Coxiella burnetii in Western Barred Bandicoots (Perameles bougainville) from Bernier and Dorre Islands in Western Australia

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Abstract

The aim of this work is to investigate the presence of *Coxiella burnetii* in *Perameles* bougainville and their ticks on two islands off Western Australia. *Haemaphysalis humerosa, Haemaphysalis ratti,* and Haemaphysalis lagostrophi were collected from *P. bougainville* on Bernier and Dorre Islands from 2005 to 2007; only *Amblyomma limbatum* was collected from humans over the same interval. One of 13 tick samples and 1 of 12 *P. bougainville* fecal samples were positive for *C. burnetii* DNA using quantitative polymerase chain reaction. DNA fragments had >99% similarity to published *C. burnetii* sequences. Three of 35 *P. bougainville* sera tested positive for anti-*C. burnetii* antibodies using enzyme-linked immunosorbent assay. *C. burnetii* was found in *P. bougainville* feces and a *H. humerosa* tick on Dorre Island and Bernier Island, respectively. This is the first reported use of enzyme-linked immunosorbent assay for screening of *P. bougainville* sera. The risk of zoonotic Q

fever infection for human visitors to these islands is considered relatively low, however, appropriate precautions should be taken when handling western barred bandicoots, their feces and their ticks on Bernier and Dorre Islands.

Keywords: *Coxiella burnetii*, Q fever, polymerase chain reaction, enzyme-linked immunosorbent assay, *Haemaphysalis humerosa*, *Perameles bougainville*

Western barred bandicoots, *Perameles bougainville*, are endangered Australian marsupials (www.iucnredlist.org, accessed 1 November, 2010) that were once widely distributed across arid southern mainland Australia. Since European colonization, they have undergone a dramatic range contraction: natural populations of *P. bougainville* now survive only on Dorre and Bernier Islands, in Shark Bay, Western Australia (Friend and Burbidge *2002*).

Q fever is caused by *Coxiella burnetii*, a Gram negative obligate intracellular bacterium classified within the gamma subdivision of the Proteobacteria (Stein et al. *1993*). Except for New Zealand, this zoonosis is globally distributed (Maurin and Raoult *1999*). 'Q fever' (query fever) was coined by E. H. Derrick in 1937 to denote an unusual febrile condition in abattoir workers from Brisbane, Australia (Maurin and Raoult *1999*). Humans in contact with infected animals (e.g., abattoir workers, veterinarians and farmers) are at increased risk of contracting Q fever (Garner et al. *1997*; Raoult et al. *2000*), usually through exposure to placentas and milk of chronically infected domestic ruminants (Berri et al. *2000*; Arricau-Bouvery et al. *2003*; Barralet and Parker *2004*; Masala et al. *2004*; Arricau-Bouvery et al. *2005*; Guatteo et al. *2006*). While many Q fever cases are clinically mild or asymptomatic, myocarditis, meningoencephalitis, and even death can occur (Raoult et al. *1989*; Helbig et al. *2005*). Infection during pregnancy may lead to low birth weight, premature birth, and abortion (Stein and Raoult *1998*).

Hosts of *C. burnetii* include mammals, birds and arthropods (Enright et al. 1971). Chronic *C. burnetii* infection in animals is usually asymptomatic, though abortion, reduced fertility and low birth weights are possible (To et al. 1988; Arricau-Bouvery et al. 2003). Early Australian Q fever research showed the northern brown bandicoot (*Isoodon macrourus*) on Moreton Island, Queensland, was susceptible to the Q fever agent, then named *Rickettsia burneti* (Derrick et al. 1939; Mackerras and Mackerras, 1960). Furthermore, the same organism was detected in *Haemaphysalis humerosa* ticks from Moreton Island, which frequently parasitized *I. macrourus* (Smith and Derrick 1940; Mackerras and Mackerras 1960). More recently, kangaroos have been implicated in the spread of Q fever in Queensland (Barralet and Parker 2004), South Australia (IMVS 1955) and Western Australia (Banazis et al. 2010).

Given the historical association between Q fever and bandicoots, and the common occurrence of ticks on *P. bougainville* from Bernier and Dorre Islands, we hypothesized that *P. bougainville* and their ticks may be hosts of *C. burnetii.*

Fifty-eight *P. bougainville* from Dorre (25°03′S; 113°06′E) and Bernier Island (24°50′S; 113°08′E) were trapped overnight using baited Sheffield and Elliot traps (approved by: Western Australian Department of Environment and Conservation; Murdoch University Animal Ethics Committee). Traps were cleared early each morning, and 12 bandicoot fecal samples were collected into 70% ethanol for subsequent DNA extraction using a PowerSoil™ DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) performed according to the manufacturer's protocol.

Bandicoots were anesthetized using isoflurane (Isorrane, Baxter Healthcare, Australia) and oxygen delivered by mask. Thorough physical examinations were conducted, ectoparasites were collected into 70% ethanol, and, where possible, blood was collected by jugular venipuncture (n = 35). Lithium

heparin anticoagulant blood tubes (Becton-Dickinson) were centrifuged, and the plasma was pipetted into Eppendorf tubes and frozen at −20°C. Ticks from 4 human volunteers working on these islands were collected daily into 70% ethanol.

Ticks were examined under a dissecting microscope and identified using appropriate keys (Roberts 1963). Three tick species were collected from 58 *P. bougainville*: 17 *H. humerosa*; 33 *Haemaphysalis ratti* and 4 *Haemaphysalis lagostrophi*. All eight ticks collected from humans were *Amblyomma limbatum*. Thirteen ticks were randomly selected and minced prior to DNA extraction with a QIAmp DNA minikit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

DNA extracts were tested by quantitative polymerase chain reaction (qPCR) targeting the *IS1111a* element and utilizing the TaqMan® fluorogenic reporter system for detection. Primers were synthesized by Invitrogen (Mount Waverley, Victoria, Australia) and oligonucleotide probes were supplied by Geneworks (Hindmarsh, South Australia, Australia). Primer and probe sequences, assay conditions, no template controls (NTCs) and the standard curve included with each run were as described previously by Banazis et al. (2010). Four microliters of template were used and all samples, NTCs and DNA standards were tested in duplicate. Valid qPCR runs showed no amplification of NTCs. One of 13 tick samples tested by qPCR was positive (estimated concentration: 2.4 genome equivalents/µl) (Table 1). This positive tick DNA extract came from a male *H. humerosa* parasitic on an adult male *P. bougainville* (ID B06B2), trapped at Red Cliffs, Bernier Island during September 2006. This bandicoot was seronegative for *C. burnetii* at the time of sample collection.

One of 12 *P. bougainville* fecal DNA extracts was positive according to qPCR (3.7 genome equivalents/µl). This fecal sample came from an adult male *P. bougainville* (ID B05Do12), trapped during July 2005 at White Beach, Dorre Island. A blood sample was not collected from this

individual. To provide template of sufficient length for sequence comparison, *C. burnetii* DNA was amplified using a conventional PCR with the OMP1/OMP2 primers (Zhang et al. 1998), with the exception that annealing was performed at 54°C. Cycling was performed on an Applied Biosystems GeneAmp® PCR System 2700 (Applied Biosystems, Foster City, California, USA). Sequencing of purified PCR products was performed using the Big Dye version 3.1 terminator kit (Applied Biosystems, Foster City, CA, USA) using the dideoxynucleotide chain termination method (Sanger et al. 1977). The sequence was determined using an ABI Prism Applied Biosystems 377 automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA) at the State Agriculture and Biotechnology Centre, Perth, Western Australia. Chromatograms were edited using Chromas Lite version 2.0 (Technelysium P/L, Helensvale, Queensland, Australia). Sequence information was compared to the GenBank archive using BLAST (http://www.ncbi.nlm.nih.gov, accessed 1 November, 2010). Amplicons from this sample (GenBank accession number HM804027) showed >99% similarity to C. burnetii Dugway strain (GenBank# CP700033.1).

Thirty-five stored *P. bougainville* plasma/serum samples from 2003 to 2007 were tested for anti-*C. burnetii* antibodies using the enzyme-linked immunosorbent assay (ELISA) and kangaroo positive and negative controls as previously described by Banazis et al. (2010). Three of 35 *P. bougainville* plasma/serum samples were clearly ELISA positive (Table 2). These positive sera were obtained from three adult male *P. bougainville* from Dorre Island in March 2003. A further two results fell in the 'suspect' range of between 30 and 39% of the absorbance of positive control values. These two sera were obtained from two adult female *P. bougainville* from Dorre Island in 2003. Thus, the seroprevalence of *C. burnetii* in *P. bougainville* from the islands is ~8.6–14%, depending on the cut-off value selected. Normalizing test serum absorbance results against positive control absorbance and then implementing a cut-off at a certain percentage of the positive control mean, as performed in this study, has been previously used in research and commercially (OIE 2000). However, selecting the cut-off value that best delineates the desired sample set requires careful consideration. Banazis et al. (2010) selected a cut-off of ≥40% of the positive control absorbance. The absorbance values of *P.*

bougainville samples ($\mu = 0.209$, $\sigma = 0.114$) were compared for differences with the absorbance values of kangaroo samples ($\mu = 0.414$, $\sigma = 0.259$) tested in Banazis et al. (2010), using an independent samples t test with the Statistical Package for Social Sciences (SPSS Inc., Chicago, USA), which showed the absorbances of *P. bougainville* samples were significantly lower than the kangaroo samples tested by Banazis et al. (2010) ($P < 0.05$). Because kangaroo sera were used as positive control samples in this study, it may be that the cut-off employed (≥40%) underestimated the true prevalence of exposure to *C. burnetii* in the bandicoots tested. Implementing a cut-off of ≥30% of the positive control absorbance yielded a further two seropositive animals and it is likely that this cut-off is more appropriate for *P. bougainville* samples, where lower background reactivity was observed.

It is presently unknown how *C. burnetii* arrived on Bernier and Dorre Islands. These islands became separated from mainland Australia ~8,000 years ago, and are thought to have been uninhabited prior to European exploration (Hancock et al. 2000). Perhaps the existence of *C. burnetii* on these islands pre-dates the separation of the islands from the mainland, or alternatively, *C. burnetii* may have been introduced more recently. Bernier Island was briefly farmed in the late 1800s: cats, mice, sheep, goats and horses were all introduced, though none of these eutherians is thought to persist (Hancock et al. 2000). It is conceivable that *C. burnetii* was brought to the islands with infected livestock or rodents, or else may have arrived via infected birds or their ticks. In 1908, the Lock hospitals were established on these islands to house indigenous Australians suffering from infectious diseases (Hancock et al. 2000). It could be hypothesized that *C. burnetii* may have been introduced during the Lock hospitals era. Further research may provide insights into the true origin of *C. burnetii* on Dorre and Bernier Islands, and potentially lead to improved understanding of the ecology and evolution of *C. burnetii*.

In contrast to the situation in humans and domestic livestock, tick-borne transmission may be epidemiologically important for *C. burnetii* infections of wild vertebrates (Maurin and Raoult 1999). Experimental transmission of *C. burnetii* has been demonstrated in guinea pigs with a variety of tick

species (reviewed by Maurin and Raoult 1999). Ticks that engorge on *C. burnetii*-bacteremic blood may become infected, support bacterial growth in their stomach and midgut, and subsequently pass phase I *C. burnetii* organisms in feces onto the skin of vertebrates (Maurin and Raoult 1999). Transovarial transmission is also possible and thus *C. burnetii* may persist across generations of ticks.

Translocation of wildlife often inadvertently causes the translocation of pathogens. Where zoonoses are involved, the public health risks may be severe, however, this issue is rarely considered (Cunningham 1996; Woodford 2001). Managers of *P. bougainville* translocations should consider the potential ecological and human health risks posed by *C. burnetii* infected individuals.

Our study detected *C. burnetii* in *P. bougainville* feces on Dorre Island and in the 'bandicoot tick' *H. humerosa* on Bernier Island. Given that *I. macrourus* is involved in the natural cycle of Q fever, more research should be conducted to determine the role of P. bougainville in a possible sylvatic cycle on Bernier and Dorre Islands. From a public health perspective, such a sylvatic cycle presents a low risk: the bandicoots are shy, nocturnal creatures; *H. humerosa* was not detected on humans; and the islands are remote and infrequently visited. Nonetheless, people should be aware of possible exposure risk for *C. burnetii*, through contact with *P. bougainville*, their feces, and the ticks that parasitize them.

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References

Arricau-Bouvery N, Souriau A, Lechopier P, Rodolakis A (2003) Experimental *Coxiella burnetii* infection in pregnant goats: excretion routes. *Veterinary Research* 34:423-433

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

Banazis MJ, Bestall AS, Reid SA, Fenwick SG (2010) A survey of Western Australian sheep, cattle and kangaroos to determine the prevalence of *Coxiella burnetii*. *Veterinary Microbiology* 143:337-345

Barralet JH, Parker NR (2004) Q fever in children: an emerging public health issue in Queensland. *Medical Journal of Australia* 180:596-597

Berri M, Laroucau K, Rodolakis A (2000) The detection of *Coxiella burnetii* from ovine genital swabs, milk and fecal samples by the use of a single touchdown polymerase chain reaction. *Veterinary Microbiology* 72:285-293

Cunningham AA (1996) Disease risks of wildlife translocations. *Conservation Biology* 10:349-353

Derrick EH, Smith DJW, Brown HE, Freeman M (1939) The role of the bandicoot in the epidemiology of "Q" fever: a preliminary study. *Medical Journal of Australia* 1:150-155

Enright JB, Franti CE, Behymer DE, Longhurst WM, Dutson VJ, Wright ME (1971) *Coxiella burneti* in a wildlife-livestock environment. Distribution of Q fever in wild mammals. *American Journal of Epidemiology* 94:79-90

Friend JA, Burbidge AA (2002) Western barred bandicoot. In: *The mammals of Australia, revised edition*, Strahan R, (editor), Frenchs Forest, New South Wales: Reed New Holland, pp 178-180

Garner MG, Longbottom HM, Cannon RM, Plant AJ (1997) A review of Q fever in Australia 1991-1994. *Australian and New Zealand Journal of Public Health* 21:722-730

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

Hancock S, Brown P, Stephens B (2000) *Shark Bay Terrestrial Reserves Management Plan 2000–2009.* Management Plan No 45, Perth: Department of Conservation and Land Management (WA)

Helbig K, Harris R, Ayres J, Dunckley H, Lloyd A, Robson J, Marmion BP (2005) Immune response genes in the post-Q-fever fatigue syndrome, Q fever endocarditis and uncomplicated acute primary Q fever. *Quarterly Journal of Medicine* 98:565-574

Institute of Medical and Veterinary Sciences (1955) *Annual Report*, Adelaide: Institute of Medical and Veterinary Sciences

Mackerras IM, Mackerras MJ (1960) Taxonomy of the common short-nosed marsupial bandicoot in eastern Queensland. *Australian Journal of Science* 23:51-52

Masala G, Porcu R, Sanna G, Chessa G, Cillara G, Chisu V, Tola S (2004) Occurrence, distribution, and role in abortion of *Coxiella burnetii* in sheep and goats in Sardinia, Italy. *Veterinary Microbiology* 99:301-305

Maurin M, Raoult D (1999) Q fever. *Clinical Microbiology Reviews* 12:518-553

Office International Des Epizooties (2000) *Manual of Standards for Diagnostic Tests and Vaccines*, Paris: Office International Des Epizooties

Raoult D, Bollini G, Gallais H (1989) Osteoarticular infection due to *Coxiella burnetii*. *Journal of Infectious Diseases* 159:1159-1160

Raoult D, Tissot-Dupont H, Foucault C, Gouvernet J, Fournier PE, Bernit E, Stein A, Nesri M, Harle JR, Weiller PJ (2000) Q fever 1985-1998. Clinical and epidemiologic features of 1,383 infections. *Medicine (Baltimore)* 79: 109-123

Roberts FHS (1963) A systematic study of the Australian species of the genus *Haemaphysalis* Koch (Acarina: Ixodidae). *Australian Journal of Zoology* 11:35-80

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Science of the United States of America* 74:5463-5467

Smith DJW, Derrick EH (1940) Studies in the epidemiology of Q fever. 1. The isolation of six strains of *Rickettsia burneti* from the tick *Haemaphysalis humerosa*. *Australian Journal of Experimental Biology and Medical Science* 18:1-8

Stein A, Saunders NA, Taylor AG, Raoult D (1993) Phylogenic homogeneity of *Coxiella burnetii* strains as determinated by 16S ribosomal RNA sequencing. *FEMS Microbiology Letters* 113:339-344

Stein A, Raoult D (1998) Q fever during pregnancy: a public health problem in southern France. *Clinical Infectious Diseases* 27: 592-596

To H, Htwe KK, Kako N, Kim HJ, Yamaguchi T, Fukushi H, Hirai K (1988) Prevalence of *Coxiella burnetii* infection in dairy cattle with reproductive disorders. *Journal of Veterinary Medical Science* 60:859-861

Woodford MH (2001) *Quarantine and health screening protocols for wildlife prior to translocation and release into the wild.* Paris, France: Office International des Epizooties

Zhang GQ, Nguyen SV, To H, Ogawa M, Hotta A, Yamaguchi T, Kim HJ, Fukushi H, Hirai K (1998) Clinical evaluation of a new PCR assay for detection of *Coxiella burnetii* in human serum samples. *Journal of Clinical Microbiology* 36:77-80

Table 1: Results of quantitative PCR to detect the *IS1111a* element of *Coxiella burnetii* in ticks of the genus *Haemaphysalis*, collected from *Pseudomys fieldi* and *Perameles bougainville* on Bernier Island

Tick species	**Sex**	**Host**	**qPCR result**
H. ratti	F	*Pseudomys fieldi*	Negative
H. humerosa	F	*Pseudomys fieldi*	Negative
H. humerosa	F	*Pseudomys fieldi*	Negative
H. humerosa	F	*Perameles bougainville*	Negative
H. humerosa	F	*Perameles bougainville*	Negative
H. humerosa	F	*Perameles bougainville*	Negative
Haemaphysalis sp.	F	*Perameles bougainville*	Negative
H. humerosa	F	*Perameles bougainville*	Negative
H. ratti	F	*Perameles bougainville*	Negative
H. humerosa	F	*Perameles bougainville*	Negative
H. humerosa	M	*Perameles bougainville*	Negative
H. humerosa	F	*Perameles bougainville*	Negative
H. humerosa	M	*Perameles bougainville*	2.4 genomes per µl

Table 2: Results of an ELISA to detect anti-*Coxiella burnetii* antibodies in the plasma/serum of *Perameles bougainville* on Bernier Island and Dorre Island

Bandicoot	**Sex**	**Location**	**Year**	**Absorbance (test/+control) (%)**
M68	M	White Beach, Dorre Island	2003	12.9
M33	M	White Beach, Dorre Island	2003	41.9
M91	M	White Beach, Dorre Island	2003	44.9
M43	M	White Beach, Dorre Island	2003	14.0
F37	F	White Beach, Dorre Island	2003	9.7
F42	F	White Beach, Dorre Island	2003	13.7
M39	M	White Beach, Dorre Island	2003	15.4
F2	F	Disaster Cove, Dorre Island	2003	35.8
M2	M	Disaster Cove, Dorre Island	2003	10.1
M3	M	Disaster Cove, Dorre Island	2003	40.7
M4	M	Disaster Cove, Dorre Island	2003	13.0
M85	M	Travertine B2, Dorre Island	2003	9.4
F63	F	Unrecorded, Dorre Island	2003	25.7
F97	F	Dune, Dorre Island	2003	26.9
F46	F	Dune, Dorre Island	2003	12.3
M99	M	White Beach, Dorre Island	2003	13.8
F48	F	White Beach, Dorre Island	2003	9.7
M36	M	White Beach, Dorre Island	2003	29.6
M96	M	White Beach, Dorre Island	2003	12.5
M97	M	White Beach, Dorre Island	2003	10.5
F45	F	White Beach, Dorre Island	2003	9.0
M95	M	White Beach, Dorre Island	2003	12.9
F5	F	Disaster Cove, Dorre Island	2003	33.8
F3	F	Disaster Cove, Dorre Island	2003	16.3
M5	M	Disaster Cove, Dorre Island	2003	14.1
F4	F	Disaster Cove, Dorre Island	2003	20.3
B05Do2	F	White Beach, Dorre Island	2005	13.9
B05Do5	M	White Beach, Dorre Island	2005	13.3
B06B1	M	Red Cliffs, Bernier Island	2006	9.7
B06B2	M	Red Cliffs, Bernier Island	2006	15.7
B06B3	F	Red Cliffs, Bernier Island	2006	24.5
B06B6	F	Red Cliffs, Bernier Island	2006	17.3
B06B7	M	Red Cliffs, Bernier Island	2006	13.0
B06B8	M	Red Cliffs, Bernier Island	2006	13.9
B06B10	F	Red Cliffs, Bernier Island	2006	12.9