

## Bacterial tick-associated infections in Australia: current studies and future directions



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**It may seem perplexing that there is any uncertainty in Australia about the existence of zoonotic tick-associated infections<sup>1–3</sup>. Outside this country, particularly in the northern hemisphere, tick-borne diseases such as human granulocytic anaplasmosis, babesiosis, Boutonneuse fever, ehrlichiosis, Lyme borreliosis, and tick-borne encephalitis, have well documented aetiologies, epidemiology, diagnostic methods, and treatments. Why is Australia different and what research is being conducted to address this issue? This article briefly addresses these questions and explains how high-throughput metagenomic analysis has started to shed light on bacterial microbiomes in Australian ticks, providing new data on the presence and distribution of potentially zoonotic microbial taxa.**

Fundamental to understanding tick-borne infections in Australia is the recognition that the tick fauna of Australia is unique and distinct. Australia's long geological isolation since the breakup of Gondwana during the Mesozoic Era has allowed its animals to evolve separately from those in other parts of the world. Of the ~896 recognised tick species worldwide<sup>4</sup>, 71 species are endemic to Australia; 66 of these occur only on the Australian continent and its islands, with a few species extant in Papua New Guinea<sup>5</sup>. The remaining five species were introduced to Australia with domestic animals (dogs, cattle and poultry) during the past 230 years, and of the 71 species, only about eight are well known to bite humans<sup>6</sup>.

Infections caused by the five introduced tick species result in economically important diseases (e.g. canine and bovine babesiosis and anaplasmosis, bovine borreliosis, bovine theileriosis and avian spirochaetosis) that are restricted to domestic animal hosts and have been well studied internationally as well as in Australia. In contrast, the same cannot be said for endemic tick species, and herein lies the knowledge gap that underpins the debate around indigenous tick-borne infections of humans (and animals) in Australia.

Zoonotic tick-borne infections occur when humans encroach into natural environments where ticks, their microbial communities and wildlife reservoir hosts co-exist within well defined (and long-evolved) ecologies. These complex interactions, sometimes referred to as tick-borne pathogen guilds<sup>7</sup>, have been the subject of research in other parts of the world for many years<sup>8</sup>, and with increased intensity since the connection was first made between tick bites and an epidemic of arthritis, in Old Lyme, Connecticut, USA, in the late 1970s<sup>9,10</sup>. However, relatively little is known about host preferences and ecologies of Australian ticks, and even less is understood about the communities of organisms within these arthropods.

With the exception of rickettsial species and *Coxiella burnetii* (the causative agent of Q fever in humans and coxiellosis in animals), there has been a dearth of research into tick-associated microorganisms in Australia, especially viruses, and a hiatus of more than 20 years between studies<sup>11,12</sup> in the 1990s and the

start of investigations using metagenomic techniques<sup>13</sup>. Our studies have been designed to address two questions. (1) Which microbes are associated with Australian ticks? (2) Are any of these microbes known pathogens, or putative pathogens?

Answering these questions requires investigation of the tick microbiome, which comprises communities of microorganisms including viruses, bacteria and eukaryotes that can be explored in a rapid and cost-effective manner by next-generation sequencing (NGS)<sup>14</sup>. However, the presence of highly abundant bacterial endosymbionts such as ‘*Candidatus* Midichloria mitochondrii’ (CMM) may challenge the effectiveness of this approach by masking less abundant bacteria, including pathogens. Application of specific CMM blocking primers to the Australian paralysis tick (*I. holocyclus*) ( $n = 196$ ) removed from various hosts, including people in eastern Australia, decreased CMM sequences by 96%, and resulted in a significantly higher taxonomic diversity (an additional 103 genera detected)<sup>13</sup>.

Metagenomic analysis reveals that Australian ticks, like their northern hemisphere counterparts, possess a rich and varied microbiome, with the tick species as the main factor influencing microbial composition (Figure 1). Novel ‘*Candidatus* Neoehrlichia’ spp., *Anaplasma* spp. and *Ehrlichia* spp. were identified in ticks ( $n = 460$ ) removed from people in Australia<sup>15</sup>. Phylogenetic characterisation of these new members of the Anaplasmataceae revealed two species; ‘*Candidatus* Neoehrlichia australis’ and

‘*Candidatus* Neoehrlichia arcana’ in 8.7% and 3.1% *I. holocyclus* ticks in New South Wales and Queensland, respectively<sup>16</sup>. Analysis of 16S rRNA and groEL gene sequences demonstrated that *Anaplasma bovis* genotype Y11 is a unique genetic variant, distinct from other *A. bovis* isolates worldwide, and the *Ehrlichia* sp. is most closely related to, but clearly distinct from, *E. ruminantium* (a bovine pathogen) and other ehrlichial species<sup>17</sup>. The zoonotic potential of these bacteria is unknown, however ‘*Candidatus* Neoehrlichia’ is a sister genus to *Anaplasma* and *Ehrlichia*, and contains ‘*Candidatus* Neoehrlichia mikurensis’, an emerging tick-borne zoonosis in Africa, Asia and Europe<sup>16</sup>.

In recent years there has been increasing debate about whether Lyme borreliosis (LB) occurs in Australia<sup>1–3</sup>. The aetiological agents of LB in North America, Asia and Europe comprise spirochaetes belonging to the genus *Borrelia* that are transmitted, together with other tick-borne agents such as anaplasmosis and babesiosis, by hard ticks of the genus *Ixodes*, including *I. scapularis*, *I. pacificus*, and *I. ricinus*<sup>18</sup>. Whilst not being the only ticks capable of zoonotic disease transmission, none of these members of the *I. ricinus* group is known to have established in Australia, a finding supported by our recent survey of 4765 ticks parasitising companion animals nationwide<sup>19</sup>.

In serological screening of 555 Australian dogs (which act as sentinels for LB in endemic regions), including foxhounds exposed to >160,000 adult *I. holocyclus* ticks for commercial antiserum

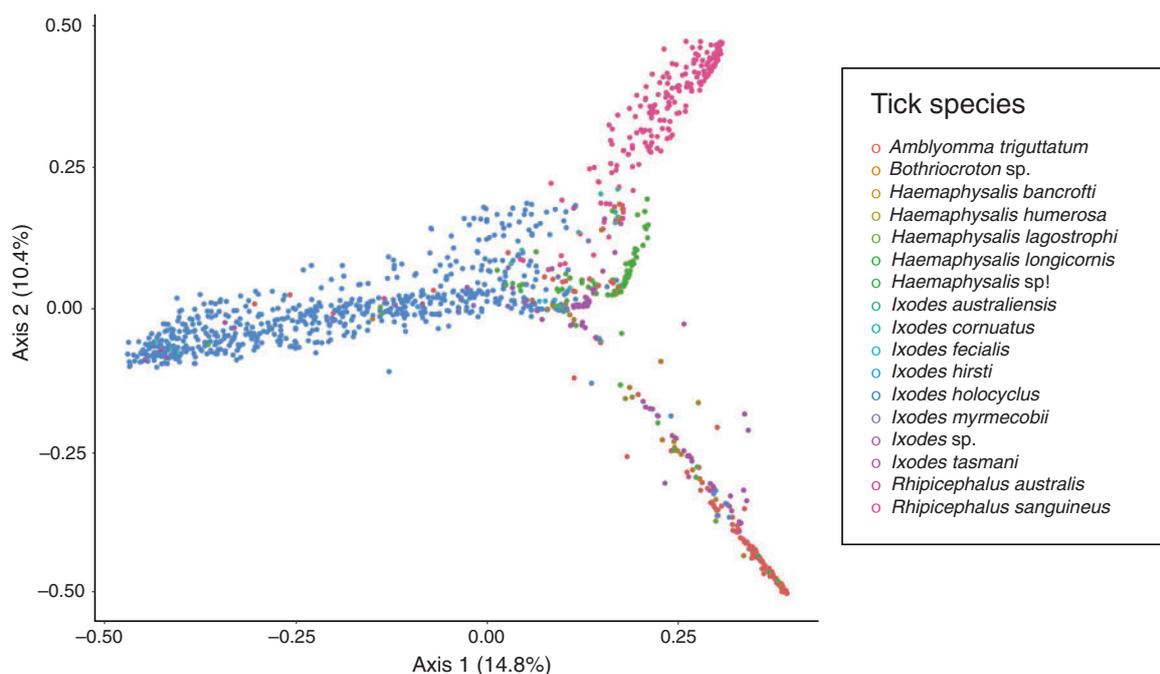


Figure 1. Multidimensional scaling plot (Bray-Curtis dissimilarity analysis) of tick 16S rRNA data from next-generation sequencing, showing that tick species is the main factor influencing microbial composition. Each point on the figure represents an individual tick sample ( $n = 1276$ ) and tick species is represented by colour. Samples of the same tick species cluster together and therefore share a similar microbial diversity. This was further supported by ANOVA testing ( $F_{16,1260} P < 0.001$ ) where grouping by tick species was the only factor that resulted in a significant difference ( $P < 0.05$ ) in the microbial diversity between samples (S. Egan, unpublished).

production, none was deemed positive for *B. burgdorferi sensu lato* infection<sup>20</sup>. However, recent studies have detected novel *Borrelia* DNA in 41% of *Bothriocroton concolor* ticks removed from echidnas<sup>21</sup>, and phylogenetic analysis of ‘*Candidatus Borrelia tachyglossi*’ indicate this antipodean *Borrelia* is closely related to, yet distinct from, the Reptile-associated and Relapsing Fever groups, and does not belong to the LB complex<sup>22</sup>.

In summary, the microbiomes of Australian ticks comprise diverse genera with similarities to those of ticks in other parts of the world. To date, no known northern hemisphere bacterial pathogens have been discovered; however, phylogenetic analysis reveals multiple organisms that are related to but distinct from known pathogens overseas, and their zoonotic potential remains unknown. Investigation of disease causation by these organisms, if any, in order to meet Koch’s postulates, is largely the direction of our future research.

## Conflicts of interest

The authors declare no conflicts of interest.

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