Rethinking *Coxiella* infections in Australia





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***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*)[10]; *Coxiella* spp. endosymbionts of ticks[11]; and more recently, '*Candidatus* Coxiella massiliensis', associated with ticks removed from humans with eschars[12]. Molecular evidence suggests that *C. burnetii* originated from an inherited symbiont in soft ticks and acquired virulence factors enabling it to infect vertebrate cells[11]. 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[11,13].

Tick-associated *Coxiella* spp. have a role in maintaining tick health and influence the vertical transmission of other tick-borne pathogens[14]. 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[14,15]. Furthermore, a retrospective study identified *Coxiella* sp. ('*Ca.* Coxiella 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[11,12]. Following the recent molecular characterisation of a *Coxiella* sp. in *R. sanguineus* ticks in Australia[16], this present study screened 41 *R. sanguineus* ticks with a *Coxiella*-specific *GroEL* PCR assay to determine the genetic relatedness to '*Ca.* Coxiella massiliensis'.

A *Coxiella*-specific PCR assay, targeting a 659 bp region of the *GroEL* gene was performed using the primers Cox-660f (GGCGCICARATGGTTAARGA) and Cox-1320r (AACATCGCTTTACGACGA) according to Angelakis *et al*.[12], with the following modifications: each 25 µL PCR reaction contained 1× Perfect Taq buffer (5 Prime, Germany), 1 mg/mL BSA (Fisher Biotech, Australia), 2.5 mM $MgCl_2$, 1 mM dNTPs, 400 nM of each primer, 1.25 U Perfect Taq polymerase (5 Prime, Germany) and 2 µL of undiluted DNA. All samples were performed under the following thermal conditions: initial

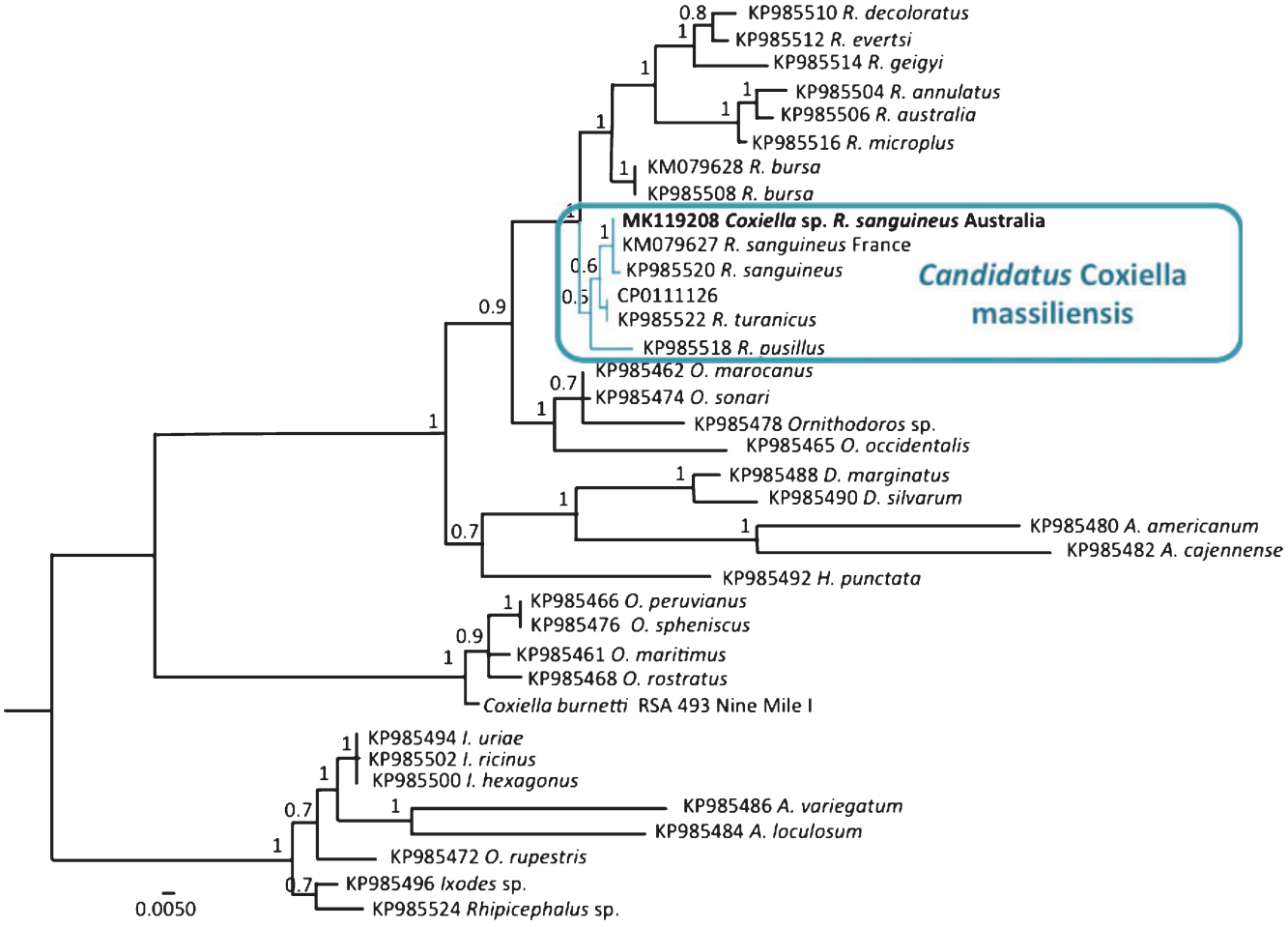

Figure 1. Phylogenetic tree based on 547 bp *GroEL* gene sequences including *Coxiella* associated with ticks, *C. burnetii* reference strain and an outgroup, *Rickettsiella gyrll* (cropped). The proposed '*Candidatus* Coxiella Massiliensis'[12] is highlighted by the teal box. The Bayesian tree was constructed using MrBayes 3.2.6[16] 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 30s, 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 (MK119208), 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. sanguineus*[12].

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

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