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Parasitic infections of brushtail possums *Trichosurus vulpecula* in urbanised environments and bushland in the greater Perth region, Western Australia

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Brushtail possums *Trichosurus vulpecula* remain in many areas of Perth, Western Australia, despite urbanisation. However, there are no data available regarding parasitic infections in this species in this locale, despite the relevance to wildlife health, and to public health when considering anthropozoonoses (infections that can spread from humans to animals, and vice versa). Further, though urbanisation is speculated to entail changes to wildlife infection epidemiology, there are few data investigating this hypothesis in marsupial populations in urbanised environments in Australia. This study aimed to measure *T. gondii* seroprevalence, gastrointestinal parasite prevalences, and macroscopic ectoparasite prevalences and intensities, in brushtail possums in the greater Perth region. It also aimed to compare infection prevalences between brushtail possum trapped in urbanised environments and bushland. As part of a cross-sectional study, 18 brushtail possums were trapped and sampled in bushland, 15 possums were trapped and sampled in urbanised environments, and 23 possum carcasses were obtained from a wildlife hospital, in the greater Perth region. This study provides parasite prevalence data, new host records for the ectoparasites *Pygiopsylla tunneyi* and *Liponyssoides* sp., and a new location record for the ectoparasite *Haemaphysalis bremneri*. Urbanised environments were inversely associated with prevalence of tick (Family Ixodidae) infections, and more specifically *Amblyomma* spp. infections. This study found no evidence that the Perth brushtail possum population is a substantial reservoir of anthropozoonotic parasites, though larger studies are required to confirm these findings.

Wildlife that survive in urbanised environments demand a "One health" combination of concerns from an infectious disease perspective (Thompson 2013). Wildlife may act as a reservoir of zoonotic infections, including infections acquired from humans and domestic pets in urban areas, which is of relevance to both public health and wildlife population health (Thompson 2013, Soulsbury and White 2016). Additionally, urbanisation is thought to impact the epidemiology of infectious disease in wildlife populations, across host populations and ecosystems (Brearley et al. 2013, Becker et al. 2015), although this is not yet a well understood phenomenon (Brearley et al. 2013).

Brushtail possums *Trichosurus vulpecula* are arboreal marsupials that remain in many urbanised areas of Australia, as well as in bushland. Brushtail possums' adaptable diet and arboreal habit may explain their success in exploiting urban environments (Kerle 2001). In non-urbanised environments brushtail possums are primarily herbivorous (How and Hillcox 2000). However, they will opportunistically utilise a variety of food resources, particularly in urbanised environments (Kerle 2001, Hillman and Thompson 2016). Current knowledge of parasitic infections in brushtail possums inhabiting urbanised environments in Australia is limited to studies focussed on the protozoan parasites *Toxoplasma gondii* (Eymann et al. 2006, Hill et al. 2008a), *Neospora caninum* (Eymann et al. 2006) and *Cryptosporidium* spp. (Hill et al. 2008b) in urban Sydney, and ectoparasites on brushtail possums in a zoological park and surrounds in Sydney (Webster et al. 2014). There is currently limited published information regarding parasites infecting brushtail possums in Western Australia (Viggers and Spratt 1995, Obendorf et al. 1998, Adams 2003, Thompson et al. 2010, Clarke 2011), and no studies have investigated parasites of brushtail possums in urban areas of the state.

This study was part of a broader project that aimed to investigate the impacts of urbanisation on the epidemiology of parasitic infections of marsupials in Perth, Western Australia, with particular consideration of parasites that may be acquired from humans and pets in urbanised environments. Objectives in sampling free-ranging brushtail

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possums in Perth were to: 1) measure *Toxoplasma gondii* seroprevalence; 2) identify and estimate the prevalence of gastrointestinal protozoan and helminth parasites; 3) identify and estimate the prevalence and intensity of macroscopic ectoparasite infections; and 4) compare parasite taxa prevalence/seroprevalence between brushtail possums trapped in urbanised environments and bushland.

Material and methods

This cross-sectional study targeted free-ranging brushtail possums inhabiting both urbanised environments and bushland within the Statistical Division of Perth. Possums were trapped using Sheffield traps, between March 2013 and July 2015, across 29 bushland sites and 35 urbanised sites (Hillman et al. 2017a). Trapping sites were classified as bushland if included in the Western Australian Planning Commission's Bush Forever policy (Department of Planning 2000). Trapping sites were classified as urbanised if they were private properties (including both residential and non-residential sites). Sites were trapped once, for up to four consecutive nights, with individual animals sampled once. Additionally, brushtail possum carcasses were obtained from a wildlife hospital between September 2013 and February 2015. Where possible, the wildlife hospital provided the location where the possum was found, the date of death and the known or suspected cause of death. The known or likely cause of death of the possum was determined (if possible) based on information provided by the wildlife hospital and gross findings on post mortem examination.

Strategies used in trapping and sampling brushtail possums are comprehensively described elsewhere (Hillman 2016, Hillman et al. 2016a, 2017b) and are summarised below.

Brushtail possum anaesthesia and age estimation

Trapped brushtail possums were briefly anaesthetised to allow thorough examination and sampling, using isoflurane (I.S.O. 1 ml ml^{-1}, V.C.A, Australia) vaporised in medical oxygen via a "Stinger" field anaesthetic machine with a Bain circuit (Advanced Anaesthesia Specialists, Sydney).

Testes measurements in males were used to classify possums as subadult (each testis $\leq$ 1.0 cm width) or adult (at least one testis $>$ 1.0 cm width). Pouch development was used to classify females as subadult (non-parous pouch) or adult (parous pouch). Additionally, assessment of the upper first molar tooth wear was used to grade the age class of the possum from 1–7, as per Cowan and White (1989) – a modification of the scale developed by Winter (1980). Adult females were classified as having an active pouch if lactating, or an inactive pouch if not lactating.

Measuring *Toxoplasma gondii* seroprevalence

From trapped possums, one millilitre of blood was taken from the lateral tail vein using a 25G needle. Blood was transferred into MiniCollect serum tubes (Interpath Services, Australia). From possum carcasses, 1 ml haemorrhagic/serohaemorrhagic fluid was collected from the chest cavity. Samples were centrifuged at 314 g for 10 min, then serum (from blood samples) or serous top layer (if available) or top half of the sample (from possum chest fluid samples) was transferred into a storage tube and frozen at –20°C until analysis.

Thirty-two serum samples and all chest fluid samples were tested for *T. gondii* IgG antibodies using a modified agglutination test kit (Toxoscreen DA, bioMérieux, France), according to manufacturer's instructions at titres of 1:40 and 1:4000. One possum serum sample was sent for the same test at the Animal Health Laboratory of the Department of Primary Industries, Parks, Water and Environment (Tasmania). Possums were considered positive for *T. gondii* antibodies if their serum tested positive at either or both titres, as validated (for specificity) in Hillman et al. (2017b).

Measuring gastrointestinal parasite prevalences

Faecal samples were collected from traps, or from the large intestine of possum carcasses. Two ml faeces were preserved in 8 ml 10% buffered formalin, and 2 ml faeces were preserved in 8 ml 70% ethanol.

Formalin-preserved faeces were used to test for gastrointestinal protozoan and helminth infections, using a faecal flotation protocol described in Hillman et al. (2017b), with the exception that screening a second sodium nitrate faecal flotation slide was not undertaken. A sample was considered positive if at least one protozoan cyst or oocyst, or helminth egg, of the respective morphological type (Supplementary material Appendix 1 Fig. A1), was identified by this protocol.

Formalin-preserved faecal samples were screened for *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts using Merifluor Cryptosporidium/Giardia kit tests (Meridian Bioscience, Inc. USA), according to manufacturer's directions for unconcentrated faecal samples. Slides were examined at 200× magnification and considered positive if at least one cyst of the respective genus was identified by the apple-green fluorescence and appropriate morphology.

Molecular characterisation of *Giardia* sp. was attempted for faecal samples positive for *Giardia* sp. on immunofluorescence microscopy. DNA was extracted from ethanol-preserved faecal samples, for amplification at the 18S rRNA, ITS1-5.8S-ITS2 and *gdh* loci, with sequencing of amplified product, using the methods described in Hillman et al. (2016b).

Measuring macroscopic ectoparasite infection prevalences and intensities

A thorough screen for macroscopic ectoparasites was undertaken on each possum, which included direct examination of the animal, a coat brush and examination of the handling bag. All macroscopic parasites detected were removed and preserved in 70% ethanol/5% glycerine. Ectoparasites were differentiated morphologically, using taxonomic keys for fleas (Dunnet and Mardon 1974), ticks (Roberts 1970) and mesostigmatid mites (Domrow 1987). A possum was considered infected with the respective ectoparasite taxa if a specimen was obtained from the possum and differentiated as such. Possums were considered infected with trombiculid mites based on the distinctive macroscopic appearance

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of trombiculid mite infections, with larval mites sampled and identified microscopically. Trombiculid mite intensity of infection was not measured.

Mapping and statistical analysis

Possum locations were mapped using qGIS ver. 2.18 (Open Source Geospatial Foundation). Trapped possums were mapped using the trap GPS location. Possum carcasses were mapped using a proxy central location of the suburb in which they were found, prior to admission to the wildlife hospital.

Parasite prevalences were calculated with Jeffrey's 95% confidence interval. Prevalences were compared between possums trapped in urbanised environments to those trapped in bushland using chi-square tests (wildlife hospital possums were excluded from these analyses).

Results

Brushtail possums sampled - descriptive findings

Fifty-six brushtail possums were sampled. This included 15 live possums trapped across nine urbanised sites and 18 live possums trapped across seven bushland sites (brushtail possums were not trapped at the additional 26 urbanised sites and 22 bushland sites where trapping was undertaken). Additionally, 23 possum carcasses were obtained from the wildlife hospital (22 frozen, one refrigerated) (Supplementary material Appendix 1 Fig. A2). Sixteen possum carcasses (70%) were known or likely to have died due to accident or injury (attacked by another animal, electrocution, vehicle strike, ingested poison or chainsaw injury). One possum had been in long term care for neurological symptoms. Six possums did not have clinical history or macroscopic pathology that suggested a likely cause of death.

Sampled possums included five subadult males, 21 adult males, two subadult females and 28 adult females (Supplementary material Appendix 1 Fig. A3) – age class distribution is summarised in Supplementary material Appendix 1 Fig. A4. Thirteen (46%) adult female possums had an active pouch. Sampling across seasons is described in Table 1.

Toxoplasma gondii seroprevalence

No trapped possums were seropositive for *Toxoplasma gondii*. One subadult male possum carcass was positive on the modified agglutination test (this was not the possum with a history of neurological symptoms) (Table 2).

Gastrointestinal parasites

Faecal samples were available from 32/33 (96.7%) trapped possums and 22/23 (95.7%) possum carcasses. Gastrointestinal parasite findings are summarised in Table 2 and Supplementary material Appendix 1 Fig. A1. The parasite eggs, cysts and oocysts observed in faeces from eleven frozen carcasses were in very good condition and readily identified. Unsporulated coccidian oocysts in 12 of the 14 positive samples had dimensions that were morphologically consistent with *Eimeria trichosuri*. The *Entamoeba* spp. cysts observed were not morphologically consistent with *Entamoeba histolytica*. The *Giardia* sp. infection amplified by PCR, with a band at the appropriate size for *Giardia* spp., but the resultant nucleotide sequence was of poor quality and was unable to be aligned with published *Giardia* sequences.

Entamoeba spp., unsporulated coccidian oocysts (Family Eimeriidae) and strongyle eggs were observed in possums trapped in urbanised environments and in bushland, in both males and females, and in subadults and adults. Other gastrointestinal parasites were relatively rare and not observed across all these groups.

Macroscopic ectoparasites

Ectoparasite results are described in Tables 1 and 3 (see also Supplementary material Appendix 1 Fig. A5). Nineteen ticks (63.3%) obtained from trapped possums and all ticks obtained from the infected possum carcass were differentiated to genus or species level, as described in Table 3. Ten ticks obtained across four possum hosts were larvae that could not be differentiated beyond Family Ixodidae, and one *Haemaphysalis* sp. tick off the possum infected with *Haemaphysalis bremneri* could not be differentiated to species level, due to specimen damage.

In possums trapped in bushland, tick (Family Ixodidae) and trombiculid mite (Suborder Prostigmata) infections

Table 1. Distribution of tick (Family Ixodidae), *Amblyomma* spp. and trombiculid mite (Suborder Prostigmata) infections in sampled brushtail possums across seasons.

Infection		Season (no. possums infected/no. possums trapped (%)) 95% CI			Total
		Spring	Autumn	Winter	
Ticks	bushland	5/6 (83.3%) 42.1–96.3%	2/9 (22.2%) 6.7–55.6%	0/3 (0%) 0–60.2%	18
	urbanised	0/14 (0%) 0–21.8%	0/1 (0%) 0–84.2%	–[1]	15
Amblyomma spp.	bushland	2/6 (33.3%) 9.9–71.0%	2/9 (22.2%) 6.7–55.6%	0/3 (0%) 0–60.2%	18
	urbanised	0/14 (0%) 0–21.8%	0/1 (0%) 0–84.2%	–[1]	15
Trombiculid mites	bushland	1/6 (16.6%) 3.7–57.9%	2/9 (22.2%) 6.7–55.6%	0/3 (0%) 0–60.2%	18
	urbanised	0/14 (0%) 0–21.8%	0/1 (0%) 0–84.2%	–[1]	15

[1]No brushtail possums were trapped in urbanised environments in winter

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Table 2. Endoparasite infection prevalences in Perth brushtail possums *Trichosurus vulpecula*. p-values compare prevalence in brushtail possums trapped in urbanised environments and bushland.

Endoparasites	Trapped possums (no. infected/no. tested) (% infected; 95% CI)			Wildlife hospital possums (no. infected/no. tested) (% infected; 95% CI)
	Urbanised environment	Bushland	p-value	
Toxoplasma gondii seroprevalence	0/15 (0%; 0–20.6%)	0/18 (0%; 0–17.6%)	–	1/22[1] (4.5%; 1.1–21.9%)
Giardia sp.	1/14 (7.1%; 1.7–31.9%)	0/18 (0%; 0–17.6%)	0.25	0/20 (0%; 0–16.1%)
Cryptosporidium sp.	1/14 (7.1%; 1.7–31.9%)	0/18 (0%; 0–17.6%)	0.25	0/20 (0%; 0–16.1%)
Coccidia (Family Eimeriidae)	4/14 (28.6%; 11.8–55.1%)	4/18 (22.2%; 9.1–45.6%)	0.68	6/22 (27.3%; 13.2–48.4%)
Entamoeba spp.[2]	1/3 (33.3%; 6.8–80.6%)	3/11 (27.3%; 9.9–57.2%)	0.47	3/22 (13.6%; 5.0–33.6%)
Strongyles (Suborder Rhabditina)	7/14 (50%; 26.6–73.4%)	9/18 (50%; 28.9–71.1%)	1.00	3/22 (13.6%; 5.0–33.6%)
Unidentified acanthocephalan (Phylum Acanthocephala)	1/14 (7.1%; 1.7–31.9%)	0/18 (0%; 0–17.6%)	0.25	0/22 (0%; 0–14.8%)

[1]Brushtail possum carcasses were tested using chest fluid; chest fluid not available from one carcass.
[2]The first 18 trapped possum samples were not screened for *Entamoeba* spp. cysts.

were identified in spring and autumn (Table 1). Tick and mite infections were not identified in possums trapped in urbanised environments. Tick infections were more prevalent in possums trapped in bushland than urbanised environments ($p = 0.007$) – attributable primarily to increased prevalence of *Amblyomma* spp. ticks on possums trapped in bushland ($p = 0.051$) (Table 3). The seven possums infected with ticks were distributed across five bushland sites, and the four possums infected with *Amblyomma* sp. were distributed across four bushland sites. Tick infections were identified in both female and male possums, and subadults and adults; flea and trombiculid mite infections were identified in both male and female possums, but only in the older age group.

One flea was obtained from each infected possum. Tick intensities ranged from two to seven ticks from infected trapped possums; the one infected possum obtained via the wildlife hospital had 37 ticks. One *Liponyssoides* sp. mite was obtained from the single infected possum.

Discussion

Parasitic infections in Perth brushtail possums

Toxoplasma gondii serological results obtained in this study reflect the low *T. gondii* seroprevalences observed in brushtail possums surveyed in other Australian locations (Cook and Pope 1959, O'Callaghan and Moore 1986, Eymann et al. 2006, Hill et al. 2008a, Parameswaran 2008, Clarke 2011, Hollings et al. 2013). Brushtail possums' diet and primarily arboreal habits may put them at relatively low likelihood of exposure to *T. gondii* (which can be transmitted by carnivory

Table 3. Macroscopic ectoparasite infection prevalences in Perth brushtail possums *Trichosurus vulpecula*. p-values compare prevalence in brushtail possums trapped in urbanised environments and bushland.

Ectoparasites	Trapped possums (no. infected/total no.) (% infected; 95% CI)			Wildlife hospital possums (no. infected/total no.) (% infected; 95% CI)
	Urbanised environment	Bushland	p-value	
Fleas				
Pygiopsylla tunneyi	1/15 (6.7%; 1.6–30.2%)	0/18 (0%; 0–7.6%)	0.27	0/23 (0%; 0–14.2%)
Choristopsylla ochi	0/15 (0%; 0–20.6%)	2/18 (11.1%; 3.4–33.1%)	0.18	0/23 (0%; 0–14.2%)
Echidnophaga myrmecobii	1/15 (6.7%; 1.6–30.2%)	1/18 (5.6%; 1.3–26.0%)	0.89	0/23 (0%; 0–14.2%)
Ticks				
Family Ixodidae[1]	0/15 (0%; 0–20.6%)	7/ 18 (38.9%; 20.3–61.6%)	0.007	1/23 (4.3%; 1.0–21.1%)
Ixodes tasmani	0/15 (0%; 0–20.6%)	1/18 (5.6%; 1.3–26.0%)	0.35	0/23 (0%; 0–14.2%)
Haemaphysalis bremneri	0/15 (0%; 0–20.6%)	1/18 (5.6%; 1.3–26.0%)	0.35	0/23 (0%; 0–14.2%)
Amblyomma spp.	0/15 (0%; 0–20.6%)	4/18 (22.2%; 9.1–45.6%)	0.051	1/23 (4.3%; 1.0–21.1%)
Mites				
Liponyssoides sp.	0/15 (0%; 0–20.6%)	1/18 (5.6%; 1.3–26.0%)	0.35	0/23 (0%; 0–14.2%)
Trombiculid mites (Suborder Prostigmata)	0/15 (0%; 0–20.6%)	3/18 (16.7%; 6.1–39.6%)	0.097	0/23 (0%; 0–14.2%)

[1]10/30 ticks, which were all larvae, were not able to be differentiated beyond Family Ixodidae.

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and exposure to oocysts originating from infected cat faeces, as well as by vertical transmission). A study of free-ranging mammalian wildlife in French Guiana found very low *T. gondii* seroprevalence in three free-ranging arboreal mammals, with relatively decreased risk of seropositivity in arboreal mammals compared to a range of sympatric terrestrial mammalian species (de Thoisy et al. 2003).

The taxa of gastrointestinal and macroscopic ectoparasites identified in trapped Perth possums, and their respective prevalences, are consistent with those of other bushland brushtail possum populations in Western Australia (Adams 2003, Thompson et al. 2010, Clarke 2011). However, they contrast to ectoparasite taxa prevalence and intensity of infection findings from brushtail possums in New South Wales, eastern Australia (Webster et al. 2014). This suggests variation in ectoparasite epidemiology across this host species in different geographical (ecological) locations.

The apparently accidental cause of death for the majority of the possum carcasses may have avoided substantial bias of endoparasite prevalence estimates towards that of 'sickly' animals. The observation of parasite eggs, cysts and oocysts in very good condition in the faeces of eleven possum carcasses suggests that obtaining the faecal samples from frozen carcasses did not substantially impact gastrointestinal parasite prevalence estimates. However, carcass ectoparasite findings are considered highly likely to have been influenced by ectoparasites abandoning dead animals.

The *Giardia* sp. and *Cryptosporidium* sp. infections are of potential anthropozoonotic significance (that is, the parasite species involved may be capable of infecting both humans and animals). However, this could not be resolved for the *Giardia* sp. infection as sequencing failed. *Cryptosporidium* spp. infections have been identified in brushtail possums in Sydney, Australia, and were considered unlikely to be of public health significance (Hill et al. 2008b).

The macroscopic ectoparasites of potential relevance to human and domestic animal infection were *Echidnophaga myrmecobii*, a flea capable of infecting a wide range of hosts including domestic poultry and mammals, and *Ixodes tasmani,* a tick that feeds on domestic animals and humans (Roberts 1970, Barker and Walker 2014, Greay et al. 2016). The *Amblyomma* spp. ticks may be of anthropozoonotic significance – this depends on the particular species involved (Barker and Walker 2014). *Amblyomma triguttatum* has previously been documented infecting brushtail possums in bushland in Western Australia (Clarke 2011). However, this study's findings suggest that brushtail possums may not be important in the epidemiology of tick infections or tick-borne zoonoses in urban Perth.

This study provides new host records for *Pygiopsylla tunneyi* and *Liponyssoides* sp. *Pygiopsylla tunneyi* has been identified infecting quenda *Isoodon obesulus*, western barred bandicoots *Perameles bougainville* and chuditch *Dasyurus geoffroii* in Western Australia (Mardon and Dunnet 1972, Bennett et al. 2007, Thomasz 2014, Hillman et al. 2017a). In Australia, *Liponyssoides* spp. have been identified infecting birds in Queensland and the Kimberley region of Western Australia (Domrow 1979, 1987). This study documents *Haemaphysalis bremneri* infecting brushtail possums in Western Australia for the first time – *H. bremneri* is known to infect brushtail possums in Queensland, Australia (Roberts 1963, Heath et al. 1986). This location record may be of importance if future research identifies these ectoparasites as vectors of infections or intermediate hosts of parasites of importance to wildlife or veterinary public health.

Comparison of parasitic infections, in urbanised environments and bushland

Due to the small sample sizes, the comparison of parasite prevalences between possums trapped in urbanised environments and bushland generally lacked power. Despite this, there was evidence of higher prevalence of ticks in brushtail possums trapped in bushland compared to urbanised environments in Perth. Due to small sample sizes and a lack of tick-infected possums identified in urbanised environments, statistical modelling was not pursued to provide a measure of association, and investigate potential data clustering and putative confounding effects – particularly that by season. However, data clustering is considered unlikely to have substantially influenced results – including tick and *Amblyomma* spp. results – given the distribution across sites of infected possums. Confounding by season may have influenced results, as nearly all possums in urbanised environments were trapped in spring, compared to a third of possums trapped in bushland (Table 1), and some marsupial tick infections, or the environmental presence of some tick species that can infect marsupials, have been shown to vary seasonally (Doube 1979, Heath et al. 1986, Murdoch and Spratt 2005, Lorch et al. 2007, Waudby and Petit 2007). Considering the distribution of these infections across season in brushtail possums trapped in bushland in this study (Table 1), if anything the relatively decreased prevalence in urbanised environments may have been underrepresented by these data.

The higher tick infection prevalence identified in possums in bushland compared to urbanised environments in this study contrasts to findings of tick parasitism between brushtail possums in urban Sydney and a New South Wales bushland population (Webster et al. 2014), and may reflect a variety of environmental and brushtail possum population heterogeneities between the states and locations. For example, brushtail possum population density is thought to be substantially higher in urban Sydney than it is in urban Perth. Correspondingly, the difference in population density between urban environments and bushland in New South Wales, may be much greater than that in Western Australia, and thus explain differing observations of tick infection epidemiology in urbanised environments compared to bushland between these states. Alternatively, as different species of ticks were identified between the Sydney study and this one, differences in tick biology may entail differing impacts of urbanisation on tick infection epidemiology. This finding in Perth brushtail possums also contrasts to tick findings from quenda sampled in the greater Perth region – quenda in Perth bushland were not more likely to be infected with *Amblyomma* spp. than those in urbanised environments (Hillman et al. 2017a). This may reflect differing habits – for example, brushtail possums are arboreal, and though brushtail possums spend some time on the ground whilst

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foraging, prior studies suggest that urban dwelling possums spend minimal time on the ground (Statham and Statham 1997). This may result in relatively lower exposure to ticks in possums in urbanised environments; whereas quenda are terrestrial in both environments. Alternatively, these differing findings may be explained by host population density – brushtail possum population density is thought to be relatively low in most areas of urbanised Perth, whereas quenda population densities appeared to be exceptionally high in the urbanised environments in which they were trapped (Hillman et al. 2017a).

The possibility that the home range of the brushtail possums trapped on bushland encompassed urbanised environments cannot be excluded. However, as discussed elsewhere (Hillman 2016, Hillman et al. 2017a) this would be expected to increase the risk of type II error in statistical comparisons, therefore maintaining the validity of differences identified between urbanised and bushland possum populations. Also discussed in detail elsewhere (Hillman 2016, Hillman et al. 2017a), by comparing possums trapped in bushland and urbanised environments within the same geographical region (the greater Perth region), potential bias in comparing urban Perth animals to geographically remote bushland (where isolation from urbanised environments is guaranteed more reliably) has been avoided.

In conclusion, this study provides novel parasite data from brushtail possums in Perth. In consideration of other available research data, study findings highlight that the impacts of urbanisation on parasitic infection epidemiology can differ between the same host species in different geographical (ecological) locations, and can differ between similar parasitic taxa infecting differing host taxa in the same geographical (ecological) location.

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Supplementary material (available online as Appendix wlb-00442 at <www.wildlifebiology.org/appendix/wlb.00442>). Appendix 1.

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