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Anthropozoonotic significance, risk factors and spatial distribution of *Giardia* spp. infections in quenda (*Isoodon obesulus*) in the greater Perth region, Western Australia

Alison E. Hillman[a,*], Amanda L. Ash[a], Alan J. Lymbery[b], R.C. Andrew Thompson[a]

[a] College of Science, Health, Engineering and Education, Murdoch University, 90 South St, Murdoch, Perth, 6150, Australia
[b] Centre for Sustainable Aquatic Ecosystems, Harry Butler Institute,Murdoch University, 90 South St, Murdoch, Perth, 6150, Australia

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ABSTRACT

Giardia spp. infections in wildlife populations have been linked to anthropogenic sources of infection and public health risk in a diversity of wildlife species and ecological locations worldwide. Quenda (*Isoodon obesulus*) remain in many urbanised areas of Perth, Western Australia, and can be gregarious in their interactions with humans and domestic animals. In a previous study, a high prevalence of *Giardia* spp. infection was identified amongst quenda trapped in urbanised environments and bushland in Perth, Western Australia. This study aimed to expand on that finding, by: identifying and estimating the prevalence of particular species of *Giardia* infecting quenda, and thus clarifying their anthropozoonotic/public health significance; identifying risk factors for *Giardia* spp. infection; and investigating putative associations between infection and indicators of ill health. *Giardia* spp. infections in Perth quenda are overwhelmingly of the host-adapted, non-zoonotic *Giardia peramelis* (apparent prevalence 22.2%; 95% CI 17.7–27.4%), indicating that quenda are not a substantial veterinary public health risk regarding this parasite genus. However, one case each of *Giardia duodenalis* and *Giardia canis* genotype D were identified in quenda trapped in urbanised environments (apparent prevalences 0.4%; 95% CI 0.1–1.9%). In quenda, *Giardia* spp. infection is associated with *Cryptosporidium* infection and flea infection intensity, which may reflect host population density, or regarding *Cryptosporidium* spp., similar transmission pathways or synergistic interactions between these taxa within the host. *Giardia* spp. infection is not associated with the measured indicators of ill health in Perth quenda, but this finding is representative of *Giardia peramelis* only, given the apparent rarity of other *Giardia* sp. infections in this study.

1. Introduction

Infection with species of the gastrointestinal protozoan parasite genus *Giardia* in wildlife populations has been linked to anthropogenic sources in a diverse range of host species and environments worldwide (e.g. Graczyk et al., 2002; Kutz et al., 2008; Teichroeb et al., 2009; Ash et al., 2010; Johnston et al., 2010; Thompson et al., 2010; Thompson, 2013; Delport et al., 2014; Vermeulen et al., 2015). The presence of anthropozoonotic species of *Giardia* in wildlife populations, particularly at wildlife-domestic animal-human interfaces, are of concern because of the potential impacts of these infections on wildlife population health, and because wildlife species may act as a reservoir of infection of public health significance (Kutz et al., 2009; Thompson, 2013).

Quenda (syn. southern brown bandicoots, *Isoodon obesulus*) exist in many urbanised areas of Western Australia, as well as in bushland. They are a small, omnivorous, terrestrial marsupial species, and can be very gregarious in interacting with humans and domestic pets (Howard et al., 2014; Hillman and Thompson, 2016). Quenda turn over up to four tonnes of soil per year each in foraging for food (Valentine et al., 2013), which may put them at particular risk of acquiring faecal-borne and soil-transmitted parasites. In urbanised environments, quenda are particularly at risk of exposure to pathogens originating from domestic pet faeces, and human faeces where septic tanks are in use, though little is known about their susceptibility to such pathogens. Quenda are known to be illegally translocated from private properties in urban Perth (Howard et al., 2014), and so may be both at risk of adverse impacts of anthropozoonotic infections and potentially act as a vector of such infections into non-urban populations.

Hillman et al. (2017b) documented a high prevalence of *Giardia* spp. infection in quenda, particularly those trapped in more urbanised environments in Perth, the capital city of Western Australia. This study aimed to expand on that finding (using data from the same sampled

* Corresponding author.
E-mail address: alison.hillman@ausvet.com.au (A.E. Hillman).

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population), by: 1) clarifying the anthropozoonotic significance of *Giardia* infections in Perth quenda, by estimating the prevalence of particular species of *Giardia*; 2) investigating risk factors for *Giardia* spp. infection in quenda, considering host factors, environmental factors, putative influences of concurrent parasitic infections, and geographical clustering of infection risk; and 3) investigating associations between *Giardia* spp. infection and indicators of ill health in Perth quenda.

2. Methods

The sampled population is the same quenda population as in Hillman et al. (2017b). Trapping and sampling methodology, parasite detection test methodology and validation of the tests, are described in detail elsewhere (Hillman, 2016; Hillman et al., 2017a, 2017b), and summarised briefly below.

2.1. Trapping and sampling quenda

A cross sectional study of free-ranging quenda in urbanised environments and bushland in the Statistical Division of Perth was undertaken across March 2013 to July 2015. Trapping of quenda was undertaken across twenty-nine bushland sites and 35 urbanised sites. Faecal samples were collected from the traps of trapped quenda, then quenda were briefly anaesthetised using isoflurane (I.S.O. 1 mL/mL, V.C.A, Australia) to enable a thorough and comprehensive examination, including collection of macroscopic ectoparasites. Biosecurity was given careful consideration in study design and methodology, to minimise the risk of cross-contamination of samples from different animals and anthropogenic spread of infection; and the risk of cross-contamination impacting study results was investigated in data analysis (and judged to have been unlikely to substantially influence study findings), as described in detail elsewhere (Hillman et al., 2016b).

2.2. Indicators of quenda health

On collection from the trap, faecal samples from quenda were classified as 'formed' if at least one discrete faecal pellet was present, or 'poorly formed' if no discrete faecal pellets were present. Fur condition of sampled quenda was graded either as 'full coat', if the animal had complete fur cover, or 'incomplete coat' if there were areas of shearing, regrowth or alopecia present. Skin condition of the quenda was graded as 'normal' if there was no evidence of gross pathology, 'scale' if scale was present, or 'exudative' if there were exudative lesions present. Body condition was assessed via two methods: 'subjective body condition' and 'objective body condition'. For the 'subjective body condition', fat and muscle mass were qualitatively palpated over the temporal fossa, scapula spine and spine: animals were graded on an ordered categorical scale: 1 (emaciated), 2 (underweight), 3 (optimal), 4 (overweight) or 5 (obese). Due to the small numbers of animals rated as emaciated or obese, these data were grouped for statistical analysis into the categories low condition (emaciated or underweight), optimal condition (optimal), or high condition (overweight or obese). The 'objective body condition' was calculated separately for adult male and adult female quenda, as the residual of observed to expected weight (female quenda with pouch young were excluded from this calculation and subsequent analysis). Expected weight was calculated by linear regression of average pes length to net weight, using Stata 14 software (StataCorp, 2015).

2.3. Parasite detection

Immunofluorescence microscopy of faeces was used to identify quenda infected with *Giardia* spp. *Giardia* spp. infections were differentiated to species level using PCR and sequencing at the 18S rRNA, ITS1-5.8s-ITS2 and *gdh* loci. Quenda were considered positive for a particular species of *Giardia* if at least one cyst of apple-green fluorescence and appropriate morphology was identified on immunofluorescence microscopy, with the species identified on PCR and sequencing at one or more loci. *Giardia* spp. prevalences were calculated with Jeffrey's 95% confidence interval.

Concurrent *Cryptosporidium* spp. infections were identified by immunofluorescence microscopy, using a commercial kit (Merifluor Cryptosporidium/Giardia, Meridian Bioscience, Inc. USA), according to manufacturer's directions for unconcentrated faecal samples. Samples were considered positive if at least one oocyst of appropriate *Cryptosporidium* spp. morphology and apple-green fluorescence was identified. Other gastrointestinal protozoan and helminth parasites were tested for using a faecal flotation protocol. Quenda were considered infected with a particular taxon if at least one protozoan cyst/oocyst or helminth egg was identified using this protocol.

Intensity of gastrointestinal protozoan and helminth infections were not estimated using cyst/oocyst or egg counts, because of concerns over both the precision of the count data and the impact of intermittent and variable shedding on intensity estimates.

All observed macroscopic ectoparasites were collected and differentiated morphologically. Quenda were classified into categories of tick (Family Ixodidae), flea (Order Siphonaptera) and mesostigmatan mite (Order Mesostigmata) intensity of infection, based on the number of specimens obtained and differentiated as such from the animal: nil = no specimens obtained; low = 1–9 specimens obtained; high = 10 + specimens obtained. Trombiculid mite (Suborder Prostigmata) infections were classified on a presence/absence basis.

2.4. Analysis of risk factors for Giardia spp. in quenda

Univariable logistic regression was undertaken using Stata 14 software (StataCorp, 2015) to assess association between *Giardia* spp. infection and a number of environmental and host risk factors. Environmental factors considered in the univariable risk analyses were trapping site (animal trapped in bushland compared to urbanised environment) and season of sampling. Host factors considered were maturity (adult or subadult), sex, the presence of an active pouch in adult females (active pouch = lactating, inactive pouch = not lactating), concurrent gastrointestinal parasitic infections (assessed on a presence/absence basis) and concurrent ectoparasitic infections (tick, flea and mesostigmatan mite infection intensities were assessed on an ordered categorical scale, and trombiculid mite infections were considered on a presence/absence basis) (Table 1).

For each univariable assessment, data clustering by trap site was assessed using the likelihood ratio test. The only evidence of clustering occurred with *Entamoeba* spp. — this association was therefore tested using mixed effects logistic regression, including trap site as a random effect. Ordinary logistic regression was used to assess all other variables. Results were reported with Wald p-values.

An explanatory approach to multivariable analysis was undertaken (Shmueli, 2010), to test the putative causal role of various risk factors in *Giardia* spp. infection. A multivariable risk factor model was built for each putative risk factor with at least weak evidence ($p \leq 0.10$) of an association with *Giardia* spp. infection. All other independent variables with at least weak evidence ($p \leq 0.10$) of an association with *Giardia* spp. infection, and maturity, were considered as putative confounders for each multivariable risk factor model. Independent variables were sequentially added to the models — they were retained if their inclusion altered the odds ratio (OR) of the risk factor of interest by ≥ 10%. Testing for interaction between each retained confounder and the risk factor of interest was undertaken using the likelihood ratio test — the interaction term was retained in the model if there was at least weak evidence ($p \leq 0.10$) of interaction. Variance inflation factors of retained independent variables were checked to ensure they were < 10.

Table 1
Univariable risk factor analysis for *Giardia* spp. infection in quenda. The crude OR (odds ratio) compares the odds of *Giardia* spp. infection between the respective subgroups of each variable. Variables with sufficient evidence of an association to be considered for multivariable analysis are shown in bold ($p \leq 0.10$).

Variable type	Variable		Crude OR	95% CI	p-value
Environmental factors	**Habitat type**	**Bushland**	**1**		
		Urbanised	**1.78**	**1.08–2.91**	**0.022**
	Season	**Autumn**	**1**		
		Spring	**2.30**	**1.35–3.91**	**0.002**
		Winter	**0.62**	**0.070–5.56**	**0.67**
Host factors	Maturity	Subadult	1		
		Adult	0.58	0.27–1.28	0.18
	Sex	Female	1		
		Male	1.31	0.80–2.15	0.29
	Active pouch[a]	No	1		
		Yes	1.80	0.66–4.93	0.25
	***Cryptosporidium* spp.**	**Not infected**	**1**		
		Infected	**17.2**	**7.33–40.6**	**< 0.001**
	Entamoeba spp.	Not infected	1		
		Infected	1.43	0.57–3.63	0.45
	Gastrointestinal coccidia (Family Eimeriidae)[b]	Not infected	1		
		Infected	1.50	0.47–4.84	0.50
	Strongyles (Suborder Rhabditina)	Not infected	1		
		Infected	1.63	0.32–8.21	0.56
	***Labiobulura* spp.**	**Not infected**	**1**		
		Infected	**3.40**	**1.46–7.93**	**0.005**
	***Linstowinema* spp.**	**Not infected**	**1**		
		Infected	**1.76**	**1.04–2.97**	**0.034**
	Physaloptera spp.	Not infected	1		
		Infected	0.93	0.31–2.80	0.90
	Strongyloids (Family *Strongyloididae*)	Not infected	1		
		Infected	0.92	0.46–1.85	0.82
	***Trichuris* spp.**	**Not infected**	**1**		
		Infected	**1.53**	**0.94–2.50**	**0.090**
	Potorolepis spp.	Not infected	1		
		Infected	0.93	0.44–1.94	0.84
	Flea (Order Siphonaptera) infection intensity	**Nil**	**1**		
		Low	**3.41**	**1.83–6.33**	**< 0.001**
		High	**5.00**	**2.48–10.1**	**< 0.001**
	Tick (Family Ixodidae) infection intensity	Nil	1		
		Low	1.02	0.59–1.75	0.95
		High	1.66	0.70–3.94	0.25
	Mesostigmatan mite (Order Mesostigmata) infection intensity	**Nil**	**1**		
		Low	**1.59**	**0.88–2.87**	**0.13**
		High	**2.14**	**1.16–3.94**	**0.014**
	Trombiculid mites (Suborder Prostigmata)	Not infected	1		
		Infected	0.96	0.49–1.89	0.91

[a] In adult females: active pouch = lactating; not active pouch = not lactating.
[b] Fresh faeces from 71 quenda were used to identify particular species of Family Eimeriidae present - all sporulated coccidian oocysts were *Eimeria* spp. (Hillman et al., 2016a).

2.5. Mapping and investigation of geographical clustering of *Giardia* spp. infection risk in quenda

Giardia spp. infection was mapped using QGIS v2.18 (QGIS Geographic Information Systems, 2018), using the GPS point of the trap in which the quenda was caught. On mapping, GPS points were displaced by 0.01, and uninfected quenda icons were set at 50% transparency, to improve visibility of the relative distribution of infected quenda.

The risk of *Giardia* spp. infection was investigated spatially using Kulldorff's spatial scan statistic (SaTScan version 9.5, www.satscan.org). Data were run as an unfocused Bernoulli model, scanning for clusters of both increased and decreased risk of *Giardia* spp. infection, utilising Gumbel approximation in the significance testing. Clusters with at least weak evidence ($p \leq 0.10$) of geographical clustering were mapped using SaTScan shapefile output in QGIS v2.18 (QGIS Geographic Information Systems, 2018).

2.6. Associations between *Giardia* spp. infection and indicators of ill health

Association between *Giardia* spp. infection and faecal condition, fur condition, skin condition and subjective body condition were tested by logistic regression, and association between *Giardia* spp. infection and objective body condition by linear regression, using Stata 14 software (StataCorp, 2015) with the methods as per Section 2.2. For the linear regression, normal distribution of the model residuals was confirmed to ensure the models were valid.

2.7. Ethics approval and sampling permits

All samples were obtained under Murdoch University Animal Ethics Permit R2530/12, and Department of Parks and Wildlife Regulation 17 (SF009640) and Regulation 4 (CE004287) permits.

3. Results

The 284 quenda included in this study comprised 11 subadult females, 17 subadult males, 116 adult females and 140 adult males (one adult female and two adult males were excluded because faeces were not recoverable from their traps, and two subadult quenda were excluded as their particularly small body sizes were judged to make them inappropriate candidates for anaesthesia). Of the adult females included in the study, 88 (76%) had an active pouch and 28 (24%) had an inactive pouch.

3.1. Giardia spp. prevalence

On immunofluorescence microscopy, 99/284 of quenda were infected with *Giardia* spp. (34.9%; 95% CI 29.6–40.6%). Of these, sixty-three infections (64%) were successfully differentiated to species level by PCR and sequencing. All 63 quenda were infected with *G. peramelis* (apparent prevalence 22.2%; 95% CI 17.7–27.4%). One quenda was concurrently infected with *G. duodenalis* (apparent prevalence 0.4%; 95% CI 0.1–1.9%), and another quenda was concurrently infected with *G. canis* genotype D (apparent prevalence 0.4%; 95% CI 0.1–1.9) — both these quenda were trapped in urbanised environments.

3.2. Giardia spp. in quenda - risk factors for infection

On univariable analysis, there was at least weak evidence ($p \leq 0.10$) of an association between *Giardia* spp. infection in quenda and habitat type, season, infection with several taxonomic groupings of gastrointestinal parasites (*Cryptosporidium* spp., *Labiobulura* spp., *Linstowinema* spp. and *Trichuris* spp.) and flea and mesostigmatan mite infection intensities (Table 1).

On multivariable analysis, quenda infected with *Cryptosporidium* spp. had over 20 times the odds of infection with *Giardia* spp. (adjusted OR = 20.4, 95% CI 8.38–49.6); and quenda with flea infections had at least three times the odds of *Giardia* spp. infection than those without (adjusted OR for low intensity infections = 3.31 (95% CI 1.61–6.81); adjusted OR for high intensity infections = 4.83 (95% CI 2.10–11.1). After controlling for confounding, there was no longer evidence of an association between *Giardia* spp. infection and the other factors associated on univariable analyses (Table 2).

3.3. Geographical clustering of Giardia spp. infection risk

Quenda infected with *Giardia* spp. were documented throughout the greater Perth region, except for the north-west aspect (Fig. 1). On Kulldorff's spatial scan statistic, a cluster of relatively decreased *Giardia* spp. infection risk was identified over this area. Quenda trapped in this area had < 0.001 times the odds of *Giardia* spp. infection than quenda trapped elsewhere in the greater Perth region ($p < 0.001$) (Fig. 1).

Table 2
Multivariable analysis of risk factors for *Giardia* spp. infection in quenda. The adjusted OR (odds ratio) compares the odds of *Giardia* spp. infection between the respective subgroups of each variable, whilst controlling for the effects of confounding variables. Variables that remained associated with *Giardia* spp. infection on multivariable analysis are highlighted in bold (95% CI does not cross the null value (1)).

Variable		Adjusted OR	95% CI
Habitat type	Bushland	1	
	Urbanised	1.30	0.69–2.45
Season	Autumn	1	
	Spring	1.83	0.89–3.77
	Winter	1.34	0.14–12.6
***Cryptosporidium* spp.**	**Not infected**	**1**	
(gastrointestinal protozoan)	**Infected**	**20.4**	**8.38–49.6**
Labiobulura spp. (gastrointestinal	Not infected	1	
nematode)	Infected	2.35	0.93–5.96
Linstowinema spp. (gastrointestinal	Not infected	1	
nematode)	Infected	1.73	0.84–3.54
Trichuris spp. (gastrointestinal	Not infected	1	
nematode)	Infected	1.05	0.58–1.88
Flea (Order Siphonaptera)	**Nil**	**1**	
infection intensity	**Low**	**3.31**	**1.61–6.81**
	High	**4.83**	**2.10–11.1**
Mesostigmatan mite (Order	Nil	1	
Mesostigmata) infection intensity	Low	0.87	0.37–2.06
	High	0.75	0.29–1.92

3.4. Associations between Giardia spp. infection and indicators of ill health

Faecal condition, skin condition and fur condition observations were available from all trapped quenda; subjective body condition was missing from one animal. There was no evidence of an association between *Giardia* spp. infection and any of these variables ($p > 0.10$; Table 3).

Forty-three out of 116 adult female quenda and 139/140 adult male quenda were included in testing the association between *Giardia* spp. infection and objective body condition. All excluded adult female quenda had pouch young, and the excluded adult male quenda did not have body weight recorded, which precluded calculation of their objective body condition scores. There was no evidence of an association between *Giardia* spp. infection and objective body condition in either adult female quenda (regression coefficient = −52.9, 95% CI -170.7 – 65.0; $p = 0.37$; adj. $R^2 = -0.0043$) or adult male quenda (regression coefficient = −10.8, 95% CI -97.2 – 75.6; $p = 0.81$; adj. $R^2 = -0.0068$).

4. Discussion

4.1. Prevalence and anthropozoonotic significance of Giardia spp. infections in Perth quenda

This study indicates that *Giardia* spp. infection in Perth quenda is typically of the host-adapted, non-zoonotic *G. peramelis*. Other *Giardia* species identified — *G. duodenalis* and *G. canis* — were rare. This study provides a new host record for *G. canis* genotype D.

Although immunofluorescence microscopy is an accurate indicator of *Giardia* spp. infection in quenda (Hillman et al., 2017a), not all infections amplified by PCR, and some infections amplified (with a band at the appropriate size for *Giardia* spp.) but the resultant nucleotide sequences were of insufficient quality to align with published *Giardia* sequences, so differentiation to species level was not possible. Hence, the prevalences of particular species of *Giardia* are likely to have been underestimated in this study. However, it is unlikely that the success of PCR and sequencing protocol would have been differentially biased based on the species of *Giardia* present, retaining validity in the proportionate representation of *Giardia* species infecting quenda in Perth.

Given that zoonotic species of *Giardia* were rarely detected in Perth quenda, this study suggests that *Giardia* spp. infections in quenda are not important from a public health point of view. *Giardia duodenalis* and *G. canis* genotype D were only found in quenda trapped in urbanised environments. It is considered likely that quenda in urbanised environments are at increased risk of acquiring these species of *Giardia* than quenda in bushland, due to increased exposure to sources of infection, but it was not possible to test this statistically in this study, given the infections' apparent rarity combined with the relatively small sample size. Thompson et al. (2009) suggest that domestic pets and/or humans are likely sources of these infections to quenda. *Giardia* spp. infections are common in domestic dogs in Australia (Palmer et al., 2008a), including *Giardia duodenalis* and *Giardia canis* genotype D (Palmer et al., 2008b).

On the basis that *G. peramelis* was the dominant species of *Giardia* isolated from Perth quenda, it is considered that the main source of *Giardia* spp. infections to quenda in this region are other quenda. *Giardia peramelis* has been previously identified in quenda in Western Australia (Adams et al., 2004; Thompson et al., 2010). *Giardia peramelis* was isolated by PCR from faeces of a northern brown bandicoot (*Isoodon macrourus*), a brush-tail rabbit-rat (*Conilurus penicillatus*) and two brushtail possums (*Trichosurus vulpecula*) in the Northern Territory, although viable cysts were not demonstrated in the faeces (Barbosa et al., 2017). Considering the potential for cross-contamination in trapping programmes of small marsupials, particularly where PCR is the sole means of detecting infection (Hillman et al., 2016b), there is uncertainty in interpretation of these findings regarding host

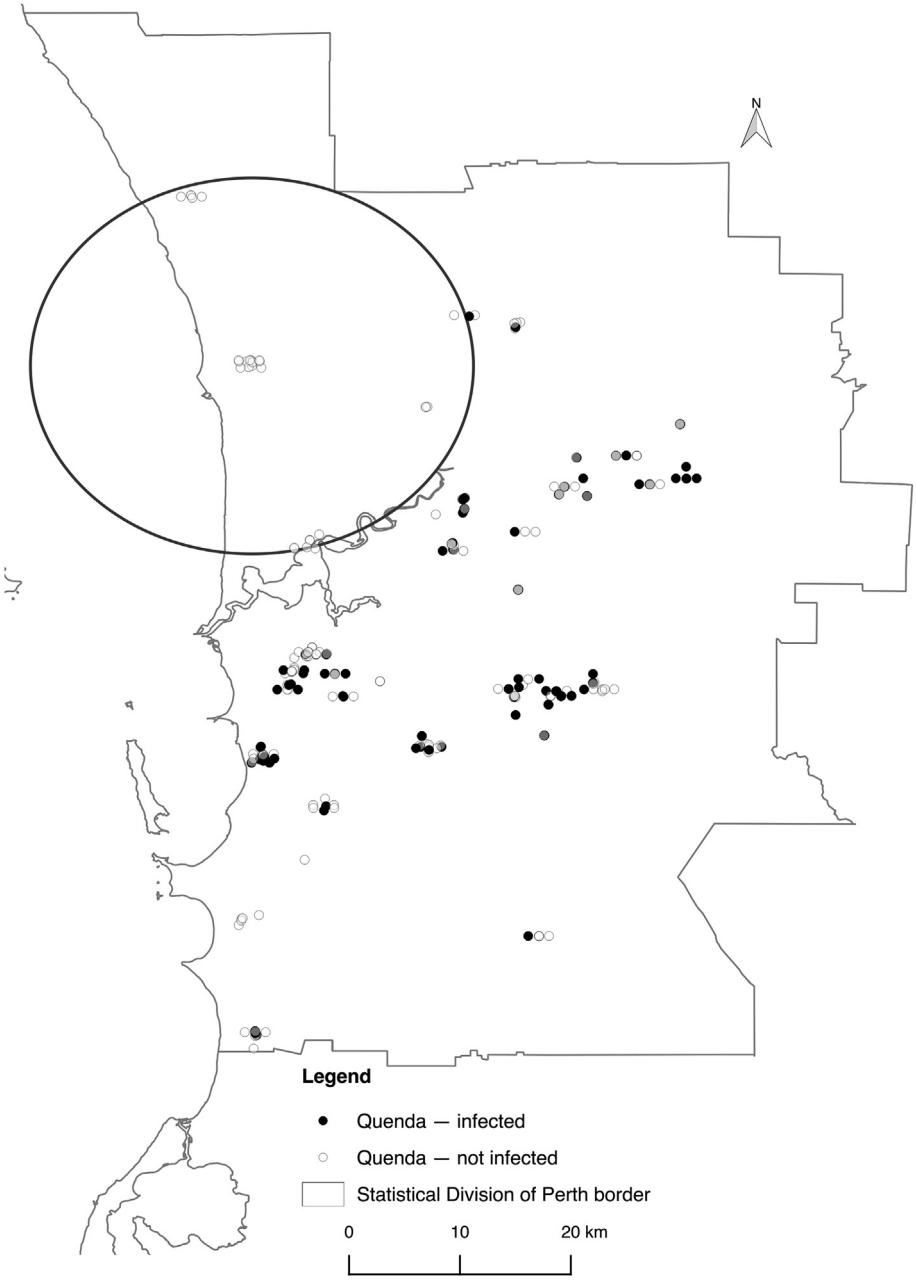

Fig. 1. Geographical distribution of *Giardia* spp. infection in Perth quenda. Quenda were mapped using the GPS position of the location at which they were trapped. GPS points are jittered, and icons for uninfected quenda are 50% transparent, to improve visualisation of the relative distribution of *Giardia* spp. infection. By Kulldorff's spatial scan statistic, the circle in the north-west region represents a cluster of relatively decreased *Giardia* spp. infection risk in Perth quenda (OR of infection < 0.001, compared to quenda trapped elsewhere in Perth; $p < 0.001$).

Table 3
Association between *Giardia* spp. infection and indicators of ill health in Perth quenda.

		Crude OR[a]	95% CI	p-value
Faecal condition	Formed	1		
	Poorly formed	1.24	0.55–2.75	0.61
Subjective body condition	Optimal	1		
	Low condition	0.75	0.23–2.44	0.63
	High condition	1.38	0.82–2.33	0.23
Fur condition	Full coat	1		
	Incomplete coat	1.40	0.86–2.29	0.18
Skin condition	Normal	1		
	Scale	0.52	0.11–2.57	0.43

[a] OR = odds ratio.

susceptibility. Of these three species, only brushtail possums are endemic to the greater Perth region. *Giardia peramelis* was not detected in the faeces of Perth brushtail possums in a related study (Hillman et al., 2018). *Giardia peramelis* has not been isolated from other endemic Australian species tested for *Giardia* spp. (McCarthy et al., 2008; Thompson et al., 2008; Thompson et al., 2010; Ng et al., 2011; Thomasz, 2014; Vermeulen et al., 2015; Wait et al., 2017).

4.2. *Giardia spp. in quenda — risk factors for infection*

An association between urban environments and *Giardia* spp. infection in quenda was previously identified by Hillman et al. (2017b). However, in exploring this association further regarding putative confounding, this association dissipated, pointing towards more specific

causal mechanisms behind the association, such as differing host population density and/or synergistic parasite infection risk across these environments, which may have been controlled for by other covariates in the multivariable analyses (as discussed further below).

Concurrent *Cryptosporidium* spp. infection was associated with *Giardia* spp. infection risk. It is possible that this reflects similar transmission pathways to *Giardia* spp. infection, or is confounded by host population density. Additionally or alternatively, synergistic interactions may occur between these parasite taxa within the quenda host — for example, through immune-mediated collaboration (Northover et al., 2018). It must be noted, that although *Cryptosporidium* spp. was the only gastrointestinal parasite that remained associated with *Giardia* spp. infection risk on multivariable analysis, associations between *Giardia* spp. and other gastrointestinal parasite taxa may not have been detected, due to a number of negative influences on the statistical power of this study to detect less marked associations. These include relatively small sample sizes (particularly for *Entamoeba* spp. infection), very high or low prevalences of some parasite taxa, and non-differential misclassification bias in the *Potorolepis* spp. infection analysis due to the utilised detection test lacking sensitivity in the detection of this parasite genus (Hillman et al., 2017a).

The association between *Giardia* spp. infection and flea infection intensity is likely to be attributable to host population density: quenda in areas of higher population density may have an increased risk of infection with fleas and increased risk of higher flea infection intensity, as well as having an increased odds of *Giardia* spp. infection, compared to quenda in areas with lower population density. Quenda population density was not measured directly in undertaking the trapping of animals, due to constraints regarding the safety of captured animals, particularly in urban environments. However, controlling for other density-dependent parasites as part of these analyses may have partially controlled for host population density. Associations between flea infection intensity and host population density have been demonstrated in other mammalian host species (e.g. Krasnov et al., 2004; Kaal et al., 2006), as have associations between *Giardia* spp. infection and host population density (e.g. Atwill et al., 1997; Claerebout et al., 2009; Dreelin et al., 2014).

4.3. Geographical clustering of Giardia spp. infection risk

The area of substantially decreased odds of *Giardia* spp. infection in quenda in north-west Perth, identified on Kulldorff's spatial scan statistic, correlates with the best available evidence (Howard et al., 2014) and our own subjective observations of an area of Perth with low density of quenda or interrupted presence of quenda over recent times, which may indicate lower levels of environmental contamination with viable *G. peramelis* cysts. However, the cluster of low infection risk may have been artificially strengthened by the edge effects of the coastline.

4.4. Association between Giardia spp. infection and indicators of ill health

There was no evidence of an association between *Giardia* spp. infection and faecal, skin, fur and body condition in this study. However, these results should be considered representative of *G. peramelis* only, as infections with other *Giardia* spp. were too rare to have impacted study findings. Though the relatively small sample sizes and a lack of temporal sequence in these observations are substantial limitations, it is reasonable to assume that a balanced host parasite relationship exists in infections with a parasite adapted to its marsupial host, particularly in animals on a good plane of nutrition.

5. Conclusions

This study finds no evidence that quenda in urbanised environments and bushland in the greater Perth region, Western Australia, are a substantial reservoir of zoonotic *Giardia* spp. infections, or of species of *Giardia* that are known to be infectious to domestic animals. In quenda, *Giardia* spp. infection is associated with *Cryptosporidium* infection and flea infection intensity, which may reflect host population density, or regarding *Cryptosporidium* spp., similar transmission pathways or synergistic interactions between these taxa within the host Although there is some evidence of spatial structuring of infection, this is not related to gradients of urbanisation and probably reflects differences in quenda population density. No associations were identified between *Giardia* spp. infection and the measured indicators of ill health, but this is representative of *G. peramelis* infection only.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2019.03.019.

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