ORIGINAL ARTICLE

Prevalence of Dog Intestinal Parasites and Risk Perception of Zoonotic Infection by Dog Owners in São Paulo State, Brazil

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Impacts

- The overall prevalence of dog intestinal parasites detected in this study was 54.33% and most dogs (31.4%) were harbouring only one parasite.
- Relevant parasites in terms of zoonotic potential were the most frequently observed, i.e. *Ancylostoma* spp. (37.8%), *Giardia* spp.(16.9%) and *Toxocara canis* (8.7%).
- The lack of knowledge showed by dog owners on the zoonotic potential of intestinal parasites of dogs, and on the methods for their control and prophylaxis seems to be the main reason for the apparent negligence in deworming their dogs.

Keywords:
Prevalence; intestinal parasites; dog; zoonoses; protozoa; helminths

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Summary

Coprological examination was used to estimate the prevalence of gastrointestinal parasites in stray and domiciled dogs from Botucatu, São Paulo State, Brazil. Risk factors for dog infection were assessed in relation to demographic, husbandry and management data. The dog owners completed a questionnaire survey on some aspects of dog parasitism such as parasite species, mechanisms of infection, awareness of zoonotic diseases and history of anthelmintic usage. Parasites were found in the faeces of 138 dogs, with an overall prevalence of 54.3%. Dogs harbouring one parasite were more common (31.4%) than those harbouring two (18.5%), three (3.2%) or four (1.2%). The following parasites and their respective frequencies were detected: *Ancylostoma* (37.8%), *Giardia* (16.9%), *Toxocara canis* (8.7%), *Trichuris vulpis* (7.1%), *Dipylidium caninum* (2.4%), *Isospora* (3.5%), *Cryptosporidium* (3.1%) and *Sarcocystis* (2.7%). Stray dogs were found more likely to be poliparasitized ($P < 0.01$) and presented higher prevalence of *Ancylostoma*, *T. canis* and *Giardia* ($P < 0.01$) than domiciled ones. *Toxocara canis* was detected more frequently in dogs with <6 months of age ($P < 0.05$) and no effect of sex or breed could be observed ($P > 0.05$). Except for *Ancylostoma*, that showed a significantly higher prevalence in dogs living in a multi-dog household ($P < 0.01$), parasite prevalences were similar in single- and multi-dog household. The answers of dog owners to the questionnaire showed that the majority does not know the species of dog intestinal parasites, the mechanisms of transmission, the risk factors for zoonotic infections, and specific prophylactic measures. The predominance of zoonotic species in dogs in the studied region, associated with the elevated degree of misinformation of the owners, indicates that the risk of zoonotic infection by canine intestinal parasite may be high, even in one of the most developed regions of Brazil.

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Introduction

Uncontrolled population of stray and semi-domesticated dogs in close proximity to increasing densities of human population in urban environments is a common fact in developing countries, which, in conjunction with the lack of veterinary attention and zoonotic awareness, increases the risks of disease transmission (Traub et al., 2005). In most Brazilian cities, government actions such as providing the population with information about the risks of zoonotic disease transmitted by domestic animals, and control of stray animals are practically non-existent, resulting in an increasing risk of exposure to zoonoses transmitted by these animals (Oliveira-Sequeira et al., 2002).

In developing countries, the risk of zoonotic infection related to domiciled dogs is also high because the obligations placed on dog owners are less restrictive (Macpherson, 2005). As a consequence, even domiciled animals go on harbouring parasitic infections, including those to which treatment and effective control methods are available. The presence of these animals in close contact with people constitutes a high potential risk, especially to children and immunocompromised individuals (Robertson et al., 2000).

Recently, the canine population of inner São Paulo state cities (the most developed state in the country) was estimated as being of one dog for each four inhabitants (1 : 4) (Alves et al., 2005), a ratio significantly above that referred to by the World Health Organization (WHO, 1992) for developing countries. In this study, another fact highlighted was that in small cities (<100 000 inhabitants), the proportion of stray dogs (approximately 9%) and of domiciled dogs that are raised with free access to the streets (approximately 35%) is higher. The high number of stray dogs was attributed to the great availability of food, probably because of the garbage scattered in the streets and the disposition of dog-loving people in feeding these animals. It is important to point out that smaller cities are exactly where the availability of health care services to humans and pet animals is scarce or even absent.

Most of the studies on human infection by canine parasitic zoonoses in Brazil are of sporadic case reports of unusual presentation, or studies of the prevalence of more common canine zoonoses, such as cutaneous larva migrans (CLM) and toxocariasis (Teixeira et al., 2006). However, several studies show high prevalence of intestinal parasites in stray and domiciled dogs (Labruna et al., 2006), as well as high rates of environmental contamination with eggs and larvae of canine intestinal parasites (Castro et al., 2005). Nevertheless, there was no information about risk perception by dog owners or continuing public education regarding the potential hazard of dogs as a source of zoonotic diseases.

Therefore, the aim of this study was to determine the prevalence of canine intestinal parasites and the possible risk factors associated with dog infection. Information obtained from a questionnaire survey was also employed to analyse the level of knowledge of dog owners on canine intestinal parasites and the extent to which they were aware of zoonotic parasitic infection.

Materials and Methods

Study area

The study was carried out in Botucatu, São Paulo State, Brazil (22°51′S, and 48°26′W), a city of 1483 km^2 and approximately 120 000 inhabitants (IBGE, 2007). The climate is subtropical humid (CWA) with an average annual temperature of 20.7°C (CEPAGRI, 2008). The Human Development Index at city level (HDI-M) is of 0.822 (UNDP, 2003). This index ranges from 0 to 1, ranking cities according to the following levels: low human development ($0 \leq HDI < 0.5$), medium ($0.5 = HDI < 0.8$) and high ($0.8 = HDI \leq 1$).

Source of samples

From October 2004 to September 2005, faecal samples from dogs were examined for the presence of parasites. Approximately, 200 samples were calculated to be a sufficient sample size to estimate general prevalence of dog intestinal parasites (De Blas et al., 2000), assuming a canine population of 30 000 [one dog for each four inhabitants (Alves et al., 2005)], and a parasite infection prevalence of 15%. The sample size of 120 animals from each group of canine population (stray and owned dogs) was calculated considering an expected difference of 20% between these two groups, and a confidence interval of 95% (Campbell and Machin, 1993).

Samples from domiciled dogs were collected by the primary investigator during morning visits to 92 randomly chosen homes, situated in medium class districts in the urban area of Botucatu. All these were semi-restricted dogs, housed indoors or in the yard, reared without free access to the street.

Samples from stray dogs were obtained from the kennel of the Universidade Estadual Paulista (UEP) and from the kennel of the Center of Zoonoses Control (CCZ) of the Botucatu City Hall. Stray dogs were caught in the urban areas by the Council Service and accommodated in separated groups according to age and gender in the CCZ kennel. These animals do not receive any kind of medicine, and those not claimed or adopted within 8 days after their arrival at the centre

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are led to the UEP kennel for scientific purpose, or are painlessly killed.

Faecal samples were collected immediately after spontaneous elimination for observation of the macroscopic characteristics, such as consistency, and the presence of parasites. Demographic (age, gender, breed) and husbandry (single or multi-dog household) data of domiciled dogs were obtained from owners. In the case of stray dogs, age was estimated by dentition analysis; animals up to 6 months of age were classified as young ones.

The parasitological diagnostics were delivered to the dog owners in a second visit when the questionnaires had been completed. The questionnaire was prepared in order to obtain answers by either 'yes' or 'no'. Participants were asked if their dogs received veterinary care, if they had dewormed their dogs within 6 months, and if they knew any of the following: any dog parasite specie; any dog intestinal parasite species; how dogs acquire intestinal parasites; any dog parasite species that infects human beings; how human contracted dog intestinal parasite and what the terms anti-helminthic, vermifuge and zoonoses meant.

Parasitological procedures

Unpreserved faecal samples were stored in closed containers (4°C) and processed within 24 h. Each sample was microscopically examined for parasite eggs, cysts and oocysts after concentration by centrifugal sedimentation (CS) technique, and by centrifugal flotation using saturated zinc sulphate (Sloss et al., 1999). A modified Ziehl-Neelsen stain (Henriksen and Pohlenz, 1981) was used to screen *Cryptosporidium* oocysts.

Statistical analysis

The observed prevalence and 95% confidence intervals (CI) were calculated for each parasite (Bush et al., 1997). Associations between parasitism and host factors (age, sex, breed, single/multi-dog household and stray/owned) were calculated for all the 254 dogs, and the association between parasitism and anti-helminthic usages was evaluated only for owned dogs (125). All the analyses were made using chi-squared tests for two independent proportions employing the PopTools software (CSIRO, 2008).

Results

Faecal samples from 254 dogs were examined for the presence of parasite. Of these samples, 125 were from domiciled dogs and 129 from stray ones. Intestinal parasites were found in the faeces of 138 dogs, with an overall prevalence of 54.3% (CI 48.3–60.3%). Dogs harbouring one parasite genera were more common (58%) than ones harbouring two (34%), three (5.8%) or four (2.2%). Most of the stray dogs (73.6%, CI 66–81%) were infected with at least one parasite, compared with 34.4% (CI 26.4–42.4%) of domiciled dogs ($P < 0.01$).

Helminthic infections (142) were more frequent ($P < 0.01$) than protozoan (67) regardless of the source of dogs (Table 1), but the proportion of domiciled dogs harbouring intestinal protozoan (23/56) was higher ($P < 0{,}01$) than stray dogs (44/153).

In both canine populations, *Ancylostoma* spp. (37.8%), *Giardia* spp. (16.9%) and *Toxocara canis* (8.7%) were the most prevalent parasites, and they were significantly more frequent in stray dogs ($P < 0.01$). In the 58 animals harbouring concurrent infection, *Ancylostoma* spp. was diagnosed in 51. The most common association was between *Ancylostoma* spp. and *Giardia* spp., diagnosed in 13 of 47 animals harbouring two, and in all animals harbouring three (8) and four (3) parasite species.

With regard to demographic factors, the frequency of parasitism in male and female adults showed no difference ($P > 0.05$), and in relation to age, the frequency of *T. canis* in young animals (4/14) was higher ($P < 0.05$) than in adult ones (18/240) (Table 2).

The influence of cohabitation with other dogs, the breed, and anti-parasitic treatment on the frequency of intestinal parasites were analysed only for domiciled dogs (125). In relation to cohabitation, these analyses revealed that only the *Ancylostoma* spp. frequency was distinctive ($P < 0.01$), being higher on the multi-dog-household dogs (60 dogs). Data from 64 pure-breed dogs and from 61 mixed-breed dogs revealed no difference between these categories ($P > 0.05$).

Table 1. Intestinal parasites diagnosed in two populations of dogs from São Paulo State, Brazil*

	Source of dogs		
Parasites	Stray (n = 129)	Domicilied (n = 125)	Total (n = 254)
Ancylostoma spp.	73 (56.6)[a]	23 (18.4)[b]	96 (37.8)
Toxocara canis	18 (13.9)[a]	4 (3.2)[b]	22 (8.7)
Trichuris vulpis	12 (9.3)	6 (4.8)	18 (7.1)
Dipylidium caninum	6 (4.6)	0 (0)	6 (2.3)
Giardia spp.	32 (24.8)[a]	11 (8.8)[b]	43 (16.9)
Isospora spp.	5 (3.9)	4 (3.2)	9 (3.5)
Cryptosporidium spp.	3 (2.3)	5 (4.0)	8 (3.1)
Sarcocystis spp.	4 (3.1)	3 (2.4)	7 (2.7)
Total parasite infection†	95 (73.6)[a]	43 (34.4)[b]	138 (54.3)

Values with different superscript letters in the same row are significantly different ($P < 0.01$).
*Values in parentheses are percentages.
†Some dogs were infected with more than one parasite.

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Table 2. Age and gender frequency of intestinal parasites in 254 dogs from São Paulo State, Brazil*

Parasites	Young† (*n* = 14)	Adult† (*n* = 240)	Adult female (*n* = 129)	Adult male (*n* = 111)
Ancylostoma spp.	5 (35.7)	91 (37.9)	44 (34.1)	47 (42.3)
Toxocara canis	4 (28.6)[a]	18 (7.5)[b]	11 (8.5)	7 (6.3)
Trichuris vulpis	1 (7.1)	17 (7.1)	11 (8.5)	6 (5.4)
Dipylidium caninum	0 (0)	6 (2.5)	4 (3.1)	2 (1.8)
Giardia spp.	6 (42.9)	37 (15.4)	21 (16.3)	16 (14.4)
Isospora spp.	1 (7.1)	8 (3.3)	5 (3.9)	3 (2.7)
Cryptosporidium spp.	0 (0)	8 (3.3)	6 (4.7)	2 (1.8)
Sarcocystis spp.	0 (0)	7 (3.0)	1 (0.8)	6 (5.4)
Infected animals‡	10 (71.4)	128 (53.3)	64 (49.6)	64 (57.7)

Values with different superscript letters in the same row are significantly different ($P < 0.05$).
*Values in parentheses are percentages.
†Total Male/female.
‡Some dogs were infected with more than one parasite.

According to information provided by owners, 95 of the 125 domiciled dogs have received some kind of anthelmintic treatment, and among these, 30 have been treated within 6 months before the collection. After stratifying the groups by last known anthelmintic treatment (either considering 30 or 95 dogs as dewormed), no difference in parasite prevalence was observed between dogs assumed to be dewormed and not-dewormed.

Seventy-seven owners (of 92), responsible for 103 domiciled animals, answered the questionnaire intended to evaluate the level of their knowledge on canine intestinal parasites. Four of the other 15 owners were moved elsewhere, and the other 11 owners refused to answer the questions. When questioned on whether they knew any canine parasite, 67.5% (52/77) of owners said no, and when asked if they knew any canine ‘worm’, this percentage dropped to 46.7% (36/77). Among the 63 (81.8%) owners who could not give the name of any parasite, 11 (17.5%) were capable of indicating at least one canine parasite, either by its vulgar name or by its aspect (tick, scabies, flea, worm, cucumber seed).

The percentage of owners [70.1% (54/77)] who said that they ignore the way dogs acquire parasite infections was equal to the owners who were unaware of the possibility of dogs harbouring parasites capable of infecting man. However, only 53.2% (41/77) of owners said they did not know the measures capable of avoiding human contamination by parasites. The most frequent answers on prophylactic measures were related to their general nature such as: cleanliness and hygiene, do not walk barefooted and treatment of dogs.

About the knowledge of owners regarding anti-parasitic drugs it was shown that 52 (67.5%) did not know the meaning of the term anthelmintic; however, when the term vermifuge was used, instead of anthelmintic, the percentage dropped 28.6% (22/77). The obtained frequency scores for owners seeking veterinarian assistance were the following: 20.7% never sought, 48.1% sought only in case of disease or for vaccines and 31.2% regularly sought.

Discussion

The overall prevalence of canine intestinal parasites found in this study (54.33%), especially in stray dogs (73.6%), revealed a very high level of infection that requires an effective anti-parasite control programme. According to the studies conducted in different countries worldwide, the estimate prevalences of dog intestinal parasites vary from 5 to 70% (Blagburn et al., 1996; Bugg et al., 1999), and some factors such as geographical location, status of animal ownership, sampling protocols, demographic factors, anthelmintic usage, and diagnostic techniques are responsible for the wide range of endoparasite prevalence.

The predominance of helminthic upon enteric protozoan infections in dogs observed here is similar to other recent observations in Brazil (Oliveira-Sequeira et al., 2002; Labruna et al., 2006), other countries of Latin America (Ramírez-Barrios et al., 2004; Ponce-Macotela et al., 2005; Fontanarrosa et al., 2006), and other places in the world (Inpankaew et al., 2007; Martínez-Moreno et al., 2007). The trend of reducing helminthic and increasing protozoan infection in Australia was attributed to an increasing routine use of anti-helminthics (Bugg et al., 1999) and to the knowledge of dog owners about potential zoonotic transmission of these agents and how to control them (Schantz, 1999). These facts stand in contrast to the current situation found in this study.

Brazil is a country of continental dimensions, whose regions present great climatic conditions, cultural and socioeconomic differences. In most developed regions, human developmental indicators as well as the veterinary services available to dogs (pets) are comparable to those found in developed countries. In less developed regions, the weak infrastructure is similar to that found in poor countries of Africa, Asia and Latin America where most of the people have no access to the services in both public health and veterinary sectors. However, the prevalence of the different species of intestinal canine parasites found in this study (one of the most developed) was similar to those recently registered, both in the developed (HDI ≥ 0.8) regions of the country (Gennari et al., 1999; Oliveira-Sequeira et al., 2002; Santos and Castro, 2006) and in less developed (HDI = 0.6) ones (Labruna et al., 2006). These data suggest that the socioeconomic differences between regions are insufficient to produce signifi-

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cant alteration on the spatial prevalence of canine intestinal parasites in this country.

Among the parasites found in our survey, *Ancylostoma* spp., *T. canis*, *Giardia* spp. and *Cryptosporidium* spp. are considered responsible for important zoonotic infections. *Ancylostoma* spp. was the most common parasite detected both in domiciled and stray dogs, occurring in single or mixed infections. Identified risk factors for dog hookworm infection were whether the dog was raised in a multi-dog household or had originated from a refuge. *Ancylostoma* spp. has been referred to as one of the most frequent intestinal parasite of dogs in Brazil (Côrtes et al., 1988; Gennari et al., 1999; Oliveira-Sequeira et al., 2002; Labruna et al., 2006) and other countries (Bugg et al., 1999; Minnaar et al., 2002; Blazius et al., 2005; Wang et al., 2006).

Besides *A. caninum*, which is one of the most pathogenic species for dogs, larvae and adults of different *Ancylostoma* species are involved in human infections. Cutaneous larva migrans or creeping eruption (Velho et al., 2003) is the most common of them. In Brazil, reliable epidemiological data about CLM in native population is scarce, but a population-based study (Heukelbach et al., 2004) demonstrated that CLM is endemic in a deprived community. The high frequency of *Ancylostoma* spp. found in this study, especially in stray dogs, suggests that this condition could be more widely distributed than it is currently believed. However, the overlooking of these diseases by the population itself and by health care professionals (Heukelbach et al., 2004) makes it difficult to evaluate the actual importance of CLM. Similarly, human infection by adults of *A. caninum*, already reported in other countries (Prociv and Croese, 1996), has not been diagnosed in Brazil.

The overall frequency of *T. canis* (8.7%) obtained here is comparable to those obtained in previous studies performed in the last 20 year in Brazil: 11.70% (Côrtes et al., 1988), 8.49% (Gennari et al., 1999), and 5.54% (Oliveira-Sequeira et al., 2002). These data suggest that there was no significant downward long-term trend in the prevalence of this parasite as reported in other countries (Robertson and Thompson, 2002).

In a recent survey on epidemiology of toxocariasis in Brazil, Muradian et al. (2005) showed a high correspondence between the frequency of infection in dogs under 1 year, soil contamination and children serology. In this study, the frequency of infected dogs (8.7%) was significantly lower than that reported by those authors, however, only 5.5% of these dogs were younger. The great fertility of these worms, associated with the great resistance of *T. canis* eggs (Jordan et al., 1993) contribute decisively for a cumulative environmental contamination, representing a higher risk of human infection than suggested by the infection rate of dogs (Oliveira-Sequeira et al., 2002). This may be one of the reasons why the human infection by *T. canis* is the most commonly acquired zoonoses from companion animals in the United States, despite decrease in the prevalence of infected dogs in the last two decades (Robertson and Thompson, 2002).

In this study, *T. canis* eggs were found in four of the 14 dogs aged <6 months, and in 18 of the 240 adult animals. All of the infected young animals were domiciled, whereas all of the adult ones were stray dogs. The infection of the young animals can be attributed to the transplacental passage of larvae, the main way of transmission of the parasite. In the case of the stray adult dogs, it is possible that the infection originated in predatory paratenic hosts, since the larvae, as soon as ingested, can originate adult worms in the intestines without undergoing further somatic migration (Parsons, 1987). Therefore, owned and stray dogs could play a role in human toxocariasis, even if the particular implication of each population is not clearly established (Eguía-Aguilar et al., 2005).

Giardia spp. was the most frequent protozoan found in dogs (16.9%), similar to what has been registered in Brazil (Gennari et al., 1999; Oliveira-Sequeira et al., 2002) and in developed countries (Bugg et al., 1999; Palmer et al., 2008). In Australia, *Giardia* spp. is the most frequent intestinal parasite in dogs. This high prevalence was attributed to the fact that *Giardia* spp. can colonize niche previously occupied by parasites such as *T. canis* and *Dipylidium caninum*, and most of the anthelmintics do not interfere in the development of *Giardia* spp. (Bugg et al., 1999). This does not seem to be a viable explanation for the high prevalence of *Giardia* spp. found in this study for two reasons. Firstly, *Giardia* was one of the most frequent in poliparasitized animals; second, the higher frequency of infection was found in stray dogs, which are not the target of anthelmintic treatment.

The clinical significance of *Giardia* spp. appears minimal, as most of dog infections are asymptomatic. Although there has been much speculation about the public health significance of companion animals (Thompson, 2004), human infections are primordially attributed to anthroponotic transmission. Nevertheless, *Giardia duodenalis* genotype A1 was reported in both child and its dog in Brazil, suggesting the putative existence of a zoonotic cycle in the studied population (Volotão et al., 2007). These findings highlight that zoonotic transmission could represent a public health problem in developing countries, especially in communities that are socioeconomically handicapped.

The low prevalence of *Cryptosporidium* spp. diagnosed here was consistent with previous studies on dog population in Brazil (Huber et al., 2005; Mundim et al., 2007)

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and worldwide (Causape et al., 1996; Fontanarrosa et al., 2006; Dubná et al., 2007). Cryptosporidiasis is a frequent cause of diarrhoeal disease in humans, and in developing countries, *Cryptosporidium* spp. infections occur mostly in children younger than 5 years. In the majority of human patients, *C. parvum* (human and cattle genotype) and *C. hominis* have been identified, but other species (*C. meleagridis*, *C. felis*) and genotypes (*C. parvum* dog genotype) were detected in a proportion of immunocompetent children (Xiao et al., 2004). The relative importance of zoonotic transmission in the epidemiology of cryptosporidiasis is not entirely clear. Recently, in Lima, Peru (Xiao et al., 2007), two children and their dog were diagnosed with *Cryptosporidium canis* infections during the same period, suggesting the possibility of the transmission of cryptosporidiasis among human and dogs.

According to their owners, most of the dogs (95 of 125) had received an anthelmintic at least once in their lives and, of these, 30 had been dewormed during the previous 6 months. However, this information could not be reliable, because during the interviews, most of owners (67.5%) did not know the meaning of the term anthelmintic, mistook vermifugation with other health care procedures, such as anti-rabic vaccinations promoted yearly in the city, and did not give any information about the anti-helminthic treatment. Indeed, many dog owners seem to feel ashamed in admitting that they had not dewormed their pets. Therefore, for most of the dogs, it was impossible to obtain correct information regarding deworming procedures (kind of drug and frequency of administration) as well as reasons for anthelmintics choosing and sources of information. Untrustworthy informations from dog owners could explain why the anti-parasitic treatment did not produce a positive effect in reducing the detection rate of helminthes in those dogs assumed to be dewormed.

It was evident from this study that most of owners are not aware of the zoonotic potential of the parasites carried by their dogs, or their mode of transmission to humans. This lack of knowledge seems to be the main reason for the apparent negligence of the owners in deworming their dogs. These findings contrasts to what was referred in Australia (Bugg et al., 1999) where the majority of owners was aware of zoonotic parasites and so, dewormed their dogs on a regular basis. Misunderstanding regarding dog parasitism, anti-helminthic drugs and health hazards associated with animals are a commonplace in Brazil, as similar problems have been reported in other investigations (Muradian et al., 2005).

During the interviews, it was possible to verify that the majority of the owners had some knowledge about other zoonotic diseases carried by dogs, like rabies and leishmaniasis. Probably, much of this information was received from media (television, radio, etc.), and by public-awareness campaigns, as only 31.2% of the owners said that they regularly sought veterinary assistance. On the other hand, the owners had demonstrated a great interest in learning about canine intestinal parasites, revealing that there is a high level of motivation within communities.

The prevalence and risk factors for canine intestinal parasite obtained in this study do not differ significantly from that previously reported in Brazil. However, for the first time the risk perception of dog owners was assessed, revealing misinformation as an important clue for the control of canine intestinal parasites in this country. It is concluded that a consistent programme of sanitary education must be included in public health government actions as a first step for the control of intestinal parasites in dogs. Finally, veterinary schools should emphasize the client education in training veterinarians as a means to prevent or minimize zoonotic disease transmissions.

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