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The veterinary and public health significance of hookworm in dogs and cats in Australia and the status of *A. ceylanicum*

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Abstract

There is no current information regarding the prevalence of hookworm in Australian dogs and cats and based on the results of studies conducted over 20 years ago, where high prevalences of helminths were recorded, the prophylactic administration of broad spectrum anthelmintics has been advocated. During this study, faecal samples were collected from dogs (*n* = 1391) and cats (*n* = 1027) across Australia. Samples were examined by microscopy and information regarding the demographics of each animal, and the management practices they experienced were recorded. A highly sensitive and species-specific PCR-RFLP technique was utilized to differentiate the various hookworm species which can infect dogs and cats directly from eggs in faeces. The prevalence of hookworm in dogs and cats was found to be 6.9% and 1.4%, respectively. *Ancylostoma ceylanicum* was detected for the first time in Australia in 10.9% of the dogs found positive for hookworm. Significantly, *A. ceylanicum* is

capable of causing a patent infection in humans. After adjusting for other factors with multiple logistic regression, dogs from refuges, dogs originating from a tropical climatic zone, dogs aged 1 year or less, and those dogs which had not received anthelmintics were significantly more likely to be parasitized. Only univariate analysis was conducted for the cats as there were too few samples positive for hookworm. Cats were more likely to be infected with hookworm if they were from refuges, originated from a tropical climatic zone, and had not received treatment with anthelmintics. The results of this study demonstrates the importance of having current information regarding the prevalence of parasites of dogs and cats and the risk factors associated with infection, as well as the need to reassess the veterinary and public health concerns regarding hookworm infection and its control, which are currently based on out-dated information.

Keywords: Hookworm; *Ancylostoma;* PCR RFLP; Australia; Dogs; Cats

1. Introduction

Hookworm infection in dogs and cats can result in serious disease and even death. Furthermore, most of the species of hookworms which infect dogs and cats are zoonotic. There are no current data available on the prevalence of hookworm in Australia, possibly because of a sense of security provided by the widespread use of broad spectrum anthelmintics. Indiscriminant use of anthelmintics without knowledge of the prevalence of the parasite in question may hasten the development of anthelmintic resistance (Thompson, 1999; Cohen, 2000; Laffont et al., 2001; Thompson and Roberts, 2001; Irwin, 2002). Moreover, knowledge of the prevalence of hookworm is important with regard to public health. Previous studies in Australia have implicated *Ancylostoma caninum* as the leading cause of human eosinophilic enteritis (EE) (Prociv and Croese, 1990). Between 1988 and 1992 an outbreak totalling 150 cases of EE was reported and the dog hookworm *A. caninum* implicated as the causal agent (Loukas et al., 1992; Croese et al., 1994).

Traditionally, identification of hookworm species in dogs and cats required coprological or post-mortem examination of the adult worms, based on morphological differences between the species

of *Ancylostoma* (Yoshida et al., 1971; Soulsby, 1982). This is very time consuming, labor intensive and requires skilled personnel (Traub et al., 2004). In some instances, the species of hookworm involved in an infection has been identified based on the morphology of the eggs recovered from the faeces (Dunsmore and Shaw, 1990), but this is not appropriate as there is overlap in egg dimensions between most species of hookworm affecting dogs and cats. However, Traub et al. (2004) recently developed a highly species-specific and sensitive PCR-RFLP technique to detect and differentiate canine *Ancylostoma* spp. directly from eggs in faeces, this obviates the requirement for the use of tedious diagnostic methods of hookworm identification. This technique was applied to a large scale epidemiological study and allowed canine hookworm identification to be conducted rapidly, with ease and accuracy (Traub et al., 2004).

The hookworm species previously thought to have been identified in Australian dogs and cats included *A. caninum*, *Ancylostoma tubaeforme*, *Ancylostoma braziliense*, *Ancylostoma ceylanicum*and *Uncinaria stenocephala*. However, the only reports of *A. ceylanicum* in Australia (Stewart, 1994; Gasser et al., 1996; Adams, 2004) have been found to be based on a misidentification of *A. braziliense* as *A. ceylanicum* (Traub et al., 2006; e Silva et al., 2006).

There is no current data on the prevalence of hookworm in Australia. High hookworm prevalence was recorded in surveys conducted over 20 years ago (Table 1). *Ancylostoma caninum* was recorded as the predominant hookworm of dogs in the warmer regions of Australia. Although *A. caninum* is regarded as an uncommon parasite of cats (Setasuban and Waddell, 1973; Kelly and Ng, 1975; Wilson-Hanson and Prescott, 1982; Beveridge, 2002), Stewart (1994) found it in 6 of 16 cats examined in Townsville. *U. stenocephala* is a common parasite of dogs in southern Australia and is less common in cats (Beveridge, 2002).

The predominant species of hookworm in cats in Australia is considered to be *A. tubaeforme* (Table 1).

Prior to 1951, *A. ceylanicum* was regarded as a synonym of *A. braziliense* (Yorke and Maplestone, 1992; Skrjabin et al., 1951), until Biocca (1951) provided morphological evidence that they were

different. *A. braziliense* has only been identified in a handful of studies and has been reported in dogs and cats in north Queensland (Heydon, 1929; Seddon, 1958; Heydon and Bearup, 1963; Setasuban and Waddell, 1973; Taveros, 1990; Stewart, 1994) with a single occurrence in a dog from Sydney (Kelly and Ng, 1975). Specimens recovered by Heydon (1929) from cats in Townsville were identified as *A. braziliense* prior to the morphological criteria established by Biocca (1951), and hence need to be reexamined.

The primary aim of this study was to establish the prevalence, species distribution and risk factors associated with hookworm infection in dogs and cats in Australia and in doing so, readdress the veterinary and public health concerns associated with these parasites as well as their control. The PCR-RFLP designed by Traub et al. (2004) was modified to allow differentiation of all hookworm species known to infect dogs and cats and was applied in the epidemiological screening of faecal samples. Material from the studies of Heydon (1929), identified as *A. braziliense*, were re-examined.

2. Material and methods

2.1. Source of faeces

Between March 2004 and September 2005 faecal samples were collected from dogs and cats from across Australia from both urban and rural locations. A total of 1391 canine faecal samples were collected from three sources: refuges (n = 568), dogs presented to veterinary clinics without gastrointestinal (GI) complaints (n = 766) and Aboriginal communities (n = 57). A total of 1027 faecal samples were collected from cats from two sources: refuges (n = 461) and cats presented to veterinary clinics without GI complaints (n = 566).

Demographic data (age, gender, neutering status, and breed) and worming history were collected for all animals sampled. A questionnaire was administered to pet owners to collect information on factors likely to impact specifically on the prevalence of GI parasites such as diet, number of dogs in a household, number of cats in a household, time spent with other dogs and cats, time spent in an area used by other dogs and cats, place of defaecation and how excrement was disposed of.

The locations of the places sampled were categorized into one of three different climatic zones (tropical, arid or temperate) (Fig. 1). These three climatic zones were a simplification of the six climatic zones depicted on a climatic map for temperature and humidity produced by the Bureau of Meteorology (Bureau of Meteorology, Commonwealth of Australia, 2003). According to the present study a 'tropical' climate included those regions on the climatic map which were classified as having a hot humid summer or a warm humid summer, while an 'arid' climate included areas which were deemed as having a hot dry summer, mild winter or hot dry summer, cold winter and a 'temperate' climate included those areas classified as warm summer, cool winter or mild/warm summer, cold winter.

2.2. Parasitological procedures

Formalized faecal samples were examined for parasites initially using a simple faecal smear followed by centrifugal flotation in saturated sodium chloride and glucose, and microscopy, as previously described by Henriksen and Christensen (1992).

2.3. Molecular methods

One hundred and 10 samples, from 96 dogs and 14 cats, which were found positive for hookworm via microscopy were then characterized using molecular methods.

2.4. DNA extraction

Two hundred micrograms of canine faeces was suspended in 1.4 ml ATL tissue lysis buffer (Qiagen, Hilden, Germany). This suspension was then subjected to five cycles of freeze-thawing at liquid nitrogen temperatures followed by boiling for 10 min. DNA was then isolated from the supernatant using the QIamp DNA Mini Stool Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Final elutions of DNA were made in 50 μl of elution buffer instead of 200 μl as recommended by the manufacturer.

PCR for differentiating *A. caninum, A. tubaeforme, A. ceylanicum*and *U. stenocephala* from *A. braziliense*.

A section of the internal transcribed spacer (ITS)-1, 5.8S and ITS-2 regions of *A. caninum*, *A. tubaeforme*, *A. ceylanicum*, *A. braziliense* and *U. stenocephala* was amplified using previously published primers, PCR reaction, and cycling conditions (Traub et al., 2004). An amendment to this PCR was however recently made by Traub et al. (2006) whereby it was recognized that the reference sequence of *A. ceylanicum* was in fact that of *A. braziliense*. Hence RTGHFI (5′-CGTGCTAGTCTTCAGGACTTTG-3′) and RTABCR1 (5′-CGGGAATTGCTATAAGCAAGTGC-3′) were used to amplify a 545 bp region of *A. caninum*, *A. tubaeforme*, *A. ceylanicum* and *U. stenocephala*. In a separate PCR a 673 bp region of *A. braziliense* was amplified using RTGHF1 and the highly specific reverse primer RTAYR1 (5′-CTGCTGAAAAGTCCTCAAGTCC-3′) (Traub et al., 2004).

2.5. PCR-linked restriction fragment length polymorphism (RFLP)

Appropriate restriction enzymes for the PCR-RFLP to differentiate the species of hookworms were selected using the PCR-RFLP MONSTER© (www.rflpmonster.com) program. PCR-RFLP Monster© is a web-based PCR-RFLP assay design program. It replaces the manual and laborious task of choosing the most suitable restriction endonuclease or combination of endonucleases to distinguish a given set of organisms.

In accordance with the outputs produced by PCR-RFLP Monster©, amplified ITS PCR products of RTGHF1-RTABCR1 were subjected to direct digestion with *Hin*F1 in order to differentiate *A. caninum* and *A. tubaeforme* from *A. ceylanicum*and *U. stenocephala* (Table 2). Restriction enzyme *RSa*I was then used to differentiate *U. stenocephala* and *A. tubaeforme*, while *A. caninum* and *A. ceylanicum* have identical cutting patterns with this enzyme (Table 2). The RFLP reaction for *Hin*FI (Promega) and *RSa*I (Promega) restriction endonuclease were identical. Ten microlitres of PCR product were digested with 0.5 μl (5 units) of a restriction endonuclease at 37 °C for 16 h in a volume of 20 μl.

2.6. Sequencing of A. ceylanicum positive samples

Ten out of the 96 canine positive hookworm samples were identified as *A. ceylanicum* from the results of the RFLP. Four of these samples were reamplified using the aforementioned PCR and the PCR products were purified using Qiagen spin columns (Qiagen) and sequenced using an ABI 3730 48 capillary DNA sequencer (Applied Biosystems) using Big Dye Version 3.1 terminators. Sequences were analyzed using Finch TV Version 1.3.1 (Geospiza Inc.) and were deposited to Genbank (Genbank Accession numbers: DQ831518, DQ831519, DQ831517 and DQ831520). Sequences were then compared with *A. ceylanicum*(Genbank Accession number: DQ381541), two of the sequences showed 100% identity (DQ831518 and DQ831519), while the other two (DQ831517 and DQ831520) were 99.8% and 99.6% similar, respectively.

2.7. Morphological identification of specimens from the Queensland Museum

Specimens collected from cats in Townsville Australia, by GM Heydon between 1923 and 1928, stored in 70% ethanol at the Queensland Museum as *A. braziliense* under the Accession numbers GL11582, GL11583 and GL11633, were identified morphologically. Identification was based on the structure of the teeth in the cutting plates, and the structure of the male bursal rays after Biocca (1951).

2.8. Statistical analysis

The prevalence and 95% confidence intervals (CI) were calculated for hookworm in both dogs and cats. Association between parasitism and host and management factors were initially made using univariate analyses of odds ratios and their 95% confidence intervals (Martin et al., 1987), the Chi-square test for independence or the analysis of variance (Martin et al., 1987). Associations between host factors (age, gender, neutering status and purebred/crossbred), management factors and climate and hookworm infection were evaluated for all dogs and cats sampled.

Multivariate logistic regression was then used where data were substantial enough to quantify the association between the presence of hookworm infection and host and management variables after adjusting for other variables. Only variables significant at $P < 0.25$ in the univariate analysis were

considered eligible for inclusion in the multiple logistic regression analysis (Hosmer and Lemeshow, 1989; Frankena and Graat, 1997). Dummy variables were generated for any categorical variable with more than two levels. Backward elimination was used to determine which factors could be dropped from the multivariable model (Hosmer and Lemeshow, 1989). The goodness of fit of the model was assessed with the Hosmer-Lemeshow statistic (Lemeshow and Hosmer, 1982).

Data were analyzed and statistical comparisons were performed using SPSS (SPSS for Windows, Version 14.0, Rainbow Technologies) and Excel 2002 (Microsoft).

3. Results

3.1. Prevalence of hookworm

The overall prevalence of infection with hookworm determined by microscopy was 6.9% (95% confidence interval [CI] 5.6, 8.2) and 1.4% (95% CI 0.7, 2.1) for dogs and cats, respectively. Dogs sourced from Aboriginal communities had the highest prevalence (14.0%) followed by those from refuges (11.4%) and from veterinary clinics (3.0%). Cats sourced from refuges had a higher prevalence (2.8%) than cats from veterinary clinics (0.2%). The prevalence of hookworm also varied greatly between geographical regions as shown in Table 3. The categorization of prevalence according to state can however be misleading, as state boundaries are merely superimposed arbitrary delineations. The variation in hookworm prevalences is best correlated with climatic zones as reflected in Table 4.

3.2. PCR-RFLP

Of the 96 microscopy positive dog hookworm samples 92 were amplified successfully by PCR. Of the 14 microscopy positive cat hookworm samples 10 were amplified successfully. The inability to amplify all of the samples was thought to be associated with inhibitory factors within the PCR reaction or an inadequate amount of DNA. No products were observed for the *A. braziliense* species-specific PCR. PCR patterns of the hookworms amplified are shown in Fig. 2.

PCR products of RTGHF1-RTABCR1 were then further subjected to the RFLP using *Hin*FI and *RSa*I (Figs. 3 and 4). Of the 92 dog samples, 70.7% were found positive for single hookworm infections with *A. caninum*, 6.5% positive for single infections with *A. ceylanicum*, 10.9% positive for single infections with *U. stenocephala*, 4.3% had mixed infections with both *A. caninum*and *A. ceylanicum* and 2.2% had mixed infections with both *A. caninum* and *U. stenocephala*. All 10-cat samples amplified had single infections; 70% were found positive for *A. tubaeforme* and 30% were found positive for *A. caninum*. The distributions of the different hookworm species identified are depicted in Tables 5 and 6.

3.3. Morphological identification of specimens from the Queensland Museum

Three species of *Ancylostoma* were identified from the Museum specimens labelled as *A. braziliense*. While *A. braziliense* was present in all three specimen containers, a single male specimen of *A. ceylanicum* was also present in GL11582 (Fig. 5), and 24 male *A. tubaeforme* in GL11633. This represents the first confirmed report of *A. ceylanicum* in Australia.

3.4. Risk factors for parasitism

3.4.1. Multivariate analysis for dogs

All variables significant at $P \leq 0.25$ on univariate analysis were included in the initial multivariable model. Dogs from refuges, dogs originating from a tropical climatic zone, dogs aged 1 year or less, and those dogs which had not received anthelmintics were significant in the final model for parasitism (Table 7).

3.4.2. Univariate analysis for dogs from veterinary clinics

There were too few dogs sourced from veterinary clinics positive for hookworm and as such only univariate analysis was conducted. Variables were considered significant if the *P*-value was ≤ 0.05. According to univariate analysis dogs from veterinary clinics were more likely to be infected with hookworm if they originated from a tropical climate and if they were male (see Table 8).

3.4.3. Univarite analysis for cats

Only univariate analysis was conducted for the cats as there were too few samples positive for hookworm to enable multivariate analysis. Variables were considered significant if the *P*-value was ≤0.05. Cats from refuges, cats originating from a tropical climatic zone, and those cats which had not received anthelmintics were all considered significant factors via univariate analysis for hookworm infection (Table 9).

4. Discussion

The prevalence of hookworm reported in this study is considerably lower than prevalences reported in previous studies (Table 1). The most obvious explanation for this result is the widespread use of anthelmintics in the last 10–15 years, not only in pet dogs and cats but also in animals kept in refuges.

Another significant finding of the present study was the identification of *A. ceylanicum* in Australia. All of the species of hookworm which may infect Australian dogs and cats are believed to have been introduced in the last 200 years with the arrival of European settlers and their domestic animals (Beveridge, 2002). The dingo was probably introduced 4000 years ago, but the parasites that accompanied it are unknown (Beveridge, 2002). It was puzzling that *A. ceylanicum* had not been found previously in Australia given that *A. braziliense*, *A. caninum* and *A. tubaeforme*have all been recovered from Australian dogs and cats in the past (Traub et al., 2006). Furthermore, the absence of *A. ceylanicum*from northern Australia was intriguing given the endemic distribution of this parasite in neighbouring regions: Malaysia (Yoshida et al., 1973; Sheikh et al., 1985), Borneo (Choo et al., 2000), Indonesia (Margono et al., 1979), Papua New Guinea (Anten and Zuidema, 1964) and the Solomon Islands (Beveridge, 2002). Finding *A. ceylanicum* in Australia is of public health significance since it is the only hookworm species capable of infecting dogs and cats which has been shown to produce both experimental and naturally patent infections in humans (Areekul et al., 1970; Chowdhury and Schad, 1972).

A. braziliense was the only species of hookworm not recovered from the present study. Although this species of hookworm is known to occur in Australia it has only rarely been reported in previous studies (Heydon, 1929; Seddon, 1958; Heydon and Bearup, 1963; Setasuban and Waddell, 1973; Kelly and Ng, 1975; Taveros, 1990).

Important risk factors for hookworm infection for both dogs and cats included climate, the age of the animal and whether the dog or cat was a pet or had originated from a refuge (Tables 7–9). A considerably higher prevalence of hookworm infection was found in those animals originating from a tropical climate (Table 4). With the exception of *U. stenocephala,* warm temperatures and adequate moisture to prevent desiccation provide the most optimal environment for hookworm egg hatching and larval development (Dunsmore and Shaw, 1990). In contrast, *U. stenocephala*requires lower temperatures for hatching and development (Dunsmore and Shaw, 1990) and this is reflected in the geographical distribution of this parasite (Table 5). Higher parasite prevalences are often noted in younger animals, most likely because of their immature immune systems and also because of transmammary infection routes of *A. caninum*. As dogs grow older, they become more resistant to hookworms whether or not they experience infection (Bowman et al., 2003).

Animals originating from refuges are at greater risk of hookworm infection for a multitude of reasons. Many refuge animals have originated from households where they were provided with minimal care, and anthelmintic administration is unlikely. These animals may also have had a history of roaming and hunting prior to being placed in the refuge and hence could have had exposure not only to contaminated environments but may have also eaten paratenic hosts. Carelessly managed refuges where faeces are allowed to accumulate have also been associated with large amounts of infective larvae (Bowman et al., 2003).

In conclusion, the results of this study demonstrate that it is imperative to have current information regarding the prevalence of parasites and the risk factors associated with infection. This will allow for the more effective implementation of strategic control programmes (Eckert, 1997).

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Table 1. Previous prevalence studies in Australia ordered chronologically

Location	Source of animals	Method of diagnosis	Dog/cat	Species of Hookworm	Prevalence (%)	Reference
Cairns	Pound	Necropsy	Dog (*n* = 10)	*A. caninum*	100	Setasuban and Waddell (1973)
Brisbane	Pound	Necropsy	Dog (*n* = 66)	*A. caninum*	67	
Sydney	Pound	Necropsy	Dog (*n* = 464)	*A. caninum*	36	Kelly and Ng (1975)
North-eastern Victoria	Pound	Necropsy	Dog (*n* = 734)	*A. caninum*	2	Blake and Overend (1982)
Central western Queensland	Sheep dogs	Faecal exam	Dog (*n* = 112)	A. caninum[a]	20	Cornack and O'Rourke (1991)
South of Sydney	Aboriginal community	Necropsy	Dog	*A. caninum*	100	Jenkins and Andrew (1993)
Kimberley	Aboriginal community	Necropsy	Dog (*n* = 188)	*A. caninum*	51	Meloni et al. (1993), Thompson et al. (1993)
Perth	Vet practices, exercise areas, pet shops, refuges, breeding kennels	Faecal exam	Dog (*n* = 421)	A. caninum[a]	2	Bugg et al. (1999)
Sydney	Pound	Necropsy	Cat (*n* = 404)	*A. tubaeforme*	35	Kelly and Ng (1975)
Brisbane	Pound	Necropsy	Cat	*A. tubaeforme*	19	Wilson-Hanson and Prescott (1982)
Brisbane	Pound	Necropsy	Cat	*A. tubaeforme*	81	Prescott (1984)
Kimberley	Aboriginal community	Necropsy	Cat (*n* = 34)	*A. tubaeforme*	20	Meloni et al. (1993), Thompson et al. (1993)
Northern Territory	Feral cats	Necropsy	Cat	*A. tubaeforme*	13	O'Callaghan and Beveridge (1996)
Sydney	Pound	Necropsy	Dog (*n* = 464) Cat (*n* = 404)	*U. stenocephala*	9.5 0.5	Kelly and Ng (1975)
Tasmania	Feral cats	Necropsy	Cat (*n* = 86)	*U. stenocephala*	2	Gregory and Munday (1976)
North-eastern Victoria	Pound	Necropsy	Dog (*n* = 734)	*U. stenocephala*	26	Blake and Overend (1982)
Adelaide	Vet practices	Faecal exam	Dog (*n* = 1614) Cat (*n* = 376)	U.stenocephala[a]	4 0.3	Moore and O'Callaghan (1985)
Perth	Private owners, refuges, boarding cats, pet shops, breeders	Faecal exam	Cat (*n* = 418)	Unknown	0.2	McGlade et al. (2003)

[a]Diagnosis of hookworm species was made according to egg measurements which is not a reliable diagnostic method.

Table 2. Restriction profile

Enzyme	Species	Band sizes
*Hin*FI	*A. tubaeforme*	7[a], 50[a], 85[a], 187, 216
	A. caninum	7[a], 49[a], 85[a], 187, 216
	A. ceylanicum	49[a], 194, 301
	U. stenocephala	40[a], 194, 303
*RSa*I	*A. tubaeforme*[b]	40[a], 77[a], 151, 277
		40[a], 77[a], 428
	A. caninum	268, 276
	A. ceylanicum	286, 276
	U. stenocephala	545

[a]Product too small to view.

[b]Allelic polymorphism produces two separate RFLP banding patterns.

Table 3. Prevalence of hookworm in dogs according to the state in Australia

State	Number of dogs positive	Total number of dogs examined	Prevalence (%)
Northern Territory	23	131	17.5
Queensland	28	274	10.2
Western Australia	26	321	8.1
Victoria	6	152	3.9
New South Wales	8	219	3.7
Australian Capital Territory	2	85	2.3
Tasmania	2	100	2.0
South Australia	1	109	0.9

Note: The prevalences of hookworm within a state tended to vary between the locations sampled, especially in those states which cover more than one climatic zone.

Table 4. Prevalence of hookworm in dogs according to climate

Climate	Prevalence (%)
Tropical	17.3 (*n* = 412)
Arid	1.7 (*n* = 120)
Temperate	2.7 (*n* = 859)

Table 5. Location of *A. caninum*, *A. ceylanicum* and *U. stenocephala*positive samples in dogs

Species of hookworm	Location of samples
Ancylostoma caninum	Brisbane, Broome, Cairns, Darwin, Derby, Gapuwiyak, Geraldton, Gold Coast, Sunshine Coast, Hobart, Melbourne, Taree, Alice Springs
A. ceylanicum	Broome, Brisbane, Sunshine Coast, Melbourne, Alice Springs
Uncinaria stenocephala	Ballarat, Canberra, Geraldton, Melbourne, Moss Vale, Perth, Port Lincoln, Sale, Sydney

Table 6. Location of *A. tubaeforme* and *A. caninum* positive samples in cats

Species of hookworm	**Location of samples**
A. caninum	Darwin, Alice Springs
A. tubaeforme	Cairns, Brisbane, Bundaberg, Darwin

Table 7. The results of the multiple logistic regression model for the relationships between the variables examined and hookworm infection in dogs

Factor	**Odds ratio (95% CI)**	***P*-value**
Dogs from refuges	2.9 (1.6–5.3)	0.001
Dogs not from a refuge	1.0	
Dogs residing in a tropical climate	5.6 (3.3–9.5)	<0.001
Dogs not from a tropical climate	1.0	
Dogs ≤1 year of age	1.7 (1.0–2.9)	0.044
Dogs >1 year of age	1.0	
Dogs which had not received anthelmintics treatment	2.6 (1.4–4.7)	0.002
Dogs which had received anthelmintic treatment	1.0	

Table 8. The results of univariate analysis for the variables likely to influence hookworm infection in dog's from veterinary clinics

Factor	**Odds ratio (95% CI)**	***P*-value**
Dogs residing in a tropical climate	3.5 (1.5, 8.1)	0.002
Dogs not from a tropical climate	1.0	
Male dogs	2.6 (1.0, 7.3)	0.049
Female dogs	1.0	

Table 9. The results of univariate analysis for the variables likely to influence hookworm infection in cats

Factor	**Odds ratio (95% CI)**	***P*-value**
Cats from refuges	16.4 (2.1, 125)	<0.001
Pet cats	1.0	
Cats residing in a tropical climate	8.4 (2.6, 26.9)	<0.001
Cats not from a tropical climate	1.0	
Cats which had not received anthelmintics treatment	22.7 (3.0, 166.7)	<0.001
Cats which had received anthelmintic treatment	1.0	

Fig. 1. Location of hookworm positive samples.

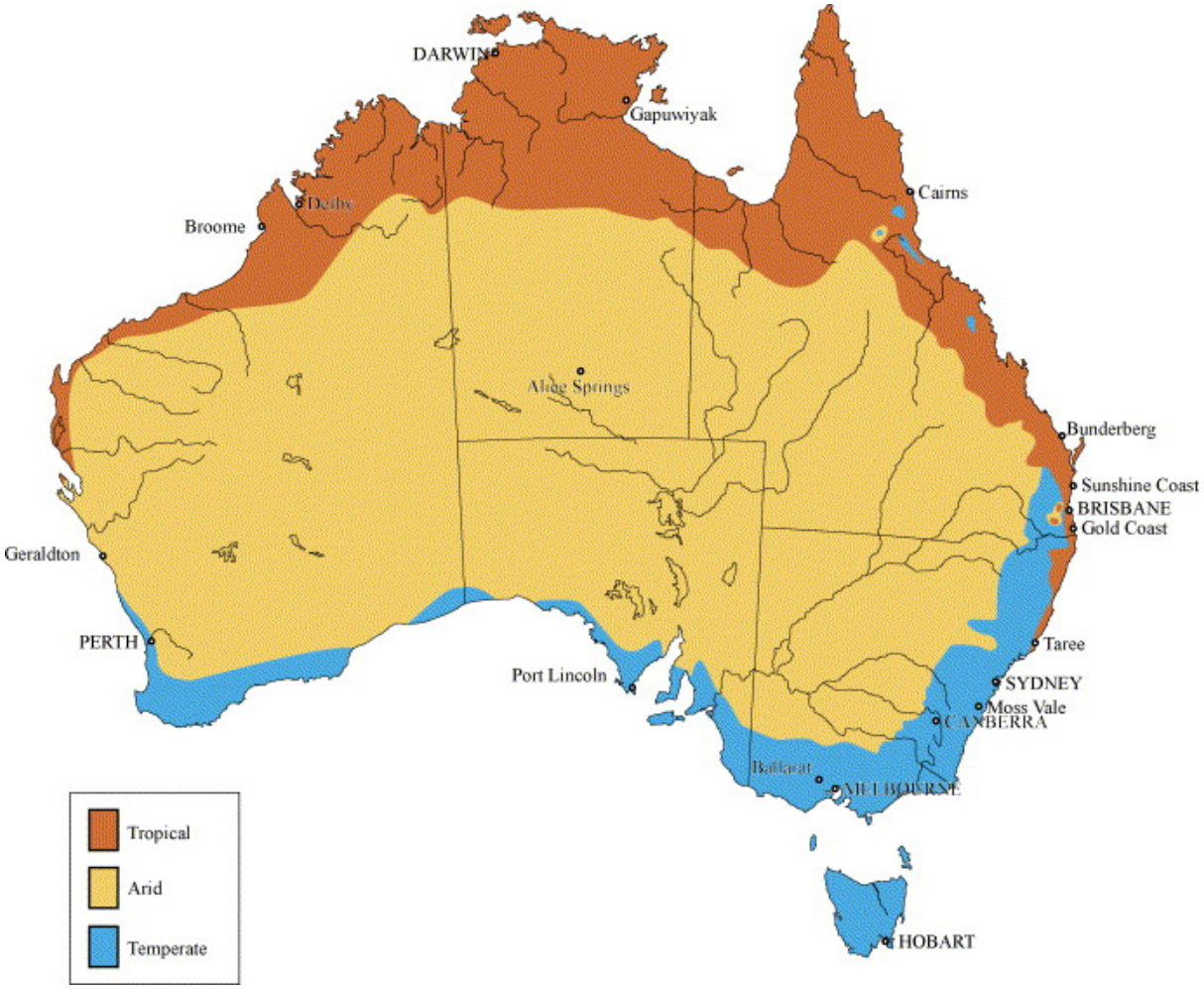

Fig. 2. PCR of the ITS of all of the canine and feline hookworm species. From left to right, lane 1 displays a 100 base pair molecular marker, lanes 2, 3, 4, 5, 6, 7 and 8 display PCR amplified products of ITS regions of *A. caninum*, *A. tubaeforme*, *A. ceylanicum*, *U. stenocephala*, mixed infection with *A. caninum* and *A. ceylanicum* and mixed infection with *A. caninum*and *U. stenocephala*, respectively (545 bp), lane 8 displays PCR product of *A. braziliense* (673 bp) and lane 9 is a negative control.

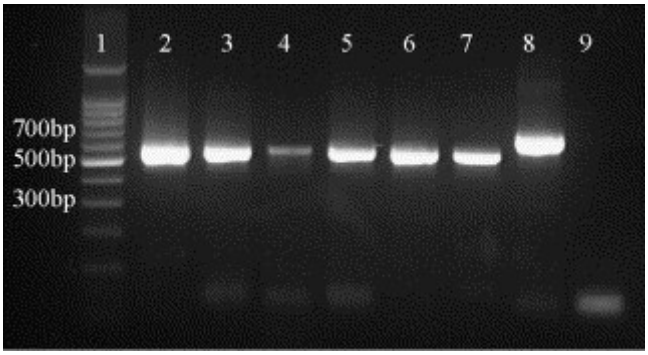

Fig. 3. RFLP of the PCR product RTGHF1-RTABCR1 following digestion with restriction endonuclease *Hin*FI. From left to right, the gel shows representatives of digested product of *A. caninum* in lane 2, digested product of *A. tubaeforme* in lane 3, digested product of *A. ceylanicum* in lane 4, digested product of *U. stenocephala* in lane 5, digested product of a mixed infection with *A. caninum* and *A. ceylanicum* in lane 6 and digested product of a mixed infection with *A. caninum* and *U. stenocephala* in lane 7.

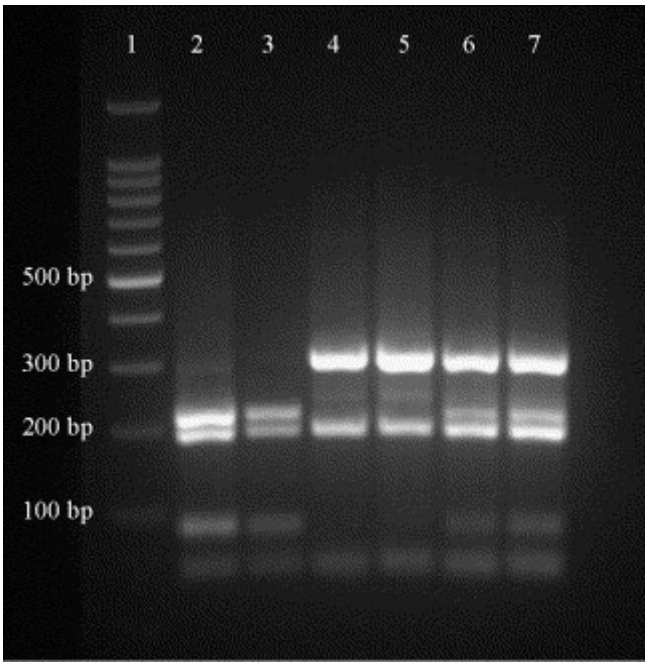

Fig. 4. RFLP of the PCR product of RTGHF1-RTABCR1 following digestion with restriction endonuclease *RSa*I. From left to right, the gel show representatives of undigested *U. stenocephala* in lane 2, digested product of *A. caninum* in lane 3, digested product of *A. tubaeforme* in lane 4, digestive product of *A. ceylanicum* in lane 5, digested product of a mixed infection with *A. caninum* and *A. ceylanicum* in lane 6 and digested and undigested product of a mixed infection with *A. caninum* and *U. stenocephala* in lane 7.

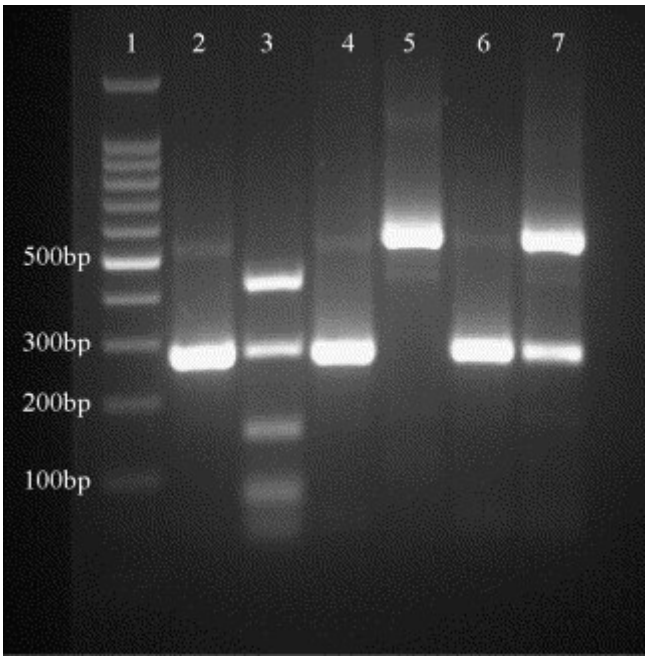

Fig. 5. Lateral view of male bursa of *A. ceylanicum* from a cat from Townsville, Australia, collected by GM Heydon in 1926 (Queensland Museum GL11582). Scalebar: 50 μm.