SHORT REPORT
Serological evidence of *Coxiella burnetii* exposure in native marsupials and introduced animals in Queensland, Australia

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SUMMARY

The state of Queensland has the highest incidence of Q fever in Australia. In recent years, there has been an increase in human cases where no contacts with the typical reservoir animals or occupations were reported. The aim of this study was to determine the seroprevalence of *Coxiella burnetii* in Australian native animals and introduced animals in northern and southeastern Queensland. Australian native marsupials sampled included the brushtail possum (*Trichosurus vulpecula*) and common northern bandicoot (*Isoodon macrourus*). Introduced species sampled included dingoes (*Canis lupus dingo*), cats (*Felis catus*), foxes (*Vulpes vulpes*) and pigs (*Sus scrofa*). Serum samples were tested by ELISA for both phase II and phase I antigens of the organism using an Australian isolate. The serological evidence of *C. burnetii* infection demonstrated in these species has public health implications due to their increasing movement into residential areas in regional Queensland. This study is the first known investigation of *C. burnetii* seroprevalence in these species in northern Queensland.

Key words: *Coxiella*, notifiable infectious diseases, Q fever, serology, zoonoses.

Coxiella burnetii is the aetiological agent of Q fever [1]. Q fever has been described as a re-emerging pathogen of increasing importance as a public health issue [2], with Australian surveys showing an increased prevalence of Q fever in humans in recent years [3–5]. Studies conducted in northern Queensland found many Q fever patients reported no contact with cattle, sheep or goats which are known to be the typical reservoirs [6, 7]. Wildlife has been proposed as a potential alternative reservoir for Q fever in these cases. In Australia, bandicoots (*Isoodon* sp.) have been found to carry the organism [8] and were associated with an outbreak of Q fever in Queensland in 1958, where there was no association found with any other potential reservoir species [9]. In the following 50 years, no further work has been performed on the role of bandicoots in the epidemiology of Q fever. To date, no evidence of *C. burnetii* has been identified in possums (*Trichosurus vulpecula*). However, possums have been identified as reservoirs of leptospirosis in Australia [9]. Many species that are reservoirs for leptospirosis are also reservoirs for Q fever [10]. Serologically leptospirosis-positive possums have also been identified in major suburban areas in Australia [11]. Therefore, there may be potential for possums to also act as reservoirs of Q fever. In Australia, feral animals and dingoes are distributed in both remote

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and peri-urban areas [12,13]. These animals may be involved in the natural cycle of *C. burnetii* in wildlife. With increased population growth in Queensland there is increasing urban development in bushland. This provides a potential conduit for the transmission of Q fever from wild and feral animals to domestic animals and humans. This study aimed to establish the prevalence of anti-*C. burnetii* antibodies in several native and non-native species in northern and south-eastern Queensland.

Bandicoots and possums sampled in northern Queensland were trapped according to procedures used by the Queensland Parks and Wildlife Service. Ethical approval was granted by the James Cook University Animal Ethics Committee. Great care was taken to reduce stress on the animals. Blood samples (equivalent to <0·5% of the body weight to a maximum 2 ml) collected from each identified animal were taken from the tail vein or other suitable site. Following blood collection, animals were released at the site at which they were captured. The approximate age, sex and capture area were recorded for each animal. Whole blood was allowed to clot and centrifuged at 1400 *g* for 10 min at room temperature. Serum removed from the samples was frozen at −20 °C prior to analysis.

Samples from introduced species were obtained for this study through a selection of pest control and eradication programmes. Dingoes, feral cats and foxes were captured humanely by professional trappers using rubber padded leg-hold traps and destroyed humanely within several hours of capture. Blood samples were collected via cardiac puncture with 18-gauge needles and 20-ml syringes and transferred to 10-ml heparinized vacutainers. Feral pigs were captured humanely by professional trappers using cage and corral traps then destroyed humanely within several hours of capture. Blood samples were obtained following severing of the jugular vein and transferred to 10-ml heparinized vacutainers. The approximate age, sex and capture area were recorded for each animal. Serum was separated and stored as described for native species.

Antigen was prepared according to the protocol described in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* [14]. Both phase II and phase I *C. burnetii* antigens were produced using an Australian *C. burnetii* isolate (Cumberland strain). Phase II *C. burnetii* was obtained by serial passage in Vero cell culture to a total of 15 passages. Phase I *C. burnetii* was maintained by animal passage in A/J

strain mice, followed by a single passage in embryonated chicken eggs. 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). NUNC™ 96-well Maxisorp plates were coated with 100 μl of phase I or phase II antigens at 50 μg/ml in carbonate/bicarbonate coating buffer (pH 9·0) and incubated overnight at room temperature in a humidified chamber. Plates were then blocked and stabilized with 100 μl post-coating buffer (TropBio, Australia), incubated at room temperature for 2 h then dried.

The enzyme-linked immunosorbent assays (ELISAs) were initially optimized and validated using serum from mice and guinea pigs experimentally infected with *C. burnetii*, PBS inoculated negative controls and confirmed Q fever patient sera. The murine ELISA was optimized using 6-week-old C57/Bl6 mice, with 10 mice injected intraperitoneally (i.p.) with 100 μl PBS containing 1×10^4 *C. burnetii* and a further 10 mice injected with 100 μl PBS. Blood was collected 2 weeks post-injection via cardiac puncture, coagulated and centrifuged to collect sera. A total of 18 guinea pigs were used to further optimize the ELISAs with five groups of three guinea pigs injected i.p. with 100 μl PBS containing 1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9 *C. burnetii*, respectively and a further three guinea pigs injected i.p. with 100 μl PBS. Blood was collected 2 weeks post-injection via cardiac puncture, coagulated and centrifuged to collect sera. This was performed under the approval of the James Cook University Animal Ethics Committee under PC3 conditions. Checkerboard ELISAs were performed to determine the best reagent concentrations and sera were also tested against *Legionella pneumophila* lysate to determine potential cross-reactivity. Using the optimized reagent concentrations a random selection of serum samples from the target species (30 canine, 30 feline, 10 porcine, 10 bandicoot, 10 possums) were selected and tested to select appropriate positive and negative control sera for each species. Using the selected positive and negative control sera, ELISAs were refined for each species and appropriate cut-offs determined through comparison with murine and cavine sera cut-offs.

Indirect ELISA were used for detection of antibodies in introduced species, with test sera added at a dilution of 1:100 and HRP-conjugated anti-species IgG (Serotec, UK) at 1:2000 for dingoes and foxes (sheep anti-canine IgG), 1:2000 for cats (goat anti-feline IgG) and 1:5000 for pigs (rabbit anti-porcine

Table 1. *Summary of seroprevalence in species sampled*

Species	No. sampled	Seropositivity (%) (95% CI)		
		Phase II	Phase I	Phase II/I
Dingo (I)	127	12·6	8·7	17·3
(*Canis lupus dingo*)		(12·5–12·8)	(8·6–8·8)	(17·2–17·5)
Feral pig (I)	50	20·0	10·0	22·0
(*Sus scrofa*)		(19·8–20·7)	(9·9–10·4)	(21·8–22·7)
Feral cat (I)	31	29·0	22·6	38·7
(*Felis catus*)		(28·6–30·6)	(22·3–23·9)	(38·0–40·6)
Fox (I)	16	37·5	18·7	43·8
(*Vulpes vulpes*)		(36·6–41·5)	(18·5–21·6)	(42·5–48·1)
Brushtail possum (N)	56	7·1	3·6	10·7
(*Trichosurus vulpecula*)		(7·1–7·5)	(3·6–3·8)	(10·6–11·1)
Northern bandicoot (N)	46	21·7	21·7	23·9
(*Isoodon macrourus*)		(21·5–22·5)	(21·5–22·5)	(23·6–24·8)

CI, Confidence interval; I, Introduced species; N, native species; II/I, antibodies to either or both phase II and I antigens.

IgG). Test sera was applied in 50-μl aliquots in duplicate and incubated at 37 °C for 1 h. Positive and negative control sera were also included in duplicate. The wells were washed three times with PBS, Tween 0·05%, after which 50 μl conjugate was applied and incubated at 37 °C for 1 h. The wells were washed again, after which 100 μl ABTS (TropBio) was applied and incubated at 37 °C for 30 min. Optical density readings were obtained using a plate reader at 414/494 nm. The S/P% was calculated for each sample using the following formula:

$$S/P\% = \frac{OD\ sample - OD\ negative\ control}{OD\ positive\ control - OD\ negative\ control} \times 100.$$

Sera with an S/P% < 50% were considered to be negative. Samples with an S/P% of between 50% and 75% were considered to be positives; those > 75% were considered strongly positive.

Competitive ELISA was used for native Australian marsupial species, with test sera added at a dilution of 1:10, indicator sera (previously defined, *C. burnetii*-positive bovine sera) at 1:200 and HRP-conjugated anti-bovine Ig at 1:1000 (Serotec, UK). Test sera was applied in 50-μl aliquots in duplicate and incubated at 37 °C for 1 h. Positive and negative control sera were also included in duplicate. Indicator serum was then applied and incubated at 37 °C for a further 1 h. The wells were washed three times with PBS-T after which 50 μl conjugate was applied and incubated at 37 °C for 1 h. The wells were washed again, after which 100 μl ABTS was applied and incubated at 37 °C for 30 min. Optical density readings were obtained using

a 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.

Percentage seropositivity was calculated by dividing the number of positive samples by the total number of samples and multiplying by 100. Comparison of seropositivity between groups was performed using Kruskal–Wallis tests. Cross-tabular analysis and Pearson χ^2 tests were performed for factors potentially associated with seropositivity for either or both antigenic phases of *C. burnetii*.

A total of 127 dingo, 56 brushtail possum, 50 feral pig, 46 bandicoot, 31 feral cat and 16 fox serum samples were screened for the presence of anti-*C. burnetii* phase II and I antibodies. Overall seroprevalence in each species was determined to be 43·8% (95% CI 42·5–48·1) in foxes, 38·7% (95% CI 38·0–40·6) in feral cats, 23·9% (95% CI 23·6–24·8) in bandicoots, 22·0% in feral pigs (95% CI 21·8–22·7), 17·3% (95% CI 17·2–17·5) in dingoes and 10·7% (95% CI 10·6–11·1) in possums. A summary of seroprevalence is included in Table 1. The only factor associated with seropositivity in dingoes was origin, with samples originating from southeastern Queensland more likely to be seropositive for phase II [relative risk (RR) 2·5, OR 2·9, $\chi^2 = 4·8$] or both antigens (RR 2·8, OR 3·6, $\chi^2 = 9·8$) than samples originating from northern Queensland. Statistically significant factors associated with seropositivity in feral cats were southeast Queensland origin (RR 3·7, OR 6·7, $\chi^2 = 8·0$) and male sex (RR 3·2, OR 5·6, $\chi^2 = 6·2$). No factors were found to have statistically

significant associations with seropositivity to either or both *C. burnetii* antigens or to each antigen separately for bandicoots and possums. Insufficient samples were available for feral pigs and foxes from different regions to identify factors associated with seropositivity.

In this study antibodies to both phase II and phase I *C. burnetii* antigens were detected using ELISA. The development of antibodies to each antigenic phase of *C. burnetii* in animal infection has not been fully established [15]. However, some studies have suggested the presence of antibodies to phase II antigen in animal sera is indicative of recent infection [16, 17]. Seropositivity to either or both antigenic phases of *C. burnetii* has been shown to vary between species in other surveys [18–20]. Serological tests for the presence of antibodies against *C. burnetii* in animals were unable to determine whether an animal is actively shedding the organism [15]. In addition, animals can seroconvert without shedding *C. burnetii* and some animals can remain seropositive for long periods after the initial infection has been cleared. Alternatively, animals may begin to shed the organism prior to the production of antibodies and some infected animals never demonstrate seroconversion [15]. The positive association with seropositivity in dingo samples originating from southeastern Queensland indicated these animals may be a potential reservoir for Q fever in peri-urban areas in this region. Studies involving GPS tracking of dingoes in this region indicated animals regularly ranged into urban areas [21]. The detection of antibodies to *C. burnetii* in a relatively large percentage of feral cat samples indicates this species may constitute an important reservoir for *C. burnetii*. The potential for feral cats as a reservoir of *C. burnetii* is considerably greater in southeastern Queensland, where seroprevalence in these animals was >50%. As only 16 fox serum samples were collected, only preliminary conclusions could be drawn from the seropositivity results for these species. The fox samples taken in this study consisted of by-catch of wild dog/dingo control works, as foxes were not the target species of the eradication programmes. However, the high seroprevalence in fox sera sampled indicates further investigation of this species as a reservoir for Q fever may be warranted. The incidence of feral pig incursion in urban areas has been increasing in Queensland [22]. Feral pigs also constitute the most popular game animal in Queensland [23]. The detection of antibodies to *C. burnetii* in these animals indicates they may be a potential reservoir for Q fever for recreational and professional pig hunters, as well as primary producers who engage in feral pig eradication measures. 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 and feral animals. In addition, some native species such as brushtail possums and bandicoots have adapted to urban habitats and are regularly observed on suburban properties. 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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DECLARATION OF INTEREST

None.

REFERENCES

1. **Angelakis E, Raoult D.** Q fever. *Veterinary Microbiology* 2010; **140**: 297–309.
2. **Arricau-Bouvery N, Rodolakis A.** Is Q fever an emerging or re-emerging zoonosis? *Veterinary Research* 2005; **36**: 327–349.
3. **Garner M, et al.** A review of Q fever in Australia 1991–1994. *Australian and New Zealand Journal of Public Health* 1997; **21**: 722–730.
4. **Islam A, et al.** Seroprevalence to *Coxiella burnetii* among residents of the Hunter New England region of New South Wales, Australia. *American Journal of Tropical Medicine and Hygiene* 2011; **89**: 318–320.
5. **Parker N, Robson J, Bell M.** A serosurvey of *Coxiella burnetii* infection in children and young adults in South West Queensland. *Australian and New Zealand Journal of Public Health* 2010; **34**: 79–82.
6. **Chong A, et al.** Q fever: a recent outbreak in Townsville. *Internal Medicine Journal* 2003; **33**: 208–210.
7. **Gale M, et al.** Q fever cases at a north Queensland centre during 1994–2006. *Internal Medicine Journal* 2007; **37**: 644–646.
8. **Derrick E, Smith D.** Studies in the epidemiology of Q fever II: the isolation of three strains of *Rickettsia*

burnetii from the bandicoot *Isoodon torosus*. *Australian Journal of Experimental Biology and Medical Science* 1940; **18**: 99–102.

9. **Derrick E.** The changing pattern of Q fever in Queensland. *Pathologica et Microbiologica* 1961; **24**: 73–79.

10. **Rabinowitz P, Conti L.** *Human-Animal Medicine: Clinical approaches to zoonoses, toxicants and other shared health risks*, 1st edn. Maryland Heights: Saunders, 2009, pp. 191–194, 222–226.

11. **Slack A, et al.** The epidemiology of leptospirosis and the emergence of *Leptospira borgpetersenii* serovar Arborea in Queensland, Australia, 1998–2004. *Epidemiology and Infection* 2006; **134**: 1217–1225.

12. **Eymann J, et al.** Leptospirosis serology in the common brushtail possum (*Trichosurus vulpecula*) from urban Sydney, Australia. *Journal of Wildlife Diseases* 2007; **43**: 492–497.

13. **Whan I.** Economic assessment of the impact of dingoes/wild dogs in Queensland. Milton, Queensland, Australia: Department of Natural Resources and Mines, 2004; Queensland Government Publication, LP02/03NRM.

14. **Rousset E, Sidi-Boumedine K, Thiery R.** Q fever. In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, chapter 2.1.12. Paris: World Organisation for Animal Health, 2010.

15. **McQuiston J, Childs J.** Q fever in human and animals in the United States. *Vector Borne and Zoonotic Diseases* 2002; **2**: 179–191.

16. **Lackmann D, et al.** Q fever studies XXIII: antibody patterns against *Coxiella burnetii*. *American Journal of Hygiene* 1962; **75**: 158–167.

17. **Sidwell R, Gebhardt L.** Q fever antibody response in experimentally infected wild rodents and laboratory animals. *Journal of Immunology* 1962; **89**: 318–22.

18. **Enright J, et al.** *Coxiella burnetii* in a wildlife-livestock environment: distribution of Q fever in wild mammals. *American Journal of Epidemiology* 1971; **94**: 79–90.

19. **Marrie T, et al.** Seroepidemiology of Q fever among domestic animals in Nova Scotia. *American Journal of Public Health* 1985; **75**: 763–766.

20. **Marrie T, Embil J, Yates L.** Seroepidemiology of *Coxiella burnetii* among wildlife in Nova Scotia. *American Journal of Tropical Medicine and Hygiene* 1993; **49**: 613–615.

21. **Allen B.** The spatial ecology and zoonoses of urban dingoes: a preliminary investigation (dissertation). Brisbane, Queensland, Australia, University of Queensland, 2006.

22. **Mitchell J.** Ecology and management of feral pigs (*Sus scrofa*) in rainforest (dissertation). Townsville, Queensland, Australia, James Cook University, 2002.

23. **Mitchell J, Merrell P, Allen L.** *Vertebrate pests of Queensland*. Stock Routes and Rural Lands Protection Board, Brisbane, Queensland, Australia: Queensland Department of Lands, 1982. Queensland Government Publication.