D., Ouh, I. O., Yun, S. H., Choi, E., . . . Kwak, D. (2016). Detection and Genotyping of *Coxiella burnetii* and *Coxiella*-Like Bacteria in Horses in South Korea. *PloS One, 11*(5), e0156710. doi:10.1371/journal.pone.0156710

Seqirus. (2016). Q-VAX® Q Fever Vaccine and Q-VAX® Skin Test (R100517 & 100518) Product Information. In *Seqirus Pty Ltd*. Parkville, Victoria, Australia: Therapeutics Goods Administration.

Seqirus. (2019). Q-VAX® Q Fever Vaccine and Q-VAX® Skin Test (AUST R 100517 & 100518) Product Information. In *Seqirus Pty Ltd* (Vol. Version 7). Australia: Therapeutic Goods Administration.

Shannon, J. G., Cockrell, D. C., Takahashi, K., Stahl, G. L., & Heinzen, R. A. (2009). Antibody-mediated immunity to the obligate intracellular bacterial pathogen *Coxiella burnetii* is Fc receptor- and complement-independent. *BMC Immunology, 10*(26). doi:10.1186/1471-2172-10-26

Shannon, J. G., Howe, D., & Heinzen, R. A. (2005). Virulent *Coxiella burnetii* does not activate human dendritic cells: role of lipopolysaccharide as a shielding molecule. *Proceedings of the National Academy of Sciences of the United States of America, 102*(24), 8722-8727. doi:10.1073/pnas.0501863102

Shapiro, A., Norris, J., Heller, J., Brown, G., Malik, R., & Bosward, K. (2016). Seroprevalence of *Coxiella burnetii* in Australian dogs. *Zoonoses and Public Health, 63*(6), 458-466. doi:10.1111/zph.12250

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), 154-161. doi:10.1016/j.vetmic.2015.02.011

Shapiro, A. J., Norris, J. M., Bosward, K. L., & Heller, J. (2016). Q Fever (*Coxiella burnetii*) Knowledge and Attitudes of Australian Cat Breeders and Their Husbandry Practices. *Zoonoses and Public Health, 64*(4), 252-261. doi:10.1111/zph.12305

Shapiro, R. A., Siskind, V., Schofield, F. D., Stallman, N., Worswick, D. A., & Marmion, B. P. (1990). A randomized, controlled, double-blind, cross-over, clinical trial of Q fever vaccine in selected Queensland abattoirs. *Epidemiology and Infection, 104*(2), 267-273. doi:10.1017/s0950268800059446

Shirangi, A., Bower, C., Holman, C. D., Preen, D. B., & Bruce, N. (2014). A study of handling cytotoxic drugs and risk of birth defects in offspring of female veterinarians. *International Journal of Environmental Research and Public Health, 11*(6), 6216-6230. doi:10.3390/ijerph110606216

Shirangi, A., Fritschi, L., & Holman, C. D. (2007). Prevalence of occupational exposures and protective practices in Australian female veterinarians. *Australian Veterinary Journal, 85*(1-2), 32-38. doi:10.1111/j.1751-0813.2006.00077.x

Shirangi, A., Fritschi, L., & Holman, C. D. (2008). Maternal occupational exposures and risk of spontaneous abortion in veterinary practice. *Occupational and Environmental Medicine, 65*(11), 719-725. doi:10.1136/oem.2007.035246

Shirangi, A., Fritschi, L., & Holman, C. D. (2009). Associations of unscavenged anesthetic gases and long working hours with preterm delivery in female veterinarians. *Obstetrics and Gynecology, 113*(5), 1008-1017. doi:10.1097/AOG.0b013e31819fe996

Shirangi, A., Fritschi, L., Holman, C. D., & Bower, C. (2009). Birth defects in offspring of female veterinarians. *Journal of Occupational and Environmental Medicine, 51*(5), 525-533. doi:10.1097/JOM.0b013e3181a01af3

Shivaprasad, H. L., Cadenas, M. B., Diab, S. S., Nordhausen, R., Bradway, D., Crespo, R., & Breitschwerdt, E. B. (2008). *Coxiella*-like infection in psittacines and a toucan. *Avian Diseases, 52*(3), 426-432. doi:10.1637/8192-120707-Reg

Signs, K. A., Stobierski, M. G., & Gandhi, T. N. Q Fever Cluster Among Raw Milk Drinkers in Michigan, 2011. *Clinical Infectious Diseases, 55*(10), 1387–1389. doi:10.1093/cid/cis690

Simon, A. K., Hollander, G. A., & McMichael, A. (2015). Evolution of the immune system in humans from infancy to old age. *Proceedings: Biological Sciences, 282*(1821), 20143085. doi:10.1098/rspb.2014.3085

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. doi:10.1016/j.onehlt.2017.03.002

Sloan-Gardner, T. S., Massey, P. D., Hutchinson, P., K.Knope, & Fearnley, E. (2017). Trends and risk factors for human Q fever in Australia, 1991. *Epidemiology and Infection, 145*(4), 787-795. doi:10.1017/S0950268816002843

Snow, J., & Rice, J. (2005). Infection Control in Veterinary Clinics. *Northwest Public Health, Fall/Winter 2005*, 22-23.

Sobotta, K., Bonkowski, K., Liebler-Tenorio, E., Germon, P., Rainard, P., Hambruch, N., . . . Menge, C. (2017). Permissiveness of bovine epithelial cells from lung, intestine, placenta and udder for infection with *Coxiella burnetii*. *Veterinary Research, 48*(1), 23. doi:10.1186/s13567-017-0430-9

Sobotta, K., Hillarius, K., Jimenez, P. H., Kerner, K., Heydel, C., & Menge, C. (2017). Interaction of *Coxiella burnetii* Strains of Different Sources and Genotypes with Bovine and Human Monocyte-Derived Macrophages. *Frontiers in Cellular and Infection Microbiology, 7*, 543. doi:10.3389/fcimb.2017.00543

Sobotta, K., Hillarius, K., Mager, M., Kerner, K., Heydel, C., & Menge, C. (2016). *Coxiella burnetii* Infects Primary Bovine Macrophages and Limits Their Host Cell Response. *Infection and Immunity, 84*(6), 1722-1734. doi:10.1128/IAI.01208-15

Soest, E. M., & Fritschi, L. (2004). Occupational health risks in veterinary nursing: an exploratory study. *Australian Veterinary Journal, 82*(6), 346-350. doi:10.1111/j.1751-0813.2004.tb11101.x

Speare, R., Mendez, D., Judd, J., Reid, S., Tzipori, S., & Massey, P. D. (2015). Willingness to Consult a Veterinarian on Physician's Advice for Zoonotic Diseases: A Formal Role for Veterinarians in Medicine? *PloS One, 10*(8), e0131406. doi:10.1371/journal.pone.0131406

Spelman, D. W. (1982). Q fever-a study of 111 consecutive cases. *Medical Journal of Australia, 1*(13), 547-548.

Stein, A., Louveau, C., Lepidi, H., Ricci, F., Baylac, P., Davoust, B., & Raoult, D. (2005). Q fever pneumonia: virulence of *Coxiella burnetii* pathovars in a murine model of aerosol infection. *Infection and Immunity, 73*(4), 2469-2477. doi:10.1128/IAI.73.4.2469-2477.2005

Stein, A., Saunders, N. A., Taylor, A. G., & Raoult, D. (1993). Phylogenic homogeneity of *Coxiella burnetii* strains as determined by 16S ribosomal RNA sequencing. *FEMS Microbiology Letters, 113*(3), 339–344. doi:10.1111/j.1574-6968.1993.tb06537.x.

Stelzner, A. a. L., W. (1968). Binary Fission in *Coxiella burneti*. *Nature, 218*(5146), 1069-1070. doi:10.1038/2181069a0

Stevenson, S., Gowardman, J., Tozer, S., & Woods, M. (2015). Life-threatening Q fever infection following exposure to kangaroos and wallabies. *BMJ Case Reports, 2015*, bcr2015210808. doi:10.1136/bcr-2015-210808

Sukocheva, O. A., Marmion, B. P., Storm, P. A., Lockhart, M., Turra, M., & Graves, S. (2010). Long-term persistence after acute Q fever of non-infective *Coxiella burnetii* cell components, including antigens. *Quarterly Journal of Medicine, 103*(11), 847-863. doi:10.1093/qjmed/hcq113

TAFE NSW. (2021). CERTIFICATE IV IN VETERINARY NURSING. Retrieved from https://www.tafensw.edu.au/course/-/c/c/ACM40418-01/Certificate-IV-in-Veterinary-Nursing

TAFE Queensland. (2021). Certificate IV in Veterinary Nursing. Retrieved from https://tafeqld.edu.au/courses/17721/certificate-iv-in-veterinary-nursing

Tan, C. K., & Owens, L. (2000). Infectivity, transmission and 16S rRNA sequencing of a rickettsia, *Coxiella cheraxi* sp. nov., from the freshwater crayfish *Cherax quadricarinatus*. *Diseases of Aquatic Organisms, 41*(2), 115-122. doi:10.3354/dao041115

Taylor, C., & Jan, S. (2017). Economic evaluation of medicines. *Australian Prescriber, 40*, 76–78. doi:doi.org/10.18773/austprescr.2017.014

Teunis, P. F. M., Schirmer, B., Notermans, D. W., Leenders, A. C. A. P., Wever, P. C., Kretzchmar, M. E. E., & Schneeberger, P. M. (2013). Time-course of antibody responses against *Coxiella burnetii* following acute Q fever. *Epidemiology and Infection, 141*(1), 62-73. doi:10.1017/S0950268812000404

Textoris, J. L. H. B., Capo, C., Raoult, D., Leone, M., & Mege, J.-L. (2010). Sex-Related Differences in Gene Expression Following *Coxiella burnetii* Infection in Mice: Potential Role of Circadian Rhythm. *PloS One, 5*(8), e12190. doi:10.1371/journal.pone.0012190.g001

Therapeutic Goods Administration. (2017). Database of Adverse Event Notifications - Medicines. Retrieved from http://apps.tga.gov.au/PROD/DAEN/daen-entry.aspx

Therapeutics Good Administration. (2020). *Database of Adverse Event Notifications - medicines: Medicine summary Q Vax*. Canberra: Australian Government Department of Health Retrieved from https://apps.tga.gov.au/PROD/DAEN/daen-entry.aspx

Therapeutics Goods Administration. (2000). *Note for Guidance on Clinical Safety Data Management: Definitions and standards for expedited reporting*. Canberra: Commonwealth Department of Health and Aged Care Retrieved from https://www.tga.gov.au/sites/default/files/ich37795.pdf

Therapeutics Goods Aministration. (2018). About the DAEN - medicines. Retrieved from https://www.tga.gov.au/about-daen-medicines

Thompson, H. A., Hoover, T. A., Vockin, M. A., & Shaw, E. I. (2003). Do Chromosomal Deletions in the Lipopolysaccharide Biosynthetic Regions Explain All Cases of Phase Variation in *Coxiella burnetii* Strains? An update. *Annals of the New York Academy of Sciences, 990*, 664–670. doi:10.1111/j.1749-6632.2003.tb07441.x

Thomson v State of Queensland & Anor, (2019).

Tigertt, W. D., Benenson, A. S., & Gochenour, W. S. (1961). Airborne Q Fever. *Microbiology and Molecular Biology Reviews, 25*(3), 285-293. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC441106/

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. doi:10.3201/eid1007.030724

Tissot-Dupont, H., & Raoult, D. (2008). Q fever. *Infectious Disease Clinics of North America, 22*(3), 505-514, ix. doi:10.1016/j.idc.2008.03.002

Tissot-Dupont, H., Vaillant, V. r., 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*, 232-237. doi:10.1086/510389

Tokarevich, N. K., Panferova, Y. A., Freylikhman, O. A., Blinova, O. V., Medvedev, S. G., Mironov, S. V., . . . Najdenski, H. (2019). *Coxiella burnetii* in ticks and wild birds. *Ticks and Tick-Borne Diseases, 10*(2), 377-385. doi:10.1016/j.ttbdis.2018.11.020

Toman, R., Heinzen, R. A., Samuel, J. E., & Mege, J.-L. (Eds.). (2012). *Coxiella burnetii: Recent Advances and New Perspectives in Research of the Q Fever Bacterium* (Vol. 984). Heidelberg; New York; London;: Springer Dordrecht.

Tozer, S. J., Lambert, S. B., Sloots, T. P., & Nissen, M. D. (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. doi:10.1007/s10096-011-1225-y

Tozer, S. J., Lambert, S. B., Strong, C. L., Field, H. E., Sloots, T. P., & Nissen, M. D. (2014). Potential Animal and Environmental Sources of Q Fever Infection for Humans in Queensland. *Zoonoses and Public Health, 61*(2), 105-112. doi:10.1111/zph.12051

Trelle, S. (2002). Accuracy of responses from postal surveys about continuing medical education and information behavior: experiences from a survey among German diabetologists. *BMC Health Services Research, 2*(1), 15. doi:10.1186/1472-6963-2-15

Tshokey, T., Stenos, J., Tenzin, T., Drukpa, K., Gurung, R. B., & Graves, S. R. (2019). Serological Evidence of *Rickettsia, Orientia*, and *Coxiella* in Domestic Animals from Bhutan: Preliminary Findings. *Vector-Borne and Zoonotic Diseases, 9*(2), 95-101. doi:10.1089/vbz.2018.2336

University of Adelaide. (2021). Guidelines for Student Vaccination. In. Adelaide, SA, Australia: School of Animal and Veterinary Sciences, The University of Adelaide.

University of Melbourne. (2021). Q Fever Screening and Vaccination. Retrieved from https://fvas.unimelb.edu.au/students/general/q-fever

University of Queensland. (2020). Vaccinations and Immunisation - Guidelines. In (Vol. 2.60.08c). Queensland, Australia: The University of Queensland.

University of Sydney. (2021, 8th January 2021). Animal and veterinary science vaccinations. Retrieved from https://www.sydney.edu.au/students/animal-veterinary-science-vaccinations.html

Valencia, M. d. C. S., Rodriguez, C. O., Puñet, O. G., & Giral, I. d. B. (2000). Q Fever Seroprevalence and Associated Risk Factors among Students from the VeterinarySchool of Zaragoza, Spain. *European Journal of Epidemiology, 16*(5), 469-476. Retrieved from http://www.jstor.org/stable/3582123

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. doi:10.1371/journal.pone.0054021

van der Hoek, W., Dijkstra, F., Schimmer, B., Schneeberger, P. M., Vellema, P., & Wijkmans, C. (2010). Q fever in the Netherlands: an update on the epidemiology and control measures. *Eurosurveillance, 15*(12). Retrieved from http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19520

van der Hoek, W., Versteeg, B., Meekelenkamp, J. C., Renders, N. H., Leenders, A. C., Weers-Pothoff, I., . . . Schneeberger, P. M. (2011). Follow-up of 686 patients with acute Q fever and detection of chronic infection. *Clinical Infectious Diseases, 52*(12), 1431-1436. doi:10.1093/cid/cir234

Vapniarsky, N., Barr, B. C., & Murphy, B. (2012). Systemic *Coxiella*-like infection with myocarditis and hepatitis in an eclectus parrot (*Eclectus roratus*). *Veterinary Pathology, 49*(4), 717-722. doi:10.1177/0300985811409251

Vishwanath, S., & Hackstadt, T. (1988). Lipopolysaccharide phase variation determines the complement-mediated serum susceptibility of *Coxiella burnetii*. *Infection and Immunity, 56*(1), 40-44. doi:10.1128/IAI.56.1.40-44.1988

Voth, D. E., & Heinzen, R. A. (2007). Lounging in a lysosome: the intracellular lifestyle of *Coxiella burnetii*. *Cellular Microbiology, 9*(4), 829-840. doi:10.1111/j.1462-5822.2007.00901.x

Wallensten, A., Moore, P., Webster, H., Johnson, C., Burgt, G. v. d., Pritchard, G., . . . Oliver, I. (2010). Q fever outbreak in Cheltenham, United Kingdom, in 2007 and the use of dispersion modelling to investigate the possibility of airborne spread separator. *Eurosurveillance, 15*(12), 19521. Retrieved from http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19521

Webster, J. P., Lloyd, G., & Macdonald, D. W. (1995). Q fever (*Coxiella burnetii*) reservoir in wild brown rat (*Rattus norvegicus*) populations in the UK. *Parasitology, 110*, 31-35. doi:10.1017/s0031182000081014

Wegdam-Blans, M. C., Wielders, C. C., Meekelenkamp, J., Korbeeck, J. M., Herremans, T., Tjhie, H. T., . . . Schneeberger, P. M. (2012). Evaluation of commonly used serological tests for detection of *Coxiella burnetii* antibodies in well-defined acute and follow-up sera. *Clinical and Vaccine Immunology, 19*(7), 1110-1115. doi:10.1128/CVI.05581-11

Weinstein, N. D., & Sandman, P. M. (1992). A model of the precaution adoption process: Evidence from home radon testing. *Health Psychology, 11*(3), 170-180.

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 infective aerosols. *Annals of the New York Academy of Sciences, 70*(3), 528-540. doi:10.1111/j.1749-6632.1958.tb35409.x

Whitney, E. A., Massung, R. F., Candee, A. J., Ailes, E. C., Myers, L. M., Patterson, N. E., & Berkelman, R. L. (2009). Seroepidemiologic and occupational risk survey for *Coxiella burnetii* antibodies among US veterinarians. *Clinical Infectious Diseases, 48*(5), 550-557. doi:10.1086/596705

Wielders, C. C. H., Boerman, A. W., Schimmer, B., Brom, R. v. d., Notermans, D. W., Hoek, W. v. d., & Schneeberger, P. M. (2015). Persistent High IgG Phase I Antibody Levels against *Coxiella burnetii* among Veterinarians Compared to Patients Previously Diagnosed with Acute Q Fever after Three Years of Follow-Up. *PloS One, 10*(1), e0116937. doi:10.1371/journal.pone.0116937

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. *Quarterly Journal of Medicine, 95*(8), 527-538. doi:10.1093/qjmed/95.8.527.

Willemsen, A., Cobbold, R., Gibson, J., Wilks, K., Lawler, S., & Reid, S. (2019). Infection control practices employed within small animal veterinary practices-A systematic review. *Zoonoses and Public Health, 66*(5), 439-457. doi:10.1111/zph.12589

Wilson, L. E., Couper, S., Prempeh, H., Young, D., Pollock, K. G., Stewart, W. C., . . . Donaghy, M. (2010). Investigation of a Q fever outbreak in a Scottish co-located slaughterhouse and cutting plant. *Zoonoses and Public Health, 57*(7-8), 493-498. doi:10.1111/j.1863-2378.2009.01251.x

Woldeyohannes, S., Perkins, N., Baker, P., Gilks, C., Knibbs, L., & Reid, S. (2020). Q fever vaccine efficacy and occupational exposure risk in Queensland, Australia: A retrospective cohort study. *Vaccine, 38*(42), 6578-6584. doi:10.1016/j.vaccine.2020.08.006

Work Safe Victoria. (2015). *Q fever prevention*. Victoria State Government Retrieved from https://www.worksafe.vic.gov.au/resources/q-fever-prevention

WorkCover-QLD. (2019, 5th February 2019). Q fever. *WorkCover Queensland.* Retrieved from https://www.worksafe.qld.gov.au/injury-prevention-safety/hazardous-exposures/biological-hazards/diseases-from-animals/q-fever

WorkSafe Tasmania. (2020, 22nd January 2020). Q fever. Retrieved from https://worksafe.tas.gov.au/topics/Health-and-Safety/safety-alerts/q-fever

World Health Organization. (2018). Causality assessment of an adverse event following immunization (AEFI): user manual for the revised WHO classification (Second edition). In (2nd ed.). Geneva, Switzerland: World Health Organization.

Worswick, D., & Marmion, B. P. (1985). Antibody Responses in Acute and Chronic Q fever and in Subjects Vaccinated Against Q fever. *Journal of Medical Microbiology, 19*(3), 281-296. doi:10.1099/00222615-19-3-281

Wright, J. G., Jung, S., Holman, R. C., Marano, N. N., & McQuiston, J. H. (2008). Infection control practices and zoonotic disease risks among veterinarians in the United States. *Journal of the American Veterinary Medicine Association, 232*(12), 1863-1872.

Yassi, A., Lockhart, K., Buxton, J. A., & McDonald, I. (2010). Vaccination of Health Care Workers for Influenza: Promote Safety Culture, Not Coercion. *Canadian Journal of Public Health, March/April 2010*, S41-S45.

Young, F. W. (1948). Q fever in Artesia California. *Journal of the California Medical Association, 69*(2), 89-90.

Zhang, G., Peng, Y., Schoenlaub, L., Elliott, A., Mitchell, W., & Zhang, Y. (2013). Formalin-inactivated *Coxiella burnetii* phase I vaccine-induced protection depends on B cells to produce protective IgM and IgG. *Infection and Immunity, 81*(6), 2112-2122. doi:10.1128/IAI.00297-13

Zhang, G., & Samuel, J. E. (2004). Vaccines against *Coxiella* infection. *Expert Review of Vaccines, 3*(5), 577-584. doi:10.1586/14760584.3.5.577

Zhang, G. Q., 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. doi:10.4049/jimmunol.179.12.8372

Zhang, Y., Zhang, G., Hendrix, L. R., Tesh, V. L., & Samuel, J. E. (2012). *Coxiella burnetii* induces apoptosis during early stage infection via a caspase-independent pathway in human monocytic THP-1 cells. *PloS One, 7*(1), e30841. doi:10.1371/journal.pone.0030841

Appendix A

National Veterinary Survey

Thank you for your interest in this research project. We are asking for responses to the following questionnaire from veterinarians and veterinary nurses. As mentioned, the questionnaire takes approximately 15 minutes to complete after which you will have the opportunity to go into a prize draw to win an iPad.

By starting the questionnaire you are confirming that you are over the age of 18, the study has been explained and acknowledge that responding is taken as consent to participate.

The School of Animal and Veterinary Sciences Ethics Committee has approved this project. If you have any complaints or reservations about the ethical conduct of this project, you may contact the Committee through the Executive Officer:
Dr Raf Freire, Chair, School of Animal and Veterinary Sciences Human Ethics Committee,
Locked Bag 588, Wagga Wagga 2678 NSW.
Telephone: (02) 6933 4451; Email: rfreire@csu.edu.au.
Any issues you raise will be treated in confidence and investigated fully and you will be informed of the outcome

For other comments or questions please contact Dr Nicholas Wood (Principal Investigator)
nicholas.wood@health.nsw.gov.au 02 9845 1429

Please click on the Next button below to start.

HOW TO PROCEED

To progress through the questionnaire please use the following navigation buttons:

Click the Next button to continue to the next page. Please note that this action will submit any responses entered on the page.

Click the Previous button to return to the previous page.

Any questions marked with an asterisk (*) require an answer in order to progress through the questionnaire.

Click the close button in the top right corner if you need to exit the questionnaire. Please note that you cannot resume the questionnaire at another time.

Click the DONE/SUBMIT button to submit the questionnaire.

Section 1 - About you and your veterinary work

Are you currently working as a veterinarian or veterinary nurse?

- ◯ No
- ◯ Yes

Section 1

Do you intend on returning to work as a veterinarian or veterinary nurse within the next 24 months?

- ◯ No
- ◯ Yes. Please consider your most recent veterinary role when answering the questions.

Section 1

Your sex

- ◯ Male
- ◯ Female

Your age

Your work postcode

What is your position in your current (or most recent) workplace?

- ◯ Veterinarian
- ◯ Veterinary Nurse

Section 1

Which of the following best describes your role in your current (or most recent) workplace?

- ◯ Practice Owner
- ◯ Veterinary Associate
- ◯ General member of veterinary staff within university, government or industry
- ◯ On plant veterinarian (OPV) at an abattoir
- ◯ Other -please specify

Section 1

At which University did you complete your veterinary degree?

- ◯ Sydney University
- ◯ Melbourne University
- ◯ Murdoch University
- ◯ Queensland University
- ◯ Charles Sturt University
- ◯ James Cook University
- ◯ Massey University New Zealand
- ◯ Adelaide University
- ◯ Other -please specify

What year did you graduate?

What is the highest level of post graduate education in veterinary science you have completed?

- ◯ No post graduate education
- ◯ Graduate certificate or diploma
- ◯ Masters Degree
- ◯ ANZCVS membership or equivalent- please specify below
- ◯ PhD or ANZCVS fellowship or equivalent- please specify below

If you have indicated "or equivalent" above please specify.

Section 1

What is the highest level of education in veterinary nursing you have completed?

- ◯ Certificate IV in Veterinary Nursing
- ◯ Diploma in Veterinary Nursing
- ◯ No formal education in Veterinary Nursing
- ◯ Other -please specify

How many years in total have you been engaged in veterinary employment where working directly with animals is part of your routine work? - include time working directly with animals in research, teaching and clinical settings

Section 1

Please estimate the number of hours per week you currently (or most recently) work directly with animals in EACH of the following veterinary environments.

Government	
Corporate practice (e.g Green Cross, Banfield)	
Group private practice / Multi-vet private practice	
Solo private practice	
Industry	
Laboratory	
University	
Abattoir	
Other- estimate hours here and specify below	

Please specify "other" as indicated above.

Section 1

ONLY RESPOND TO THIS QUESTION IF YOU ARE CURRENTLY WORKING IN CLINICAL VETERINARY PRACTICE OR ARE RETURNING TO CLINICAL PRACTICE AFTER A SHORT BREAK EG MATERNITY LEAVE

Thinking of your current (or most recent) veterinary workplace where you spend the most time working directly with animals,please enter the total number of staff, including yourself.

Veterinarians	
Veterinary nurses	
Kennel hands, animal attendants	
Administrative staff	

Section 1

Thinking of your current (or most recent) veterinary workplace where you spend the most time working directly with animals, please estimate the proportion of time spent on each animal species. Please only enter whole numbers- no decimals. (responses should total 100%)

If no animal handling - Please enter 100% here.	
Dogs	
Cats	
Horses	
Dairy cattle	
Beef cattle	
Sheep	
Goats	
Pigs	
Poultry / other birds	
Pocket pets (guinea pigs, ferrets, rabbits etc)	
Fish	
Australian wildlife	
Zoo animals	
Other (please enter percentage here and specify species below)	

Please specify "other" as indicated above.

Section 1

Throughout the course of your veterinary career, on average what do you think your level of exposure to the causative agent for Q fever (Coxiella burnetii) has been?

Don't know	No exposure	Very low exposure	Low exposure	Moderate exposure	High exposure	Very high exposure
◯	◯	◯	◯	◯	◯	◯

Section 2 - Attitudes

The next series of questions focuses on your attitudes towards the Q fever illness and Q fever vaccination. Some of the questions are general in nature while others are asking for a more personal perspective.

Thinking about vaccination for Q fever across each occupation group within each practice type, what would be your recommendations for Q fever vaccination?

Please use a scale of 1 to 7 where;

1. Strongly recommend against vaccination
2. Moderately recommend against vaccination
3. Slightly recommend against vaccination
4. No recommendation either way
5. Slightly recommend vaccination
6. Moderately recommend vaccination
7. Strongly recommend vaccination

VETERINARIANS

	1	2	3	4	5	6	7
Cat and dog only practice	◯	◯	◯	◯	◯	◯	◯
Mixed practice	◯	◯	◯	◯	◯	◯	◯
Farm animal only practice	◯	◯	◯	◯	◯	◯	◯
Equine only practice	◯	◯	◯	◯	◯	◯	◯
Wildlife practice	◯	◯	◯	◯	◯	◯	◯

VETERINARY NURSES

	1	2	3	4	5	6	7
Cat and dog only practice	◯	◯	◯	◯	◯	◯	◯
Mixed practice	◯	◯	◯	◯	◯	◯	◯
Farm animal only practice	◯	◯	◯	◯	◯	◯	◯
Equine only practice	◯	◯	◯	◯	◯	◯	◯
Wildlife practice	◯	◯	◯	◯	◯	◯	◯

KENNEL HANDS, ANIMAL ATTENDANTS AND OTHER ANIMAL HANDLERS

	1	2	3	4	5	6	7
Cat and dog only practice	◯	◯	◯	◯	◯	◯	◯
Mixed practice	◯	◯	◯	◯	◯	◯	◯
Farm animal only practice	◯	◯	◯	◯	◯	◯	◯
Equine only practice	◯	◯	◯	◯	◯	◯	◯
Wildlife practice	◯	◯	◯	◯	◯	◯	◯

ADMINISTRATIVE STAFF WITH NO DIRECT ANIMAL HANDLING

	1	2	3	4	5	6	7
Cat and dog only practice	◯	◯	◯	◯	◯	◯	◯
Mixed practice	◯	◯	◯	◯	◯	◯	◯
Farm animal only practice	◯	◯	◯	◯	◯	◯	◯
Equine only practice	◯	◯	◯	◯	◯	◯	◯
Wildlife practice	◯	◯	◯	◯	◯	◯	◯

Section 2

For this group of statements we are interested in your feelings and would like you to indicate your level of agreement with each of the following.

	Strongly Disagree	Disagree	Slightly Disagree	Slightly Agree	Agree	Strongly Agree
If a vaccine exists for a certain disease, then vaccination is usually a good way to protect someone against this disease	◯	◯	◯	◯	◯	◯
I am convinced of the importance of the Q fever vaccine	◯	◯	◯	◯	◯	◯
I worry that the Q fever vaccine will do more harm than good	◯	◯	◯	◯	◯	◯
It is difficult to get vaccinated for Q fever	◯	◯	◯	◯	◯	◯

Section 2

Again, thinking about your current (or most recent) veterinary workplace from a personal perspective, how concerned are you that.....

	Not concerned	Slightly Concerned	Moderately Concerned	Very Concerned
...you could be exposed to the bacteria causing Q fever	◯	◯	◯	◯
...your co-workers could be exposed to the bacteria causing Q fever	◯	◯	◯	◯
... your family or your co-workers' family could be exposed to the bacteria causing Q fever	◯	◯	◯	◯

Section 2

The following statements are more general in nature, please indicate your level of agreement with each of them.

	Strongly Disagree	Disagree	Slightly Disagree	Slightly Agree	Agree	Strongly Agree	Don't know
Q fever is a serious illness with significant health consequences	◯	◯	◯	◯	◯	◯	◯
The Q fever vaccine is safe if appropriately administered	◯	◯	◯	◯	◯	◯	◯
The Q fever vaccine is effective in preventing Q fever	◯	◯	◯	◯	◯	◯	◯
The Q fever vaccine is too expensive	◯	◯	◯	◯	◯	◯	◯

Section 3 - Q fever exposure

Have you ever had Q fever?

◯ No

◯ Yes

Section 3

What year did you have Q fever? Please estimate if unsure.

How was the diagnosis made?

- ◯ Self diagnosis
- ◯ Medical practitioner -no laboratory testing
- ◯ Medical practitioner -laboratory testing
- ◯ Other -please specify

Did you take time off work as a result of your illness?

- ◯ No
- ◯ Yes

Section 3

Please indicate the number of days or weeks taken off work.

Days OR

Weeks

Section 3

Were you hospitalised during the illness?

- ◯ No
- ◯ Yes

Section 3

Please indicate the number of days or weeks that you were hospitalised.

Days OR

Weeks

To what extent did you experience each of the following:

	Did not experience	Very mild	Mild	Moderate	Severe	Very severe
Fever and chills	◯	◯	◯	◯	◯	◯
Sweats	◯	◯	◯	◯	◯	◯
Headaches	◯	◯	◯	◯	◯	◯
Muscle and joint pains	◯	◯	◯	◯	◯	◯
Fatigue	◯	◯	◯	◯	◯	◯
Endocarditis	◯	◯	◯	◯	◯	◯
Hepatitis	◯	◯	◯	◯	◯	◯
Post-Q fever fatigue syndrome	◯	◯	◯	◯	◯	◯
Pneumonia	◯	◯	◯	◯	◯	◯
Other- please indicate severity here and specify below	◯	◯	◯	◯	◯	◯

Other -please specify

Section 3

Do you personally know anyone who has been diagnosed with Q fever?

- ◯ No
- ◯ Yes

Section 3

How many people do you personally know who have had Q fever?

Number of people

How many of these people (that you know of) experienced a severe complication of Q fever ? eg extended time off work, endocarditis, hepatitis, post-Q fever fatigue syndrome, pneumonia

Number of people

Section 4 - Q fever vaccination

Which of the following best describes your Q fever vaccination status?

- ◯ I have been vaccinated
- ◯ I have not been vaccinated
- ◯ I cannot recall if I have been vaccinated

Section 4

Which of the following best describes your reason for being vaccinated for Q fever?

- ◯ I was vaccinated as part of my university course
- ◯ I was vaccinated as a requirement of my job
- ◯ I actively sought vaccination although it wasn't a specific requirement of my job or university course
- ◯ Other- please specify below

Please specify "other" as indicated above

Section 4

In what year did you receive your Q fever vaccination? Please estimate if unsure.

Please enter year as four digits eg 2002

If you are unable to estimate the year of your Q fever vaccination (above) please select "Don't know"

- ◯ Don't know

Did you experience any adverse effects after Q fever vaccination?

- ◯ No
- ◯ Yes
- ◯ Don't recall

Section 4

Were these adverse effects;

- ◯ Mild
- ◯ Moderate
- ◯ Severe

Did you seek medical attention as a result of any adverse effects of the Q fever vaccine?

- ◯ No
- ◯ Yes
- ◯ Don't recall

Section 4

Was the medical attention received from;

- ◯ General practitioner
- ◯ Hospital emergency room
- ◯ Admitted to hospital
- ◯ Other -please specify

Section 4

Before today, were you aware that there was a Q fever vaccine?

- ◯ No
- ◯ Yes

Section 4

Is the reason you have not been vaccinated for Q fever because you were ineligible as a result of pre-vaccination screening process?

- ◯ No
- ◯ Yes

Section 4

Please rate the extent to which each of the following had an influence on you not being vaccinated to date.

	No influence	Minor influence	Moderate influence	Major influence	Sole reason
I've not been able to access a service provider trained to provide Q fever vaccination	◯	◯	◯	◯	◯
The pre-screening and vaccination process is too time consuming	◯	◯	◯	◯	◯
I cannot afford the financial cost of getting vaccinated	◯	◯	◯	◯	◯
I think the Q fever vaccine may harm my health	◯	◯	◯	◯	◯
I think the Q fever vaccine may not be effective in preventing Q fever	◯	◯	◯	◯	◯
I think I won't be seriously affected by Q fever.	◯	◯	◯	◯	◯
Other - please indicate influence here and specify below	◯	◯	◯	◯	◯

Please specify "other" as indicated above.

Section 5 - Knowledge of disease risk

Please answer this group of questions based on your existing understanding of Q fever.

How would you rate your current level of knowledge of Q fever?

Please use a scale of 1 to 10 where 1 is very low (minimum) level and 10 is very high (maximum) level of knowledge.

1	2	3	4	5	6	7	8	9	10
◯	◯	◯	◯	◯	◯	◯	◯	◯	◯

Section 5

Thinking about each occupation group within each practice type in Australia, what is the level of risk of exposure to the bacterium (Coxiella burnetii) that causes Q fever ?

Please use a scale of 0 to 5 where 0 is no risk and 5 is maximum possible risk.

VETERINARIANS

	0	1	2	3	4	5
Cat and dog only practice	◯	◯	◯	◯	◯	◯
Mixed practice	◯	◯	◯	◯	◯	◯
Farm animal only practice	◯	◯	◯	◯	◯	◯
Equine only practice	◯	◯	◯	◯	◯	◯
Wildlife practice	◯	◯	◯	◯	◯	◯

VETERINARY NURSES

	0	1	2	3	4	5
Cat and dog only only practice	◯	◯	◯	◯	◯	◯
Mixed practice	◯	◯	◯	◯	◯	◯
Farm animal only practice	◯	◯	◯	◯	◯	◯
Equine only practice	◯	◯	◯	◯	◯	◯
Wildlife practice	◯	◯	◯	◯	◯	◯

KENNEL HANDS, ANIMAL ATTENDANTS AND OTHER ANIMAL HANDLERS

	0	1	2	3	4	5
Cat and dog only practice	◯	◯	◯	◯	◯	◯
Mixed practice	◯	◯	◯	◯	◯	◯
Farm animal only practice	◯	◯	◯	◯	◯	◯
Equine only practice	◯	◯	◯	◯	◯	◯
Wildlife practice	◯	◯	◯	◯	◯	◯

ADMINISTRATIVE STAFF WITH NO DIRECT ANIMAL HANDLING

	0	1	2	3	4	5
Cat and dog only practice	◯	◯	◯	◯	◯	◯
Mixed practice	◯	◯	◯	◯	◯	◯
Farm animal only practice	◯	◯	◯	◯	◯	◯
Equine only practice	◯	◯	◯	◯	◯	◯
Wildlife practice	◯	◯	◯	◯	◯	◯

Section 5

If an animal that was infected with the bacterium Coxiella burnetii (the cause of Q fever in humans) was presented to your clinic, please indicate the risk of transmission to someone when performing the following procedures without the implementation of any biosecurity measures (eg. PPE).

Please use a scale of 0 to 5, where 0 is no risk and 5 is maximum possible risk

	0	1	2	3	4	5
Routine physical examination	◯	◯	◯	◯	◯	◯
Faecal flotation	◯	◯	◯	◯	◯	◯
Assisting with parturition (giving birth)	◯	◯	◯	◯	◯	◯
Performing CPR (cardiopulmonary resuscitation)	◯	◯	◯	◯	◯	◯
Collecting and processing blood	◯	◯	◯	◯	◯	◯
Post mortem examination	◯	◯	◯	◯	◯	◯
Cystocentesis	◯	◯	◯	◯	◯	◯
Admitting the patient to hospital cage/stable	◯	◯	◯	◯	◯	◯
Simply being present in the room with the animal	◯	◯	◯	◯	◯	◯
Cleaning cages	◯	◯	◯	◯	◯	◯

Section 6 - Biosecurity practices

Using the broad definition of biosecurity as "a set of preventative measures designed to reduce the risk of transmission of infectious diseases" how would you rate your current level of knowledge of biosecurity as it relates to the work you undertake?

Please use a scale of 1 to 10 where 1 is very low (minimum) level and 10 is very high (maximum) level of knowledge.

1	2	3	4	5	6	7	8	9	10
◯	◯	◯	◯	◯	◯	◯	◯	◯	◯

Do you wash your hands before treating patients?

◯ Never ◯ Rarely ◯ Sometimes ◯ Often ◯ Always

Do you wash your hands after treating patients?

◯ Never ◯ Rarely ◯ Sometimes ◯ Often ◯ Always

Section 6

When performing* your usual veterinary work, what level of personal protective equipment (PPE) do you currently (or most recently) use in the following situations? - Please select all that apply.

***If you are not involved in a listed procedure, please select "Do not perform" for that procedure.**

	Do not perform	No special precautions taken	Protective clothing	Gloves	Surgical Mask	Goggles/face shield	P2/N95 respirator
Routine physical examination	☐	☐	☐	☐	☐	☐	☐
Faecal flotation	☐	☐	☐	☐	☐	☐	☐
Assisting with parturition (giving birth)	☐	☐	☐	☐	☐	☐	☐
Performing CPR (cardiopulmonary resuscitation)	☐	☐	☐	☐	☐	☐	☐
Collecting and processing blood	☐	☐	☐	☐	☐	☐	☐
Post mortem examination	☐	☐	☐	☐	☐	☐	☐
Cystocentesis	☐	☐	☐	☐	☐	☐	☐
Admitting the patient to hospital/stable	☐	☐	☐	☐	☐	☐	☐
Simply being present in the room with the animal	☐	☐	☐	☐	☐	☐	☐
Cleaning cages	☐	☐	☐	☐	☐	☐	☐

Section 6

On average how frequently does your current (or most recent) job require you to perform resuscitation ?

Never - please enter 0

Times per month OR

Times per year

If yes, when resuscitating animals in general, do you use:

	Never	Rarely	Sometimes	Often	Always
Mouth to nose/mouth resuscitation	◯	◯	◯	◯	◯
Breathing bag / resuscitation mask / oxygen mask / ET tube	◯	◯	◯	◯	◯
Other -please select frequency here and specify below	◯	◯	◯	◯	◯

Please specify "other" as indicated above

Section 6

On average how frequently does your current (or most recent) job require you to resuscitate non breathing neonatal animals (eg puppies or kittens) after caesarian birth or difficult births?

Never - please enter 0	
Times per month OR	
Times per year	

If yes, when resuscitating non breathing puppies or kittens after caesarian birth or difficult births, do you use:

	Never	Rarely	Sometimes	Often	Always
Mouth to nose / mouth resuscitation	◯	◯	◯	◯	◯
Breathing bag / resuscitation mask / oxygen mask / ET tube	◯	◯	◯	◯	◯
Other, please select frequency here and specify below	◯	◯	◯	◯	◯

Please specify "other" as indicated above

Section 6

Which of the following information sources are your main source of influence regarding information about work related biosecurity?

	No influence	Minor influence	Moderate influence	Major influence	Sole influence
Protocols established by the employer/practice	◯	◯	◯	◯	◯
Veterinarians within your practice	◯	◯	◯	◯	◯
Veterinarians outside your practice	◯	◯	◯	◯	◯
Veterinary nurses within your practice	◯	◯	◯	◯	◯
Veterinary nurses outside your practice	◯	◯	◯	◯	◯
My personal research through veterinary journals and textbooks, websites etc	◯	◯	◯	◯	◯
My personal research through the internet	◯	◯	◯	◯	◯
Australian Veterinary Association Biosecurity Guidelines	◯	◯	◯	◯	◯
Government or Health authority	◯	◯	◯	◯	◯
Workplace culture	◯	◯	◯	◯	◯
Other- please select influence here and specify below.	◯	◯	◯	◯	◯

Please specify "other" as indicated above

Section 6

What is your level of responsibility within your workplace with respect to workplace health and safety (WHS).

Please use a scale of 1 to 5 where 1 is not at all and 5 is completely responsible.

	1	2	3	4	5
Training of other staff in WHS in my workplace	◯	◯	◯	◯	◯
Ensuring that staff comply with WHS requirements or established protocols in my workplace	◯	◯	◯	◯	◯
Establishing WHS protocols for my workplace	◯	◯	◯	◯	◯
Other - please select level here and specify below	◯	◯	◯	◯	◯

Please specify "other" as indicated above

THANK YOU FOR TAKING THE TIME TO COMPLETE THE QUESTIONNAIRE. YOUR PARTICIPATION IS APPRECIATED.

After you submit the questionnaire you will be redirected to the prize draw for an iPad. You can choose whether you would like to enter this. Please note that this is done via a separate link and will not be linked to your responses to this questionnaire.

IF YOU WOULD LIKE TO OBTAIN MORE INFORMATION ABOUT Q FEVER OR Q FEVER VACCINATION PLEASE VISIT THE FOLLOWING WEBSITES.

Australian Government Department of Health and Ageing- The Australian Immunisation Handbook 10th Edition 2013
www.health.gov.au

CSL Q Vax-R Q fever vaccine
www.csl.com.au

Appendix B

Blood Donor Survey

Thank you for agreeing to participate in our survey.

We are interested in gaining further information on the immune status for Q Fever within the veterinary community of Australia and the longevity of immunity afforded from **Q-VAX**®, the Q Fever Vaccination currently used in Australia.

We are a collegial group of veterinary and medical professionals who are passionate about advancing our understanding of Q fever especially as it relates to veterinarians and veterinary nurses but also the general public. This project is part of a National Health and Medical Research Council Grant (AP10499558)

On average, this survey takes about 4 minutes to complete.

Personal information:

If you would like to receive your Q fever blood test results, please complete your name and address or email address below. Results will be analysed and published as de-identified data, so your personal information will be kept confidential.

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* | - please see research staff for your correct lab ID

Name: _____

Email OR postal address: (*This is the preferred address for correspondence of your lab results*)

_____

_____

Attach ID label here

Q FEVER VACCINATION: HOW LONG DOES PROTECTION LAST?

Section 1: About you and your veterinary work

1. Are you currently working as a veterinarian?

☐ Yes
☐ No → ***In what year were you last working as a veterinarian?*** _____

2. Your sex
☐ Male
☐ Female

3. Your age_____

4. Your current work postcode_____

5. Which **University** did you graduate from for your veterinary degree? _____

6. How many **years in total** have you been **engaged in veterinary employment** where **working directly with animals** is part of your routine work? - *include time working directly with animals in research, teaching and clinical settings* _____

7. Please estimate the number of **hours per week** you **currently** (or most recently) **work directly with animals** in each of the following veterinary environments:

___ Government
___ Private Practice
___ Industry
___ Laboratory
___ On plant veterinarian at an abattoir
___ Other: _____

8. Thinking of your **complete work history** as a veterinarian, please estimate the proportion of time you have spent **working directly with each animal species** (responses should total 100%).

SPECIES	**%**				
No animal handling	_____	Sheep	_____	Fish	_____
Dogs	_____	Goats	_____	Australian wildlife	_____
Cats	_____	Pigs	_____	Zoo animals	_____
Horses	_____	Poultry/Other Birds	_____	Other	_____
Dairy cattle	_____	Pocket pets (guinea pigs, ferrets, rabbits etc)	_____	**Total**	**100%**
Beef cattle	_____				

Section 2 - Q fever vaccination status

9. Which of the following best describes your **Q fever vaccination status**?

   ☐ Yes, I **have been** vaccinated ⟶ Go to Q 11

   ☐ No, I have **NOT** been vaccinated ⟶ Go to Q 10 (next question)

   ☐ I **cannot recall** if I have been vaccinated ⟶ Go to Q 14

10. 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 11 (next question)

    ☐ No ⟶ go to Q14

11. In what **year** did you receive your Q fever screening +/- vaccination? Please estimate if unsure or enter "don't recall" if unable to estimate _____

12. Where did you receive your Q fever screening +/- vaccination?

    ☐ **University** provided health service as a requirement for my veterinary or other animal course

    ⟶ At **which University** was this vaccination administered? _____

    ☐ At a **private general practitioner**

    ☐ **I do not recall**

13. Is your Q fever screening / vaccination history recorded on the **Q fever register**?

    ☐ Yes

    ☐ No

    ☐ I do not recall

Section 3- Q fever disease

14. Have you ever had **Q fever disease**? *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 Q17

15. What **year** did you have Q fever? Please estimate if unsure _____

16. How was the **diagnosis** made?

    ☐ Self diagnosis
    ☐ Medical practitioner - no laboratory testing
    ☐ Medical practitioner - laboratory testing
    ☐ Other _____

THANK YOU FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE.
YOUR PARTICIPATION IS APPRECIATED.

Appendix C

Q-VAX® Product Information

AUSTRALIAN PRODUCT INFORMATION – Q-VAX® Q fever Vaccine and Q-VAX® SKIN TEST Q fever Skin Test (inactivated *Coxiella burnetii*) suspension for injection

1. NAME OF THE MEDICINE

Inactivated *Coxiella burnetii* as active ingredient.

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

Q-VAX® is a purified suspension of formalin-inactivated, *Coxiella burnetii* prepared from the Phase I Henzerling strain of the organism grown in the yolk sacs of embryonated eggs. Trace amounts of ovalbumin (<1 microgram) may also be present.

Q-VAX® Vaccine contains ≥ 25µg of antigen in 0.5 mL of aqueous solution.

Q-VAX® Skin Test contains ≥ 2.5 µg of antigen per 0.5 mL of aqueous solution. **Prior to administration**, Q-VAX® Skin Test is diluted with Sodium Chloride injection to ensure that 16.7 ng (nanograms) of antigen is delivered per 0.1 mL intradermal dose. (see **SECTION 4.2 – DOSE AND METHOD OF ADMINISTRATION**).

Each 0.5 mL Q-VAX® Vaccine also contains sodium chloride 4.1 mg, monobasic sodium phosphate dihydrate 120 microgram, dibasic sodium phosphate dodecahydrate 245 microgram thiomersal as preservative 50 microgram and water for injections to 0.5 mL.

Each 0.1 mL Q-VAX® Skin Test dose after dilution also contains sodium chloride 0.9 mg, monobasic sodium phosphate dihydrate 0.8 microgram, dibasic sodium phosphate dodecahydrate 1.6 microgram, thiomersal as preservative 333 nanogram and water for injections to 0.1 mL.

3. PHARMACEUTICAL FORM

Q-vax® Q-Fever Vaccine is a clear to slightly opaque, colourless suspension for subcutaneous injection.
Q-vax® Skin test is a clear to slightly opaque, colourless suspension for dilution prior to intradermal injection.

4. CLINICAL PARTICULARS

4.1 THERAPEUTIC INDICATIONS

Q-VAX® Vaccine is indicated for the immunisation of susceptible adults at identifiable risk of infection with Q fever.

Abattoir workers (and those closely associated with the meat industry), farmers, veterinarians, stockyard workers, shearers, animal transporters and many others exposed to cattle, sheep or goats or their products should be considered for vaccination.

Note also that Q fever has occurred among persons culling and processing kangaroos and that laboratory personnel handling potentially infected veterinary specimens, or visiting abattoirs, are at risk.

Q-VAX® Skin Test is indicated for the pre-screening of potential vaccine recipients for prior sensitisation to Q fever antigens.

It is essential to test for sensitisation to Q fever antigens using Q-VAX® Skin Test in every individual prior to immunisation (see **SECTION 4.4 – SPECIAL WARNINGS AND PRECAUTIONS FOR USE**).

4.2 DOSE AND METHOD OF ADMINISTRATION

Q-VAX® Vaccine:

Q-VAX® Vaccine should not be administered until the results of serology and skin testing are known (see **SECTION 4.4 – SPECIAL WARNINGS AND PRECAUTIONS FOR USE).** Q-VAX® should be given only to those who have no demonstrable evidence of sensitisation to Q fever antigens.

The dose of Q-VAX® Vaccine is 0.5 mL given by subcutaneous [NOT INTRAMUSCULAR] injection. The container should be gently shaken before use.

The vaccine should never be administered intravenously.

No information is available on paediatric use.

Revaccination must never be undertaken due to the possibility of severe hypersensitivity reactions.

Q-VAX® Skin Test:

Preparation: Skin Test solution should be prepared by diluting 0.5 mL of the Q-VAX® Skin Test in 14.5 mL of Sodium Chloride Injection (to a final volume of 15 mL). The **diluted** Q-VAX® Skin Test should be freshly prepared, stored at 4°C and used within six hours**.**

Administration: The dose administered for skin testing is 0.1 mL of the **diluted** Q-VAX® Skin Test. This should be injected intradermally into the volar surface of the mid-forearm.

4.3 CONTRAINDICATIONS

Q-VAX® should not be administered to:

- Persons who have a history of Q fever
- Persons who have been previously vaccinated with Q fever vaccine
- Persons who have a history of likely exposure followed by an illness strongly suggestive of Q fever
- Persons with positive serology for Q fever antibody or a positive Q fever skin test
- Persons with known hypersensitivity to egg proteins or any component of the medicinal

product.

4.4. SPECIAL WARNINGS AND PRECAUTIONS FOR USE

Prior to immunisation, all potential vaccines must have a serum antibody estimation and a skin test reported; administration of Q-VAX® to those who are already sensitised to Q fever antigens can cause serious hypersensitivity reactions.

As with other injectable vaccines, including Q-VAX® Skin Test solution, appropriate medical treatment and supervision should always be available in case of anaphylactic reactions. Adrenaline should always be readily available whenever the injection is given.

Q-VAX® should never be administered intravenously.

There is no information available on the efficacy and safety of Q-VAX® in immunodeficient or immunosuppressed individuals.

Those who have a confirmed positive antibody test or a positive skin reaction must not be given Q-VAX® (see Pre-vaccination testing).

If the skin test is negative or equivocal and antibodies are present at low titres (reported as a borderline laboratory test result), it cannot be concluded that the subject has adequate *protective* immunity against Q fever. The low-level presence of antibodies may be non-specific or due to technical factors of the assay. The risk-benefit decision of being vaccinated or not should be individually assessed and discussed with the subject, in order to decide whether potential adverse events following vaccination outweigh the potential risk to that subject from Q fever infection and its associated complications.

It should be noted that a very small number of people may have had Q fever in the past and yet show no response to serological or skin testing. Such persons may have severe reactions to Q-VAX®. For this reason, subjects should be carefully questioned regarding the possibility of previous exposure to Q fever and the duration of such exposure.

Workers who are at risk of contracting Q fever should be immunised prior to commencement of work or as soon as possible after they commence work as the risk of infection is highest in the first few years.

Vaccination during the incubation period of Q fever does not prevent the onset of the disease.

Despite the significant efficacy of Q-VAX® in clinical trials, cases of Q fever following vaccination have been reported (see **SECTION 5.1 – PHARMACODYNAMIC PROPERTIES, Clinical Trials**).

Pre-vaccination Testing

Serology: People who are being considered for Q fever vaccination must have serum antibody testing. Subjects in whom antibodies are unequivocally positive should not be given Q-VAX® (see **SECTION 4.4 – SPECIAL WARNINGS AND PRECAUTIONS FOR USE**).

Skin Test:

Preparation: Skin Test solution should be prepared by diluting 0.5 mL of the Q-VAX® Skin Test in 14.5 mL of Sodium Chloride Injection (to a final volume of 15 mL). The diluted Q-VAX® Skin Test should be freshly prepared, stored at 4°C and used within six hours**.**

Administration: The dose administered for skin testing is 0.1 mL of the diluted Q-VAX® Skin Test. This should be injected intradermally into the volar surface of the mid-forearm.

A positive reaction is indicated by any induration at the site of injection read seven days after the test dose. Any person with a positive reaction must not be vaccinated.

Use in the elderly

No data available

Paediatric use

No data available

Effects on laboratory tests

No data available

4.5 INTERACTIONS WITH OTHER MEDICINES AND OTHER FORMS OF INTERACTIONS

No data available.

4.6 FERTILITY, PREGNANCY AND LACTATION

Effects on fertility

No data available.

Use in pregnancy (Category B2)

Safety of use in pregnancy has not been established. Deferral of vaccination is recommended.

Use in lactation

No data available.

4.7 EFFECTS ON ABILITY TO DRIVE AND USE MACHINES

The effect of this medicine on person's ability to drive and use machines were not assessed as part of its registration

4.8 ADVERSE EFFECTS (UNDESIRABLE EFFECTS)

Vaccination of already immune subjects may result in severe local or general reactions, with the possibility of local abscess formation.

Clinical trial data
In a clinical trial in South Australia the following adverse events were recorded amongst 464 persons who received Q-VAX®.

Table 1 Q-VAX® vaccine Clinical Trial Adverse Events

Reaction	Frequency of vaccine reactions (%)
Local	
Tenderness	48
Erythema	33
Induration/oedema	< 1
Systemic	
Headache	9
Fever	0.2

There was a single case report of abscess formation at the injection site.

Post-marketing data

A range of adverse reactions has been reported with clinical use of Q-VAX®. The reactions are summarised below and categorised by frequency according to the following definitions. Very common: ≥ 1/10; common: <1/10 and ≥ 1/100; uncommon: <1/100 and ≥ 1/1000, rare: <1/1000 and ≥ 1/10,000 and very rare: <1/10,000.

Blood and Lymphatic System Disorders
Very rare: Lymphadenopathy

Nervous System Disorders
Common: Headache
Very rare: Dizziness

Gastrointestinal Disorders
Uncommon: Nausea, vomiting and diarrhoea

Skin and subcutaneous tissue Disorders
Common: Delayed skin reaction (presenting up to 6 months after vaccination) at injection site (either vaccination and/or skin test site)
Uncommon: Hyperhidrosis

Musculoskeletal and connective tissue Disorders
Uncommon: Myalgia
Very rare: Arthralgia

General disorders and administration site conditions
Very common: Injection site inflammation (e.g. erythema, pain, warmth and swelling).

Uncommon: Injection site induration and/or oedema, pyrexia, malaise, fatigue
Rare: Injection site abscess formation, granuloma
Very rare: Chills, chronic fatigue syndrome

Reporting suspected adverse effects

Reporting suspected adverse reactions after registration of the medicinal product is important. It allows continued monitoring of the benefit-risk balance of the medicinal product. Healthcare professionals are asked to report any suspected adverse reactions at www.tga.gov.au/reporting-problems.

4.9 OVERDOSE

For information on the management of overdose, contact the Poisons Information Centre on 13 11 26 (Australia).

5. PHARMACOLOGICAL PARTICULARS

5.1 Pharmacodynamic properties

Mechanism of action

Q fever is caused by *Coxiella burnetii*, an obligate, intracellular, Gram-negative coccobacillus. The *C. burnetii* is shed in the products of conception, and on the neonate of the infected animal. It may also be present in the udder and milk of infected animals and is passed on within their faeces and urine. Infection is transmitted to humans primarily by inhalation of infected airborne particles or dust during the handling or processing of these materials or by close proximity to infected animals and their products.

Administration of inactivated *Coxiella burnetii* in Q-vax vaccine stimulates production of an immune response in the vaccinated individual. The immune response provides protection against clinical illness in a high proportion of vaccinated individuals, but may not be effective in some individuals.

Early antibody response to the vaccine is predominantly with the IgM subclass; IgG antibodies appear later. Although the seroconversion rate is low (50-80%) and antibody levels are transient, cell mediated immunity develops. Clinical trials have demonstrated a high degree of efficacy (see **SECTION 5.1 – PHARMACODYNAMIC PROPERTIES, Clinical Trials**). As Q fever is often asymptomatic or misdiagnosed due to its non-specific nature, many abattoir workers develop immunity to Q fever without an obvious illness.

The duration of protective immunity following immunisation is unknown, but is believed to be in excess of five years.

Revaccination must never be undertaken due to the possibility of severe hypersensitivity reactions (see SECTION 4.3 – CONTRAINDICATIONS).

Clinical trials

A randomised, blind, controlled study comparing Q-VAX® and influenza vaccine for the prevention of Q fever amongst 200 workers in three Queensland abattoirs was undertaken, using sequential analysis for determining the efficacy of Q-VAX®. A statistically significant difference in the incidence of symptomatic Q fever was noted 15 months after commencement of vaccination, with 7 cases in those given the control vaccine and no cases in those given Q-VAX®. At 15 months, 24% of those who had not been vaccinated and had not developed symptomatic infection had serological evidence of exposure to Q fever, indicating subclinical infection.

A retrospective cohort study in three South Australian abattoirs was undertaken to compare the incidence of Q fever in vaccinated and unvaccinated subjects between 1985 and 1990. There were two cases of Q fever amongst 2555 vaccinated employees compared with 55 cases in 1365 unvaccinated subjects. Both cases of Q fever in the vaccinated group occurred within two weeks of receiving the vaccine. For workers who were vaccinated, the mean duration of employment following vaccination was 1.9 years; 203 workers were employed for all five years of the study. Protection against clinical infection over this period was demonstrated.

Although the dose in each of these studies was nominally 30 µg, one batch which contained only 20 µg in each dose was shown to be as effective. However, as with all vaccines, 100% effectiveness for generation of protective immunity against Q fever cannot be guaranteed (see **SECTION 4.4 – SPECIAL WARNINGS AND PRECAUTIONS FOR USE**).

5.2 PHARMACOKINETIC PROPERTIES

Not applicable

5.3 PRECLINICAL SAFETY DATA

Genotoxicity

No data available

Carcinogenicity

No data available

6. PHARMACEUTICAL PARTICULARS

6.1 LIST OF EXCIPIENTS

Refer to **SECTION 2 – QUALITATIVE AND QUANTITATIVE COMPOSITION**.

6.2 INCOMPATIBILITIES

Incompatibilities were either not assessed or not identified as part of the registration of this

medicine.

6.3 SHELF LIFE

In Australia, information on the shelf life can be found on the public summary of the Australian Register of Therapeutic Goods (ARTG). The expiry date can be found on the packaging.

6.4 SPECIAL PRECAUTIONS FOR STORAGE

Q-VAX® Vaccine and Q-VAX® Skin Test should be protected from light and stored at 2°- 8°C. Refrigerate. DO NOT FREEZE.

6.5 NATURE AND CONTENTS OF CONTAINER

AUST R 100517

Q-VAX® Vaccine is available as a pre-filled syringe containing ≥ 25 µg of antigen, in 0.5 mL solution.
The syringe and all associated syringe components do not contain natural rubber latex. The Q-VAX® Vaccine syringe is supplied in a moulded plastic blister with peel-off paper cover. Do not use if the blister pack encasing the syringe is damaged or missing.

AUST R 100518

Q-VAX® Skin Test is available as a pre-filled vial containing ≥ 2.5 µg of antigen, in 0.5 mL solution. **Q-VAX® Skin Test must be diluted prior to use in pre-vaccination screening (see SECTION 4.2 – DOSE AND METHOD OF ADMINISTRATION).** The vial and all associated components do not contain natural rubber latex. The Q-VAX® Skin Test vial is packaged with a plastic tear away cap covering the vial septum. Do not use if the tear away cap on the vial is damaged or missing.

6.6 SPECIAL PRECAUTIONS FOR DISPOSAL

In Australia, any unused medicine or waste material should be disposed of in accordance with local requirements,

6.7 PHYSICOCHEMICAL PROPERTIES

Not applicable

7. MEDICINE SCHEDULE (POISONS STANDARD)

Prescription Only Medicine (S4)

8. SPONSOR

Seqirus Pty Ltd
ABN 26 160 735 035
63 Poplar Road
Parkville, VIC 3052
Australia

9. DATE OF FIRST APPROVAL

9 July 1999

10. DATE OF REVISION

26 August 2019

SUMMARY TABLE OF CHANGES

Section Changed	Summary of new information
2	Replace 'Excess egg proteins are removed by fractionation and ultracentrifugation' by "**Trace amounts of ovalbumin (<1 microgram) may also be present."**
6.2	Update ingredient names for compliance with AAN
6.5	Addition of latex statement
All	Updated as per TGA Form for providing PI dated Mar 2018

Appendix D

Adverse Events Following Immunisation Survey

THE UNIVERSITY OF SYDNEY

Discipline of Paediatrics and Child Health
Sydney Medical School

ABN 15 211 513 464

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PARTICIPANT CONSENT FORM

I, ...[PRINT NAME], give consent to my participation in the research project

TITLE: **Adverse events following Q fever vaccination**

In giving my consent I acknowledge that:

1. The procedures required for the project and the time involved have been explained to me and any questions I have about the project have been answered to my satisfaction.

2. I have read the Participant Information Statement and have been given the opportunity to discuss the information and my involvement in the project with the researcher/s.

3. I understand that being in this study is completely voluntary – I am not under any obligation to consent.

4. I understand that my involvement is strictly confidential. I understand that any research data gathered from the results of the study may be published however no information about me will be used in any way that is identifiable.

5. I understand that I can withdraw from the study at any time, without affecting my relationship with the researcher(s) or the University of Sydney now or in the future.

.....
Signature

....
Please PRINT name

Date.....

Email: _____ **Age** _____**years**

Q fever vaccination survey

1. What is your gender?

- ◯ Female
- ◯ Male

2. Did you experience pain at the injection site?

- ◯ yes
- ◯ no

If yes (please specify) 1 = pain on touch, 2 = pain when limb moved, 3= pain with no movement

3. Did you experience redness at the injection site?

- ☐ yes
- ☐ No

If yes, please measure largest diameter in mm using a ruler

4. Did you experience swelling at the injection site?

- ◯ yes
- ◯ no

If yes, please specify largest diameter of swelling in mm using a ruler

5. How many days after the vaccine did the injection reaction begin?

- ☐ 1 day
- ☐ 2-5 days
- ☐ >5 days

6. Did you seek medical attention for the injection site reaction?

- [ ] No
- [ ] Yes, University health service
- [ ] Yes, My GP
- [ ] Yes, Emergency department

7. Did you experience fever in the 7 days following the vaccine?

- [ ] yes
- [ ] no

If yes, what was the highest temperature

8. Did you experience headache in the 7 days following the vaccine?

- [ ] yes
- [ ] no

If yes, did you seek medical attention and to whom

9. Did you experience lethargy or weakness in the 7 days following the vaccine?

- [ ] yes
- [ ] no

If yes, did you seek medical attention and to whom

10. Did you experience joint pain in the 7 days following the vaccine?

- [ ] yes
- [ ] no

If yes, did you seek medical attention and to whom