IJP: Parasites and Wildlife 7 (2018) 197–203

Contents lists available at ScienceDirect

IJP: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Molecular surveillance of piroplasms in ticks from small and medium-sized urban and peri-urban mammals in Australia

Siew-May Loh[a], Siobhon Egan[a], Amber Gillett[b], Peter B. Banks[c], Una M. Ryan[a], Peter J. Irwin[a], Charlotte L. Oskam[a,*]

[a] Vector and Water-Borne Pathogens Research Group, School of Veterinary and Life Sciences, Murdoch University, Perth, Western Australia, Australia
[b] Australia Zoo Wildlife Hospital, Beerwah, Queensland, Australia
[c] School of Life and Environmental Sciences, The University of Sydney, Sydney, New South Wales, Australia

ARTICLE INFO

Keywords:
Piroplasm
Bandicoot
Brush-tailed possum
Red fox
Australia

ABSTRACT

Natural landscape alterations as a consequence of urbanisation are one of the main drivers in the movements of wildlife into metropolitan and peri-urban areas. Worldwide, these wildlife species are highly adaptable and may be responsible for the transmission of tick-borne pathogens including piroplasms (*Babesia*, *Theileria* and *Cytauxzoon* spp.) that cause piroplasmosis in animals and occasionally in humans. Little is known about piroplasms in the ticks of urban wildlife in Australia. Ticks from long-nosed bandicoots (*Perameles nasuta*; n = 71), eastern-barred bandicoots (*Perameles gunnii*; n = 41), northern-brown bandicoots (*Isoodon macrourus*; n = 19), southern-brown bandicoots (*Isoodon obesulus*; n = 4), bandicoot sp. (n = 2), flying foxes (*Pteropus* sp.; n = 3), black rats (*Rattus rattus*; n = 7), bush rats (*Rattus fuscipes*; n = 4), brushtail possums (*Trichosurus vulpecula*; n = 19), ringtail possums (*Pseudocheirus peregrinus*; n = 12), short-eared possums (*Trichosurus caninus*; n = 6), possum sp. (*Trichosurus* sp.; n = 8), and red foxes (*Vulpes vulpes*; n = 12) were analysed using piroplasm-specific 18S primers and Sanger sequencing. Seven *Ixodes tasmani* ticks from long-nosed bandicoots and bandicoots sp., three *I. tasmani* ticks and one *Ixodes holocyclus* tick from brushtail possums, and one *Haemaphysalis longicornis* tick from a red fox were positive for piroplasms. New genotypes, with sequences sharing 98% nucleotide similarities with *Theileria* sp. K1 detected in a burrowing bettong (*Bettongia lesueur*), were identified from bandicoot ticks. New genotypes were detected in ticks from brushtail possums, which shared 98% similarity with a *Babesia* sp. (JQ682877) previously identified in marsupials. *Theileria orientalis* was identified in the *H. longicornis* tick from the red fox. *Babesia* and *Theileria* spp. in the ticks parasitizing bandicoots and brushtail possums clustered closely with respective *Babesia* and *Theileria* clades derived from Australian marsupials. This represents the first detection of piroplasms in ticks parasitizing brushtail possums and a red fox in Australia.

1. Introduction

Deforestation, habitat fragmentation, and increases in human populations associated with urbanisation inevitably decrease the natural flora and fauna biodiversity (Mackenstedt et al., 2015). While some wildlife species remain urbanophobes, some have emerged as urban adapters or exploiters, and over the years have become familiar inhabitants of our towns and cities. There are various factors that favour the urban-adaptation of certain wildlife species, including the availability of anthropogenic food resources (Oro et al., 2013), shelter (Parris and Hazell, 2005), and reduction in threats from natural predators (Bateman and Fleming, 2012). Constant supplies of season-independent resources enable many successful urban-adapted wildlife species to attain higher population densities compared to their rural counterparts (Bradley and Altizer, 2007). Consequently, the growth in populations of competent hosts, reservoirs and amplifiers of vector-borne pathogens, increases the prevalence of those pathogens and the frequency of human-wildlife interactions, potentially leading to higher rates of zoonotic disease transmission (Bradley and Altizer, 2007).

Although many mammal populations have declined as a consequence of landscape alterations (Baker and Harris, 2007), others have benefitted, especially from the creation of urban environments. In Australia, the most common mammals in urban areas include native brushtail possums (*Trichosurus vulpecula*), ringtail possums (*Pseudocheirus peregrinus*) (Hill et al., 2007), and bandicoots (*Isoodon* and *Perameles* spp.) (Fitzgibbon et al., 2011); as well as introduced wildlife such as European red foxes (*Vulpes vulpes*) (Marks and Bloomfield, 1999). In addition, bats (*Pteropus* sp.) and rats (*Rattus* spp.) also reside

* Corresponding author.
E-mail address: C.Oskam@murdoch.edu.au (C.L. Oskam).

https://doi.org/10.1016/j.ijppaw.2018.05.005
Received 7 February 2018; Received in revised form 22 May 2018; Accepted 24 May 2018

in Australian metropolitan areas (Plowright et al., 2011; Tait et al., 2014; Banks and Smith, 2015). Individuals of these species make use of urban areas either exclusively or by nesting in remnant bushland and regularly visiting nearby urban habitats (Harper, 2005).

Ticks are one of the most competent arthropod vectors that transmit a vast number of pathogenic bacteria, haemoparasites and viruses that significantly affect the health of human, livestock and companion animals worldwide (Dantas-Torres et al., 2012). Ticks are responsible for a variety of emerging zoonotic diseases and wildlife are often the amplifying hosts for these pathogens (Colwell et al., 2011). The encroachment of wild animals into urban areas as a result of urban development, brings wildlife and their ticks into close proximity with humans, consequently introducing possible spill-over of tick-borne pathogens (Banks and Smith, 2015).

A range of haemoparasites, i.e. piroplasms, have been identified and described in Australian native wildlife (Paparini et al., 2012), and in addition, unnamed species of piroplasm have been detected in brushtailed bettongs (*Bettongia penicillata*), burrowing bettongs (*Bettongia lesueur*), and bandicoots (Paparini et al., 2012; Barbosa et al., 2017). In general, however, knowledge about the piroplasms in Australian wildlife ticks remains scarce and their zoonotic significance is far from being understood at the current time. There have been increasing reports of tick-associated illnesses in Australian humans, including a case of babesiosis reported in an Australian patient who lived in a peri-urban area and had encountered tick bites, but who had not travelled to endemic countries (Senanayake et al., 2012). This emphasises the importance of understanding and examining every aspect of tick-borne disease (TBD), particularly the potential for wildlife ticks to act as reservoirs for human disease.

Urbanised wildlife contribute to the importance and health impact of TBD worldwide, both as transporters of ticks, as well as acting as reservoirs of TBD pathogens (Bradley and Altizer, 2007; Pfäffle et al., 2013; Rizzoli et al., 2014). The occurrence of wildlife in urban and peri-urban settings is becoming more evident (Harper, 2005), increasing the chances of human-wildlife interactions and the potential for human illness via tick bites. Hence, further research into TBD's in wildlife that reside in urban areas together with their ticks is essential. The objective of the present study was to conduct a survey of ticks parasitizing small and medium-sized mammalian wildlife species that are most common in urban environments in Australia, and to identify and characterise any piroplasms present.

2. Materials and methods

2.1. Ethics statements

This study was conducted under the compliance of the *Australian Code for the Responsibility Conduct of Research (2007)* and *Australian Code for the Care and Use of Animals for Scientific Purposes, 2013*. Tick collection was carried out opportunistically with the approval from the Murdoch University Animal Ethics Committee.

2.2. Sample collection and tick identification

Ticks from 18 long-nosed bandicoots (*P. nasuta*; n = 71), two eastern-barred bandicoots (*P. gunnii*; n = 41), five northern-brown bandicoots (*I. macrourus*; n = 19), four southern-brown bandicoots (*I. obesulus*; n = 4), two bandicoot sp. (n = 2), three bat sp. (*Pteropus* sp.; n = 3), three black rats (*R. rattus*; n = 7), four bush rats (*R. fuscipes*; n = 4), seven brushtail possums (*T. vulpecula*; n = 19), six ringtail possums (*P. peregrinus*; n = 12), two short-eared possums (*T. caninus*; n = 6), two possum sp. (*Trichosurus* sp.; n = 8), and one red fox (*V. vulpes*; n = 12) were sampled by veterinarians, wildlife rescuers, and the public from urban and peri-urban areas across Australia. Ticks were preserved in 70% ethanol before shipment to Murdoch University and the species was subsequently identified using morphological keys (Roberts, 1970).

2.3. DNA extraction and amplification

The external tick surface was washed with solutions of 10% sodium hypochlorite followed by 70% ethanol. Genomic DNA (gDNA) was extracted using the DNeasy Blood and Tissue kit (Qiagen) in parallel with the extraction reagent blank (EXB) controls (Loh et al., 2016).

The BTF1/BTR1 and BTF2/BTR2 primers, targeting a 850 bp region of the 18S rRNA gene, were used in a nested-PCR as described previously (Jefferies et al., 2007). All PCR reactions were conducted in parallel with EXB and no-template controls, as well as *Babesia gibsoni* gDNA extracted from a canine blood sample as a positive control. Each 25 μL PCR reaction contained 1x Green GoTaq Flexi buffer, 1.5 mM $MgCl_2$, 1 mM dNTPs, 400 nM of each primer, 1.25U of GoTaq DNA polymerase (Promega, Madison, WI, USA), and 2 μL undiluted DNA. A second set of 18S primers, BT18SF1/BT18SR1 and BT18SF2/BT18SR2 that amplify a longer 18S gene fragment (1466 bp), were used for phylogenetic purposes, on the samples positive in the initial screening, following the conditions described in Paparini et al. (2012). Amplified DNA products were visualised on 1% agarose gels containing SYBR safe under a blue light transilluminator. Bands were then excised and purified using an in house filter tip method (Yang et al., 2013) before Sanger sequencing was carried out using each forward and reverse primer at the Western Australian State Agricultural Biotechnology Centre (SABC).

2.4. Phylogenetic analyses

Raw sequences were trimmed using Geneious version 8.1.9 and MAFFT v7.017 was used to construct multiple alignments with previously described piroplasm nucleotide sequences retrieved from GenBank (Katoh et al., 2002; Kearse et al., 2012). Alignments were then refined using MUSCLE (Edgar, 2004). Alignments of 1701 bp and 856 bp were generated for the reconstruction of the phylogenetic tree and the inset trees, respectively. MEGA6 was used to determine the best nucleotide substitution model on the basis of Bayesian Information Criterion (Tamura et al., 2013). Finally, Bayesian analyses were used for phylogenetic reconstruction of the piroplasm 18S trees, with invgamma rate variation, Gamma categories of five, MCMC length of 1,100,000, burn-in length of 10,000, and subsampling frequency of 200 (Huelsenbeck and Ronquist, 2001). *Plasmodium falciparum* (JQ627152) was used as an outgroup. 18S sequenes generated in this study were deposited into GenBank under the accession numbers: MG251435-MG251440.

3. Results

Of the 137 ticks collected from bandicoots, seven ticks (Table 1) were positive for both short and long piroplasm 18S fragments. Of these, five were from *I. tasmani* nymphs from two long-nosed bandicoots, and a single *I. tasmani* female removed from a bandicoot sp., at the Australia Zoo Wildlife Hospital in Queensland (QLD); and a single female *I. tasmani* from a bandicoot sp. from a veterinary clinic in Port Sorell, Tasmania (TAS). Using the shorter 18S gene primers, amplicons were generated in four brushtail possum ticks; comprising one *I. tasmani* female tick from the Royal Botanical Gardens in New South Wales (NSW), and one *I. holocyclus* and two *I. tasmani* ticks from two brushtailed possums at the Australia Zoo Wildlife Hospital, QLD. Among the 12 tick samples from a red fox from Grosevale, NSW, only a single *H. longicornis* nymph was positive.

Sequence analysis identified three unique genotypes in the bandicoot ticks, designated as *Theileria* sp. B16, *Theileria* sp. B43, and *Theileria* sp. B60. *Theileria* sp. B16 was identified in the *I. tasmani* tick from TAS, and shared 97.1% and 99.9% similarity with *Theileria* sp. B43 and *Theileria* sp. B60, respectively (Supplementary Table S1). Genotype

Table 1
Ticks identified in this study and number of positive samples for piroplasm 18S. Parentheses indicate percentage positive.

Hosts	Tick species	Region	Instar	n	No. of positives
Long-nosed bandicoot *Perameles nasuta*	*Ixodes holocyclus*	Sydney, NSW	Nymph	8	–
		Castlecrag, NSW	Nymph	1	–
			Female	2	–
			Male	2	–
		Boorie Creek, NSW	Female	10	–
			Male	14	–
		Boxhill, NSW	Female	1	–
		Manly, NSW	Nymph	4	–
		Murrah, NSW	Nymph	2	–
		Beerwah, QLD	Nymph	5	–
			Female	1	–
	Ixodes tasmani	Sydney, NSW	Nymph	5	–
		Beerwah, QLD	Nymph	11	5
	Haemaphysalis bancrofti	Castlecrag, NSW	Nymph	1	–
		Stony Chute, NSW	Nymph	1	–
			Female	1	–
		Murrah, NSW	Nymph	1	–
	Haemaphysalis humerosa	Stony Chute, NSW	Nymph	1	–
Eastern-barred bandicoot *Perameles gunnil*	*Ixodes tasmani*	Devonport, TAS	Nymph	20	–
			Female	19	–
		Ridgeway, TAS	Nymph	2	–
Northern-brown bandicoot *Isoodon macrourus*	*Ixodes holocyclus*	Beerwah, QLD	Female	3	–
	Haemaphysalis humerosa	Palmerston, NT	Nymph	1	–
			Female	2	–
			Male	3	–
		Bees Creek, NT	Nymph	2	–
			Female	7	–
		Beerwah, QLD	Female	1	–
Southern-brown bandicoot *Isoodon obesulus*	*Ixodes fecialis*	Albany, WA	Female	2	–
		Maida Vale, WA	Female	1	–
	Ixodes australiensis	Albany, WA	Female	1	–
Bandicoot sp.	*Ixodes tasmani*	Port Sorell, TAS	Female	1	1
		Beerwah, QLD	Female	1	1
Total bandicoot ticks				**137**	**7 (5.1%)**
Bat sp.	*Rhipicephalus sanguineus*	Darwin, NT	Female	1	–
Flying fox *Pteropus* sp.	*Ixodes holocyclus*	Beerwah, QLD	Female	1	–
Black flying fox *Pteropus alecto*	*Ixodes tasmani*	Beerwah, QLD	Female	1	–
Total bat ticks				**3**	**0**
Black rat *Rattus rattus*	*Ixodes holocyclus*	Castlecrag, NSW	Nymph	1	–
			Female	1	–
		Sydney, NSW	Nymph	1	–
	Ixodes tasmani	Sydney, NSW	Nymph	4	–
Bush rat *Rattus fuscipes*	*Ixodes tasmani*	Pearl Beach, NSW	Nymph	4	–
Total rat ticks				**11**	**0**
Brushtail possum *Trichosurus vulpecula*	*Ixodes holocyclus*	Beerwah, QLD	Female	7	1
			Male	1	–
	Ixodes tasmani	Royal Botanic Gardens, NSW	Female	1	1
		Beerwah, QLD	Female	7	2
	Ixodes trichosuri	Beerwah, QLD	Female	3	–
Ringtail possum *Pseudocheirus peregrinus*	*Ixodes holocyclus*	Beerwah, QLD	Female	1	–
	Ixodes tasmani	Neutral Bay, NSW	Female	1	–
		Turramurra, NSW	Female	6	–
		Beerwah, QLD	Female	3	–

(*continued on next page*)

Table 1 (*continued*)

Hosts	Tick species	Region	Instar	n	No. of positives
		Sandy Bay, TAS	Nymph	1	–
Short-eared possum *Trichosurus caninus*	*Ixodes holocyclus*	Beerwah, QLD	Female	4	–
			Male	1	–
	Ixodes tasmani	Beerwah, QLD	Female	1	–
Possum sp.	*Ixodes holocyclus*	Beerwah, QLD	Nymph	1	–
			Female	1	–
	Haemaphysalis bremneri	Beerwah, QLD	Female	4	–
	Haemaphysalis humerosa	Beerwah, QLD	Female	2	–
Total possum ticks				**45**	**4 (8.9%)**
Red fox *Vulpes vulpes*	*Ixodes holocyclus*	Grosevale, NSW	Female	1	–
	Haemaphysalis longicornis	Grosevale, NSW	Nymph	3	1
			Female	8	–
Total fox ticks				**12**	**1 (8.3%)**
Grand total				**205**	**12 (5.9%)**

Theileria sp. B60 consisted of five identical sequences and shared 97.2% similarity with *Theileria* sp. B43, both of which were sourced from QLD. NCBI BLAST analyses revealed that *Theileria* sp. B16 (1371 bp), *Theileria* sp. B43 (1368 bp), and *Theileria* sp. B60 (1371 bp), shared 98% sequence homology with *Theileria* sp. K1 (JQ682879) detected in a burrowing bettong (*Bettongia lesueur*) (Paparini et al., 2012). Of the four sequences detected in brush-tailed possum ticks, all three sequences from QLD were identical, designated as *Babesia* sp. BP7, whereas the single sequence from NSW, designated as *Babesia* sp. BP1, shared 98% similarity with the QLD genotype (Supplementary Table S1). According to NCBI BLAST, both *Babesia* sp. BP1 (1313 bp) and *Babesia* sp. BP7 (1312 bp) showed 98% similarity with *Babesia* sp. detected in marsupials (JQ682877) (Paparini et al., 2012). The sequence (1349 bp) in the *H. longicornis* tick from a red fox was identical to *T. orientalis* (XR_696404) (Hayashida et al., 2012).

The Bayesian phylogenetic tree constructed using a multiple nucleotide alignment of 1701 bases (Fig. 1) revealed *Babesia* sp. BP1 and *Babesia* sp. BP7 were grouped in a clade with other *Babesia* spp. previously identified in marsupials. *Theileria* sp. B16, *Theileria* sp. B43, and *Theileria* sp. B60 from the bandicoot ticks grouped closely with the marsupial *Theileria* clade along with the *Theileria* sp. derived from *I. australiensis* ticks from kangaroos and the *Theileria* sp. from burrowing bettongs with a high posterior probability value (Fig. 1). Likewise, the *Theileria* sequence derived from the *H. longicornis* tick from the red fox clustered closely with the *T. orientalis*, *T. buffeli*, and *T. sergenti* clade with a high posterior probability value of 1.0.

A shorter nucleotide alignment (856 bp) was used to construct inset trees in order to incorporate species (*Babesia macropus*, *Theileria fuliginosa*, *Theileria brachyuri*, and *Theileria penicillata*) for which longer sequences were not available (Fig. 1a and b). In the case of the marsupial *Babesia* group, the inset tree generated from the shorter alignment exhibited a polytomy topology (i.e. the relationships could not be fully resolved to dichotomies). However, the *Babesia* sequences from the brushtail possum ticks remained clearly distinct from *B. macropus* and other *Babesia* spp. (Fig. 1a). In this analysis, *B. macropus* (KM206778; KM206780; KM206783) (Donahoe et al., 2015) showed 94.6%–94.8% and 92.8%–92.9% similarity with *Babesia* sp. BP1 and *Babesia* sp. BP7, respectively (Supplementary Table S2). Similarly, *Theileria* sp. B16, *Theileria* sp. B43, and *Theileria* sp. B60 shared over 95% similarities with *T. brachyuri* (95.4%, 95.1%, and 95.4%, respectively) and *T. penicillata* (97.6%, 95.8%, and 97.6%, respectively); and lowest similarities with *T. fuliginosus* (90.8%, 89.7%, and 90.8%, respectively) (Supplementary Table S2). Consistent with the larger phylogenetic tree topology, the *Theileria* spp. identified in the present study branched out to form a subgroup with a strong posterior probability (0.99), and with *T. penicillata* and *Theileria* sp. from the burrowing bettong as a sister subgroup (Fig. 1b).

4. Discussion

The present study provides the first molecular survey and characterisation of the genera *Babesia* and *Theileria* in ticks from Australian brush-tailed possums and bandicoots, respectively. Additionally, to the best of our knowledge, this is also the first report of the presence of *T. orientalis* in a *H. longicornis* tick parasitizing a red fox in Australia. To date, very few studies have explored the presence of piroplasms in Australian wildlife ticks, therefore the current findings contribute further to our understanding of the capacity of wildlife ticks to harbour microorganisms in Australia.

Although the classification of Piroplasmida is yet to be fully resolved, this order is currently divided into several clades on the basis of concatenated mitochondrial and 18S sequences. Five distinct clades have been established, comprising *Babesia* sensu stricto., *Theileria* and *Cytauxzoon*, *Theileria equi*, the Western *Babesia* group, and the *Babesia microti* group (Schreeg et al., 2016). Previous analyses have also identified a novel marsupial piroplasm clade (Paparini et al., 2012). Results from the present study have further confirmed that the piroplasm species derived from marsupial ticks are closely related and able to form their own respective clades, thus, increasing the sample size in future studies may further support the hypothesis that marsupial piroplasms are unique from other well described Piroplasmida groups.

In Australia, haemoprotozoan infections are frequently identified in possums (Hill et al., 2008; Paparini et al., 2011; Barbosa et al., 2017); however, to the best of our knowledge, piroplasms have not been detected in possum ticks. This study represents the first detection of a *Babesia* sp. from a tick from a brushtail possum at the Royal Botanical Gardens. This landmark in Sydney is a popular tourist attraction with an estimated 3 million visitors per annum (Pautasso and Parmentier, 2007). As a consequence, there is potential for humans to interact with these possums, and indirectly with their ticks. Unlike their rural counterparts, urban possums have been reported to host more than three ectoparasites species, and can have significant tick infestations (Webster et al., 2014; Hillman et al., 2017). In these environments, possums frequently inhabit food spaces in homes and damage gardens (Hill et al., 2007). This close association with people increases the chances of exposure by possum ticks to humans and companion

Fig. 1. Bayesian phylogenetic reconstruction of the novel *Babesia* and *Theileria* spp. identified in ticks from brushtail possums and bandicoots using a multiple nucleotide alignment of 1701 bp at the 18S locus. Inset trees were produced on the basis of a shorter alignment (856 bp) with the inclusion of (a) *B. macropus* and (b) *T. penicillata, T. brachyuri*, and *T. fuliginosus*. Bold represents sequences identified in this study. GenBank accession numbers are shown in parentheses. Node labels represent posterior probabilities.

animals. *Ixodes holocyclus* ticks, in particular, possess a liberal feeding behaviour (Roberts, 1970; Barker and Walker, 2014) and may be associated with the transmission of TBD.

Anecdotal evidence suggests that damaged lawns and tick transmission caused by bandicoots are the biggest human-wildlife conflicts in Australian metropolitan areas (FitzGibbon and Jones, 2006; Dowle and Deane, 2009). Thus, urban bandicoots are often considered a nuisance or pest for some residents. In one study, urban residents had interactions with urban bandicoots at least once or more on a daily basis (Dowle and Deane, 2009). Again, as with the brush-tailed possums, piroplasms are well studied in bandicoot hosts, but less so in bandicoot ticks. Similar to the *I. holocyclus* tick, *I. tasmani*, the common marsupial tick, parasitises a wide range of hosts including humans (Roberts, 1970; Barker and Walker, 2014). This tick species was previously reported to harbour a *Hepatozoon* sp. from Tasmanian devil (*Sarcophilus harrisii*) (Vilcins et al., 2009). *Theileria peramelis* has also been identified in bandicoots with *I. tasmani* as its associated vector (Mackerras, 1959; Weilgama, 1986). Unfortunately, nucleotide sequences for the type species of *T. peramelis* are unavailable, therefore it is not possible to determine if the *Theileria* sp. detected in the present study is *T. peramelis*.

The *Theileria orientalis* species complex, vectored by *H. longicornis* ticks, causes bovine theileriosis worldwide. Based on the sequence analysis of the major piroplasm surface protein (MPSP) gene, at least 11 distinct genotypes of the *T. orientalis* (types 1–8 and N1–N3) have been recognized globally (Sivakumar et al., 2014). The genotypes Ikeda and Chitose have been considered to be the pathogenic strains responsible for several outbreaks in the Eastern states of Australia (Izzo et al., 2010; Kamau et al., 2011). Outbreaks in Victoria were associated with the introduction of cattle from NSW (where the disease is endemic in some regions) to a beef cattle farm near Seymour, Victoria (Cufos et al., 2012). Theileriosis is now considered as an emerging TBD of high

significance in the beef and dairy industries (Rogers and Callow, 1966; Izzo et al., 2010; Islam et al., 2011). *Haemaphysalis longicornis* is a non-native tick in Australia and is suspected to have been introduced from Japan via livestock importation, along with the *Theileria* species it harbours (Seddon, 1952; Hoogstraal et al., 1968). In the 1870s, red foxes were also imported into Australia for recreational hunting (Rolls, 1977; Mulley and Starr, 1984; Marks and Bloomfield, 1999). In Europe, *B. microti*-like and *Theileria annae* are commonly reported in red foxes and their ticks (Cardoso et al., 2013; Najm et al., 2014; Farkas et al., 2015; Bartley et al., 2016). Therefore, further research is essential to better determine the range of piroplasms in foxes and their ticks in Australia.

The data from the present study emphasises the significance of tick studies in Australia and the importance of determining whether these newly characterised *Babesia* and *Theileria* spp. can cause disease. Piroplasm-positive ticks in the present study originated from the eastern states of Australia. There is evidence from previous studies that kangaroos infected with *B. macropus* in QLD and NSW may exhibit clinical babesiosis in some situations (Dawood et al., 2013; Donahoe et al., 2015). In the present study, the health of the animals from which the ticks were removed was not recorded. In future studies, additional details on host condition at the time of sample collection and examination of blood smears could be incorporated for a more comprehensive investigation. In addition, the biology and the relationship between the tick and the protozoa, as well as the competence of the tick as a possible vector should to be investigated. Further epidemiological studies are required on piroplasms in wildlife living at the interface of urban settlements to gain further knowledge about potential emerging TBD and to provide new opportunities for targeted wildlife management.

Acknowledgements

The authors would like to acknowledge the clinicians and volunteers who have provided tick samples for this study. The authors would also like to thank Frances Brigg and the Western Australia State Agricultural Biotechnology Centre for Sanger sequencing. This research was part-funded by the Australian Research Council (LP160100200) and Bayer HealthCare (Germany) and Bayer Australia.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijppaw.2018.05.005.

References

Baker, P.J., Harris, S., 2007. Urban mammals: what does the future hold? An analysis of the factors affecting patterns of use of residential gardens in Great Britain. Mamm Rev. 37, 297–315.
Banks, P.B., Smith, H.M., 2015. The ecological impacts of commensal species: black rats, *Rattus rattus*, at the urban-bushland interface. Wildl. Res. 42, 86–97.
Barbosa, A., Reiss, A., Jackson, B., Warren, K., Paparini, A., Gillespie, G., Stokeld, D., Irwin, P., Ryan, U., 2017. Prevalence, genetic diversity and potential clinical impact of blood-borne and enteric protozoan parasites in native mammals from northern Australia. Vet. Parasitol. 238, 94–105.
Barker, S.C., Walker, A.R., 2014. Ticks of Australia. The species that infest domestic animals and humans. Zootaxa 3816, 1–144.
Bartley, P.M., Hamilton, C., Wilson, C., Innes, E.A., Katzer, F., 2016. Detection of *Babesia annae* DNA in lung exudate samples from Red foxes (*Vulpes vulpes*) in Great Britain. Parasites Vectors 9, 84.
Bateman, P.W., Fleming, P.A., 2012. Urban carnivores. J. Zool. 287, 1–23.
Bradley, C.A., Altizer, S., 2007. Urbanization and the ecology of wildlife diseases. Trends Ecol. Evol. 22, 95–102.
Cufos, N., Jabbar, A., de Carvalho, L.M., Gasser, R.B., 2012. Mutation scanning-based analysis of *Theileria orientalis* populations in cattle following an outbreak. Electrophoresis 33, 2036–2040.
Cardoso, L., Cortes, H.C.E., Reis, A., Rodrigues, P., Simões, M., Lopes, A.P., Vila-Viçosa, M.J., Talmi-Frank, D., Eyal, O., Solano-Gallego, L., Baneth, G., 2013. Prevalence of *Babesia microti*-like infection in red foxes (*Vulpes vulpes*) from Portugal. Vet. Parasitol. 196, 90–95.
Colwell, D.D., Dantas-Torres, F., Otranto, D., 2011. Vector-borne parasitic zoonoses: emerging scenarios and new perspectives. Vet. Parasitol. 182, 14–21.
Dantas-Torres, F., Chomel, B.B., Otranto, D., 2012. Ticks and tick-borne diseases: a One Health perspective. Trends Parasitol. 28, 437–446.
Dawood, K.E., Morgan, J.A.T., Busfield, F., Srivastava, M., Fletcher, T.I., Sambono, J., Jackson, L.A., Venus, B., Philbey, A.W., Lew-Tabor, A.E., 2013. Observation of a novel *Babesia* spp. in eastern grey kangaroos (*Macropus giganteus*) in Australia. Int. J. Parasitol. Parasites Wildl 2, 54–61.
Donahoe, S.L., Peacock, C.S., Choo, A.Y., Cook, R.W., O'Donoghue, P., Crameri, S., Vogelnest, L., Gordon, A.N., Scott, J.L., Rose, K., 2015. A retrospective study of *Babesia macropus* associated with morbidity and mortality in eastern grey kangaroos (*Macropus giganteus*) and agile wallabies (*Macropus agilis*). Int. J. Parasitol. Parasites Wildl 4, 268–276.
Dowle, M., Deane, E.M., 2009. Attitudes to native bandicoots in an urban environment. Eur. J. Wildl. Res. 55, 45–52.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.
Farkas, R., Takács, N., Hornyák, A., Nachum-Biala, Y., Hornok, S., Baneth, G., 2015. First report on *Babesia* cf. *microti* infection of red foxes (*Vulpes vulpes*) from Hungary. Parasites Vectors 8.
FitzGibbon, S.I., Jones, D.N., 2006. A community-based wildlife survey: the knowledge and attitudes of residents of suburban Brisbane, with a focus on bandicoots. Wildl. Res. 33, 233–241.
Fitzgibbon, S.I., Wilson, R.S., Goldizen, A.W., 2011. The behavioural ecology and population dynamics of a cryptic ground-dwelling mammal in an urban Australian landscape. Austral Ecol. 36, 722–732.
Harper, M.J., 2005. Home range and den use of common brushtail possums (*Trichosurus vulpecula*) in urban forest remnants. Wildl. Res. 32, 681–687.
Hayashida, K., Hara, Y., Abe, T., Yamasaki, C., Toyoda, A., Kosuge, T., Suzuki, Y., Sato, Y., Kawashima, S., Katayama, T., Wakaguri, H., Inoue, N., Homma, K., Tada-Umezaki, M., Yagi, Y., Fujii, Y., Habara, T., Kanehisa, M., Watanabe, H., Ito, K., Gojobori, T., Sugawara, H., Imanishi, T., Weir, W., Gardner, M., Pain, A., Shiels, B., Hattori, M., Nene, V., Sugimoto, C., 2012. Comparative genome analysis of three eukaryotic parasites with differing abilities to transform leukocytes reveals key mediators of *Theileria*-induced leukocyte transformation. mBio 3, e00204–00212.
Hill, N.J., Carbery, K.A., Deane, E.M., 2007. Human-possum conflict in urban Sydney, Australia: public perceptions and implications for species management. Hum. Dimens. Wildl. 12, 101–113.
Hill, N.J., Deane, E.M., Power, M.L., 2008. Prevalence and genetic characterization of *Cryptosporidium* isolates from common brushtail possums (*Trichosurus vulpecula*) adapted to urban settings. Appl. Environ. Microbiol. 74, 5549–5555.
Hillman, A.E., Lymbery, A.J., Elliot, A.D., Andrew Thompson, R.C., 2017. Urban environments alter parasite fauna, weight and reproductive activity in the quenda (*Isoodon obesulus*). Sci. Total Environ. 607–608, 1466–1478.
Hoogstraal, H., Roberts, F.H., Kohls, G.M., Tipton, V.J., 1968. Review of *Haemaphysalis (kaiseriana) longicornis* Neumann (resurrected) of Australia, New Zealand, New Caledonia, Fiji, Japan, Korea, and Northeastern China and USSR, and its parthenogenetic and bisexual populations (Ixodoidea, Ixodidae). J. Parasitol. 54, 1197–1213.
Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Islam, M.K., Jabbar, A., Campbell, B.E., Cantacessi, C., Gasser, R.B., 2011. Bovine theileriosis – an emerging problem in south-eastern Australia? Infect. Genet. Evol. 11, 2095–2097.
Izzo, M.M., Poe, I., Horadagoda, N., De Vos, A.J., House, J.K., 2010. Haemolytic anaemia in cattle in NSW associated with *Theileria* infections. Aust. Vet. J. 88, 45–51.
Jefferies, R., Ryan, U.M., Irwin, P.J., 2007. PCR-RFLP for the detection and differentiation of the canine piroplasm species and its use with filter paper-based technologies. Vet. Parasitol. 144, 20–27.
Kamau, J., de Vos, A.J., Playford, M., Salim, B., Kinyanjui, P., Sugimoto, C., 2011. Emergence of new types of *Theileria orientalis* in Australian cattle and possible cause of theileriosis outbreaks. Parasites Vectors 4 22–22.
Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30, 3059–3066.
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.
Loh, S.M., Gofton, A.W., Lo, N., Gillett, A., Ryan, U.M., Irwin, P.J., Oskam, C.L., 2016. Novel *Borrelia* species detected in echidna ticks, Bothriocroton concolor, in Australia. Parasit. Vectors 9.
Mackenstedt, U., Jenkins, D., Romig, T., 2015. The role of wildlife in the transmission of parasitic zoonoses in peri-urban and urban areas. Int. J. Parasitol. Parasites Wildl 4, 71–79.
Mackerras, M.J., 1959. The haematozoa of Australian mammals. Aust. J. Zool. 7, 105–135.
Marks, C.A., Bloomfield, T.E., 1999. Distribution and density estimates for urban foxes (*Vulpes vulpes*) in Melbourne: implications for rabies control. Wildl. Res. 26, 763–775.
Mulley, R.C., Starr, T.W., 1984. Dirofilaria immitis in red foxes (*Vulpes vulpes*) in an endemic area near Sydney, Australia. J. Wildl. Dis. 20, 152–153.
Najm, N.A., Meyer-Kayser, E., Hoffmann, L., Herb, I., Fensterer, V., Pfister, K., Silaghi, C., 2014. A molecular survey of *Babesia* spp. and *Theileria* spp. in red foxes (*Vulpes vulpes*) and their ticks from Thuringia, Germany. Ticks Tick-Borne Dis 5, 386–391.
Oro, D., Genovart, M., Tavecchia, G., Fowler, M.S., Martínez-Abraín, A., 2013. Ecological and evolutionary implications of food subsidies from humans. Ecol. Lett. 16, 1501–1514.

Paparini, A., Irwin, P.J., Warren, K., McInnes, L.M., de Tores, P., Ryan, U.M., 2011. Identification of novel trypanosome genotypes in native Australian marsupials. Vet. Parasitol. 183, 21–30.
Paparini, A., Ryan, U.M., Warren, K., McInnes, L.M., de Tores, P., Irwin, P.J., 2012. Identification of novel *Babesia* and *Theileria* genotypes in the endangered marsupials, the woylie (*Bettongia penicillata ogilbyi*) and boodie (*Bettongia lesueur*). Exp. Parasitol. 131, 25–30.
Parris, K.M., Hazell, D.L., 2005. Biotic effects of climate change in urban environments: the case of the grey-headed flying-fox (*Pteropus poliocephalus*) in Melbourne. Australia. Biol. Conserv 124, 267–276.
Pautasso, M., Parmentier, I., 2007. Are the living collections of the world's botanical gardens following species-richness patterns observed in natural ecosystems? Bot. Helv. 117, 15–28.
Pfäffle, M., Littwin, N., Muders, S.V., Petney, T.N., 2013. The ecology of tick-borne diseases. Int. J. Parasitol. 43, 1059–1077.
Plowright, R.K., Foley, P., Field, H.E., Dobson, A.P., Foley, J.E., Eby, P., Daszak, P., 2011. Urban habituation, ecological connectivity and epidemic dampening: the emergence of Hendra virus from flying foxes (*Pteropus* spp.). P. Roy. Soc. B-Biol. Sci. 278, 3703.
Rizzoli, A., Silaghi, C., Obiegala, A., Rudolf, I., Hubálek, Z., Földvári, G., Plantard, O., Vayssier-Taussat, M., Bonnet, S., Špitalská, E., Kazimírová, M., 2014. *Ixodes ricinus* and its transmitted pathogens in urban and peri-urban areas in Europe: new hazards and relevance for public health. Front. Public Health 2, 251.
Roberts, F.H.S., 1970. Australian Ticks. CSIRO, Melbourne.
Rogers, R.J., Callow, L.L., 1966. Three fatal cases of *Theileria mutans* infection. Aust. Vet. J. 42, 42–46.
Rolls, E.C., 1977. They All Ran Wild. Angus & Robertson, London.
Schreeg, M.E., Marr, H.S., Tarigo, J.L., Cohn, L.A., Bird, D.M., Scholl, E.H., Levy, M.G., Wiegmann, B.M., Birkenheuer, A.J., 2016. Mitochondrial genome sequences and structures aid in the resolution of Piroplasmida phylogeny. PLoS One 11, e0165702.
Seddon, H.R., 1952. Diseases of Domestic Animals in Australia. Commonwealth of Australia Department of Health, Canberra.
Senanayake, S.N., Paparini, A., Latimer, M., Andriolo, K., Dasilva, A.J., Wilson, H., Xayavong, M.V., Collignon, P.J., Jeans, P., Irwin, P.J., 2012. First report of human babesiosis in Australia. Med. J. Aust. 196, 350–353.
Sivakumar, T., Hayashida, K., Sugimoto, C., Yokoyama, N., 2014. Evolution and genetic diversity of *Theileria*. Infect. Genet. Evol. 27, 250–263.
Tait, J., Perotto-Baldivieso, H.L., McKeown, A., Westcott, D.A., 2014. Are flying-foxes coming to town? Urbanisation of the spectacled flying-fox (*Pteropus conspicillatus*) in Australia. PLoS One 9.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
Vilcins, I.M., Old, J.M., Deane, E., 2009. Detection of a *Hepatozoon* and spotted fever group *Rickettsia* species in the common marsupial tick (*Ixodes tasmani*) collected from wild Tasmanian devils (*Sarcophilus harrisii*). Tasman. Vet. Parasitol. 162, 23–31.
Webster, K.N., Hill, N.J., Burnett, L., Deane, E.M., 2014. Ectoparasite infestation patterns, haematology and serum biochemistry of urban-dwelling common brushtail possums. Wildl. Biol. 20, 206–216.
Weilgama, D.J., 1986. Transmission of *Theileria peramelis* mackerras, 1959 by *Ixodes tasmani*. In: Cremin, M., Dobson, C., Moorhouse, D.E. (Eds.), Parasite Lives: Papers on Parasites, Their Hosts and Their Associations. University of Queensland Press, Brisbane, pp. 174–178.
Yang, R., Murphy, C., Song, Y., Ng-Hublin, J., Estcourt, A., Hijjawi, N., Chalmers, R., Hadfield, S., Bath, A., Gordon, C., Ryan, U., 2013. Specific and quantitative detection and identification of *Cryptosporidium hominis* and *C. parvum* in clinical and environmental samples. Exp. Parasitol. 135, 142–147.