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Coxiella burnetii seroprevalence and Q fever in Australian wildlife rehabilitators

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ABSTRACT

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

1. Introduction

Coxiella burnetii is the causative agent of the zoonotic disease Q fever, which is recognised as a public health concern globally [46]. Infection is typically acquired via the inhalation of aerosols contaminated with the bacterium. Although domestic ruminants are the main reservoirs of human disease [70], direct evidence of *C. burnetii* infection has also been identified in a variety of wild and domestic animal species including: dogs [63], cats [42], horses [44] birds [1] and macropods [10,52,61]. Following human infection, clinical outcomes vary in severity, ranging from asymptomatic infection with seroconversion, to a flu-like illness. In some instances, Q fever may progress to chronic forms including endocarditis that may result in death [55]. Additionally, post Q fever fatigue syndrome is a relatively common clinical sequela to Q fever disease [45]. The economic impact of Q fever disease in Australia is considerable with the cost of compensation alone estimated to exceed $AU1.3 million ($US960 000) annually [41].

In Australia, Q fever has been a notifiable human disease in all states

Abbreviations: Australian Statistical Geography Standard, ASGS; Australian Wildlife Rehabilitation Conference, AWRC; Australian wildlife rehabilitators, AWRs; Indirect immunofluorescence assay, IFA; Personal protection equipment, PPE; Q fever disease, QFD; Q fever disease status, QFDS; Q fever serostatus, QFSS; Q fever vaccination, QFV; Q fever vaccination status, QFVS.

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and territories since 1977 [28]. It is the most frequently reported directly transmitted zoonosis [47] with the highest Q fever notification rates typically associated with livestock/meat industry workers in New South Wales (NSW) and Queensland (QLD) [28]. A safe and highly effective human Q fever vaccine (Q-Vax®; Seqirus, Parkville, Vic.) has been available in Australia since 1989, and vaccination is recommended for high-risk occupational groups such as veterinary personnel, and abattoir and livestock workers [8]. Recently, the recommendation for Q fever vaccination (QFV), has been extended to wildlife and zoo workers, with kangaroos particularly mentioned amongst the list of 'high risk' animals [8].

Over the past decade in Australia, there has been an increased incidence in Q fever notifications with minimal known exposure to well-documented risk factors [3,14–16,26,39,50,66], and there is a growing body of evidence suggesting macropods, in particular kangaroos, represent a potential source of *C. burnetii* infection for humans. *Coxiella burnetii* has been isolated from the ticks of infected kangaroos [52], and *C. burnetii* DNA has been identified in kangaroos [10,17,53,61] and other wildlife including bandicoots [12] and their associated ticks [12,17]. A Western Australian study found *C. burnetii* DNA in the faeces of kangaroos co-grazing with livestock, along with a *C. burnetii* seroprevalence of 33% in these same animals [10]. Furthermore, *C. burnetii* DNA was recently detected in samples of raw meat containing kangaroo sold for pet consumption [61]. Ongoing occupational exposure to kangaroo and wallaby carcasses was postulated as a possible source of *C. burnetii* infection for a Queensland park ranger who contracted Q fever in 2015 [65]. Q fever has also been reported in individuals working in outdoor environments inhabited by kangaroos, or on grounds heavily contaminated with kangaroo faeces, and in those handling juvenile joeys [25]. Although molecular evidence of *C. burnetii* was not found in any of the kangaroo samples tested, the association with macropods in these cases was still considered a plausible risk factor for *C. burnetii* transmission. Combined, these studies suggest that wildlife rehabilitators can potentially acquire Q fever by handling sick, injured and orphaned wildlife.

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

2. Materials and methods

2.1. Study design and recruitment

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

2.2. Sample size calculation

Since the population size of wildlife rehabilitators across the whole of Australia was not available, an estimation based on the known number of rehabilitators in NSW was made. The population of NSW was 7.7 million of which approximately 4600 (0.06%) [56] people engaged in wildlife rehabilitation. The relative proportion of wildlife rehabilitators residing in other Australian states and territories was presumed to be similar to NSW and were subsequently calculated using the Australian Bureau of Statistics 2016 population figures [4]. The population estimates from each jurisdiction were summated to give an estimated national wildlife rehabilitator population size of 14,358. The sample size for this study was calculated using Statulator software [20]. Assuming a nationwide average of 3% seroprevalence of Immunoglobulin G (IgG) antibodies to *C. burnetii* [30], an expected response rate of 15% [based on a serosurvey of veterinary workers [58]] and a national wildlife rehabilitator population size of 14,358, this study would require a sample size of 117 AWRs for estimating *C. burnetii* seroprevalence with 8% absolute precision and 95% confidence.

2.3. Questionnaire

Participants completed a questionnaire to accompany their blood sample (Supplement 1). A unique identification number assigned to each participant was used to label their questionnaire and corresponding blood collection tube. The questionnaire consisted of 32 questions (24 closed and eight Likert scale) and was divided across four sections containing questions on (i) demographics of the rehabilitator and where they rehabilitated wildlife, (ii) the type of wildlife they rehabilitated and other animals located on or nearby to the caring residence (iii) their rehabilitation and husbandry practices, (iv) a history of Q fever disease (QFD) and vaccination status. Each participant was provided with an information statement explaining the purpose and expected outcomes of the research, and written consent was obtained prior to study participation. At the end of the questionnaire, participants could opt to be notified of their individual serological results, as well as a receive a summary of the study outcomes.

2.4. Laboratory methods

2.4.1. Blood sample collection

Approximately 8 mL of blood was drawn from the median cubital vein of each participant into serum separator tubes (Interpath, Victoria, Australia) by a certified venepuncturist or registered doctor. The blood was centrifuged at 4000 $\times g$ for 5 min, after which the serum was removed and stored at 20 °C until transportation to the laboratory.

2.4.2. Indirect immunofluorescence antibody testing

The serum samples were analysed at the Australian Rickettsial Reference Laboratory (ARRL), Geelong, Australia using an in-house indirect immunofluorescence assay (IFA) accredited by the *National Association of Testing Authorities* (accreditation No. 14342). Initial screening of serum samples was conducted using a 1/25 and a 1/400 (to detect prozone phenomenon) dilution of sera in 2% casein. Approximately 2 μL of diluted serum was spotted in duplicate onto a glass slide coated with *C. burnetii* Phase I or Phase II antigen (Virion/Serion, Germany). After incubation at 35 °C for 40 min, the slides were washed with PBS (diluted 1/10) and air-dried before adding a combined conjugate containing fluorescein-labelled goat anti-human IgA + IgG + IgM (H + L). The incubation and wash steps were repeated, the slides were dried, mounted with a cover slip and read using a fluorescent microscope (400×; Axioskop 40; Zeiss). Positive sera underwent a doubling dilution series (1/25 to 1/3200 in 2% casein) with and without rheumatoid factor removal reagent (Virion/Serion, Germany) to reduce non-specific binding. Each serum dilution was tested against three fluorescein labelled goat anti-human conjugates, anti-IgM, anti-IgG anti-IgA, and total conjugate containing anti- IgA + IgG + IgM (H + L) using the methodology described above. For both screening and titration, positive and negative human serum samples were included on each slide as controls, and serum was considered positive if fluorescence was observed at a dilution of 1/25 or greater. All antibodies were manufactured by KPL/ SeraCare (USA). Criteria adapted from Healy et al. [37] was used to classify exposure with relatively recent exposure considered if Phase I and/or phase II IgG $\geq$1/50 and phase II IgM $\geq$1/50 and past exposure if Phase I and/or phase II IgG $\geq$1/50 and phase II IgM $<$1/50.

2.5. Statistical analysis

2.5.1. Data management

Participants completed paper questionnaires, and the data were manually entered into the secure online platform REDCap (Research Electronic Data Capture) [35,36], hosted at The University of Sydney, Australia. A subset (10%) of randomly selected questionnaires were checked for transcription errors, after which the data were exported into Microsoft® Excel® (Microsoft Corporation, Washington, USA) for cleaning and processing. Data analysis was performed using R statistical program® (R [54]).

2.5.2. Outcome variables and risk factors

The primary outcome variable was whether the rehabilitator was *C. burnetii* seropositive or seronegative [Q fever serostatus (QFSS)]. Secondary outcome variables included whether the participant had or had not been vaccinated against Q fever [Q fever vaccination status (QFVS)] and whether or not the participant had been medically diagnosed with Q fever disease [(Q fever disease status (QFDS)]. All outcome variables were dichotomous. Descriptive statistics (mean, median and range for continuous variables, bar charts for categorical variables) were generated to obtain information regarding the distribution of each variable. The continuous variables age and number of animals cared for per year were categorised and then collapsed into condensed categories for further analyses. Questions regarding animal exposure were collapsed into four groups as follows: ruminants (cattle, goats, sheep), macropods (kangaroos and wallabies), domestic species (dogs, cats, pigs, horses, poultry) and other wildlife species (bandicoots, possums, flying foxes, koalas, wombats and other wildlife). Responses to questions which utilised a Likert scale (frequently, occasionally, rarely, never) were collapsed and categorised as 'yes' if the response was frequently or occasionally, and 'no', if the response was rarely or never. Variables with 10% missing data were not included in the statistical analysis. Participants unsure of their QFVS (5/165) were excluded from the final data set.

Two additional variables were generated from postcode of residence: (1) Australian state of residence and (2) Australian Statistical Geography Standard (ASGS) Remoteness Area. Participant's postcodes were matched to the corresponding remoteness area, according to the Australian Bureau of Statistics (ABS) remoteness structure, which divides Australia into five geographic regions of relative remoteness (Major cities of Australia, Inner Regional Australia, Outer Regional Australia, Remote Australia and Very Remote Australia) [5]. Postcodes spanning more than one remoteness category, were allocated to the category that contained the majority of the geographic area of the postcode.

Biosecurity practices were based on two questions in which participants indicated how frequently ('always', 'frequently', 'occasionally', 'rarely' or 'never') they utilised the following infection control practices while handling animals and cleaning enclosures: overalls/protective outerwear, disposable gloves, safety glasses, face mask, and prompt hand washing. Biosecurity practices were deemed inadequate if participants 'never' used any form of PPE when handling animals or cleaning enclosures. The use of each type of infection control was considered adequate if 'always' or 'frequently' was selected. A participant's biosecurity practice was considered adequate if they 'always' or 'frequently' used overalls/protective outerwear and practiced prompt hand washing when handling animals, and additionally wore disposable gloves when cleaning enclosures. Respondents were considered to practice enhanced biosecurity when handling animals if they 'always' or 'frequently' used overalls/protective outerwear, practiced prompt hand washing and wore disposable gloves, or if all five methods of infection control were practiced when cleaning enclosures. This assessment and classification of adequate and enhanced biosecurity were established by the authors, using recommendations from the Australian Veterinary Association Guidelines for Veterinary Personal Biosecurity [9], in combination with National Wildlife Biosecurity Guidelines (Wildlife Health [69]).

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

2.5.3. Modelling

Univariable logistic regression was conducted to identify associations between potential risk factors and the outcome variables QFSS and QFVS. Risk factors with $p < 0.3$ in the univariable analysis were progressed to multivariable analysis after evaluation of the strength of association between these risk factors using the Cramer's V statistic. When the correlation coefficient for a pair of risk factors was >0.7 only the variable deemed more biologically plausible was included in subsequent multivariable analysis. Multivariate modelling was performed using backward selection where the variable with the least significance (Wald test) was removed sequentially. Variables with p-values <0.1 were retained in the final model. Occupational animal contact was considered a potential confounder and included in the multivariable model for the outcome variable QFSS a priori due to its association with positive serology.

3. Results

3.1. Responses

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

3.2. Demographics of Australian wildlife rehabilitators

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

Participants were predominantly from NSW (53.8%; 86/160) followed by Western Australia (WA; 13.1%, 21/160), Victoria (VIC; 12.5%, 20/160), QLD (9.4%; 15/160), South Australia (SA; 6.3%, 10/160), Tasmania (TAS; 1.9%, 3/160), Northern Territory (NT; 1.3%, 2/160) and the Australian Capital Territory (ACT;1.9%, 3/160). Although all Australian states and territories were represented, the proportion of AWRs residing in NSW was higher, and the proportions in VIC and QLD were lower (53.8%, 12.5% and 9.4% respectively), compared to the available total national population estimates for these states (32%, 26% and 20% respectively) [6]. The proportions within the remaining jurisdictions of WA, SA, TAS, ACT and NT (combined 24.4%) were

comparable to the Australian population distribution. According to the available data on population distribution via state and remoteness area (National Rural Health [49]), the proportion of the cohort living in major cities was lower (48% vs 70%), while the proportion living in inner regional Australia was higher (39% vs 18%) than the distribution of the general Australian population. Thirteen percent (20/160) of AWRs resided in outer regional/remote areas, which was similar to the population distribution for these remoteness categories (11%).

3.3. Wildlife rehabilitating practices

Of the 160 AWRs, 98.1% (157/160) who rehabilitated animals in the same state as their residence, 83.7% (134/160) resided in the same geographical postcode in which their wildlife rehabilitation was undertaken, and 78.6% (125/159) of rehabilitators spent more than 30 weeks per year caring for wildlife. The number of animals cared for per year ranged from 2 to 1500, with three rehabilitators reporting having cared for over 1000 animals per year. Of the 93.1% (149/160) of AWRs who rehabilitated animals on their own property, 20.1% (30/149) housed animals exclusively within their home, 8.7% (13/149) used outdoor enclosures and 71.1% (106/149) practiced both housing arrangements. Regarding the primary location at which rehabilitation was undertaken, 89.4% (143/160) of respondents rehabilitated wildlife primarily in their home or someone else's home, 25.0% (40/160) in a wildlife rescue centre/dedicated wildlife hospital, 13.8% (22/160) in a veterinary clinic that also treats wildlife and 3.8% (6/160) primarily rehabilitated wildlife in a zoo. Of the 58.8% (94/160) of AWRs who reported occupational contact with animals, 37.2% (35/94) had been exposed to ruminants, 78.7% (74/94) to domestic animals, 53.2% (50/94) to macropods and 71.3% (67/94) to other animals including wildlife. Over half of the participants had frequently rehabilitated macropods throughout their wildlife rehabilitation career and in the year prior (61.9%; 99/160 and 52.9%; 83/157 respectively). Overall, the most commonly and frequently rehabilitated species, over the duration of their rehabilitation career and in the year prior (data not shown) were possums and gliders followed by macropods (kangaroos and wallabies). Almost all (96.2%, 152/158) had handled orphaned joeys, 43.8% (70/160) had been bitten by a tick, and 27.5% (44/160) had been present or assisted with a non-human birth.

Biosecurity practices adopted when handling animals and cleaning enclosures are presented in Table 1. While the majority of AWRs practiced prompt hand washing after handling animals and cleaning enclosures, 3.2% (5/158) of AWRs indicated that they did not practice any form of biosecurity during or after either activity. Disposable gloves were worn more frequently when cleaning enclosures than when handling animals ($p = 0.002$), however the vast majority of AWRs did not meet adequate biosecurity requirements in either situation, and only 5.1% (8/158) and 1.9% (3/158) practiced enhanced biosecurity when handling animals and cleaning enclosures respectively.

Table 1
Biosecurity practices reported by 158 Australian wildlife rehabilitators when handling animals and cleaning animal enclosures. Results obtained from a survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018.

	Number (%) of participants when handling animals	Number (%) of participants when cleaning enclosures
Biosecurity practice		
No PPE	5 (3.2)	5 (3.2)
Prompt hand washing	153 (96.8)	153 (96.8)
Overalls/ protective outerwear	20 (12.7)	29 (18.4)
Disposable gloves	36 (22.8)	61 (38.6)
Safety glasses	5 (3.2)	10 (6.3)
Face mask	4 (2.5)	8 (5.1)
Level of biosecurity practice[a]		
Inadequate	138 (87.3)	136 (86.1)
Adequate	12 (7.6)	19(12.0)
Enhanced	8 (5.1)	3 (1.9)

[a] Level of biosecurity practice was based on reported personal protection equipment (PPE) use and recommendations from the Australian Veterinary Association Guidelines for Veterinary Personal Biosecurity [9] and National Wildlife Biosecurity Guidelines (Wildlife Health [69]).

3.4. Q fever serostatus and investigated potential risk factors

Serological results of vaccinated and unvaccinated rehabilitators are presented in Table 2. Nine (6.1%; 95% CI 2.8%–11.3%) of the 147 unvaccinated participants were *C. burnetii* seropositive, and all except one rehabilitator resided in either NSW or QLD. The two participants whose serological response was classified as 'recent exposure' also resided in NSW and QLD, were unvaccinated and one described themselves as a wildlife rehabilitator, and the other a wildlife researcher/student. Seven of the nine (77.7%) seropositive participants had rehabilitated macropods, 5/9 (55.6%) had been present at, or assisted with non-human births, and 7/9 (77.7%) had been exposed to animals through their occupation.

Of the 18 potential risk factors investigated for association with positive *C. burnetii* serostatus amongst the 147 unvaccinated AWRs, six had a $p < 0.3$ in the univariable analyses (Table 3). Participants were more likely (OR 3.7 95%; CI 0.92–15.60) to be seropositive if they had been present at, or assisted with non-human births, and participants residing in QLD were twice as likely (OR 2.3; 95% CI 0.38–14.54) to be seropositive than those living in NSW. All measures of association returned a Cramer's V p value <0.7; therefore all six variables were included in the multivariable model. Although not significant ($p = 0.535$) in the univariable analysis, 'occupational animal contact' was considered a confounder, and therefore included in the model. Multivariable analysis was unsuccessful in producing a final model of risk factors associated with a positive QFSS.

3.5. Q fever diagnosis

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

3.6. Q fever vaccination and investigated potential risk factors

Thirteen (8.1%; 95% CI 4.4%–13.5%) of the 160 participants self-reported having been vaccinated against Q fever and all reported never being diagnosed with QFD. The majority (84.6%; 11/13) of the 13 vaccinated participants resided in NSW (7/13) or QLD (4/13) and reported having received the Q fever vaccine through their General

Table 2
Serological results, and self-reported Q fever vaccination of, and Q fever disease in, wildlife rehabilitators participating in a *Coxiella burnetii* seroprevalence survey at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018.

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

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

Table 3
Univariable logistic regression analysis of positive serological result for *C. burnetii* exposure amongst Australian wildlife rehabilitators participating in a survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. ($p < 0.3$).

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

* $p > 0.3$ but considered a confounder a priori and therefore included in the multivariable analysis.

Practitioner (GP) (6/13) or a workplace/university vaccination program (7/13). All rehabilitators who had been vaccinated through a vaccination program, reported occupational contact with ruminants. Of the 26 (26/160; 15.5%) veterinary nurses participating in this study, 93.3% (24/26) were not vaccinated two (8.3%) of whom were seropositive.

Univariate logistic regression identified six risk factors (out of 14) that were associated with having received QFV ($p < 0.3$) (Table 4). Of these, 'occupational animal contact' was highly significant; AWRs reporting occupational contact with ruminants were six times more likely to have received QFV (OR 6.2; 95% CI 1.66–30.09) than those reporting no occupational contact with animals. The risk factor 'state of residence' was also significant; AWRs residing in QLD were four times

Table 4
Univariable logistic regression analysis of Q fever vaccination amongst Australian wildlife rehabilitators participating in a survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018 ($p < 0.3$).

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

more likely to have been vaccinated (OR 4.26; 95% CI 0.99–16.68) than those residing in NSW or other Australian jurisdictions. Of the six risk factors considered in the multivariable analysis, three were retained in the final model (Table 5). After accounting for the state of residence, and the total number of animals rehabilitated per year, 'occupational animal contact' was the only significant risk factor for QFV; AWRs reporting occupational contact with ruminants were eight times more likely to have received QFV (OR 8.1; 95% CI 1.85–45.09) than those who had no occupational contact with animals.

4. Discussion

This is the first study to investigate *C. burnetii* exposure amongst Australian wildlife rehabilitators. We report an overall seroprevalence for this cohort of 6.1%, which is 70% greater than the recently reported 3.6% in an Australian study of Red Cross blood donors from non-metropolitan NSW and QLD (using the same laboratory techniques as the current study) [30]. Two other Australian studies (also employing the same laboratory methods as the current study), have reported higher general population *C. burnetii* seroprevalence: Islam et al. [39] estimated an overall seroprevalence of 7% in the Hunter New England region of NSW, and more recently Gidding et al. [31] reported a 5.6% nationwide *C. burnetii* seroprevalence, however these two studies used archived sera that were opportunistically obtained from pathology laboratories. We believe that the Red Cross blood donor study is a closer approximation to the assumed healthy participants in the current study, as blood donors must be of good general health and meet specific eligibility criteria to donate blood [7]. Furthermore, participants in the current study, and the blood donor study, completed a questionnaire which accompanied their blood sample that contained specific questions regarding their demographic details, QFDS, QFVS and potential exposure history. This enabled a detailed analysis of the respective data sets to identify potential risk factors associated with seropositivity and having received Q fever vaccination. The 6.1% seroprevalence observed in the current study is lower than the 19% *C. burnetii* seroprevalence reported for a cohort of unvaccinated Australian veterinary workers, where increasing exposure to ruminants was identified as a significant risk factor for seropositivity [58]. International studies of livestock veterinarians have reported seroprevalence for *C. burnetii* as high as 65.1% [67]. Our findings suggest that rehabilitators of Australian wildlife are almost twice as likely to be exposed to *C. burnetii* compared to the general population, but only a third as likely to be exposed as Australian veterinarians associating with ruminants.

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

Table 5
Final multivariable logistic regression analysis of Q fever vaccination amongst Australian wildlife rehabilitators participating in a survey conducted at the Australian Wildlife Rehabilitation Conference in Sydney in July 2018. ($p < 0.1$).

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

diagnosis [46], due to temporal decline in antibody levels and the variability in immune responses between individuals [58], the 6.1% seroprevalence observed in this study likely represents the minimum level of *C. burnetii* exposure amongst this cohort. This heterogeneity in antibody titres is demonstrated by the finding that only 30.7% (4/13) of vaccinated participants and two of the three participants with medically diagnosed QFD were seropositive at the time of blood collection (Table 2). Similarly, in the study by Gidding et al. [30], only 10% of vaccinated blood donors and 39% of donors with a history of QFD were seropositive. Additional AWRs with previous *C. burnetii* exposure may have been identified via intradermal skin testing or the measurement of interferon gamma production in response to *C. burnetii* antigenic stimulation [57], however such tests were beyond the scope of this study.

Currently there is limited information available on the demographics of AWRs, however one recent study on NSW rehabilitators reported that 79% were female and over half were > 50 years [34], which was reflected in the findings of this study. A potential source of bias in this study is the representativeness of the cohort with respect to the proportion of wildlife rehabilitators in Australia and notifications across states. In this study the state of NSW was overrepresented by 22%, which could have resulted in a higher number of seropositive participants given that 43% of the national Q fever notifications in 2018 were from NSW [48]. However, this was offset to some extent by an 11% underrepresentation of participants from QLD which has similarly high notification rates as NSW [48]. The higher number of participants from NSW compared to other states was not unexpected given it was the host state, making travel and attendance easier for these participants. It is recognised that there may be self-selection bias with rehabilitators choosing to participate in the study because of previous experience or association with Q fever, however with half the participants of the conference engaging in the study, the impact of this potential bias is likely limited.

While this study demonstrated that *C. burnetii* exposure was higher in AWRs compared to the general population, and although QFD notification data suggests that macropods are potential sources of infection [15,16,32], we were unable to demonstrate a positive correlation between *C. burnetii* seropositivity and exposure to macropods (adults or juveniles) within this cohort based on the responses to the questionnaire. Nor were we able to identify that exposure to ruminants, other domestic animals or other wildlife, or being present at non-human births were risk factors for *C. burnetii* seropositivity. This was surprising given that the majority of QFD notifications are ruminant associated [15,16,32], and that birth products of infected animals, particularly ruminants, can potentially contain high levels of *C. burnetii* [46,68]. In this study, seropositivity was also not associated with tick bites. Similarly, a study of Q fever in Belgian veterinarians also reported a lack of association between tick bites and *C. burnetii* exposure [18]. Early investigations by Pope et al. [52] in which *C. burnetii* was isolated from the ticks of infected kangaroos, and more recent Australian studies which detected *C. burnetii* DNA in several wildlife-associated tick species including *A. triguttatum* (ornate kangaroo tick), [17] and *Ixodes holocyclus* (paralysis tick) [17,33], suggests that a tick-wildlife transmission cycle exists. It is therefore possible that spillover from infected kangaroos to humans may occur, however whether ticks are a direct source of *C. burnetii* infection for humans has not yet been demonstrated. In reports of cases of Q fever in which tick bites were a part of the clinical history, it was hypothesised that the tick was the source of infection for the affected patients, however, infection from other sources, particularly contaminated aerosols could not be discounted [11,24]. The link between *C. burnetii* and ticks has long been established [19], and given that ticks can excrete large amounts of *C. burnetii* organisms in their faeces during feeding [51], it is plausible that direct transmissions to humans could potentially occur via inhalation of aerosolised tick excreta, or through direct contamination of the bite site. More research focussing on ticks as a direct source of *C. burnetii* infection for humans is needed. The discovery that many tick species harbour genetically-related *Coxiella*-like endosymbionts [23], further complicates the role of ticks in *C. burnetii* transmission, and highlights the need for robust serological [2] and molecular [22] assays, which are able to definitively differentiate between these two *Coxiella burnetii* and non-*burnetii* species.

While the source of infection for the seropositive participants in this study remains unknown, the possibility that macropods can occasionally be an infection source for AWRs cannot be ruled out given the serological evidence that macropods can become infected with *C. burnetii* [10,52], and potentially shed the bacterium [10,53,61]. Further research is required to determine whether macropods are reservoirs for *C. burnetii*, and whether they are capable of shedding viable organism which can subsequently infect humans and cause QFD. This is particularly important, given the growing number of Q fever notifications citing exposure to macropods, without exposure to other well-known infection sources such as ruminants.

Overall, three (2%) out of the 147 unvaccinated AWRs in this cohort self-reported having been medically diagnosed with QFD. This finding is similar to what was found in a study on Australian veterinary workers by Sellens et al. [58], where 2% (4/192) of the cohort reported having QFD, but lower in comparison to another study of Australian cat breeders in which 6% (7/123) of the study population reported having had medically diagnosed QFD [62]. In all three studies, the level of QFD is substantially higher than the Australian annual notification rate of 0.002% [48]. Due to the non-specific symptoms, many cases of Q fever go undiagnosed [64]. It has been suggested that occurrence of QFD could be more than three times higher than that recorded in the notification data [40]. Interestingly, one of the participants in the current study reported being ineligible for the Q fever vaccine due to a positive pre-vaccination screening result. Patients such as these who have been exposed to *C. burnetii* but are not medically diagnosed, contribute to the underestimation of the actual QFD burden. Although age and gender breakdown of Q fever notifications reveal an overrepresentation of males in the 40–59 year age group [64], given the elevated seroprevalence in this cohort of AWRs, who were predominantly female, practitioners should not discount the possibility of QFD as a differential diagnosis in female AWRs presenting with an acute febrile illness.

Multivariable modelling for QFVS revealed that the strongest predictor of having been vaccinated against Q fever in this study was occupational animal contact, in particular ruminant contact, (Table 5) with rehabilitators reporting occupational contact with ruminants eight times more likely to have been vaccinated against Q fever compared to those reporting no contact with animals. Rehabilitators residing in QLD were more likely to have been vaccinated against Q fever than those residing in NSW (OR 2.04) or other Australian jurisdictions (OR:0.231). This was not unexpected given the majority of vaccinated (11/13) participants in this study resided in NSW and QLD, and that the vast majority Q fever notifications originate in these states [48].

Currently, the Australian Immunisation Handbook recommends QFV for wildlife and zoo workers who have contact with at-risk animals, including kangaroos and bandicoots [8], however only 8.1% (13/160) AWRs in this study had undergone vaccination. This is consistent with other Australian studies which have also reported low levels of vaccine uptake in groups for whom vaccination is recommended [30,38,43,60], Similarly, this handbook recommends QFV for veterinary nurses, but alarmingly 93.3% (24/26) of the veterinary nurses participating in this study were not vaccinated. Furthermore, evidence of *C. burnetii* antibody was observed in two of the 24 (8.3%) unvaccinated veterinary nurses (Table 2) indicating exposure to *C. burnetii* and reinforcing the need for QFV amongst this group. This low rate of vaccination is consistent with the findings of Sellens et al. [60] who surveyed Australia's veterinary workforce and found that only 29% veterinary nurses had sought QFV, compared to 74% of veterinarians. Poor knowledge and awareness of QFD and vaccination were cited as notable barriers for not having sought the Q fever vaccine amongst the veterinary nurse cohort. From a workplace health and safety (WH&S) perspective, veterinary employers and veterinarians have a legal and ethical responsibility to reduce or eliminate hazards or threats within the workplace such as those posed by

diseases such as Q fever [59]. Low rates of Q fever vaccine uptake in 'at-risk' groups such as AWRs and veterinary nurses, places them at unnecessary risk of *C. burnetii* infection. Overall these findings reinforce the need for greater Work Health & Safety promotion amongst employers by the delivery of targeted education programs to 'at-risk' groups regarding the risks of *C. burnetii* exposure, and appropriate risk prevention strategies, the most important of which is vaccination. The need for a national Q fever vaccine register was highlighted by five study participants stating that they were 'unsure' of their vaccination status. Given previous QFV is a contraindication for subsequent vaccination due to serious adverse events in those previously exposed to the vaccine, knowledge of vaccination status is vital. None of the vaccinated participants reported having been diagnosed with QFD, which supports the effectiveness of the vaccine [29].

Comprehensive National Wildlife Biosecurity Guidelines issued by Wildlife Health Australia (Wildlife Health [69]) state that wildlife rehabilitators should be aware of, and implement, basic biosecurity practices at all times regardless of the animal species or perceived disease risk, and, in particular for Q fever, recommend that biosecurity practices include ventilation controls, P2/N95 particulate respirator, dust management, and QFV. Although approximately 95% of AWRs in this study reported practicing appropriate hand hygiene, a finding which is consistent with other studies of wildlife health professionals [13,27], overall we discovered a shortfall in the biosecurity practices within this cohort according to these guidelines. Given that wildlife can serve as reservoirs of known and potentially novel zoonotic pathogens which can be transmitted to humans and domestic animals through bites, scratches and contact with bodily fluid such as urine and faeces [27], it is essential for wildlife rehabilitators to adopt appropriate biosecurity practices (including the use of PPE) to help mitigate the risk of contracting Q fever and other zoonotic diseases. The reasons for the deficiency in biosecurity practices amongst this cohort are unclear. Significant knowledge gaps regarding Q fever have been identified in Australian cat breeders [62] and Australian veterinary personnel [60]. A study of Australian veterinarians reported that a lack of perceived risk of zoonotic disease exposure and awareness of industry guidelines contributed to poor infection control practices and insufficient PPE usage [21]. It is anticipated that wildlife rehabilitators may have similar knowledge gaps regarding the availability of the National Wildlife Biosecurity Guidelines document, the health risks posed by zoonotic diseases, and what constitutes high-risk activities when rehabilitating wildlife. Future studies investigating the knowledge, attitudes and practices regarding zoonotic diseases amongst AWRs are required for the development and delivery of targeted education programs, aimed at improving biosecurity practices and preventing zoonotic disease transmission to this population. Although ensuring best practice biosecurity will aid in the prevention of many zoonotic diseases, the risk of contracting Q fever from infected animals is still possible due to the transmission mode and environmental persistence of *C. burnetii* [46,55]. Therefore, it is recommended that vaccination is a major component of the Q fever prevention strategy for at-risk populations.

5. Conclusion

This is the first study to investigate the level of *C. burnetii* exposure in rehabilitators of Australian wildlife and correlate seroprevalence with potential risk factors. We observed elevated *C. burnetii* seroprevalence and a higher rate of self-reported QFD in this cohort compared to the general Australian population, however only 8.1% of the cohort had received QFV. Although the source of their increased *C. burnetii* seropositivity requires further clarification, the increased exposure rates, and the finding that wildlife rehabilitators as a group have a broad range of animal exposures suggest that rehabilitators of Australian wildlife would benefit from QFV. Therefore, as per national guidelines, QFV is recommended for this group [8], and efforts are needed to increase their awareness and uptake of the vaccine. Shortfalls in the biosecurity practices employed by AWRs identified in this study has important implications, not only for Q fever, but for a range of zoonotic diseases.

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CRediT authorship contribution statement

Karen O. Mathews: Conceptualization, Writing - original draft, Formal analysis, Investigation, Methodology. **Jenny-Ann Toribio:** Writing - review & editing, Supervision, Methodology, Formal analysis. **Jacqueline M. Norris:** Conceptualization, Writing - review & editing, Methodology, Investigation, Funding acquisition. **David Phalen:** Conceptualization, Writing - review & editing, Methodology, Funding acquisition. **Nicholas Wood:** Funding acquisition, Writing - review & editing, Methodology, Investigation. **Stephen R. Graves:** Funding acquisition, Writing - review & editing, Methodology. **Paul A. Sheehy:** Conceptualization, Writing - review & editing, Methodology. **Katrina L. Bosward:** Conceptualization, Funding acquisition, Investigation, Writing - review & editing, Supervision, Methodology, Project administration.

Declaration of Competing Interest

The authors declare no conflict of interest (document uploaded).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2020.100197.

References

[1] J.S. Agerholm, *Coxiella burnetii* associated reproductive disorders in domestic animals-a critical review, Acta Vet. Scand. 55 (2013) 13.
[2] E. Angelakis, O. Mediannikov, S.-L. Jos, J.-M. Berenger, P. Parola, D. Raoult, *Candidatus Coxiella massiliensis* infection, Emerg. Infect. Dis. 22 (2016) 285–288.
[3] B.N. Archer, C. Hallahan, P. Stanley, K. Seward, M. Lesjak, K. Hope, A. Brown, in: Australian Government Department of Health (Ed.), Atypical outbreak of Q fever affecting low-risk residents of a remote rural town in New South Wales, 2017, pp. E125–E133.
[4] Australian Bureau of Statistics, Australian demographic statistics, Jun 2016. https://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/3101.0Jun%202016, 2016.
[5] Australian Bureau of Statistics, Australian Statistical Geography Standard (ASGS) Remoteness Structure July 2016. http://www.abs.gov.au/AUSSTATS/abs@.nsf/Lookup/1270.0.55.005Main+Features1July%202016?OpenDocument, 2016 (Accessed July 2018).
[6] Australian Bureau of Statistics, Australian demographic statistics, Jun 2018, 2018. https://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/3101.0Jun%202018?OpenDocument (August 2019).
[7] Australian Red Cross Blood Service, Blood Donation. https://www.betterhealth.vic.gov.au/health/conditionsandtreatments/blood-donation, 2019 (Accessed February, 2020).
[8] Australian Technical Advisory Group on Immunisation, The Australian Immunisation Handbook, Australian Government Department of Health, Canberra, 2018. https://immunisationhandbook.health.gov.au. (Accessed November 2019).
[9] Australian Veterinary Association, Guidelines for Veterinary Personal Biosecurity. https://www.ava.com.au/library-resources/other-resources/veterinary-personal-biosecurity/, 2017 (Accessed July 2019).

[10] M.J. Banazis, A.S. Bestall, S.A. Reid, S.G. Fenwick, A survey of Western Australian sheep, cattle and kangaroos to determine the prevalence of *Coxiella burnetii*, Vet. Microbiol. 143 (2010) 337–345.

[11] M.H. Beaman, J. Hung, Pericarditis associated with tick-borne Q fever, Aust. NZ J. Med. 19 (1989) 254–256.

[12] M.D. Bennett, L. Woolford, M.J. Banazis, A.J. O'Hara, K.S. Warren, P.K. Nicholls, C. Sims, S.G. Fenwick, *Coxiella burnetii* in western barred bandicoots (*Perameles bougainville*) from Bernier and Dorre Islands in Western Australia, EcoHealth 8 (2011) 519–524.

[13] S.A. Bosch, K. Musgrave, D. Wong, Zoonotic disease risk and prevention practices among biologists and other wildlife workers–results from a national survey, US National Park Service, 2009, J. Wildl. Dis. 49 (2013) 475–585.

[14] A.K.H. Chong, J. La Brooy, R. Norton, J. Masson, Q fever: a recent 'outbreak' in Townsville, Intern. Med. J. 33 (2003) 208.

[15] N.J. Clark, S. Tozer, C. Wood, S.M. Firestone, M. Stevenson, C. Caraguel, A. L. Chaber, J. Heller, R.J. Soares Magalhães, Unravelling animal exposure profiles of human Q fever cases in Queensland, Australia, using natural language processing, Transbound. Emerg. Dis. 67 (5) (2020) 2133–2145.

[16] H.C. Clutterbuck, K. Eastwood, P.D. Massey, K. Hope, S.M. Mor, Surveillance system enhancements for Q fever in NSW, 2005-2015, Commun. Dis. Intell. 2018 (2018) 42.

[17] A. Cooper, J. Stephens, N. Ketheesan, B. Govan, Detection of *Coxiella burnetii* DNA in wildlife and ticks in northern Queensland, Australia, Vector-Borne and Zoonotic Diseases 13 (2012) 12–16.

[18] F. Dal Pozzo, L. Martinelle, P. Leonard, B. Renaville, R. Renaville, C. Thys, F. Smeets, G. Czaplicki, M. Van Esbroeck, C. Saegerman, Q fever serological survey and associated risk factors in veterinarians, southern Belgium, 2013, Transbound. Emerg. Dis. 64 (2017) 959–966.

[19] G.E. Davis, H.R. Cox, R.R. Parker, R.E. Dyer, 1938. A filter-passing infectious agent isolated from ticks. I isolation from *Dermcentor andersoni,* reactions in animals and filtration experiments, Public Health Rep. 53 (1896-1970) 2259–2267.

[20] N.K. Dhand, M.S. Khatkar, Statulator: An Online Statistical Calculator. Sample size Calculator for Estimating a Single Proportion. http://statulator.com/SampleSize/ss1P.html, 2014 (Accessed May 2018).

[21] K. Dowd, M. Taylor, J.A. Toribio, C. Hooker, N.K. Dhand, Zoonotic disease risk perceptions and infection control practices of Australian veterinarians: call for change in work culture, Prev. Vet. Med. 111 (2013) 17–24.

[22] O. Duron, The IS1111 insertion sequence used for detection of *Coxiella burnetii* IS widespread in Coxiella-like endosymbionts of ticks, FEMS Microbiol. Lett. 362 (2015) fnv132.

[23] O. Duron, V. Noël, K.D. McCoy, M. Bonazzi, K. Sidi-Boumedine, O. Morel, F. Vavre, L. Zenner, E. Jourdain, P. Durand, C. Arnathau, F. Renaud, J.-F. Trape, A. S. Biguezoton, J. Cremaschi, M. Dietrich, E. Léger, A. Appelgren, M. Dupraz, E. Gómez-Díaz, G. Diatta, G.-K. Dayo, H. Adakal, S. Zoungrana, L. Vial, C. Chevillon, The recent evolution of a maternally-inherited endosymbiont of ticks led to the emergence of the Q fever pathogen, *Coxiella burnetii*. PLoS Pathog. 11 (2015), e1004892.

[24] C.M. Eklund, R.R. Parker, D.B. Lackman, 1947. A case of Q fever probably contracted by exposure to ticks in nature, Public Health Rep. 62 (1896-1970) 1413–1416.

[25] J. Flint, C.B. Dalton, T.D. Merritt, S. Graves, J.K. Ferguson, M. Osbourn, K. Eastwood, D.N. Durrheim, Q fever and contact with kangaroos in New South Wales, in: G.D.O. Health (Ed.), Communicable Diseases Intelligence, 2016, pp. 202–203.

[26] M. Gale, N. Ketheesan, B. Govan, R.L. Kennedy, R. Norton, Q fever cases at a North Queensland Centre during 1994–2006, Intern. Med. J. 37 (2007) 644–646.

[27] G. Garland-Lewis, C. Whittier, S. Murray, S. Trufan, P.M. Rabinowitz, Occupational risks and exposures among wildlife health professionals, EcoHealth 14 (2017) 20–28.

[28] M.G. Garner, H.M. Longbottom, R.M. Cannon, A.J. Plant, A review of Q fever in Australia 1991-1994. Aust. N. Z. J, Public Health 21 (1997) 722–773.

[29] G. Gefenaite, J.M. Munster, R. van Houdt, E. Hak, Effectiveness of the Q fever vaccine: a meta-analysis, Vaccine 29 (2011) 395–398.

[30] H.F. Gidding, H.M. Faddy, D.N. Durrheim, S.R. Graves, C. Nguyen, P. Hutchinson, P. Massey, N. Wood, Seroprevalence of Q fever among metropolitan and non-metropolitan blood donors in New South Wales and Queensland, 2014-2015, Med. J. Aust. 210 (2019) 309–315.

[31] H.F. Gidding, C.Q. Peng, S. Graves, P.D. Massey, C. Nguyen, J. Stenos, H.E. Quinn, P.B. McIntyre, D.N. Durrheim, N. Wood, Q fever seroprevalence in Australia suggests one in twenty people have been exposed, Epidemiol. Infect. 148 (2020), e18.

[32] S.R. Graves, A. Islam, Endemic Q fever in New South Wales, Australia: a case series (2005-2013), Am. J. Trop. Med. Hyg. 95 (2016) 55.

[33] S.R. Graves, C. Jackson, H. Hussain-Yusuf, G. Vincent, C. Nguyen, J. Stenos, M. Webster, *Ixodes holocyclus* tick-transmitted human pathogens in north-eastern New South Wales, Australia, Trop Med Infect Dis 1 (2016) 4.

[34] R. Haering, V. Wilson, A. Zhuo, P. Stathis, Towards a more effective model of wildlife care and rehabilitation: a survey of volunteers in New South Wales, Australia. Aust. Zool. 40 (2020) 605–627.

[35] P.A. Harris, R. Taylor, B.L. Minor, V. Elliott, M. Fernandez, L. O'Neal, L. McLeod, G. Delacqua, F. Delacqua, J. Kirby, S.N. Duda, The REDCap consortium: building an international community of software platform partners, J. Biomed. Inform. 95 (2019) 103208.

[36] P.A. Harris, R. Taylor, R. Thielke, J. Payne, N. Gonzalez, J.G. Conde, Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support, J. Biomed. Inform. 42 (2009) 377–381.

[37] B. Healy, H. van Woerden, D. Raoult, S. Graves, J. Pitman, G. Lloyd, N. Brown, M. Llewelyn, Chronic Q fever: different serological results in three countries–results of a follow-up study 6 years after a point source outbreak, Clin. Infect. Dis. 52 (2011) 1013–1019.

[38] M. Irwin, P. Massey, D.N. Durrheim, Enhanced Q fever risk exposure surveillance may permit better informed vaccination policy. Commun. Dis. Intell, Quarterly Report 33 (2009) 41–45.

[39] A. Islam, J. Ferguson, R. Givney, S. Graves, Seroprevalence to *Coxiella burnetii* among residents of the hunter New England region of New South Wales, Australia. Am. J. Trop. Med. Hyg. 84 (2011) 318–320.

[40] H.W. Kaufman, Z. Chen, J. Radcliff, H.J. Batterman, J. Leake, Q fever: an under-reported reportable communicable disease, Epidemiol. Infect. 146 (2018) 1240–1244.

[41] M. Kermode, K. Yong, S. Hurley, B. Marmion, 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. Aust. N. Z. J, Public Health 27 (2003) 390–398.

[42] L. Kopecny, K.L. Bosward, A.J. Shapiro, J.M. Norris, Investigating *Coxiella burnetii* infection in a breeding cattery at the Centre of a Q fever outbreak, J. Feline Med. Surg. 15 (2013) 1037–1045.

[43] T. Lower, P. Corben, P. Massey, J. Depczynski, T. Brown, P. Stanley, M. Osbourn, D. Durrheim, Farmers' knowledge of Q fever and prevention approaches in New South Wales. Aust. J, Rural Health 25 (2017) 306–310.

[44] M.L. Marenzoni, V. Stefanetti, P. Papa, P. Casagrande Proietti, A. Bietta, M. Coletti, F. Passamonti, K. Henning, Is the horse a reservoir or an indicator of *Coxiella burnetii* infection? Systematic review and biomolecular investigation, Vet. Microbiol. 167 (2013) 662–669.

[45] B.P. Marmion, O. Sukocheva, P.A. Storm, M. Lockhart, M. Turra, T. Kok, J. Ayres, H. Routledge, S. Graves, Q fever: persistence of antigenic non-viable cell residues of *Coxiella burnetii* in the host—implications for post Q fever infection fatigue syndrome and other chronic sequelae, QJM 102 (2009) 673–684.

[46] M. Maurin, D. Raoult, Q Fever, Clin. Microbiol. Rev. 12 (1999) 518–553.

[47] National Notifiable Diseases Surveillance System, National Notifiable Diseases Surveillance System, 1 July to 30 September 2016, Communicable Diseases Intelligence. http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi4004n.htm, 2016 (Accessed July 2019).

[48] National Notifiable Diseases Surveillance System, Notifications of Selected Diseases by State Territory and Year. http://www9.health.gov.au/cda/source/cda-index.cfm, 2019 (Accessed 30 October 2019).

[49] National Rural Health Alliance, 2011, The little book of rural health numbers, https://www.ruralhealth.org.au/book/demography, (Accessed February, 2020).

[50] N. Parker, J. Robson, M. Bell, A serosurvey of *Coxiella burnetii* infection in children and young adults in south West Queensland, Aust. N. Z. J. Public Health 34 (2010) 79–82.

[51] C.B. Philip, Observations on experimental Q fever, J. Parasitol. 34 (1948) 457–464.

[52] J.H. Pope, W. Scott, R. Dwyer, *Coxiella burnetii* in kangaroo and kangaroo ticks in Western Queensland, Aust J Exp Biol Med 38 (1960) 17–27.

[53] A.S. Potter, M.J. Banazis, R. Yang, S.A. Reid, S.G. Fenwick, Prevalence of *Coxiella burnetii* in Western Grey kangaroos (*Macropus fuliginosus*) in Western Australia, J. Wildl. Dis. 47 (2011) 821–828.

[54] R. Core Team, R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria, https://www.R-project.org/, Accessed 2019 (2019).

[55] D. Raoult, T. Marrie, J. Mege, Natural history and pathophysiology of Q fever, Lancet Infect. Dis. 5 (2005) 219–226.

[56] C. Savage, Knowledge, Attitude and Practices of Australian Wildlife Rehanilitators in Relation to Q Fever, School of Veterinary Science. The University of Sydney, 2017, p. 91.

[57] T. Schoffelen, L. Joosten, T. Herremans, A. de Haan, A. Ammerdorffer, H. Rümke, C. Wijkmans, H. Roest, M. Netea, J. van Der Meer, T. Sprong, M. van Deuren, Specific interferon [gamma] detection for the diagnosis of previous Q fever, Clin. Infect. Dis. 56 (2013) 1742.

[58] E. Sellens, K.L. Bosward, J.M. Norris, N. Wood, J. Heller, S. Graves, H.F. Gidding, *Coxiella burnetii* seroprevalence in unvaccinated veterinary workers in Australia: evidence to support Q fever vaccination, Zoonoses Public Hlth 67 (2020) 79–88.

[59] E. Sellens, J. Norris, N.K. Dhand, J. Heller, L. Hayes, H. Gidding, H. Willaby, N. Wood, K. Bosward, Willingness of veterinarians in Australia to recommend Q fever vaccination in veterinary personnel: implications for workplace health and safety compliance, PLoS One 13 (2018), e0198421.

[60] E. Sellens, J.M. Norris, N.K. Dhand, J. Heller, L. Hayes, H.F. Gidding, H. Willaby, N. Wood, K.L. Bosward, Q fever knowledge, attitudes and vaccination status of Australia's veterinary workforce in 2014, PLoS One 11 (2016), e0146819.

[61] A.J. Shapiro, K.L. Bosward, K.O. Mathews, G. Vincent, J. Stenos, M. Tadepalli, J. M. Norris, Molecular detection of *Coxiella burnetii* in raw meat intended for pet consumption, Zoonoses Public Hlth 67 (2020) 443–452.

[62] A.J. Shapiro, J.M. Norris, K.L. Bosward, J. Heller, Q fever (*Coxiella burnetii*) knowledge and attitudes of Australian cat breeders and their husbandry practices, Zoonoses Public Hlth 64 (2017) 252–261.

[63] A.J. Shapiro, J.M. Norris, J. Heller, G. Brown, R. Malik, K.L. Bosward, Seroprevalence of *Coxiella burnetii* in Australian dogs, Zoonoses Public Hlth 63 (2016) 458–466.

[64] T.S. Sloan-Gardner, P.D. Massey, P. Hutchinson, K. Knope, E. Fearnley, Trends and risk factors for human Q fever in Australia, 1991-2014, Epidemiol. Infect. 145 (2017) 787–795.

[65] S. Stevenson, J. Gowardman, S. Tozer, M. Woods, Life-threatening Q fever infection following exposure to kangaroos and wallabies, BMJ Case Rep. 2015 (2015).
[66] S. Tozer, S.B. Lambert, T.P. Sloots, M.D. Nissen, Q fever seroprevalence in metropolitan samples is similar to rural/remote samples in Queensland, Australia, European journal of clinical microbiology and infextious diseases 30 (2011) 1287–1293.
[67] R. Van den Brom, B. Schimmer, P.M. Schneeberger, W.A. Swart, W. van der Hoek, P. Vellema, Seroepidemiological survey for *Coxiella burnetii* antibodies and associated risk factors in Dutch livestock veterinarians, PLoS One 8 (2013), e54021.
[68] H.H. Welsh, E.H. Lennette, F.R. Abinanti, J.F. Winn, Air-borne transmission of Q fever: the role of parturition in the generation of aerosols, Ann. N. Y. Acad. Sci. 70 (1958) 528–540.
[69] Wildlife Health Australia, National Wildlife Biosecurity Guidelines, Sydney, NSW, 2018. https://www.wildlifehealthaustralia.com.au/Portals/0/Documents/ProgramProjects/National_Wildlife_Biosecurity_Guidelines.PDF.
[70] Thomas Marrie J, Q fever — a review, The Canadian Veterinary Journal 31 (8) (1990) 555–563.