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Occurrences of gastrointestinal parasites in fecal samples from domestic dogs in São Paulo, SP, Brazil

Ocorrência de parasitos gastrintestinais em amostras fecais de cães domiciliados em São Paulo, SP, Brasil

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Abstract

Occurrences of gastrointestinal parasites were assessed in fecal samples from 3,099 dogs in the metropolitan region of São Paulo, SP, that were treated at the Veterinary Hospital of the University of São Paulo Veterinary School. The samples were analyzed using the flotation and centrifugal sedimentation methods. The results were compared with those from previous studies (at different times). The frequency of each parasite was correlated with the dogs' ages, breeds and gender, as well as the occurrences of diarrhea and the use of anthelmintics, by means of the chi-square or Fisher exact test. Partitioned chi-square tests were used to compare occurrences of each parasite and the times analyzed. Out of the total number of samples, 20.5% were positive and 16.1% (102/635) of these presented more than one genus of parasites. *Ancylostoma* spp. (7.1%) and *Giardia* spp. (5.5%) were the most frequent helminths and protozoa, respectively. *Ancylostoma* spp. was associated (p<0.05) with age (over one year), mixed breeds, sex (male) and no use of anthelmintics. Dogs under one year and mixed breeds were associated with occurrences of *Toxocara canis*; and younger dogs with *Giardia* spp., *Cryptosporidium* spp. and *Cystoisospora* spp. *Giardia* spp. were also associated with dogs with a defined breed (p<0.05). All the parasites analyzed presented lower incidence in the last period analyzed than in the previous periods.

Keywords: Dogs, helminths, protozoa, São Paulo, Brazil.

Resumo

A ocorrência de parasitos gastrintestinais foi estudada em amostras fecais de 3099 cães, da região metropolitana de São Paulo, SP, tratados no Hospital Veterinário da Faculdade de Medicina Veterinária da Universidade de São Paulo. As amostras foram analisadas por métodos de flutuação e centrífugo-sedimentação. Os resultados foram comparados com estudos prévios (diferentes períodos). Associação entre as frequências de cada parasito e idade, raça, sexo, ocorrência de diarreia e uso de anti-helmínticos foram analisadas pelo teste do qui-quadrado ou exato de Fisher. O qui-quadrado estratificado foi utilizado para comparar a ocorrência de cada parasito e os períodos analisados. Do total 20,5% das amostras eram positivas e 16,1% destas (102/635) apresentaram mais de um gênero de parasitos. *Ancylostoma* spp. (7,1%) e *Giardia* spp. (5,5%) foram os mais frequentes helminto e protozoário, respectivamente. *Ancylostoma* spp. foi associado (p<0,05) com idade (> 1 ano), animais sem raça definida, sexo (macho) e a não utilização de anti-helmínticos. Cães < 1 ano e mestiços apresentaram associação com ocorrência de *Toxocara canis* e cães jovens com *Giardia* spp, *Cryptosporidium* spp. e *Cystoisospora* spp. *Giardia* spp. também apresentou associação com cães de raças definidas (p<0,05). Para todos os parasitas analisados a incidência diminuiu no último período analisado quando comparado aos anteriores.

Palavras-chave: Cães, helmintos, protozoários, São Paulo, Brasil.

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Introduction

The growing numbers of pets, especially dogs and cats, in large urban centers are associated with human exposure to various zoonotic agents, including parasites. Brazil has the fourth largest pet population in the world and has the second largest number of dogs and cats (ABINPET, 2015). According to the Brazilian Institute for Geography and Statistics (IBGE, 2015) there are 52.2 million domestic dogs and 22.1 million domestic cats in Brazil.

Parasitic diseases caused by gastrointestinal helminths and protozoa are among the most common diseases in dogs and cats worldwide. These parasites have a prominent role within public health and are responsible for important zoonoses. They include larval forms of *Ancylostoma* spp., *Toxocara* spp., *Echinococcus granulosus*, *Dipylidium caninum* and *Strongyloides stercoralis* and protozoa such as *Giardia* spp. and *Cryptosporidium* spp. (GENNARI et al., 1999, 2001). Not only stray dogs and cats, but also domestic animals are important in the epidemiology of such zoonosesand play an important role, since they generally frequent public places that are also visited by other animals and humans, thus leading to a high likelihood of infection by parasitic agents (LABRUNA et al., 2006).

Considering the close coexistence between pets and humans, it becomes essential to diagnose and adequately control endoparasites in order to reduce environmental contamination with infective forms of these parasites, thereby minimizing the risks of human and animal infections (HARVEY et al., 1991; LABRUNA et al., 2006).

This study aimed to evaluate occurrences of intestinal parasites in fecal samples from pet dogs treated at the Veterinary Hospital of the Faculty of Veterinary Medicine (FMVZ), University of São Paulo (USP), between January 2005 and December 2014, and to compare the findings with data from the same diagnostic service that were published previously, going back to 1991 (GENNARI et al., 1999, 2001; FUNADA et al., 2007).

Materials and Methods

Animals

Results from fecal samples from dogs that were received for parasitological diagnosis by the Parasitic Diseases Laboratory, Department of Preventive Veterinary Medicine and Animal Health, of the Faculty of Veterinary Medicine, University of São Paulo (FMVZ-USP), São Paulo, SP, Brazil, between January 2005 and December 2014, were analyzed in this study. Veterinarians at the Veterinary Hospital (HOVET) of FMVZ-USP sent the samples for examination.

A total of 3,099 fecal samples from domestic dogs living in different areas of the metropolitan region of São Paulo were examined. Information related to gender, breed, age, presence of diarrhea and use of anthelmintics was available, although not for all dogs. Out of the total number of samples analyzed, 1,339 were from males and 1,419 were from females; 2,102 of the dogs belonged to a defined breed and 782 did not; 314 were young (< 1 year old) and 2,248 were adults (≥ 1 year old). Information regarding presence of diarrhea and use of anthelmintics was also available for most of the samples analyzed.

Diagnostic techniques

Fecal samples were examined by means of three methods: centrifugal flotation in sucrose solution (d = 1.203 g/cm^3), as described by Ogassawara et al. (1986); centrifugal sedimentation in water-ether (FERREIRA et al., 1962), and flotation in saturated sodium chloride. Diagnosis of *Cryptosporidum* spp was also based on sucrose flotation, as recommended by Ogassawara et al. (1986). All of these methods were qualitative, and the results were expressed as presence or absence of parasites. Diagnosis of *Dipylidium caninum* was based on visual observation of proglottids in feces or on detection of ovigerous capsules by means of centrifugal sedimentation methods. Because of the impossibility of morphologically differentiating between oocysts of *Hammondia heydorni* and *Neospora caninum* in the feces of dogs, the oocysts that were found were named *Neospora-Hammondia* oocysts.

Statistical analysis

The frequency of positive animals and the respective binomial 95% confidence intervals were calculated for each parasite. Associations between the frequencies of positivity for each parasite and the variables of age, breed, gender, occurrence of diarrhea and anthelmintic treatment were investigated using the chi-square test or the Fisher's exact test (ZAR, 1999). A partitioned chi-square test was used to compare four periods (1991-1995, 1996-1999, 2000-2004 and 2005-2014) in relation to positivity for each parasite. P values < 0.05 were considered significant. All analyses were performed using the SPSS for Windows software, version 20.

Results

Out of the 3,099 fecal samples examined, 20.5% (635) were positive for protozoa and/or helminths (Table 1), and 16.1% (102/635) were infected with more than one genus of parasites. The most common mixed infections found were *Giardia* spp. + *Cystoisospora* spp. (24.5%; 25/102) and *Ancylostoma* spp. + *Trichuris vulpis* (10.8%; 11/102). *Ancylostoma* spp. were the most frequent nematodes, found in 7.1% of all the samples examined (221/3099), and in 34% (221/635) of the positive samples. *Giardia* spp., the most frequent protozoa, was found in 5.5% of the samples (169/3099) and in 26.6% of the positive samples (169/635).

Table 2 shows the associations presented by each parasite with the variables analyzed. Dogs over one year of age, mixed breed, males and no use of anthelmintics were associated with occurrences of *Ancylostoma* spp. ($p < 0.05$). Younger dogs (< 1 year) and mixed breed were associated with occurrences of *T. canis* ($p < 0.05$). Younger dogs were also associated with presence of *Giardia* spp., *Cystoisospora* spp. and *Cryptosporidium* spp. ($p < 0.05$). An association was also observed between positivity for *Giardia* spp. and dogs belonging to a defined breed ($p < 0.05$).

Table 3 shows the results from the most common nematodes and protozoa found in this study and comparisons with values previously published by the same laboratory service using the same techniques on samples from dogs seen at the HOVET. Statistical differences in the results obtained were found ($p < 0.001$) between the periods and between the frequencies of all the parasites analyzed

Table 1. Frequencies of helminths and protozoa in 3,099 fecal samples from dogs in the city of São Paulo, SP, Brazil, from 2005 to 2014.

Parasite	No. positive	Occurrences (%)	$CI_{95\%}$ [%]
Helminths			
Ancylostoma spp.	221	7.1	[6.2; 8.0]
Toxocara canis	23	0.7	[0.4; 1.0]
Trichuris vulpis	15	0.5	[0.2; 0.7]
Strongyloides stercoralis	3	0.1	[0.0; 0.21]
Dipylidium caninum	3	0.1	[0.0; 0.21]
Protozoa			
Giardia spp.	169	5.5	[4.7; 6.3]
Cystoisospora spp.	46	1.5	[1.1; 1.9]
Cryptosporidium spp.	28	0.9	[0.6; 1.2]
Sarcocystis spp.	16	0.5	[0.3; 0.8]
Neospora-Hammondia	2	0.06	[0.02; 0.24]

(*Ancylostoma* spp., *T. canis, T. vulpis, Giardia* spp., *Cryptosporidium* spp. and *Cystoisospora* spp.). The occurrences found in the last period (2005-2014) were lower ($p < 0.05$) than those observed over the last two previous periods (1996-1999, 2000-2004).

Discussion

Ancylostoma spp., *T. canis* and *T. vulpis* were the most common nematodes and *Giardia* spp., *Cryptosporidium* spp. and *Cystoisospora* spp., the most frequent protozoa found in the present study, as observed in previous studies by the same parasitological service (GENNARI et al., 1999, 2001; FUNADA et al., 2007). However, the occurrence rate of each parasitic agents was lower over the most recent period evaluated, thus indicating that effective control measures were implemented. Administration of anthelminthics to

Table 2. Occurrences (%) of helminth and protozoan parasites in feces from 3,099 dogs, according to age, breed, sex, presence of diarrhea and use of anthelmintics. São Paulo, 2005-2014.

Parasites	Age		Breed		Sex		Diarrhea		Anthelmintic use	
	< 1 year	≥ 1 year	Pure	Mixed	Male	Female	No	Yes	No	Yes
	Nº (%)	Nº (%)	Nº (%)	Nº (%)	Nº (%)	Nº (%)	Nº (%)	Nº (%)	Nº (%)	Nº (%)
Single infection	116 (36.9)*	340 (15.1)*	407 (19.4)	165 (21.1)	268 (20)*	213 (15)*	96 (18.3)	147 (20.8)	75 (19.8)	183 (18.9)
Mixed infection	24 (7.6)*	37 (1.6)*	69 (3.3)	24 (3.1)	25 (1.9)	37 (2.6)	13 (2.5)	22 (3.1)	7 (1.9)	27 (2.8)
Helminth										
Ancylostoma spp.	6 (1.9)*	181 (8.1)*	133 (6.3)*	68 (8.7)*	131 (9.8)*	71 (5.0)*	38 (7.2)	54 (7.6)	40 (10.6)*	60 (6.2)*
Toxocara canis	8 (2.5)*	13 (0.6)*	10 (0.5)*	11 (1.4)*	14 (1)	9 (0.6)	6 (1.1)	4 (0.6)	3 (0.8)	8 (0.8)
Trichuris vulpis	1 (0.3)	13 (0.6)	9 (0.4)	6 (0.8)	9 (0.7)	6 (0.4)	2 (0.4)	6 (0.8)	1 (0.3)	6 (0.6)
Strongyloides stercoralis	1 (0.3)	2 (0.1)	2 (0.1)	1 (0.1)	1 (0.1)	2 (0.1)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
Dipylidium caninum	0 (0.0)	2 (0.1)	2 (0.1)	1 (0.1)	3 (0.2)	0 (0)	0 (0)	1 (0.1)	1 (0.3)	0 (0.0)
Protozoa										
Giardia spp.	37 (11.8)*	46 (2.0)*	119 (5.7)*	26 (3.3)*	44 (3.3)	43 (3)	17 (3.2)	29 (4.1)	11 (2.9)	43 (4.5)
Cystoisospora spp.	25 (8.0)*	14 (0.6)*	31 (1.5)	10 (1.3)	22 (1.6)	17 (1.2)	7 (1.3)	15 (2.1)	3 (0.8)	19 (2.0)
Cryptosporidium spp.	9 (2.9)*	14 (0.6)*	16 (0.8)	10 (1.3)	8 (0.6)	15 (1.1)	5 (1.0)	7 (1.0)	2 (0.5)	11 (1.1)
Sarcocystis sp.	3 (1.0)	12 (0.5)	8 (0.4)	7 (0.9)	8 (0.6)	7 (0.5)	5 (1.0)	6 (0.8)	4 (1.1)	7 (0.7)
Hammondia-Neospora	0 (0.0)	2 (0.08)	0 (0.0)	2 (0.2)	2 (0.2)	0 (0.0)	1 (0.2)	1 (0.1)	0 (0.0)	1 (0.1)

* ($P<0.05$) between the categories within the same variable.

Table 3. Occurrences of nematodes and protozoa in dog fecal samples diagnosed in the Parasitic Diseases Laboratory, Veterinary Hospital, Faculty of Veterinary Medicine, University of Sao Paulo, Sao Paulo, SP, Brazil, from 1991 to 2014.

Parasites	Periods Positive (%)			
	1991-1995[1] (n = 353)	1996-1999[2] (n = 871)	2000-2004[3] (n = 1755)	2005-2014[4] (n = 3099)
Helminths				
Ancylostoma spp.	72 (20.4)[a]	118 (13.5)[b]	223 (12.7)[b]	221 (7.1)[c]
Toxocara canis	30 (8.5)[a]	48 (5.5)[a]	46 (2.6)[b]	23 (0.7)[c]
Trichuris vulpis	1 (0.3)[a]	21 (2.4)[b]	31 (1.8)[b]	15 (0.5)[a]
Protozoa				
Giardia spp.	27 (7.6)[ab]	111 (12.7)[c]	149 (8.5)[a]	169 (5.5)[b]
Cryptosporidium spp.	10 (2.8)[a]	31 (3.5)[a]	43 (2.4)[a]	28 (0.9)[b]
Cystoisospora spp.	9 (2.5)[ab]	52 (5.9)[c]	77 (4.4)[ac]	46 (1.5)[b]

Different letters in the same row indicate significant difference ($p<0.05$). [1]Gennari et al. (1999), [2]Gennari et al. (2001), [3]Funada et al. (2007), [4]Present study, n = number of dogs examined.

the dogs of the present study significantly reduced occurrences of *Ancylostoma* spp. Given that this was the most prevalent nematode (7.1%; CI: 6.2-8.0%), and because the dogs' immunity has low effect on the development of this nematode, which can be found in both adult and young dogs, the efficacy of treatments can clearly be seen.

Other surveys, conducted in different regions of Brazil on domestic and feral dogs (BLAZIUS et al., 2005; LABRUNA et al., 2006; BRESCIANI et al., 2008; KATAGIRI & OLIVEIRA-SEQUEIRA, 2008; OLIVEIRA et al., 2009; PRATES et al., 2009; KLIMPEL et al., 2010; FERREIRA et al., 2013), also found that *Ancylostoma* spp. had the highest prevalence. Thus, the present study confirms previous observations that *Ancylostoma* is the most common nematode genus in dogs in Brazil (reviewed by LABRUNA et al., 2006).

Recent surveys on dogs in other parts of the world have also shown that *Ancylostoma* spp, *T. canis* and *T. vulpis* are the most prevalent nematodes. In the Czech Republic (DUBNÁ et al., 2007), Nigeria (UGBOMOIKO et al., 2008), USA (MOHAMED et al., 2009) and Argentina (SORIANO et al., 2010), all three of these nematodes were present, but *T. canis* showed the highest prevalence. In Venezuela (RAMÍREZ-BARRIOS et al., 2004), Colombia (GIRALDO et al., 2005) and Argentina (FONTANARROSA et al., 2006), like in the present study, *Ancylostoma* spp. were the nematodes most frequently found.

Comparisons between the results from different studies should be made carefully, given that diagnostic techniques with different sensitivities were used. The use of fecal flotation coupled with centrifugation is recommended for routine examination of fecal samples, as this provides significantly greater sensitivity than the flotation alone (MOHAMED et al., 2009). The centrifugal flotation technique in association with simple flotation and centrifugal sedimentation methods were used in this and in all previous studies (GENNARI et al., 1999, 2001; FUNADA et al., 2007) that are compared here. Combined use of these techniques improves the diagnostic of helminths and protozoa in dogs and cats.

All intestinal helminths detected in this study have a zoonotic potential. *A. caninum* and *A. braziliense* are responsible for cutaneous larva migrans in humans. This disease, despite its low importance in comparison with other parasitic zoonosis, can cause clinical complications. In a study conducted in Germany on 98 patients with cutaneous larva migrans, which had been acquired after these individuals had traveled to tropical and subtropical areas, 28.9% reported having symptoms for more than one month, and one patient had symptoms for nine months (JELINEK et al., 1994).

Toxocariasis is also common in humans and is one of the most prevalent zoonosis transmitted by dogs around the world. The serological prevalence of toxocariasis in humans, in Brazil, ranges from 7% to 54.8% (reviewed by CARVALHO & ROCHA, 2011), and this reflects the high level of environmental contamination by *Toxocara* spp. eggs.

Trichuris vulpis, S. stercoralis and *D. caninum* were also found in the present survey. Although *T. vulpis* is rarely found in humans, this species can cause an uncommon and severe zoonosis (MÁRQUEZ-NAVARRO et al., 2012). *S. stercoralis* infects humans percutaneously and can replicate within the host causing persistent infection and chronic disease with a wide variation of clinical manifestations, depending on the host immune status (OLSEN et al., 2009). *D. caninum* is another zoonotic agent (NEIRA et al., 2008; CABELLO et al., 2011) with worldwide prevalence. In many surveys on dogs and cats, the frequency of this parasite has been underestimated because the diagnosis is based on fecal flotation methods, which are inefficient to detect proglottids (KLIMPEL et al., 2010). The best means of detection is the observation of proglottids in the fecal samples (GENNARI et al., 1999). In addition to being zoonotic agents, those nematodes may also be very pathogenic to dogs, especially to young ones, and in cases of heavy infections (SOULSBY, 1982).

Giardia spp. was the most prevalent protozoa found in the present study, as observed in the previous studies (GENNARI et al., 1999, 2001; FUNADA et al., 2007). *Giardia* spp. cysts are eliminated through feces intermittently, but this sporadic shedding of cysts by dogs may still give rise to zoonotic risk (TRAUB et al., 2004). Most epidemiological surveys of infections with gastrointestinal parasites in dogs and cats are based on examination of a single stool sample, which may provide underestimation of occurrences of this parasite (THOMPSON et al., 2008).

Molecular studies on *G. duodenalis* have defined seven genetic assemblages and not all of them were found to affect both humans and animals. However, the role of dogs and cats in transmitting this infection cannot be conclusively ruled out (BALLWEBER et al., 2010). In Brazil, Volotão et al. (2007) described a case of *G. duodenalis* infection associated with a child and her dog¸ in which both isolates had the same assemblage (A1). In a rural community in India, Traub et al. (2004) also recovered genetically similar isolates of *Giardia* from dogs and humans living in the same household. In Germany, Leonhard et al. (2007) found 60 *Giardia*-positive samples in dogs, among which 60% had the zoonotic assemblage A, 12% had the dog-specific assemblages C and D, and 28% had mixed infections.

The occurrence rates of *Cryptosporidium* spp, *Sarcocystis* spp. and *Neospora-Hammondia* oocysts were lower than 1%. *Cryptosporidium* spp. is considered to be a zoonotic agent and is rarely presented in surveys on dog feces in Brazil. However, it was described infecting domestic dogs in Botucatu, also in the state of São Paulo (KATAGIRI & OLIVEIRA-SEQUEIRA, 2008), in Rondônia (LABRUNA et al., 2006), in Rio de Janeiro (HUBER et al., 2005) and in the previous surveys on dogs at the same veterinary service as in the present study (GENNARI et al., 1999, 2001; FUNADA et al., 2007), as well as the dogs of this present study. In all of these surveys, the occurrence values were higher than 0.9%, as observed in the present study.

Thompson et al. (2008) predominantly found host-adapted species (*C. canis* and *C. felis*). Nonetheless, based on a few studies on genotyping oocysts from dogs, they considered that companion animals were a potential source of human *Cryptosporidium* infection. This infection is more common in young and immunosuppressed animals, as well as in humans (THOMPSON et al., 2008). In Peru, Xiao et al. (2007) diagnosed *C. canis* in two children who lived in the same house, and in their dog, thus indicating the possibility that dog-adapted *Cryptosporidium* species were able to infect humans.

Although virtually all parasitic agents observed in this study are zoonotic, a Brazilian study showed that dog owners knew very little about the importance of parasites carried by dogs. Thus, tackling the lack of information among dog owners will be an important step towards parasite control in Brazil (KATAGIRI & OLIVEIRA-SEQUEIRA, 2008).

The association observed in the present study between age < 1 year and parasitic infections was expected and it was related to the biology of the parasite and the host immune status (FONTANARROSA et al., 2006; LABRUNA et al., 2006; KATAGIRI & OLIVEIRA-SEQUEIRA, 2008; UGBOMOIKO et al., 2008; MOHAMED et al., 2009). The factors of age over one year, mixed breed, male gender and lack of anthelmintic use were associated with occurrences of *Ancylostoma* spp. ($p<0.05$). *Ancylostoma* spp. was prevalent in dogs of all ages, but higher occurrences were observed in adult dogs (> one year). In tropical or semitropical countries, and especially in Brazil, where *Ancylostoma* spp. is the most prevalent nematode in dogs, the use of anthelminthics is recommended not only in juveniles but also in adult dogs, throughout their lives.

Many of the positive samples were not from dogs with diarrhea, including samples positive for *Giardia* spp., *Cryptosporidium* spp. and *Cystoisospora* spp., although all these agents can cause diarrhea in dogs. These findings draw attention to the importance of fecal examination within the routine of veterinary practice. Pet owners need to be properly educated about the risks of zoonoses and about the importance of sending samples for parasitological examination regularly and also of collecting and properly discarding the feces from their animals. Some anthelminthics formulated for dogs do not show efficacy against protozoan parasites, for which different treatments are indicated.

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