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Research paper

Substrate type and age are risk factors for gastrointestinal parasitism in greyhound kennels

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ABSTRACT

The control of parasitic infections is particularly challenging in environments that are conducive to the maintenance of parasite lifecycles, such as the greyhound kennel, where the long-term breeding and rearing of dogs is common. The prevalence of gastrointestinal (GI) parasites within the Australian greyhound population has never previously been assessed, which seriously constrains the implementation of effective control measures. The aims of this study were to determine the prevalence and risk factors for GI parasites in Australian greyhounds, identify parasites which may be detrimental to the health and performance of dogs, and evaluate the likelihood of zoonotic transmission to kennel staff. Faecal samples were collected from 721 individual greyhounds situated in kennels across five states of Australia; Western Australia, Queensland, New South Wales, Victoria and Tasmania. Animal husbandry and current parasite control protocols were obtained from each kennel and analysed in conjunction with the detected level of parasitism. Overall parasite prevalence was approximately 60%, ranging from 50 to 70% between states. Eleven parasite genera were identified, with *Sarcocystis*, hookworm, *Giardia* and *Toxocara* detected most frequently. Generalised linear mixed model analyses found the major risk factors associated with parasitism were: a) the type of substrate which dogs were housed; b) age of dogs; and c) geographic region. Parasitism was associated most frequently with young dogs housed on grass/sand substrates, which allowed parasite lifecycles to continue, with constant reinfection the likely outcome. Routine treatment with broad-spectrum anthelmintics did not provide effective control in these environments and the adoption of alternate parasite control strategies is recommended. A substantial risk from zoonotic parasites was also identified, with six of the eleven parasite genera detected considered to be zoonotic and a poor understanding of zoonotic transmission among kennel managers.

1. Introduction

The control of parasitic infections can be challenging, particularly in environments that are conducive to the maintenance of parasite lifecycles, such as those that house large numbers of a single animal species in a confined space. These conditions provide an abundance of susceptible hosts, which can intensify the parasite load and allow for quick and efficient parasite transmission (Anderson and May, 1978; May and Anderson, 1978). Dog kennels can present such a challenging environment, particularly facilities that are involved in the breeding and long-term housing of dogs (Jacobs and Prole, 1976; Overgaauw and Boersema, 1998). Whelping bitches are prone to the reactivation of *Toxocara canis* and hookworm spp. infections (Burke and Roberson, 1985), while puppies, often infected with these and other parasites, provide a constant source of infective parasite stages for all other long-term residents. An example of this scenario is found in the greyhound kennel, where it is common to house dogs across all life stages such as breeding bitches, pups, juveniles and adult racing dogs in the same kennel environment.

While parasitic infections in dogs are common, the athletic requirements of the racing greyhound necessitate optimal health and growing conditions, and gastrointestinal (GI) parasites can be detrimental to both. Infections with nematodes such as hookworm species can lead to severe anaemia and sometimes death in young animals (Miller, 1966), while chronic infection with species of *Trichuris*, *Toxocara* and the protozoan *Giardia* can result in ill thrift and growth

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retardation (Buret, 2007; Hayden and Van Kruiningen, 1975; Parsons, 1987; Traversa, 2011), all of which can have a negative effect on the development of a physically fit racing animal. In addition to the implications for animal health there is also a risk of transmission of zoonotic parasites to racing personnel including veterinarians, trainers and kennel hands. The major canine GI parasites that pose a zoonotic risk are species of hookworm, *Toxocara canis* and *Giardia* spp. (Palmer et al., 2007; Robertson and Thompson, 2002), although *Strongyloides stercoralis and Trichuris vulpis* have also been implicated in zoonotic transmission (Dunn et al., 2002; Robertson and Thompson, 2002).

Australia has an extensive greyhound industry with racing bodies in all states and territories, and as result there are numerous kennelling facilities across the country. Current information on parasitism in the Australian greyhound population is not available and indeed recent data are lacking for the racing greyhound worldwide. Studies which have investigated parasitism outside Australia reported prevalences of GI parasites of 46% in English greyhound kennels (Jacobs and Prole, 1976) and 40% in American greyhound kennels (Ridley et al., 1994). While data are lacking for Australian greyhounds, there have been studies investigating GI parasites in pet dogs, dog refuges and breeding kennels within Australia (Bugg et al., 1999; Palmer et al., 2008). Palmer et al., (2008) reported an overall parasite prevalence of 16% within pet dogs and 34.6% in refuge dogs, while Bugg et al., (1999) reported a prevalence of 37% in dog refuges and 32.7% in breeding kennels.

Due to the lack of information pertaining to parasitism in greyhounds and related animal health concerns, a parasite survey was conducted across several states within Australia. The aims for the study were to: a) ascertain the overall prevalence of GI parasites existing within Australian greyhounds; b) identify the major risk factors for these parasitic infections; c) evaluate the zoonotic risk from the GI parasites detected; and d) ascertain the level of understanding of zoonotic transmission among kennel managers. Information obtained in this study could then be used by both greyhound and pet dog breeding personnel to develop effective parasite control strategies within the kennel environment and ultimately improve the overall health standards for dogs and related zoonotic risks for kennel personnel.

2. Methodology

2.1. Ethics approval

Ethics approval was granted from the Animal Ethics Committee (permit R2604/13) and the Human Research Ethics Committee (permit 2015/103) at Murdoch University, Perth, Western Australia.

2.2. Study design

Faecal samples were obtained from greyhound kennels during November-December 2013 in Western Australia and June-July 2015 in Queensland, New South Wales, Victoria and Tasmania. The timing of these samples was determined by access to the kennels and because of operational constraints, it was not possible to sample on the western and eastern sides of Australia in the time period. Participation in the study was voluntary and recruitment of kennel owners for the study was guided by greyhound veterinarians able to identify kennels containing dogs across all life-stages. A maximum of 160 faecal samples were collected from each state and consisted of randomly selected animals representing racing, breeding, juvenile and pup life-stages (Table 1). Classification of life-stage for this study was based on industry standards, where pups are generally considered to be < 12 months, juveniles (also considered as pre-trainers) between 12–18 months and racing dogs > 18 months. A minimum of 5 g of faeces was collected from the ground soon after defaecation and stored at 4 °C until fixed and later analysed. Samples collected in Western Australia and analysed immediately (unfixed). Samples collected in other states were required to be preserved in 10% formalin (2 parts faeces/8 parts 10% formalin) and couriered to the laboratory for later analysis. The recovery rate of parasite eggs/cysts is known to be reduced in formalin fixed samples, however this largely affects estimates of the intensity of infection, rather than the presence of parasite species (Foreyt, 1986). Upon inclusion of a dog into the study, the owner/trainer of the animal provided written consent and completed a short questionnaire designed to obtain information relating to each dog (age, sex, life-stage), animal husbandry (kennel surface, faecal collection, diet) and parasite control. General awareness of zoonotic parasitic infections transmitted from dogs was also obtained from each participant.

Table 1
The number of kennels sampled from each state with the breakdown of faecal samples obtained from greyhounds within the different life-stages.

Number sampled	*WA*	*QLD*	*NSW*	*VIC*	*TAS*	*Total*
Kennels	27	15	11	11	17	81
Racing	109	69	58	71	66	373
Juvenile	0[a]	30	23	22	24	99
Pup	34	34	35	28	28	159
Brood bitch	14	10	11	12	12	59
Retired	3	6	13	8	1	31
Total dogs	160	149	140	141	131	721

(Abbreviations for states WA = Western Australia; QLD = Queensland; NSW = New South Wales; VIC = Victoria; TAS = Tasmania).

[a] Juveniles were not sampled in WA as dogs of this age are generally reared in the eastern states of Australia and subsequently not encountered in the sampled group.

2.3. Parasitological analysis

Two parasitological tests were applied to each sample: (1) a wet malachite-stained smear, often used to detect *Cryptosporidium* species (Elliot et al., 1999) but also to detect protozoan species that may be adversely affected by saturated salt solutions; and (2) a zinc sulphate centrifugation flotation method, which concentrates parasite eggs/oocysts, allowing for the detection of both light and heavy infections (Zajac and Conboy, 2006). All parasite ova/cysts were identified to genus and/or species level where possible. Detected parasites are referred to here at the genus level only, except for hookworms where genera cannot be distinguished for all infections (e.g. *Ancylostoma* and *Uncinaria*). The methods used were not able to detect lungworm, as larval cultures from fresh faeces was required, and have a low sensitivity for detecting parasites with intermittent shedding such as taeniid tapeworms.

2.4. Molecular characterisation

A subset of samples representing all kennels which were positive for hookworm, *Giardia*, *Taenia* and *Neospora/Hammondia* were characterised molecularly to ascertain which species were present. PCR amplifying the ITS1-5.8S-ITS2 region was used for differentiation of hookworm species with slight modifications from Traub et al. (2008), as described in Ash et al. (2017). *Giardia* positive samples were amplified at the 18 s rRNA locus and ITS1-5.8s-ITS2 region as per protocols described in Ash et al. (2010) and Cacciò et al. (2010). *Taenia* positive samples were amplified at the 12S rRNA locus as per Trachsel et al. (2007) and *Neospora/Hammondia* positive samples were amplified by PCR at the internal transcribed spacer region (ITS-1) described by King et al. (2010) and Ash et al. (2015).

PCR products were purified using an Agencourt AMPure XP system (Beckman Coulter Inc., Brea USA) and sequencing reactions were performed using the Big Dye Terminator Version 3.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions. Reactions were electrophoresed on an ABI 3730 48 capillary machine with resultant nucleotides compared with published sequences on NCBI

Table 2
The percentage prevalence (95% CI intervals in parentheses) of gastrointestinal parasites identified from 721 individual greyhounds.

	WA (N = 160)	QLD (N = 149)	NSW (N = 140)	VIC (N = 141)	TAS (N = 131)	Total sampled (N = 721)
Total Prevalence %	70 (62-77)	51 (43-60)	50 (41-60)	64.5 (56-75)	66 (56-75)	**60.3 (57-64)**
			Helminths			
Hookworm**	30.6 (24-38)	18.1 (12-25)	10.7 (6-17)	9.9 (6-16)	17.5 (12-25)	**17.8 (15-21)**
Toxocara**	18.7 (13-25)	1.3 (0.2-5)	5.7 (2.7-11)	14.2 (9-21)	29 (21-37)	**14.1 (11-16)**
Toxascaris	1.9 (0.05-5)	0	0 (0-0.2)	0 (0-0.2)	0.8 (0.04-4)	**0.6 (0.1-1.4)**
Trichuris	1.3 (0.02-4)	0 (0-0.3)	0.7 (0.04-4)	0.7 (0.04-4)	10.7 (6-17)	**2.5 (1.6-4)**
Strongyloides**	1.9 (0.5-5)	0 (0-0.3)	2.1 (0.6-6)	0 (0-0.2)	2.3 (0.6-7)	**1.2 (0.6-2.3)**
Dipylidium**	0 (0-0.2)	1.3 (0.2-5)	0 (0-0.2)	1.4 (0.2-5)	0 (0-0.2)	**0.6 (0.2-1.4)**
Taenia**	0 (0-0.2)	0.7 (0.04-3.6)	0 (0-0.2)	0.7 (0.04-3.8)	0 (0-0.2)	**0.3 (0.05-1)**
			Protozoans			
Sarcocystis	32.5 (25-40)	17.4 (12-24)	32.9 (25-41)	43.3 (35-52)	35.9 (28-44)	**32.2 (29-36)**
Giardia**	21.8 (16-29)	9.3 (5-15)	15.7 (10-24)	17 (10-26)	16.0 (10-24)	**16.1 (13-19)**
Cystoisospora	3.7 (1-8)	4 (1-9)	6.4 (3.2-12)	7.1 (3-14)	8.4 (5-16)	**5.8 (4.3-8)**
Neospora/Hammondia	1.3 (0.2-4.5)	0 (0-0.3)	1.4 (0.2-5)	3.5 (1.4-8)	3.8 (1.5-8)	**1.9 (1.1-3.2)**

** Parasites with zoonotic potential.

using the basic alignment search tool (BLAST). Further sequence analysis was conducted using the sequence alignment program Sequencer™ 5.4.6 (Genes Codes Corporation, Ann Arbor, USA).

Nucleotide sequence data representing each parasite species genotyped in this paper are available in the GenBank database under the accession numbers: MK203853 through to MK203863.

2.5. Data analysis

Parasitological data were analysed to estimate the prevalence and diversity of parasite species detected overall and within each sampled state and age groups. Parasite prevalences were expressed as a percentage of greyhounds infected with one or more parasite genera, with 95% confidence intervals calculated assuming a binomial distribution, using the software Quantitative Parasitology 3.0 (Rozsa et al., 2000).

To determine which factors were important in influencing infection patterns among greyhounds, we used generalised linear mixed models (GLMM). We modelled overall parasitism (i.e. infection with any parasite) and the five main parasite genera/species present separately (*Sarcocystis*, *Giardia*, Hookworm, *Toxocara canis* and *Cystoisospora*), as binomial dependent variables (the animal either had the parasite detected or did not) in separate model sets. For each parasite, we considered six different predictor variables that reflected hypotheses about the factors (predictors) that influenced infection patterns. The predictor variables used were states (five categories: WA, QLD, NSW, VIC and TAS), number of dogs owned, exposure to a dominant surface type (five categories: concrete, concrete/grass, grass, grass/sand and sand), treatment frequency (number of weeks between treatments) and age (juvenile or adult). For modelling purposes, pups were grouped with the juvenile life-stage as housing and husbandry conditions were the same. Diet was not included as a predictor variable because of insufficient variation among kennels; all greyhounds were fed raw meat (see Results). Within kennel variation was not examined. A random term was included in all models to account for the nesting of observations within kennels.

We used a model selection approach to determine which combination of predictors were most important in explaining infection patterns. All predictor variables were included in a single global model and compared against all possible subsets of that model. Collinearity between predictor variables was checked visually and by calculating variance inflation factors (VIF); VIFs greater than three were considered to have significant collinearity problems. To determine what influence age had on infection patterns, we reran the model selection process for dogs housed on concrete only (controlling for surface type), considering the influence of age and state (since the other predictors (number of dogs owned and treatment frequency) were found to be unimportant in initial modelling).

Akaike's Information Criteria (AIC) was used to assess the fit of each model, with smaller values reflecting a more parsimonious fit to the data that were observed. We derived variable importance for each predictor variable (the sum of the Akaike weights across all models in which a predictor variable was included) from each model set to indicate which of those factors were weighted most strongly in the model selection. The fit of the global model was calculated using the marginal R^2 (variance explained by the predictor variables alone) and the conditional R^2 (variance explained by both the predictors and random effects). Models, model selection and R^2 values were calculated using the packages lme4 (Bates et al., 2015) and MuMIn (Barton, 2016) in R 3.3.2 (R Core team, 2016).

3. Results

3.1. Overall prevalence and diversity of GI parasites within the sampled greyhound population

A total of 721 individual greyhounds were sampled from across five states of Australia. The sampled group came from a total of 81 kennels and consisted of 373 racing dogs, 99 juveniles, 159 pups, 59 breeding dams and 31 retired dogs (Table 1)

The total parasite prevalence observed within the sampled population was 60.3% and ranged from 50 to 70% between states (Table 2). Eleven parasite genera were detected, with *Sarcocystis*, hookworm, *Giardia* and *Toxocara* identified most frequently (Table 2). These results were compared to published prevalence studies on UK/USA greyhounds

Table 3
Prevalence (%) of the most common GI parasites obtained in the current study and those from similar prevalence studies.

	Total prevalence	Hookworm	*Toxocara*	*Giardia*	*Sarcocystis*	Reference
This study	**60.3**	**17.8**	**14.1**	**16.1**	**32.1**	
UK greyhounds	48	31.5	12	na	na	Jacobs and Prole, 1976
USA greyhounds	40.4	16	5	0.4	0.4	Ridley et al., 1994
Australian dog refuges	34.6	10.7	2.4	14.4	3.2	Palmer et al., 2008
Australian veterinary clinics	16	3.9	0.4	5.5	3.9	Palmer et al., 2008

Table 4
Molecular characterization of a subset of samples positive for species of hookworm, *Giardia*, *Taenia* and *Neospora/Hammondia* found in each geographical region.

	WA	*QLD*	*NSW*	*VIC*	*TAS*	*Total*
Hookworm sp.	8	2	5	3	5	23
*A. caninum***	–	2	–	1	–	3
*A. braziliense***	–	1	–	–	–	1
*A. ceylanicum***	–	–	–	–	1	1
U. stenocephala						
***Giardia* sp.**	15	3	7	8	9	42
G. canis						
***Taenia* sp.**	–	–	–	1	–	1
T. ovis						
***Neospora/Hammondia* sp.**	2	–	–	–	3	5
H. heydorni	–	–	–	1	–	1
N. caninum						

** Parasites with zoonotic potential.

and Australian dogs in Table 3.

3.2. Molecular characterisation

Molecular characterisation provided identification of species for a subset of samples positive for hookworm, *Taenia*, *Giardia* and *Neospora/Hammondia* (Table 4). From 50 hookworm samples, 35 were successfully amplified and sequenced. The dominant species detected was *Ancylostoma caninum* (n = 29); however, *A. braziliense* (n = 3), *A. ceylanicum* (n = 2) and *Uncinaria stenocephala* (n = 1) were also detected. Forty-two of 45 *Giardia* positive samples were sequenced at either the 18S or ITS2 regions, with all typing as *G. canis*. One of the two *Taenia* positive samples was successfully sequenced and identified as *T. ovis* and six of 14 samples positive for *Neospora/Hammondia* were sequenced with five identifying as *H. heydorni* and one as *N. caninum*.

3.3. Kennel management

The number of dogs housed within kennels ranged from two to more than 100, however the general housing of the different life-stages was largely the same (Table 5). Kennel managers advised they housed adult racing greyhounds individually in concrete kennels with minimal time spent on grass/sand surfaces, while juveniles/pups were housed in group runs (2–8 dogs), which were predominantly sand/grass surfaces. Dogs of all ages (post weaning) were provided a diet of raw meat and kibble. The removal of faeces from dog runs was reported as at least daily in most kennels (84%), with the others collected 4–5 times a week. All participants reported the regular use of anthelmintics to control gastrointestinal parasites within their kennels, with the most common treatment regime between 4–6 weeks (Table 5). A wide variety of anthelmintic products were used, with both canine-specific products and off-label use of livestock-specific products (Fig. 1).

3.4. Factors influencing parasitism

Model selection identified the major risk factors associated with parasitism as the surface on which dogs were housed, state of residence and, when controlling for surface type, age of dog (Table 6). The dominant surface on which dogs were housed was featured in 90–100% of all model weights for total parasite prevalence and the prevalence of all parasite groups except hookworms (Table 6). A greater proportion of dogs housed on substrates consisting of grass and/or sand were infected with all groups of parasites than those housed on concrete (Table 7). State of residence featured in 100% of all models for *Toxocara canis* (Table 6), with a greater prevalence in WA, Victoria and Tasmania (see Table 2). The prevalence of *Trichuris* spp. was also notably higher in Tasmania (10.7%) than other states (0.7–1.3%; Table 2), although this

Table 5
Husbandry procedures reported from kennel managers (n = 81) across all sampled regions.

Husbandry	*Age group*	*Factor*	*% kennels*
Dominant kennel surface	Adult	concrete	46
		concrete/sand/	53
		grass	1
		sand/grass	
	Juvenile/pup	concrete	2
		concrete/sand/	11
		grass	87
		sand/grass	
Number housed together	Adult	Individual	99
		Group	1
	Juvenile/pup	Group	100
Exposure to raw meat diet	Adult		100
	Pups post weaning		100
Removal of faeces	All ages	More than daily	27
		daily	57
		Less than daily	16
Frequency of anti-parasitic treatment	Adult/juvenile	4-6 weeks	57
		6-12 weeks	33
		< 12 weeks	8
		Unknown	2
	Pups < 8wks	2 weeks	100

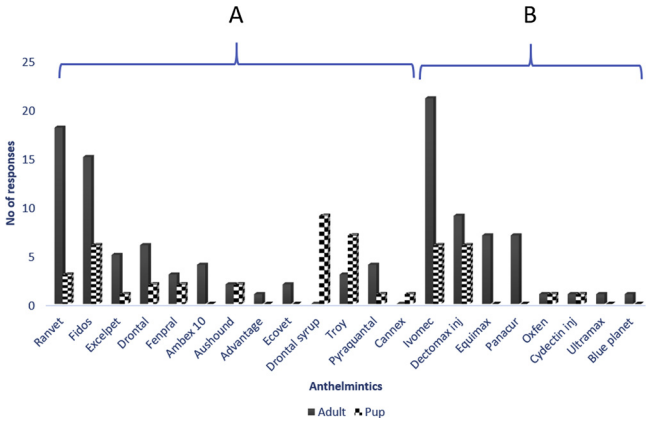

Fig. 1. Reported useage of anthelmintic by brand* for adult dogs and pups. Products registered for use on dogs (A) and those registered for use in livestock (B).
*active ingredients for each anthelmintic brand can be found in supplementary data Table1.

genus was not included in the GLMM analyses. Juvenile dogs had a greater prevalence overall and for all parasite groups than adult dogs (Fig. 2a), and also had a greater incidence of polyparasitism (presence of more than one parasite group in an individual host; Fig. 2b). However, when the model selection was rerun for dogs housed on concrete only (n = 372, controlling for surface type) to allow testing for an effect of age independent of surface type, age was only found to be an important risk factor for *Sarcocystis* spp. (Table 6). Interestingly, hookworm prevalence did not appear to be influenced by any of the environmental factors tested, however the high conditional R^2 indicates that there is more variation in infection prevalence at the kennel level that is not explained by any of the predictor variables included in the modelling.

Table 6
Association between environmental variables and prevalence of the most common parasite genera detected within the sampled greyhound population. R^2 values are reported that indicate the amount of variance explained by the fixed effects on their own (marginal) and the variance explained by both the fixed and random (kennel) effects (conditional). Importance values from the model selection analysis are provided for each of the predictor variables for each parasite. Values above 0.8 are bolded to indicate that they were strongly weighted (i.e. considered important) in the model selection analysis.

	Total prevalence	*Sarcocystis*	*Giardia*	Hookworm	*Toxocara canis*	*Cystoisospora*
R2 (marginal)	0.19	0.13	0.28	0.09	0.41	0.00
R2 (conditional)	0.34	0.34	0.37	0.58	0.49	0.00
Dominant Surface	**1.00**	**0.92**	**1.00**	0.49	**1.00**	**0.96**
State	0.43	0.22	0.59	0.20	**1.00**	0.05
Treatment Freq	0.68	0.65	0.55	0.30	0.30	0.43
No of Dogs Owned	0.31	0.42	0.38	0.27	0.41	0.68
Concrete only						
Age	0.54	**0.90**	0.30	0.27	0.37	*NA
State	0.20	0.08	0.74	0.18	**0.99**	

*NA - only adult dogs infected.

3.5. Awareness of zoonotic parasites

Of the 79 completed questionnaires, 54% (n = 43) of participants were aware of zoonotic parasites, 38% (n = 30) were not and 8% (n = 6) were unsure. From a list of potential zoonotic parasites, most respondents identified hydatid tapeworm (63%) as having zoonotic potential followed by hookworm (39%), roundworm (37%); flea tapeworm (16%), *Giardia* (11%), whipworm (9%) and *Cryptosporidium* (4%). When given a list of possible infection sources, most respondents identified removal of dog faeces (58%), followed by contact with soil the dog has had contact with (42%) and touching dog bedding (39%). The final question asked where participant obtained this information from, with most respondents identifying a veterinarian (35%) followed by friends/family (18%) and television (14%).

4. Discussion

The prevalence GI of parasites (60.3%) detected in this study was higher than reported in UK and USA greyhound studies (Jacobs and Prole, 1976; Ridley et al., 1994) and the most recent investigation into Australian dogs in refuges and veterinary clinics (Palmer et al., 2008). In addition, this study detected higher prevalences of all major parasite groups except hookworms, for which only the UK greyhound study reported a higher rate of infection.

The main risk factor associated with parasitism within the kennel environment in this study was the surface on which dogs were housed, with a greater parasite prevalence in dogs housed on grass/sand substrates. These substrates, as opposed to concrete, provide suitable conditions for the survival and development of the infective stages of parasites such as hookworm, and species of *Toxocara, Giardia*, *Strongyloides* and *Trichuris*, allowing for continual transmission to occur (Dunsmore et al., 1984; O'Lorcain, 1994;Paquet-Durand et al., 2007). The importance of kennel surface was obscured to some extent by the tendency to house susceptible animals (juvenile aged dogs) on substrates conducive to parasite transmission (grass/sand substrates), while adult dogs were usually housed in a concrete kennel environment. Young animals are often predisposed to a higher level of parasitism due to an under-developed immune system, and immediate

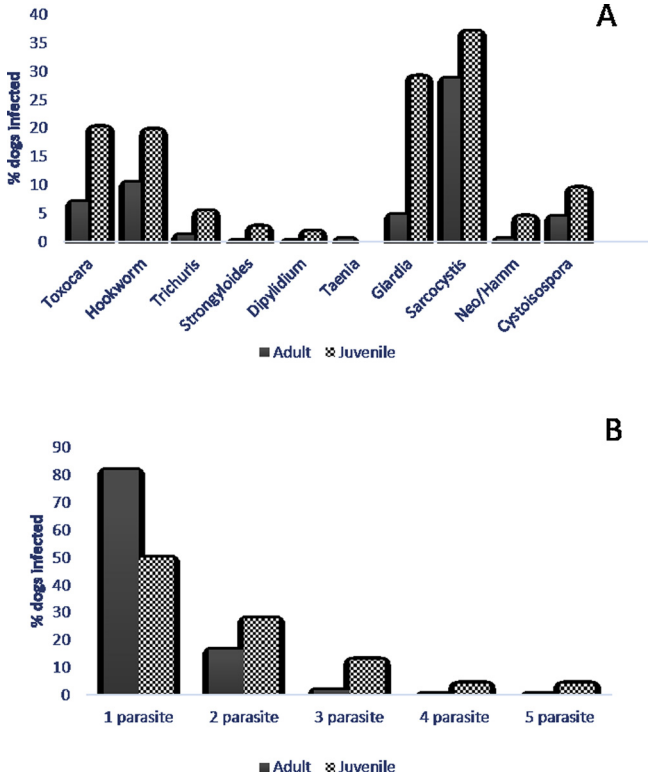

Fig. 2. a, b. Percentage prevalence of GI parasites detected between the adult (> 18 months) and juvenile (< 18 months) age group. Data presented for each parasite genera detected (2a) and for polyparasitism (2b).

Table 7
Prevalence (%) of GI parasite detected within dogs housed on the five dominant kennel surfaces identified within the study. Total prevalence (with 95% confidence intervals) of the five main parasite genera are presented for each substrate type.

Dominant surface	Total prev		Sarcocystis		Giardia		Hookworm		Toxocara canis		Cystoisospora	
	Prev	CI	Prev	CI	Prev	CI	Prev	CI	Prev	CI	Prev	CI
Concrete	56	50 – 61	36	31 – 41	7	04 – 10	17	14 – 21	10	07 – 13	3	01 – 05
Concrete/Grass	48	33 – 63	10	03 – 23	10	03 – 23	27	15 – 42	4	01 – 14	4	01 – 14
Grass	61	52 – 70	20	14 – 29	30	22 – 38	19	12 – 27	17	11 – 25	7	03 – 13
Grass/Sand	88	76 – 96	65	50 – 78	25	14 – 40	18	08 – 31	33	21 – 48	16	07 – 29
Sand	92	80 – 98	35	22 – 51	48	33 – 63	25	14 – 40	29	17 – 44	17	07 – 30

exposure in utero or at birth to parasites such as hookworm and *Toxocara* spp. (Burke and Roberson, 1985; Kalkofen, 1974). In the current study, we found a higher prevalence of all parasite groups in juvenile greyhounds, however, when controlling for kennel surface, age was an important risk factor only for *Sarcocystis* spp. *Sarcocystis* was the only parasite group tested that cannot be influenced by a contaminated surface, as transmission is only through ingestion of raw meat. While it is not possible to truly disentangle the influence of age and substrate on infection patterns in our study, our results suggest that surface substrates which facilitate parasite transmission may be more important when ascertaining the risk of parasitic infections than age. However, age related immunity is an important factor in host/parasite interactions and it is probable that the two variables, age and substrate, are acting synergistically to increase the incidence of infection within the younger age group.

Geographic region had an apparent influence on the prevalence of specific parasite genera across the different states sampled in this study. *Toxocara canis* infections were more prevalent in Victoria, Tasmania and Western Australia, while *Trichuris* spp. infections were more prevalent in Tasmania. This variation in prevalence could be due to climatic conditions allowing for different parasite genera to be maintained in the environment. While all regions sampled experience seasonal fluctuations and most samples were collected in winter the regions sampled from Queensland and New South Wales usually experience longer periods of warm moist weather compared to the more southern states of Victoria and Tasmania, which experience longer periods of cool and wet weather, the latter conducive for the survival of *Toxocara* and *Trichuris* ova (O'Lorcain, 1994; Traversa, 2011). Interestingly geographic region was not influential for hookworm, with reasonably high prevalence levels detected across all states. This lack of climatic influence on prevalence levels is most likely due to the collection of samples in winter in all regions except Western Australia. Hookworm infections are generally lower in winter (Becker et al., 1977; Croese, 1995) and a spring/summer sampling regime may have revealed significant differences in prevalence levels between regions. Nevertheless, the presence of different species of hookworm in different states did suggest a climatic influence was at play (Beveridge, 2002). Genotyping revealed *A. caninum* as the dominant species present and was detected in all geographic regions, reflecting its cosmopolitan distribution (Miller, 1971). However, *A. braziliense* and *A. ceylanicum* which both favour a warm moist environment were detected in Queensland, while *Uncinaria stenocephala* which prefers cooler temperatures for larval development was only found in Tasmania (Levine, 1968; Provic, 1998; Beveridge, 2002; Palmer et al., 2008). While one Victorian greyhound was found to be infected with *A. braziliense*, it is possible this animal was recently imported from another state, as is common in the greyhound industry. The high levels of hookworms detected in winter would suggest that these parasites may be a significant problem in high-risk regions and warrant targeted control strategies.

Diet was not tested as a variable influencing parasite infection as all dogs in the study were fed a diet of raw meat and kibble from weaning and throughout adulthood. However, this diet explains the high prevalence of *Sarcocystis* sp. (32.2%) and the other food-born parasites *Taenia* spp. and *Neospora/Hammondia*-like species, both of which were detected at much lower levels (0.3% and 1.9% respectively).

The health implications of chronic parasitic infections for a growing dog are significant. Perhaps even more important than the acute symptoms of parasitic infections are the sub-clinical effects of chronic infections which result in ill thrift and failure to thrive, greatly reducing the ability of a greyhound to meet its full athletic potential. Young animals are particularly susceptible to hookworm infections and the anaemia caused by heavy infections can be severe and sometimes fatal (Miller, 1966). Heavy infections with species of *Toxocara* and *Trichuris* can also be hazardous, with the potential development of intestinal obstruction or rupture and bloody diarrhoea (Soulsby, 1985; Traversa, 2011). In addition, both hookworm and *Toxocara* larvae have a migratory period through the host body leading to disruption and damage of organs such as the liver and lungs, which in chronic infection may be a continuous occurrence (Dillon et al., 2013). *Giardia* spp. which were also commonly detected in this study, are often related to diarrhoea, with young dogs again at most risk (Epe et al., 2010). The detection of *Neospora caninum* in one kennel is of particular importance for breeding kennels, as infected dams can transmit the infection to their litters, resulting in neurological disease and often death of the pups (Dubey et al., 1988). The high prevalence of *Sarcocystis* spp. is not considered to be of clinical concern, with most signs occurring in the intermediate herbivore host.

A significant risk from zoonotic parasites was identified within the sampled kennels, with six of the eleven parasite genera considered zoonotic. Of particular concern are the levels detected in each state for *T,canis.* (1.3%–30%), hookworm (10%–30%) and *Giardia* spp. (9–22%). Ingestion of *T. canis* eggs obtained from a contaminated environment can lead to visceral and/or ocular larva migrans involving the liver, lungs and eyes of humans (Beaver et al., 1952). The larvae of all canid hookworm species can penetrate the skin causing cutaneous larva migrans in humans (Bowman et al., 2010). More importantly, *A. ceylanicum* can result in patent intestinal infections (Carroll and Grove, 1984). While genotyping of a subset of *Giardia*-positive samples detected only *G. canis*, which is not thought to be zoonotic, it is possible that the zoonotic species *G. duodenalis* and *G. enterica* could be present at a low prevalence. The potential for zoonotic transmission may be increased by the finding that 46% of questionnaire participants indicated they were unaware or unsure of any zoonotic parasites and therefore not taking appropriate precautionary measures.

Parasite control within kennels is heavily reliant on chemotherapy, with all trainers practicing regular anthelmintic treatment programmes; the most commonly reported treatment frequency of once every 4–6 weeks (56.5%). This frequent use of anthelmintics and the high level of GI parasites detected would suggest that chemotherapy is not controlling parasitism within the kennel environment. The GLMM analysis supported this assumption, as no influence of treatment frequency on any of the parasite genera was detected. This apparent lack of efficacy is most likely related to the habit of continuously housing young dogs on contaminated environments which allow for constant reinfection to occur within most deworming periods. It is common practice in the Australian greyhound kennel to rear pup/juveniles on large grassy runs while racing age dogs are brought into the kennel (concrete surface) for close monitoring of diet and training requirements. Anecdotally, veterinary personnel within the greyhound industry considered the possibility of anthelmintic resistance, however ascertaining anthelmintic efficacy in this study was inhibited by a number of factors, such as the almost complete use of broad-spectrum anthelmintics, which greatly hinders the identification of which active ingredient may or may not be effective; the large range of products used, particularly the array of off-label product use; and the possibility of inefficient administration of treatment to dogs. Anthelmintic resistance among canine parasites has rarely been established, but is considered a serious risk (Kopp et al., 2007; Ridley et al., 1994). The high-level and indiscriminate use of broad-spectrum anthelmintics in dog populations experiencing continual parasitic infection pressure, as identified in this study, certainly provides suitable conditions for the development of chemical resistance (Wolstenholme et al., 2004).

The issue of parasitism is not specific to greyhound kennels, but rather a problem faced by all intensive animal housing facilities. Livestock farmers have been plagued with parasite control issues for decades and as such have had to develop a range of methods to control parasite infections within their animals (Chandrawathani et al., 1998; Barger, 1996; Waller, 1993). Of major significance is the recognition within the livestock industry that total reliance on anthelmintics is no longer viable, and integrated pest management strategies are now frequently employed to reduce the level of exposure animals have to the infective stages of parasites (Krecek and Waller, 2006; Waller, 2003). In

this study the greatest risk factors for parasitism were young animals on grass/sand substrates. Strategies that could mitigate these high-risk environments include providing clean runs on which to rear pups and juvenile dogs; resting runs for long periods to be used in rotation; moving dogs post-treatment to clean runs to avoid immediate reinfection; and developing strategic anthelmintic treatment regimens based on known parasitic infections, rather than the indiscriminate use of broad-spectrum anthelmintics.

5. Conclusions

Surface of which dogs were housed was found to be the major risk factor associated with parasitic infections in the kennel environment, which is exacerbated by the practice of continuously rearing young dogs on the same contaminated grass/sand surfaces. Reliance on chemotherapy for complete parasite control is not effective and alternate control strategies are required. Many parasites with zoonotic potential were detected, however general awareness of zoonotic transmission among kennel personnel appears to be low.

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Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetpar.2018.11.013.

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