Rethinking *Coxiella* infections in Australia

*Coxiella burnetii* is the causative agent of coxiellosis in animals and Q fever in humans. Despite being a vaccine preventable disease, Q fever remains a frequently reported zoonotic infection in Australia. Recently, a *Coxiella* species was identified in brown dog ticks (*Rhipicephalus sanguineus*) in urban and rural regions of Australia. Further molecular characterisation revealed that it is genetically identical to *Candidatus Coxiella massiliensis* (KM079627) described in *R. sanguineus* ticks removed from humans with eschars in France and serologic cross-reactivity among *Ca. Coxiella massiliensis* and *C. burnetii* may occur. This report highlights the need for molecular testing of seropositive companion animals and humans to determine which species of *Coxiella* they are infected with, in order to further assess *Coxiella* species associated with *Coxiella* infections in Australia.

*Coxiella burnetii* is a small, obligate intracellular, Gram-negative coccobacillus found worldwide (except in New Zealand) and has a sylvatic lifecycle involving wildlife and domestic mammals, birds, and arthropods\(^1\)\(^2\). *Coxiella burnetii* was first described in the 1930s as the causative agent of Q (query) fever in abattoir workers in Brisbane, Queensland, Australia\(^3\). *Coxiella burnetii* is also the known cause of coxiellosis in animals and is persistently shed by infected animals in secretions and parturient by-products. Transmission occurs predominantly through direct or indirect contact with infected tissues from domestic ruminants and companion animals, rather than as a consequence of tick bite\(^4\). Clinical presentations of Q fever range from acute to chronic, and can lead to post-Q fever fatigue syndrome, although asymptomatic Q fever represents >54–60% of infections\(^3\). High annual reports of human Q fever in Australia persist despite a readily available vaccine\(^5\); over 4800 cases were reported between 2007 and 2017, with 716 notifications of Q fever in the past 18 months\(^6\).

Australian serological surveys have reported the number of infected dogs with *C. burnetii* has increased over 26 years to nearly 22%\(^7\), with free-roaming dogs within Indigenous communities having the highest seroprevalence compared with breeding, pet, or shelter dogs, in a most recent study\(^8\). It has been proposed that dogs become infected with *C. burnetii* through consumption of infected raw meat, hunting, and scavenging wildlife, or due to heavy tick infestations\(^8\), most commonly with *Rhipicephalus sanguineus* ticks\(^9\). While our knowledge about the epidemiology of *C. burnetii* in companion animals continues to increase, it is unclear whether the high *C. burnetii*-seropositivity observed in these animals contributes to increasing reports of Q fever cases in humans.
In addition to *C. burnetii*, several other *Coxiella* species and subtypes of the genus have been identified in a range of different hosts, including *C. cheraxi*, the cause of mass mortalities in Australian redclaw crayfish, (*Cherax quadricarinatus*); *Coxiella* spp. endosymbionts of ticks; and more recently, ‘*Candidatus Coxieilla massiliensis*’, associated with ticks removed from humans with eschars. Molecular evidence suggests that *C. burnetii* originated from an inherited symbiont in soft ticks and acquired virulence factors enabling it to infect vertebrate cells. To date, over 40 tick species have been associated with *C. burnetii* and *Coxiella* spp. *Amblyomma*, *Dermacentor*, *Ixodes*, and *Rhipicephalus* species are the most frequently implicated vectors.

Tick-associated *Coxiella* spp. have a role in maintaining tick health and influence the vertical transmission of other tick-borne pathogens. Due to their symbiotic role in ticks, *Coxiella* spp. endosymbionts of ticks are considered non-pathogenic to vertebrates, however, the dogma of what is considered an endosymbiont versus a pathogen has been challenged recently though the observation of serological reactions to a number of tick-associated endosymbionts in people following a tick bite. Furthermore, a retrospective study identified *Coxiella* sp. (*Ca. Coxieilla massiliensis*) in several tick species, including *R. sanguineus* ticks removed from patients presenting with scalp eschars, cervical lymphadenopathy, fever, increased C-reactive protein and thrombocytopenia. Following the recent molecular characterisation of a *Coxiella* sp. in *R. sanguineus* ticks in Australia, this present study screened 41 *R. sanguineus* ticks with a *Coxiella*-specific GroEL PCR assay to determine the genetic relatedness to *Ca. Coxieilla massiliensis*.

A *Coxiella*-specific PCR assay, targeting a 659 bp region of the GroEL gene was performed using the primers Cox-660f and Cox-1320r according to Angelakis et al., with the following modifications: each 25 μL PCR reaction contained 1× Perfect Taq buffer, 1 mg/mL BSA, 2.5 mM MgCl₂, 1 mM dNTPs, 400 nM of each primer, 1.25 U Perfect Taq polymerase, and 2 μL of undiluted DNA. All samples were performed under the following thermal conditions: initial...

Figure 1. Phylogenetic tree based on 547 bp GroEL gene sequences including *Coxiella* associated with ticks, *C. burnetii* reference strain and an outgroup, *Rickettsiella gyril* (cropped). The proposed ‘*Candidatus Coxieilla Massiliensis*’ is highlighted by the teal box. The Bayesian tree was constructed using MrBayes 3.2.6 with posterior probabilities and the following parameters were used: substitution model GTR, gamma category 5, chain length 1,100,000, sampling every 200 trees and burn-in length 100,000. Bold type indicates the consensus sequence from this study. Abbreviations: A., *Amblyomma*; D., *Dermacentor*; I., *Ixodes*; O., *Ornithodoros*; R., *Rhipicephalus*. 
denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for
30 s, annealing at 52°C for 30 s, extension at 72°C for 1 min, and a final
extension at 72°C for 5 min. A phylogenetic tree was constructed with
a 547 bp trimmed alignment of all known Coxiella GroEL
sequences, including those obtained in this study, with MrBayes
3.2.6.17

DNA was successfully amplified in 80% (33/41) of the R. sanguineus
ticks and Sanger sequencing was conducted on 10 positive samples
according to Oskam et al.16. All 10 sequences were identical to each
other (MK19208), and 100% similar to ‘Ca. Coxiella massiliensis’
isolated from R. sanguineus in France (KM079627). Phylogenetic
analysis revealed the ‘Ca. Coxiella massiliensis’ identified in this
study had high support (posterior probability 1.0) to ‘Ca. Coxiella
massiliensis’ found within other R. sanguineus ticks (Figure 1).12
The prevalence of ‘Ca. Coxiella massiliensis’ in this study was
higher than the ‘Ca. Coxiella massiliensis’ prevalence of 35%
(7/20) reported by Angelakis et al. in R. sanguineus12

It is still unknown whether ‘Ca. Coxiella massiliensis’ can be
transmitted to humans via tick bite or aerosol inhalation in
Australia, however it prompts further investigation to determine
if cross-reactions can occur among other Coxiella sp. in Q fever
serological tests. This study highlights the need for molecular
testing of companion animals and humans that are seropositive
for C. burnetii to determine which species of Coxiella they are
infected with and to comprehensively assess all species of Coxiella
in Australia for health risks.

Conflicts of interest
The authors declare no conflicts of interest.

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Biographies
Dr Charlotte Oskam is a senior lecturer and team leader in the
Vector and Waterborne Pathogens Research Group at Murdoch University. Her research interests extend from ancient DNA, micro-
bioorganisms, ticks, to zoonoses.

Jadyn Owens is a Murdoch University graduate in Molecular
Biology and completed an independent study contract supervised
by Dr Oskam in the Vector and Waterborne Pathogens Research
Group at Murdoch University.

Annachiara Codello was a research assistant during this project in
the Vector and Waterborne Pathogens Research Group at Murdoch University.

Alexander Gofton is a PhD student in the Vector and Waterborne
Pathogens Research Group at Murdoch University. His research interests are in tick microbiomes and tick-borne pathogens of
animals and humans.

Telleasha Greay is a PhD student in the Vector and Waterborne
Pathogens Research Group at Murdoch University. Her research interests are in tick microbiomes and tick-borne pathogens of
companion animals.

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