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Authors: Alanna Cooper, Tamsin Barnes, Abbey Potter, Natkunam Ketheesan, Brenda Govan

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Determination of *Coxiella burnetii* seroprevalence in macropods in Australia

COOPER, Alanna[a]*

BARNES, Tamsin[b]

POTTER, Abbey[c]

KETHEESAN, Natkunam[a]

GOVAN, Brenda[a]

[a] Department of Microbiology and Immunology, School of Veterinary and Biomedical Sciences, James Cook University, Douglas 4811, Queensland, Australia

[b] School of Veterinary Science, University of Queensland, Gatton 4343, Queensland, Australia

[c] School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch 6150, Western Australia, Australia

*Corresponding author:

Alanna Cooper

Phone: +61 0747 816 915

Fax: +61 0747 791 526

Alanna.Cooper@jcu.edu.au

ABSTRACT

Many animal species, including macropods, have the potential to act as atypical reservoirs of the causative agent of Q fever, *Coxiella burnetii*. The objective of this study was to determine the seroprevalence of *C. burnetii* in various macropod species in Australia. Competitive and indirect ELISAs were developed for the testing of macropod sera for antibodies to phase II and I *C. burnetii* antigens separately. A total of 500 macropod serum samples from selected species sampled in eastern and western coastal states of Australia were screened for the presence of anti-*C. burnetii* antibodies. An overall seroprevalence of 20.8% (95% CI 20.8-20.9%) was observed with 30.4% (30.2-30.9%) in northern Queensland, 13.0% (12.9-13.1%) in southern Queensland, 7.1% (7.1-8.0%) in western Queensland and 22.8% (22.7-22.9%) in south-western Western Australia. These data indicated macropods represented a potential reservoir for zoonotic transmission of *C. burnetii* to domestic animals and the human population.

KEYWORDS

Zoonoses, *Coxiella burnetii*, Q fever, serology, macropods, Australia

INTRODUCTION

Coxiella burnetii is an obligate intracellular pathogen with worldwide distribution and a wide host range (Babudieri, 1959). In the natural lifecycle of the organism, it is transmitted between wild animals and their ticks. Many serological surveys and bacterial isolations have indicated the extent of wildlife coxiellosis worldwide. In Australia, bandicoots (Smith and Derrick, 1939; Smith 1942) and macropods (Pope *et al*., 1960; Banazis *et al*., 2010) have been identified as potential reservoirs. Macropods are marsupials belonging to the Family Macropodidae, which includes kangaroos, wallabies, pademelons and bettongs. The bacterium can also be transmitted to livestock and other domestic animals via ticks. Once in livestock, *C. burnetii* primarily infects the female reproductive

tract, resulting in the shedding of a spore-like form of the organism in parturient fluids and milk (Lang, 1990). Q fever is more commonly contracted following exposure to infected livestock such as cattle, sheep and goats. Studies conducted in Queensland found many Q fever patients had no known contact with the typical animal reservoirs. Two patients reported contact with wallabies (Chong *et al*., 2003; Gale *et al*., 2007).

In Q fever patients, seroconversion to the various antigens post-infection is relatively well characterised, with an initial rise in antibodies to phase II antigen generally followed by antibodies to phase I antigen (Maurin and Raoult, 1999). Differences in titres of immunoglobulin isotypes to phase II or phase I antigens also form the basis for diagnosis of acute or chronic Q fever. Antibodies to phase II and I antigen of IgM isotype, and antibodies to phase II of IgG and IgA isotypes are associated with acute Q fever; whereas, antibodies to phase I of IgG and IgA isotypes are associated with chronic Q fever (Capo *et al*., 1998; Fournier and Raoult, 1999; Camacho *et al*., 2000). However, the process of seroconversion is not well characterised in animals and seropositivity to either or both antigenic phase of *C. burnetii* has been shown to vary between species (Enright *et al*., 1971; Marrie *et al*., 1985; Marrie *et al*., 1993). Some studies have suggested the presence of antibodies to phase II antigen in animal sera is indicative of recent infection (Lackman *et al*., 1962; Sidwell and Gebhardt, 1962). The current study aimed to determine the potential for native Australian macropods to act as reservoirs of Q fever in Australia using two ELISA methods for the detection of antibodies to *C. burnetii* in macropod sera.

MATERIALS AND METHODS

Sample collection

Study populations

A list of species sampled, numbers and common names is included in Table 1. A breakdown of study populations, including species, region and sample methods is included in Table 2. Samples collected

within southern Queensland originated from properties at three sites, including Warwick, Injune and Roma. Samples collected within northern Queensland originated from properties at three sites, including Townsville, Greenvale and Richmond. Samples collected within western Queensland originated from properties at three sites, including Longreach, Thurles Park and Winton. A number of samples from Townsville and western Queensland were donated from a serum bank for an unrelated study and capture dates were not available. Samples collected within south-western Western Australia originated from properties at five sites including Whiteman Park, Preston Beach, Capel, Nannup and Eneabba. Site, species, sex and age data for each animal sampled were recorded. Sites included in the survey are shown in Supplementary Figure 1.

Trapping

Trapped animals were captured according to procedures used by the Queensland Parks and Wildlife Service using Treadle traps lined with foam and shade cloth. All care was taken to reduce stress on the animals. Blood samples (equivalent to less than 0.5% of the body weight to a maximum 2 mL) collected from each identified animal were taken the lateral coccygeal vein of the tail with the animal manually restrained in a Hessian sack. Following blood collection, animals were released at the site at which they were captured. Whole blood was collected in a clot activating tube and centrifuged at 1,400 ×*g* for 10 min at 4°C. Serum removed from the samples was frozen at -20°C prior to analysis.

Post mortem

Blood samples were collected via cardiac puncture from animals shot by licensed kangaroo shooters during routine culling expeditions. Harvesters held a commercial wildlife harvesting licence. Serum was separated from whole blood and stored as described previously for trapped animals.

Preparation of ELISA antigens

Antigen was prepared according to the protocol described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2004). Both phase I and phase II *C. burnetii* antigen were produced using an Australian *C. burnetii* isolate (Cumberland strain). This isolate was obtained from the Australian Rickettsial Reference Laboratory (Geelong, Victoria), which was isolated from a patient who contracted Q fever through contact with beef cattle. Phase II *C. burnetii* was obtained by serial passage in vero cell culture to a total of 15 passages. Cell suspensions and supernatants were pooled and centrifuged at 1,000 ×*g* at 20°C for 10 min. Supernatants were discarded and the cells resuspended in PBS, pH 7.4. Host cells were disrupted by sonication and bacteria were separated from cell debris by centrifugation at 550 ×*g* at 20°C for 10 min. The resultant supernatant was layered over 25% sucrose and centrifuged at 4,500 ×*g* at 20°C for 20 min. The supernatant was discarded and the purified *C. burnetii* resuspended in PBS, pH 7.4. The bacteria were inactivated with 1% formalin for 48 hrs at 4°C, pelleted at 10,000 ×*g* at 20°C for 10 min and washed three times. The inactivated cells were resuspended in sterile ddH_2O.

Phase I *C. burnetii* antigen was produced via animal passage in A/J mice (Inbred albino: Tyr^{c}, $Tyrp1^{b}$, a, $Cdh23^{ahl}$, H-2^{a}) followed by a single passage in the yolk sac of embryonated chicken eggs. Mice were inoculated with 1×10^{4} *C. burnetii intra peritoneal* and maintained for 7 days at which time the spleens were removed for bacterial extraction as described above for phase II antigen. Bacteria were separated from egg yolk and formalin-inactivated as described above for phase II antigen.

Phase II and I antigenicity was confirmed by complement block titration using commercial anti-*C. burnetii* phase II and I control sera and antigens (Virion/Serion, Germany). Commercially available phase II and I *C. burnetii* antigen and control sera (Virion\Serion, Germany) was initially used for comparison and standardisation of the whole cell antigen. Absorbance values for control sera against commercial antigen were comparable to those obtained with control sera against prepared whole cell antigen. Both the competitive and indirect ELISAs were initially optimised and validated using serum

from mice experimentally infected with *C. burnetii* and PBS inoculated negative controls. A total of 30 macropod samples were randomly selected and screened using reagent concentrations determined using murine optimisation to indentify *C. burnetii*-positive and negative samples for subsequent use as control sera. Positive thresholds were validated against murine and canine *C. burnetii*-positive and negative sera.

Competitive ELISA

NUNC™ 96-well Maxisorp plates were coated with 50 µL of phase II or phase I antigen at 25 µg mL^{-1} in carbonate/bicarbonate coating buffer, pH 9.0 and incubated overnight at 37°C. Plates were coated with 50 µL post-coating buffer (Tropbio, Australia), incubated at room temperature for 2 hr then dried. Test sera were applied at a dilution of 1:10 in 50 µL aliquots in duplicate and incubated at 37°C for 1 hr. Positive and negative control sera were also included in duplicate. Indicator serum (previously defined, *C. burnetii*-positive bovine sera) was then applied at a dilution of 1:200 for both antigenic phases and incubated at 37°C for a further 1 hr. The wells were washed three times with PBS-T after which 50 µL horse-radish peroxidase (HRP)-conjugated rabbit anti-bovine Ig (Serotec, UK) at 1:1,000 was applied and incubated at 37°C for 1 hr. The wells were washed again, after which 50 µL 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) was applied and incubated at 37°C for 30 min. Optical density readings were obtained using a Multiskan Ascent plate reader at 414/494 nm. A reduction in optimal density of ≥ 70% from that of the indicator serum alone was considered to be a positive result. Results of duplicates for each sample were averaged to produce a mean result for each animal. A diagrammatic representation of the competitive ELISA is included in Figure 1. A distribution of optical density values for the cELISA is included in Supplementary Figure 2.

147 **Indirect ELISA**

148 NUNC™ 96-well Maxisorp plates were coated with phase II or phase I antigen and stabilised as
149 described previously for the competitive ELISA (cELISA). Test sera were applied at a dilution of
150 1:100 in 50 µL aliquots in duplicate and incubated at 37°C for 1 hr. Positive and negative control sera
151 were also included in duplicate. The wells were washed three times with PBS-T after which 50 µL of
152 chicken anti-macropod IgG polyclonal antibody (in-house production) was applied at a dilution of
153 1:250 for both antigenic phases and incubated at 37°C for a further 1 hr. The wells were then washed
154 four times with PBS-T after which 50 µL HRP-conjugated rabbit anti-chicken IgY (Jackson, USA) at
155 1:2,000 was applied and incubated at 37°C for 1 hr. The wells were washed again, after which 50 µL
156 ABTS was applied and incubated at 37°C for 30 min. Optical density readings were obtained using a
157 Multiskan Ascent plate reader at 414/494 nm. The Sample/Positive Ratio (S/P%) was calculated for
158 each sample using the following formula: S/P% = (OD sample – OD negative control) ÷ (OD positive
159 control – OD negative control) × 100. Sera with an S/P% less than 50% were considered to be
160 negative. Samples with an S/P% of between 50% and 75% were considered to be positives; those
161 greater than 75% were considered strongly positive. Results of duplicates for each sample were
162 averaged to produce a mean result for each animal. A diagrammatic representation of the indirect
163 ELISA is included in Figure 1. A distribution of optical density values for the cELISA is included in
164 Supplementary Figure 2. Chicken anti-macropod IgG polyclonal antibodies were produced following
165 immunization of white leghorn chickens with purified macropod IgG according to standard protocols.

166

167 **Statistical analyses**

168 Percentage seropositivity was calculated by dividing the number of positive samples by the total
169 number of samples and multiplying by 100. Comparison of seropositivity between sites and regions
170 was performed using single factor ANOVA tests. Agreement between (cELISA and iELISA) and
171 within ELISAs (Phase II and Phase I) was determined by kappa measurement of agreement. A kappa
172 (*K*) value of 1 indicates perfect agreement and a value of 0 indicates no agreement. Excellent

agreement is indicated by values greater than 0.75, fair to good agreement by values of 0.4-0.75 and poor agreement by values less than 0.4.

An ordinal logistic generalised linear model was constructed using SPSS Statistics 19 (IBM, USA) to identify factors associated with seropositivity for each antigenic phase separately, and seropositivity to either or both antigens combined in macropod samples. All data was transformed into numerical values prior to statistical analysis. Type III analysis was performed with Wald Chi-Square test and 95% Profile Likelihood Confidence Intervals calculated. Factors modelled included, sex, age, species, sample site and region. Age was subjectively categorised as adult (approximately three years and older) or juvenile (under three years including pouch young) based on size and apparent sexual maturity. Species were categorised according to phylogenetic similarity and were divided into genera and sub-genera (Table 1). The eastern grey kangaroo (*Macropus giganteus*) and western grey kangaroo (*Macropus fuliginosus*) samples were considered separately despite their phylogenetic similarity due to the geographical separation of these species. Sample sites were grouped according to statistical division (region), with each site defined as within a 100 km radius of the major locality in the area.

RESULTS

The current study demonstrated that the seroprevalence of *C. burnetii* in the macropod populations sampled from southern Queensland (n=200), northern Queensland (n=120), western Queensland (n = 28) and southern Western Australia (n=180) were 13.0% (95% CI 12.9-13.1%), 30.4% (95% CI 30.2-30.9%), 3.6% (95% CI 3.6-4.3%) and 22.8% (95% CI 22.7-22.9%) respectively. Overall seroprevalence in the 500 macropod samples tested was 20.8% (95% CI 20.8-20.9%). Sites with significantly higher seroprevalence included Greenvale and Richmond in northern Queensland with 58.8% (95% CI 56.9-63.6%), and 46.2% (95% CI 44.7-51.9%) respectively and Capel and Whiteman

198 Park in southern Western Australia with 43.2% (95% CI 42.5-44.9%) and 37.5% (95% CI 36.8-
199 39.3%) respectively.

200

201 Reactivity to phase II and phase I antigens in serum samples varied both within and between the two
202 ELISA methods performed. The number of samples determined to be positive for antibodies to both
203 phase II and phase I antigens varied between sites and regions (Table 3). The number of samples
204 determined to be positive for antibodies against either or both phase II and phase I using the different
205 ELISA methods also varied between sites and regions (Table 3). Reactivity to phase II and phase I
206 antigens in serum samples varied according to species and site (Table 4). Species with relatively high
207 seroprevalence included *M. giganteus*, *M. fuliginosus*, *M. robustus* and *M. dorsalis* (Table 4).
208 However, seroprevalence varied between sites for the same species.

209

210 Despite variable kappa agreement within each ELISA method, good agreement was found between
211 phase II and I seropositivity using the competitive ELISA (cELISA) (K<0.6) and fair agreement using
212 the polyclonal indirect ELISA (iELISA) (K>0.4). Overall, 43.2% of macropod samples testing
213 positive for antibodies to phase II antigen, also tested positive for phase I antigen. This differed by
214 region, with 64.3% of samples positive for phase II antigen also reacting against phase I antigen in the
215 northern Queensland samples and 57.7% in southern Queensland samples, with only 19.5% of
216 samples from Western Australia and 0% of western Queensland samples reacting against both
217 antigens. Kappa measurement of agreement between ELISA methods was minimal, with only 7 of the
218 500 samples (1.4%) determined to be positive using both ELISA methods (K<0.4). Overall
219 seropositivity in macropod samples in the various regions tested, as determined using both ELISA
220 methods is displayed in Table 3.

221

Statistically significant factors associated with seropositivity to phase II antigen using both ELISAs included Sex ($P<0.05$), Site ($P<0.01$) and Region ($P<0.01$) with male animals, animals from Greenvale, Whiteman Park and animals from northern Queensland found more likely to be positive (Table 5). Both site and region were significantly associated with seropositivity to phase I antigen using both ELISAs ($P<0.01$). Specifically, animals from Richmond, Greenvale and animals from northern Queensland were found more likely to be positive (Table 5). Statistically significant factors associated with seropositivity to either or both *C. burnetii* antigens were similar to those for each antigen individually. These factors included Site ($P<0.01$) and Region ($P<0.01$). Within these it was found that animals from Greenvale, Capel, Whiteman Park and animals from northern Queensland were more likely to have antibodies to *C. burnetii* (Table 5). No significant associations were found between seropositivity and age or species.

DISCUSSION

Seropositivity rates found in the current study are similar to those reported in previous studies in Queensland (Pope *et al*., 1960) and Western Australia (Banazis *et al*., 2010). Several sites were found to have higher than expected numbers of seropositive macropods. These included Greenvale (QLD), Richmond (QLD), Capel (WA) and Whiteman Park (WA). Northern Queensland as a whole also had higher than expected numbers of seropositive animals. The relatively high seroprevalence found at Whiteman Park is noteworthy due to its location in an urban area.

Seropositivity varied for phase II and phase I antigens depending on the ELISA method used. Combined seropositivity for either or both phase II and I antigens also varied between ELISA methods. In the current study, seropositivity was generally higher for phase II antigen than phase I antigen, with the exception of the northern Queensland macropod cohort.

247 Seropositivity was similar for phase II and phase I antigen using the cELISA for all species tested,
248 with many of these samples testing positive for both antigens (K=0.6). This ELISA method also
249 resulted in the highest seropositivity values for the various species. The greater number of positive
250 samples detected by the cELISA is thought to be due to the choice of indicator sera and conjugate.
251 Strongly reacting bovine sera was used as the indicator, combined with anti-bovine Ig conjugate, as
252 bovine sera was found to have no cross-reactivity with the IgG of other species tested. As the
253 conjugate was not immunoglobulin isotype specific, it would be able to detect bovine
254 immunoglobulin of all potential isotypes. It is hypothesised that due to this factor, prevention of the
255 indicator sera from binding to *C. burnetii* antigens by the test sera would indicate the presence of any
256 potential immunoglobulin isotypes in the test sera. Immunoglobulin isotypes associated with Q fever
257 in human serology include IgM and IgG predominately, but also IgA (Angelakis and Raoult, 2010).
258 Therefore, it is thought that the seropositivity determined using the cELISA, may represent antibodies
259 to *C. burnetii* from these three immunoglobulin isotypes. However, the only antibodies that would be
260 detected in the test sera using cELISA are those that bind to the same epitopes in the antigen
261 preparation as the indicator sera. Conversely, it is thought that the positive samples detected by the
262 iELISA may mainly represent IgG_1 antibody as this would have been the predominant isotype present
263 in the inoculum used to produce the polyclonal antibody. The poor kappa agreement between the
264 ELISA methods is also thought to be due to epitope differences, as IgG_1 is protein specific and IgG_2
265 carbohydrate specific. As whole cell antigen preparations were used in the ELISAs, both protein and
266 liopolysaccaride antigens would be present.

267

268 Two ELISA methods were developed for the detection of antibodies to *C. burnetii* in native
269 Australian marsupials. As can be seen in the current study, the choice of ELISA method and
270 conjugate greatly affects the detection of antibodies. Antibody responses to *C. burnetii* in the animals
271 tested were highly heterogeneous, a finding which was consistent with human Q fever serology. A
272 similar discrepancy in the estimation of seroprevalence in a population was demonstrated in a recent
273 human study using two different serological assays (Blaauw *et al*., 2011). The heterogeneity of the

antibody response to *C. burnetii* infection complicates serological investigation and epidemiological studies. The development of diagnostic tests for native wildlife is complicated by a lack of diagnostic reagents for these animals. In serosurveys, the cELISA developed in the current study would be of greatest advantage, whereas, in isotype specific studies the iELISA may be of greater worth. Further work would be required in animal serology in order to determine the pattern of antibody production in response to *C. burnetii*. Such patterns may differ between species and may indicate relative recentness of infection and whether animals are chronically infected. Additional work would also be required to establish the identity of the immunoglobulin subsets detected in the current study, as isotype-specific and subclass-specific regents are not currently available for native Australian marsupials. However, this work would require the experimental infection of Australian native marsupials with *C. burnetii*, a process that is unlikely to be approved under existing animal ethics regulations. Ultimately, the aim of this project was to detect circulating antibodies to *C. burnetii* in native Australian marsupials and the subsequent detection of antibodies of multiple isotypes and subclasses, while confusing, provides evidence of prior infection with *C. burnetii* in these species.

In conclusion, it was found that antibodies to *C. burnetii* were detected in all native macropod species tested in this study. This result indicates these animals are potential and highly probable reservoirs of *C. burnetii*. The increasing incidence of human Q fever cases where no contact with more typical reservoir species is present may be attributable to contact with atypical reservoirs, such as marsupials. Housing shortages in Queensland have resulted in residential areas expanding into wildlife habitats throughout the State. There has also been an increase in demand for semi-rural housing estates in northern Queensland. These developments would increase the exposure of the human population and companion animals to wildlife. The close association these species have with human habitation, combined with the evidence of exposure to *C. burnetii* may have important public health implications.

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CONFLICTS OF INTEREST

No competing financial interests exist.

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Figure 1: Diagrammatic representation of the competitive (cELISA) and indirect (iELISA) ELISAs developed for the screening of macropod sera for antibodies to *Coxiella burnetii*.

Suppl Figure 1: Origin of macropod samples included in the survey

Suppl Figure 2: Distribution of optical density values for phase II and phase I ELISAs using both ELISA methods. [A] Competitive ELISA, [B] Indirect ELISA.

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414 **Table 1: Macropod species included in the survey and common names**

GROUP	SPECIES INCLUDED	NUMBER
Subgenus *Notamacropus*	*Macropus agilis* (Agile wallaby)	21
	Macropus dorsalis (Black-striped wallaby)	10
	Macropus rufogriseus (Red-necked wallaby)	9
	Macropus parryi (Whiptail wallaby)	2
Subgenus *Osphranter*	*Macropus robustus* (Common wallaroo)	16
	Macropus rufus (Red kangaroo)	12
	Macropus antilopinus (Antilopine kangaroo)	9
Subgenus *Macropus* 1	*Macropus giganteus* (Eastern grey kangaroo)	164
Subgenus *Macropus* 2	*Macropus fuliginosus* (Western grey kangaroo)	180
Genus *Petrogale*	*Petrogale penicillata* (Brush-tailed rock wallaby)	68
Genus *Thylogale*	*Thylogale stigmatica* (Red-legged pademelon)	5
Genus *Aepyprymnus*	*Aepyprymnus rufescens* (Rufous bettong)	4

415

Table 2: Macropod species and sites included in the survey

SITE	SPECIES	SAMPLES (n)	SAMPLE METHOD
Southern Queensland	*M. giganteus*	114	*p.m.*
	P. penicillata	64	trap
	M. robustus	13	*p.m.*
	M. rufogriseus	9	trap
Northern Queensland	*M. giganteus*	30	*p.m.*
	M. agilis	21	*p.m.*
	M. antilopinus	9	*p.m.*
	M. dorsalis	9	trap
	M. rufus	5	*p.m.*
	T. stigmatica	5	trap
	P. penicillata	4	trap
	A. rufescens	4	trap
	M. robustus	3	*p.m.*
	M. parryi	2	trap
Western Queensland	*M. giganteus*	20	*p.m.*
	M. rufus	7	*p.m.*
	M. dorsalis	1	trap
Western Australia	*M. fuliginosus*	180	*p.m.*

[nb] *p.m.* represents post mortem sampling

422 **Table 3: Seroprevalence of anti-*Coxiella burnetii* antibodies in macropods using two ELISA methods**

REGIONS SITES	SAMPLES (n)	SEROPREVALENCE % (95%CI)								
		PHASE II			PHASE I			PHASE II AND/OR I		
		cELISA	iELISA	BOTH	cELISA	iELISA	BOTH	cELISA	iELISA	BOTH
Southern Queensland (SQ) (Warwick, Injune, Roma)	200	8.0 (7.9-8.1)	5.5 (5.5-5.6)	13.0 (12.9-13.1)	6.5 (6.3-6.6)	1.0 (1.0-1.0)	7.5 (7.5-7.6)	8.5 (7.5-9.5)	5.5 (5.5-5.6)	13.0 (12.9-13.1)
Northern Queensland (NQ) (Townsville, Greenvale, Richmond)	92	16.3 (16.2-16.6)	7.6 (7.6-7.8)	21.7 (21.6-22.1)	20.7 (20.5-21.0)	9.8 (9.7-10.0)	28.3 (28.0-31.2)	22.8 (22.7-23.2)	10.9 (10.8-11.1)	30.4 (30.2-30.9)
Western Queensland (WQ) (Longreach, Thurles Park, Winton)	28	0.0 (0.0-0.4)	3.6 (3.6-4.3)	3.6 (3.6-4.3)	0.0 (0.0-0.4)	0.0 (0.0-0.4)	0.0 (0.0-0.4)	0.0 (0.0-0.4)	3.6 (3.6-4.3)	3.6 (3.6-4.3)
South-western Western Australia (WA) (Preston Beach, Capel, Nannup, Whiteman Park, Eneabba)	180	8.3 (8.3-8.4)	16.8 (16.6-16.8)	17.8 (17.7-17.9)	5.0 (5.0-5.1)	5.0 (5.0-5.1)	9.4 (9.4-9.5)	11.7 (11.6-11.8)	18.4 (18.3-18.5)	22.8 (22.7-22.9)
Total	500	9.2 (9.19-9.22)	9.8 (9.79-9.83)	15.8 (15.77-15.84)	8.2 (8.19-8.22)	4.0 (4.0-4.01)	11.6 (11.58-11.63)	9.8 (9.79-9.83)	11.0 (10.98-11.03)	20.8 (20.8-20.9)

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427 **Table 4: Seroprevalence of anti-*C. burnetii* antibodies in macropod species sampled**

SITE	SPECIES	SAMPLES (n)	POSITIVE (n)	SEROPREVALENCE (95% CI)
Warwick (SQ)	*P. penicillata*	64	7	10.9% (10.9-11.3%)
	M. robustus	13	3	23.1% (22.7-27.2%)
	M. rufogriseus	9	0	0.0% (0-3.8%)
	M. giganteus	8	2	25.0% (24.6-33.1%)
Injune (SQ)	*M. giganteus*	83	12	14.5% (14.4-14.7%)
Roma (SQ)	*M. giganteus*	23	2	8.7% (8.6-9.9%)
Townsville (NQ)	*M. agilis*	16	1	6.3% (6.2-8.1%)
	M. giganteus	13	5	38.5% (37.4-43.7%)
	M. antilopinus	9	1	11.1% (11.1-16.4%)
	M. dorsalis	8	2	25.0% (24.6-33.1%)
	T. stigmatica	5	2	40.0% (38.9-57.0%)
	P. penicillata	4	0	0.0% (0.0-15.1%)
	A. rufescens	4	0	0.0% (0.0-15.1%)
	M. parryi	2	0	0.0% (0.0-42.1%)
Richmond (NQ)	*M. giganteus*	5	2	40.0% (38.9-57.0%)
	M. rufus	5	1	20.0% (19.9-34.3%)
	M. robustus	3	2	66.7% (63.5-99.7%)
	M. dorsalis	1	1	100% (97.5%-100%)
Greenvale (NQ)	*M. giganteus*	12	7	58.3% (56.0-65.4%)
	M. agilis	5	3	60.0% (57.1-78.9%)
Longreach (WQ)	*M. giganteus*	17	1	5.9% (5.9-7.6%)
	M. dorsalis	1	0	0.0% (0.0-97.5%)
Thurles park (WQ)	*M. rufus*	7	0	0.0% (0.0-5.9%)
	M. giganteus	1	0	0.0% (0.0-97.5%)
Winton (WQ)	*M. giganteus*	2	0	0.0% (0.0-42.1%)
Preston Beach (WA)	*M. fuliginosus*	60	10	16.7% (16.5-17.2%)
Capel (WA)	*M. fuliginosus*	37	16	43.2% (42.5-44.9%)
Nannup (WA)	*M. fuliginosus*	34	3	8.8% (8.8-9.5%)
Whiteman Park (WA)	*M. fuliginosus*	32	12	37.5% (36.8-39.3%)
Eneabba (WA)	*M. fuliginosus*	17	0	0.0% (0.0-1.1%)

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430 **Table 5: Factors associated with seropositivity to *C. burnetii* in macropods**

FACTOR	Risk Ratio	Odds Ratio	*P*
SEROPOSITIVITY TO PHASE II ANTIGEN			
Greenvale origin	2.7	3.9	<0.05
Whiteman Park origin	2.3	3.0	<0.05
Northern Queensland origin	1.5	1.6	<0.01
Male	1.5	1.6	<0.05
SEROPOSITIVITY TO PHASE I ANTIGEN			
Greenvale origin	9.2	20.8	<0.01
Richmond origin	6.5	11.1	<0.05
Northern Queensland origin	7.7	10.3	<0.01
SEROPOSITIVITY TO EITHER/BOTH PHASE II/I ANTIGEN			
Greenvale origin	3.4	6.8	<0.01
Capel origin	2.6	3.8	<0.05
Whiteman Park origin	2.1	2.8	<0.05
Northern Queensland origin	1.9	2.3	<0.01

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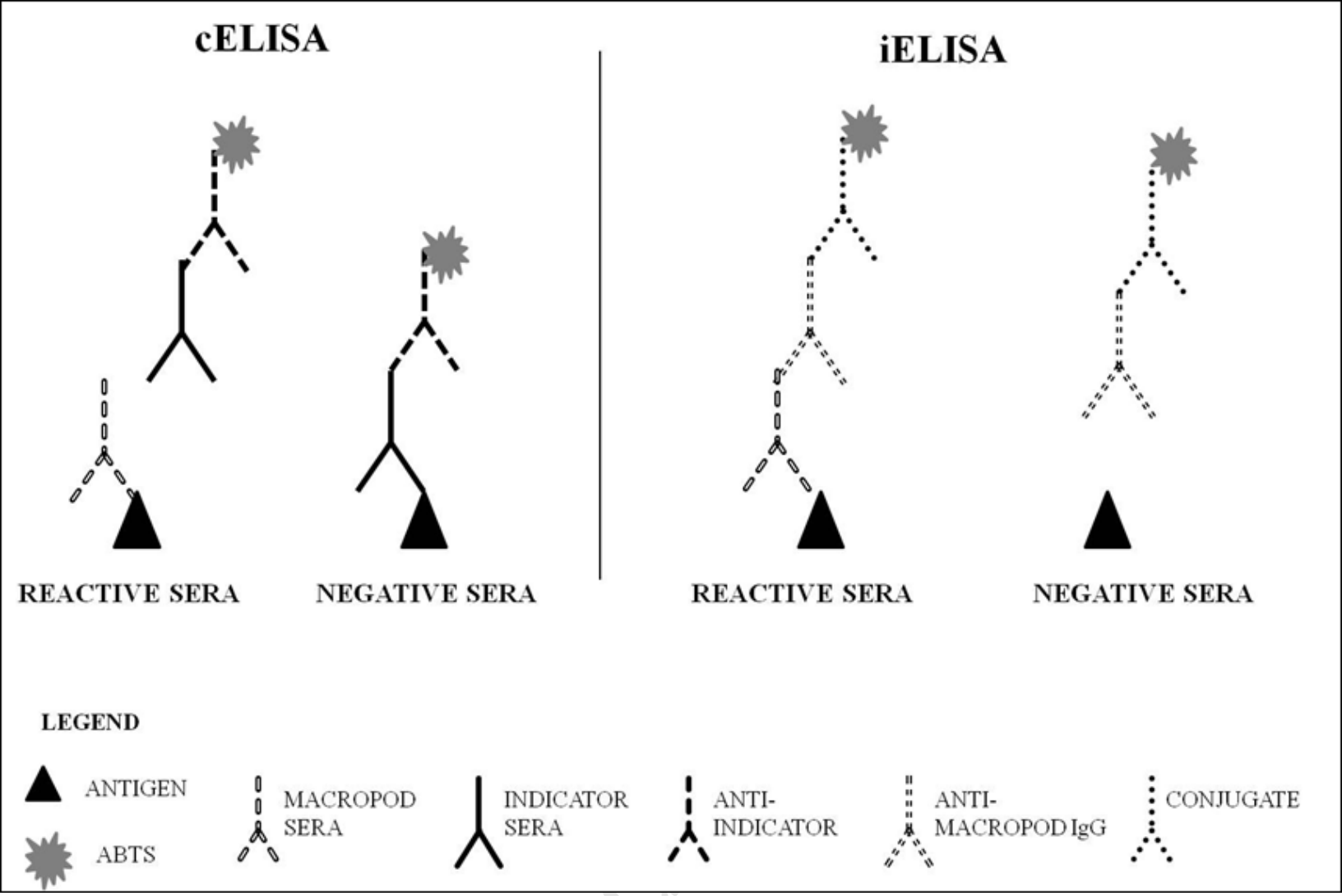
cELISA
iELISA
REACTIVE SERA
NEGATIVE SERA
REACTIVE SERA
NEGATIVE SERA
LEGEND
ANTIGEN
ABTS
MACROPOD SERA
INDICATOR SERA
ANTI-INDICATOR
ANTI-MACROPOD IgG
CONJUGATE