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Molecular identification of *Ancylostoma* species from dogs and an assessment of zoonotic risk in low-income households, São Paulo State, Brazil

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Abstract

Hookworm infection stands out for its worldwide distribution and for its veterinary and public health relevance. Based on copromicroscopic examinations and polymerase chain reaction (PCR) amplification of the ITS1–5.8S–ITS2 region, we assessed, respectively, the prevalence of intestinal parasites and the identification of canine hookworm species in faeces recovered from 278 dogs living in households of an inland municipality of São Paulo State, Brazil. Intestinal parasites were found in 67.3% of dogs and hookworm infection was found at the highest prevalence rate (56.6%), followed by *Toxocara canis* (11.9%), *Isospora* spp. (11.9%), *Giardia* spp. (5.8%), *Sarcocystis* spp. (4.0%), '*Hammondia*-like' (1.4%), *Dipylidium caninum* (1.1%) and *Trichuris vulpis* (0.7%). Of 158 samples positive for hookworm eggs, 106 (67.1%) were amplified by PCR and, of those, 88 (55.7%) were successfully sequenced for species identification. Single infections with *Ancylostoma caninum* and *Ancylostoma braziliense* were recorded in 61.4% and 12.5%, respectively, and mixed infections were found in 26.1%. The nucleotide sequences of both species showed high identity rates (98–100%) when compared with reference sequences. Although *A. caninum* was the most prevalent hookworm in the dogs assessed, the occurrence of both *A. caninum* and *A. braziliense* in single and/or mixed infections poses a potential risk for the local population in a low-income area, especially children, to acquire cutaneous larva migrans (CLM).

Introduction

Lack of canine population management in developing countries has led to the overpopulation that emerges as a public health problem since dogs can harbour infections by pathogens potentially transmissible to humans (Katagiri & Oliveira-Sequeira, 2008). Among the zoonotic infections, those caused by hookworms stand out for their worldwide distribution and for veterinary and public health relevance. Canine hookworms include the species *Ancylostoma caninum*, *Ancylostoma braziliense, Ancylostoma ceylanicum* and *Uncinaria stenocephala*, and, of these, *A. caninum* is one of the most prevalent and pathogenic

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gastrointestinal parasites, causing an acute or chronic haemorrhagic anaemia, particularly severe in young pups (Bowman *et al.*, 2010). From a public health perspective, the most common situation related to dog hookworms is cutaneous larva migrans (CLM), a neglected parasitic skin disease commonly found in resource-poor communities in tropical countries (Lesshafft *et al.*, 2012). The skin lesions, also called creeping eruptions or sand-worm disease, are due to the penetration and migration of canine hookworm infective larvae, producing an erythematous, linear or serpiginous track that is intensely pruritic and self-limiting (Bowman *et al.*, 2010). Even though both *A. caninum* and *A. braziliense* are the most common canine hookworms, there is evidence that the latter is most often responsible for limiting CLM (Traub *et al.*, 2005; Palmer *et al.*, 2007; Bowman *et al.*, 2010).

Despite the recognized importance of hookworms in low-income settings in the world, there is still limited information to date on the occurrence of these species in South America. However, investigations have provided data on the overall prevalence of *Ancylostoma* spp. infection in dogs (Fontanarrosa *et al.*, 2006; Katagiri & Oliveira-Sequeira, 2008; Gingrich *et al.*, 2010; Mandarino-Pereira *et al.*, 2010; Soriano *et al.*, 2010; Heukelbach *et al.*, 2012) and on soil contamination by hookworm eggs (Mandarino-Pereira *et al.*, 2010; Marques *et al.*, 2012; Sprenger *et al.*, 2014; La Sala *et al.*, 2015). To a lesser extent, adults of both *A. caninum* and *A. braziliense* have been reported to infect dogs in Brazil but, as far as we know, in surveys based on parasitological findings at necropsy (Heukelbach & Feldmeier, 2008; Klimpel *et al.*, 2010; Coelho *et al.*, 2011). With the advent of molecular biology, polymerase chain reaction (PCR)-based methods have allowed us to differentiate canine *Ancylostoma* species in microscopic-positive stool samples, resulting in improvement of the accuracy of diagnosis as well as in the elucidation of epidemiological aspects (Traub *et al.*, 2004a). Therefore, based on microscopy and molecular analyses, we aimed here to assess the occurrence and the frequency of infection of hookworm species in dogs living in households of an inland municipality in São Paulo State, Brazil. To the best of our knowledge, this is the first survey conducted in South America in which the identification of hookworm species in a canine population was based on a PCR technique instead of examination of adult worms recovered during necropsy of euthanized animals.

Materials and methods

Collection and processing of faecal samples

Dogs from Pratânia, a small municipality with both low economic and social development located in São Paulo State, Brazil (22°48′30″S and 48°39′58″W), were enrolled in the study. All dogs found living in households with schoolchildren were screened for a coproparasitological survey (David *et al.*, 2011). A single faecal sample from each dog was recovered immediately after a warm-water enema-induced evacuation. Unpreserved faecal samples were stored in plastic vials (at 4°C) and sent to the laboratory at the Department of Parasitology, Universidade Estadual Paulista (UNESP) for analysis within 24 h. All samples were examined microscopically for parasite eggs, cysts and oocysts after concentration by centrifugal sedimentation and by centrifugal flotation using saturated zinc sulphate (specific gravity 1.18) (Garcia, 2001). Data regarding age, gender, breed and urban or rural location were obtained for all the dogs sampled. The age of dogs was either provided by the owners or estimated by dentition analysis into three categories, namely pups less than 6 months, juveniles from 6 months to less than 1 year and adults more than 1 year old.

Molecular analysis

For molecular characterization, all hookworm eggs from microscopically positive faecal samples were submitted to DNA extraction using the QIAamp®DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions, and the DNA elution obtained was stored at −20°C until use. To optimize disruption of eggs, prior to DNA extraction, samples were suspended in 1.4 ml ATL tissue lysis buffer (Qiagen) and the suspension was then subjected to three cycles of freezing at liquid nitrogen temperatures followed by thawing at 96–98°C (Traub *et al.*, 2004a). Amplification of the ITS1–5.8S–ITS2 region was carried out according to previously published protocols (Traub *et al.*, 2004a; Palmer *et al.*, 2007). For species identification, the primers RTGHFI (5′-CGTGCTAGTCTTCAGGAC-TTTG-3′) and RTABCR1 (5′-CGGGAATTGCTATAAGC-AAGTGC-3′) were employed to amplify a 545-bp DNA fragment of *A. caninum*, *A. ceylanicum* and *U. stenocephala* hookworms. Then, in a separate PCR, a 673-bp fragment of *A. braziliense* was amplified using RTGHF1 and the highly specific reverse primer RTAYR1 (5′-CTGCTGAA-AAGTCCTCAAGTCC-3′). Positive and negative controls were included in all PCR experiments and the amplicons were visualized on 1.5% agarose/ethidium bromide gels.

For determining the nucleotide sequences, PCR products were excised from the agarose gel, purified using the Ultrafree® DA kit (Millipore Corp., Billerica, Massachusetts, USA) and both strands were sequenced by Macrogen (Seoul, South Korea) using the PCR primers. The resulting nucleotide sequences were analysed using Chromas Lite 2.01 (Technelysium Pty Ltd, South Brisbane, Brisbane, Australia) and nBLAST searches of the GenBank database, and they were aligned against reference sequences using the Clustal X2 sequence alignment program (Larkin *et al.*, 2007). Neighbour-joining analyses were performed using Tamura–Nei parameter distance estimates (Tamura *et al.*, 2011), and a phylogenetic tree was constructed by using MEGA 5.0 (www.megasoftware.net). The bootstrap values were obtained using 1000 replicates.

Data analysis

Prevalence was calculated for each enteric parasite on the basis of microscopic examination. Regarding hookworm species, the prevalence was determined considering PCR results, and associations with host factors (age, sex, breed, households) were evaluated. All analyses were made using the Student's *t*-test ($P < 0.05$) and employing SAS® software, Version 9.1.3 (Cary, North Carolina, USA).

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Results

Single faecal samples were collected from 278 dogs, comprising 143 males and 135 females, with the majority (212) being adults and all (278) of mixed-breeding. Up to 160 dogs lived in urban households compared with 118 from rural areas.

Microscopic examination revealed that 67.3% of dogs were positive for at least one parasite species, with the most prevalent parasite being the hookworm *Ancylostoma* spp. (56.6%) followed by the ascarid nematode *Toxocara canis* (11.9%), *Isospora* spp. (11.9%), *Giardia* spp. (5.8%), *Sarcocystis* spp. (4.0%), '*Hammondia*-like' (1.4%), *Dipylidium caninum* (1.1%) and *Trichuris vulpis* (0.7%). No statistically significant differences were detected between the overall prevalence of intestinal parasites and the variables analysed.

Of 158 samples positive for hookworm eggs, 106 (67.1%) were amplified by PCR and, of those, 88 (55.7%) were successfully sequenced for species identification. Molecular analyses revealed that single infections with *A. caninum* or with *A. braziliense* were recorded in 61.4% and 12.5% of the dogs, respectively. Mixed infections with both species were identified in 26.1% of dogs. Both species were found among dogs living in urban and rural zones; however, in the latter, *A. braziliense* was detected only in dogs harbouring mixed infections (table 1). Analysis of prevalence of each hookworm species according to age, gender, sex and household location showed no statistically significant differences. No *A. ceylanicum* infection was detected in this population.

For each species, comparative analysis revealed that retrieved sequences were closely related to reference sequences, exhibiting high identity rates of 98–100% and 99–100%, respectively, for *A. caninum* and *A. braziliense*. No novel sequence was described. Phylogenetic trees were constructed based on 33 nucleotide sequences randomly selected. Among these sequences, 23 were related to mixed infections and the remaining to single infections with *A. caninum* (AC46, AC47, AC87, AC130 and AC133) or with *A. braziliense* (AB26, AB40, AB159, AB191 and AB201). The phylogenetic tree demonstrated a distinct separation of the sequences into two major clusters (fig. 1).

Discussion

Dogs often harbour several enteric parasites and most of them have potential for transmission to humans, representing a public health concern. Still today, the high level of environmental contamination with canine faeces exposes both dogs and human beings to infective parasitic elements, particularly in low-income areas (Traversa *et al.*, 2014). In the present study, a high overall prevalence (67.3%) of canine gastrointestinal parasites was recorded and zoonotic parasites were more frequent, highlighting infections by the Ancylostomatidae (56.6%), followed by *Toxocara canis* (11.9%) and *Giardia* spp. (5.8%). These findings agree with recent data reported in different parts of the world (Little *et al.*, 2009; Scorza *et al.*, 2011; Riggio *et al.*, 2013; Cardoso *et al.*, 2014), including in Brazil (Heukelbach & Feldmeier, 2008; Katagiri & Oliveira-Sequeira, 2008; Klimpel *et al.*, 2010).

Hookworms have been found to be a common intestinal parasite infecting owned and stray dogs (Traversa *et al.*, 2014). Although no statistically significant differences were observed between hookworm prevalence rate and variables such as age, gender, breed and household location, some interesting findings deserve comments. In general, higher prevalence rates of infection have been commonly registered in stray dogs and also in young dogs. The present results did not fit this pattern as all dogs had an owner and the population consisted mostly of adult dogs. Although hookworm infections are more frequently detected in young dogs, as they grow they continue to be susceptible and can be infected. In the present study, the fact that up to 70% of the dogs were adults and the high frequency of Ancylostomatidae eggs recorded reinforce this observation. Furthermore, according to almost all owners, the majority of these dogs had never been dewormed in their lives which, probably, contributed to the high frequency of hookworm infection. Another aspect to be stressed is that although the dogs had an owner, they did not live in restricted conditions, since most of them were reared with free access to the streets in both urban and rural areas. Thus, dogs were allowed to defecate outside their households, leading to environmental contamination, even in urban zones where, due the lack of infrastructure, a considerable part of the streets were unpaved.

Hookworms are the only helminths producing strongyle-type eggs in dogs, and these are easily identifiable by standard flotation and microscopy. So, PCR is not necessary to determine the prevalence of hookworms *per se*, but is necessary to differentiate between the species of *Ancylostoma*. Based on molecular methods, the present analyses demonstrated that of 158 microscopy-positive hookworm samples, 67% were amplified successfully by PCR. Amplification was not observed for all samples, and similar findings have been reported by other researchers

Table 1. Hookworm infections in dogs from Pratânia, São Paulo State, relative to household location; $N =$ number of dogs sampled.

Household	N	No. of positives/ microscopy	No. of PCR positives	Hookworm species (%)*		
				A. caninum[1]	*A. braziliense*[2]	Mixed infections[3]
Urban	160	88	63	38.9	100	78.3
Rural	118	70	43	61.1	–	21.7
Total	278	158	106	61.4*	12.5*	26.1*

*Prevalence of infection (%) from 88/106 PCR products successfully sequenced for species identification.
Number of nucleotide sequences retrieved: [1]$n = 54$; [2]$n = 11$; [3]$n = 23$.

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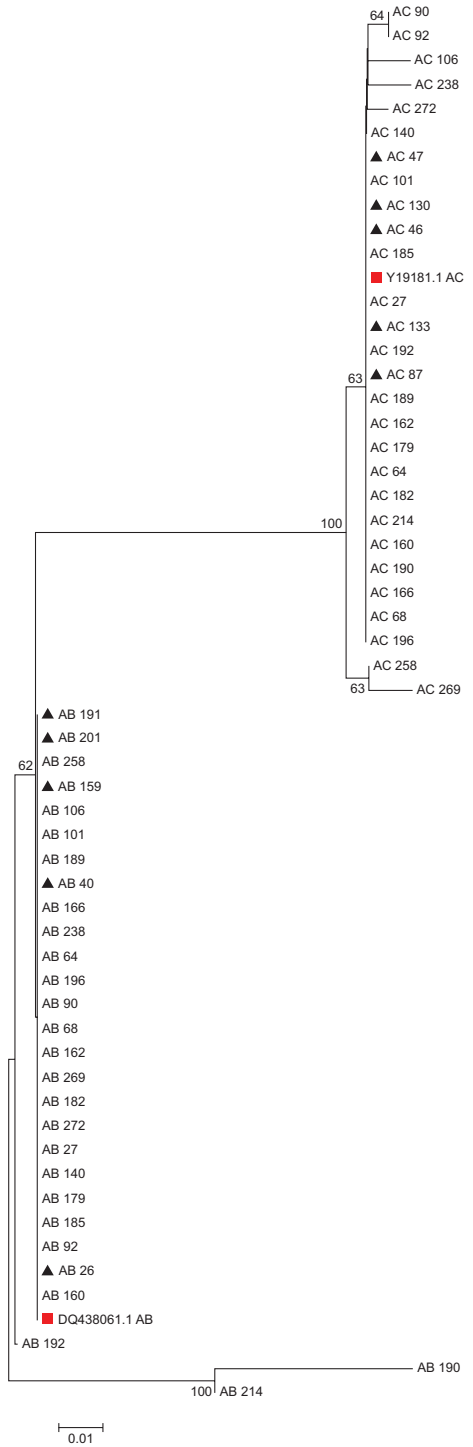

Fig. 1. Phylogenetic tree of canine hookworm isolates based on the ITS1 and 5.8S rRNA sequences retrieved from single infections with *A. caninum* ($n = 5$) or with *A. braziliense* ($n = 5$) and from mixed infections with both species ($n = 23$); bootstrap values obtained from 1000 replicates (>50% shown) with single infections (red squares) and reference sequences (black triangles) highlighted.

(Palmer *et al.*, 2007; Mahdy *et al.*, 2012). According to some authors this can be associated with the presence of inhibitors in faeces or the low number of eggs in the stool, resulting in a low amount of DNA (Palmer *et al.*, 2007). Despite this, out of 88 hookworm-positive faecal samples that were both amplified and sequenced; only *A. caninum* and *A. braziliense* were isolated from dogs – 61.4% were found to be infected with *A. caninum*, 12.5% with *A. braziliense* and 26.1% with mixed infections. These results are consistent with previous surveys based on PCR methods, which also demonstrated the predominance of *A. caninum* among dogs in regions of Asia (Traub *et al.*, 2004b, 2008; Mahdy *et al.*, 2012), Australia (Palmer *et al.*, 2007) and the USA (Liotta *et al.*, 2012). In Brazil, prevalence data of each species have been recorded only by post-mortem examination and *A. caninum* had been reported to be the predominant hookworm, often as the only one identified (Heukelbach & Feldmeier, 2008; Klimpel *et al.*, 2010; Coelho *et al.*, 2011). In the present investigation, *A. braziliense* was also detected but few molecular studies have reported the occurrence of this species in dogs (Traub *et al.*, 2004a; Liotta *et al.*, 2012; Ngui *et al.*, 2012).

In a public health context, notwithstanding the widespread distribution of *A. caninum*, it seems that the occurrence of CLM follows the geographical distribution of *A. braziliense* and most reported cases have been in tourists who have visited regions where this species is endemic in dogs and cats (Bowman *et al.*, 2010). However, it is not easy to establish an association because in many areas the animals can harbour mixed infections (Bowman *et al.*, 2010).

Although CLM has been reported sporadically in low-income countries, it is known that low socio-economic status and certain behaviour, such as walking barefoot on contaminated soil, are probably risk factors for the occurrence of this syndrome (Heukelbach & Feldmeier, 2008). A relevant study carried out in deprived communities of India showed that *A. braziliense* was detected in up to 60% of dogs (Traub *et al.*, 2004b, 2005) and in these areas most of the population (64%) admitted to walking barefoot outdoors (Traub *et al.*, 2004b). In this scenario, the authors believe that these findings would account for the high incidence of creeping eruptions observed among the human population (Traub *et al.*, 2004b). In the present study, no association could be made between the presence of dogs infected with hookworms, especially harbouring *A. braziliense*, and the occurrence of CLM. During the survey, in visits to the local primary healthcare centre, the workers were informed that no patients were registered as CLM cases. However, the habit of walking barefoot outside the household was very common, particularly among children living in the rural zone, who are at a constant risk of infection by hookworm larvae.

To our knowledge, the present study is the first parasitological survey and, so far, the only one in South America in which the PCR technique was employed for differential diagnosis of canine hookworm species directly from eggs in stool samples, avoiding post-mortem examination. The findings assembled herein confirm that molecular methods have clearly enabled further improvements in parasite diagnosis and epidemiology, while providing interesting insights on canine

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hookworm prevalence and reinforcing the need for future studies that focus on clarifying the relationship between species distribution and the occurrence of cutaneous larva migrans.

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Conflict of interest

None.

Ethical standards

All procedures were conducted according to the animal protocols reviewed and approved by the Ethics Committee on Animal Experimentation (CEEA) of the Bioscience Institute, IBB-UNESP (Protocol number 027/06CEEA). Permission forms were handed out to dog owners, who signed them before animal sampling was done.

References

Bowman, D.D., Montgomery, S.P., Zajac, A.M., Eberhard, M.L. & Kazacos, K.R. (2010) Hookworms of dogs and cats as agents of cutaneous larva migrans. *Trends in Parasitology* **26**, 62–67.

Cardoso, A.S., Costa, I.M.H., Figueiredo, C., Castro, A. & Conceição, M.A.P. (2014) The occurrence of zoonotic parasites in rural dog populations from northern Portugal. *Journal of Helminthology* **88**, 203–209.

Coelho, W.M.D., Amarante, A.F.T., Apolinário, J.D.C., Coelho, N.M.D. & Bresciani, K.D.S. (2011) Occurrence *Ancylostoma* in dogs, cats and public places from Andradina city, São Paulo state, Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo* **53**, 181–184.

David, E.B., Coradi, S.T., Oliveira-Sequeira, T.C.G., Ribolla, P.E.M., Katagiri, S. & Guimarães, S. (2011) Diagnosis of *Giardia* infections by PCR-based methods in children of an endemic area. *Journal of Venomous Animals and Toxins including Tropical Diseases* **17**, 209–215.

Fontanarrosa, M.F., Vezzani, D., Basabe, J. & Eiras, D.F. (2006) An epidemiological study of gastrointestinal parasites of dogs from Southern Greater Buenos Aires (Argentina): age, gender, breed, mixed infections, and seasonal and spatial patterns. *Veterinary Parasitology* **136**, 283–295.

Garcia, L.S. (2001) *Diagnostic medical parasitology*. 4th edn. 1092 pp. Washington, DC, ASM Press.

Gingrich, E.N., Scorza, A.V., Clifford, E.L., Olea-Popelka, F.J. & Lappin, M.R. (2010) Intestinal parasites of dogs on the Galapagos Islands. *Veterinary Parasitology* **169**, 404–407.

Heukelbach, J. & Feldmeier, H. (2008) Epidemiological and clinical characteristics of hookworm related cutaneous larva migrans. *Lancet Infectious Diseases* **8**, 302–309.

Heukelbach, J., Frank, R., Ariza, L., Lopes, I.S., Silva, A.D.A., Borges, A.C., Limongi, J.E., De Alencar, C.H.M. & Klimpel, S. (2012) High prevalence of intestinal infections and ectoparasites in dogs, Minas Gerais State (southeast Brazil). *Parasitology Research* **111**, 1913–1921.

Katagiri, S. & Oliveira-Sequeira, T.C.G. (2008) Prevalence of dog intestinal parasites and risk perception of zoonotic infection by dog owners in Sao Paulo State, Brazil. *Zoonoses Public Health* **55**, 406–413.

Klimpel, S., Heukelbach, J., Pothmann, D. & Rückert, S. (2010) Gastrointestinal and ectoparasites from urban stray dogs in Fortaleza (Brazil): high infection risk for humans? *Parasitology Research* **107**, 713–719.

Larkin, M.A, Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., & Higgins, D.G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947–2948.

La Sala, L.F., Leiboff, A., Burgos, J.M. & Costamagna, S.R. (2015) Spatial distribution of canine zoonotic enteroparasites in Bahía Blanca, Argentina. *Revista Argentina de Microbiologia* **47**, 17–24.

Lesshafft, H., Schuster, A., Reichert, F., Talhari, S., Ignatius, R. & Feldmeier, H. (2012) Knowledge, attitudes, perceptions, and practices regarding cutaneous larva migrans in deprived communities in Manaus, Brazil. *Journal of Infection in Developing Countries* **6**, 422–429.

Liotta, J.L., Koompapong, K.N., Yaros, J.P., Prullage, J. & Bowman, D.D. (2012) Prevalence of *Ancylostoma braziliense* in cats in three northern counties of Florida, United States. *Journal of Parasitology* **98**, 1032–1033.

Little, S.E., Johnson, E.M., Lewis, D., Jaklitsch, R.P., Payton, M.E., Blagburn, B.L., Bowman, D.D., Moroff, S., Tams, T., Rich, L. & Aucoin, D. (2009) Prevalence of intestinal parasites in pet dogs in the United States. *Veterinary Parasitology* **166**, 44–52.

Mahdy, M.A., Lim, Y.A., Ngui, R., Fatimah, M., Choy, S.H., Yap, N.J., Al-Mekhlafi, H.M., Ibrahim, J. & Surin, J. (2012) Prevalence and zoonotic potential of canine hookworms in Malaysia. *Parasites & Vectors* **5**, 88.

Mandarino-Pereira, A., Souza, F.S., Lopes, C.W.G. & Pereira, M.J.S. (2010) Prevalence of parasites in soil and dog feces according to diagnostic tests. *Veterinary Parasitology* **170**, 176–181.

Marques, J.P., Guimarães, C.D.R., Boas, A.V., Carnaúba, P.U. & Moraes, J. (2012) Contamination of public parks and squares from Guarulhos (São Paulo State, Brazil) by *Toxocara* spp. and *Ancylostoma* spp. *Revista do Instituto de Medicina Tropical de Sao Paulo* **54**, 267–271.

Ngui, R., Lim, Y.A., Traub, R., Mahmud, R. & Mistam, M.S. (2012) Epidemiological and genetic data supporting the transmission of *Ancylostoma ceylanicum* among human and domestic animals. *PLoS Neglected Tropical Diseases* **6**, 1–7.

Palmer, C.S., Traub, R.J., Robertson, I.D., Hobbs, R.P., Elliot, A., While, L., Rees, R. & Thompson, R.C.A. (2007) The veterinary and public health significance of hookworm in dogs and cats in Australia and the status of *A. ceylanicum*. *Veterinary Parasitology* **145**, 304–313.

Riggio, F., Mannella, R., Ariti, G. & Perrucci, S. (2013) Intestinal and lung parasites in owned dogs and cats from central Italy. *Veterinary Parasitology* **193**, 78–84.

Scorza, A.V., Duncan, C., Miles, L. & Lappin, M.R. (2011) Prevalence of selected zoonotic and vector borne agents in dogs and cats in Costa Rica. *Veterinary Parasitology* **183**, 178–183.

Soriano, S.V., Pierangeli, N.B., Roccia, I., Bergagna, H.F.J., Lazzarini, L.E., Celescinco, A., Saiz, M.S., Kossman, A., Contreras, A.P., Arias, C. & Basualdo, J.A. (2010) A wide diversity of zoonotic intestinal parasites infects urban and rural dogs in Neuquén, Patagonia, Argentina. *Veterinary Parasitology* **167**, 81–85.

Sprenger, L.K., Green, K.T. & Molento, M.B. (2014) Geohelminth contamination of public areas and epidemiological risk factors in Curitiba, Brazil. *Revista Brasileira de Parasitologia Veterinária* **23**, 69–73.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28** 2731–2739.

Traub, R.J., Robertson, I.D., Irwin, P., Mencke, N. & Thompson, R.C.A. (2004a) Application of a species specific PCR-RFLP to identify *Ancylostoma* eggs directly from canine faeces. *Veterinary Parasitology* **123**, 245–255.

Traub, R.J., Robertson, I.D., Irwin, P., Mencke, N. & Thompson, R.C.A. (2004b) The prevalence, intensities and risk factors associated with geohelminth infection in tea-growing communities of Assam, India. *Tropical Medicine and International Health* **9**, 688–701.

Traub, R.J., Robertson, I.D., Irwin, P.J., Mencke, N. & Thompson, R.C.A. (2005) Canine gastrointestinal parasitic zoonoses in India. *Trends in Parasitology* **21**, 42–48.

Traub, R.J., Inpankaew, T., Sutthikornchai, C., Sukthana, Y. & Thompson, R.C.A. (2008) PCR-based coprodiagnostic tools reveal dogs as reservoirs of zoonotic ancylostomiasis caused by *Ancylostoma ceylanicum* in temple communities in Bangkok. *Veterinary Parasitology* **155**, 67–73.

Traversa, D., Frangipane di Regalbono, A., Di Cesare, A., La Torre, F., Drake, J. & Pietrobelli, M. (2014) Environmental contamination by canine geohelminths. *Parasites & Vectors* **7**, 67.

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