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Frequency of gastrointestinal parasites in cats seen at the University of São Paulo Veterinary Hospital, Brazil

Frequência de parasitos gastrintestinais em gatos atendidos no Hospital Veterinário da Universidade de São Paulo, Brasil

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Abstract

The frequency of gastrointestinal infections in 502 cats seen at the Veterinary Hospital of the University of São Paulo, SP, Brazil, between 2005 and 2014, was measured. The samples were analyzed using methods of flotation and sedimentation. The results were compared with those from studies published previously using fecal samples from the same hospital at different times. Associations between the frequency of positivity for each parasite and age, breed, sex, diarrhea and use of anthelmintic were investigated (chi-square or Fisher's exact tests). A partitioned chi-square test was used to compare different periods. *Cryptosporidium* spp., *Giardia* spp., *Cystoisospora* spp. and *Sarcocystis* spp. were the most common parasites, followed by *Toxocara cati* and *Ancylostoma* spp. *Cryptosporidium* spp. presented higher frequency in young cats and *Sarcocystis* spp. with the presence of diarrhea ($p < 0.05$). Results from this study with previous periods showed that the frequencies of *Cryptosporidium* spp., *Cystoisospora* spp. and *T. cati* were lower ($p < 0.05$) than those observed in previous periods. The frequencies of *Giardia* spp. and *Ancylostoma* spp. were similar to the results found in the preceding period and lower than the values found for the other periods ($p < 0.05$). The reasons for these changes should be investigated.

Keywords: Cat, gastrointestinal parasites, São Paulo, Brazil.

Resumo

A frequência de infecções gastrintestinais foi avaliada em 502 gatos atendidos no Hospital Veterinário da Universidade de São Paulo, SP, Brasil, entre 2005 e 2014. As amostras foram analisadas utilizando-se os métodos de flutuação e sedimentação. Os resultados foram comparados com os de estudos previamente publicados com amostras de fezes oriundas do mesmo hospital, em momentos diferentes. As associações entre a frequência de positividade para cada parasito e idade, raça, sexo, ocorrência de diarreia e uso de anti-helmínticos foram investigadas (teste de qui-quadrado ou teste exato de Fisher). Um teste de qui-quadrado estratificado foi utilizado para comparar os diferentes períodos (1991-1995; 1996-1999; 2000-2004 e 2005-2014). *Cryptosporidium* spp., *Giardia* spp., *Cystoisospora* spp. e *Sarcocystis* spp. foram os parasitos mais comuns, seguidos por *Toxocara cati* e *Ancylostoma* spp. *Cryptosporidium* spp. apresentou maior frequência em gatos jovens e *Sarcocystis* spp. em animais com diarreia ($p < 0,05$). Comparação das frequências obtidas neste estudo com os de períodos anteriores mostrou que as frequências de *Cryptosporidium* spp., *Cystoisospora* spp. e *T. cati* foram menores ($p < 0,05$) que as observadas nos períodos anteriores. As frequências de *Giardia* spp. e *Ancylostoma* spp. foram semelhantes ($p > 0,05$) aos resultados encontrados no período de 2000 a 2004 e menores do que os valores encontrados para os outros períodos ($p < 0,05$). Os motivos dessas alterações devem ser investigados.

Palavras-chave: Gatos, parasitas gastrintestinais, São Paulo, Brasil.

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Introduction

Felines play an essential role in the epidemiology of gastrointestinal parasites causing zoonosis, including *Ancylostoma tubaeforme*, *Ancylostoma braziliense*, *Toxocara cati*, *Dipylidium caninum* and protozoa such as *Giardia* spp. and *Cryptosporidium* spp. (GENNARI et al., 2001; FUNADA et al., 2007).

According to the Brazilian Institute for Geography and Statistics (IBGE, 2015), there are 22.1 million domestic cats in Brazil, thus representing approximately 1.9 cats per household. However, in some regions of the country, studies on the prevalence of gastrointestinal parasites of cats are scarce or absent, and veterinarians have very little information for developing strategic control measures and treatments.

The aims of this study were to determine the frequency of gastrointestinal parasites in fecal samples from cats seen at a veterinary teaching hospital, in the metropolitan region of São Paulo, SP, Brazil, from 2005-2014, and compare this with results published previously (GENNARI et al., 1999, 2001; FUNADA et al., 2007), from the same diagnostic service since 1991.

Materials and Methods

Animals

The data used in the present study were obtained from single fecal samples from all cats examined for gastrointestinal parasites at the Parasitology Laboratory Service of the Veterinary Hospital (HOVET), University of São Paulo Veterinary School, São Paulo, SP, Brazil, from January 2005 to December 2014. The samples were collected by the owners, kept refrigerated and send to the laboratory within 12 hours after collection.

A total of 502 samples from domestic cats (i.e. with an owner) living in the metropolitan region of São Paulo were examined. Information relating to sex, breed (belonging or not belonging to a breed), age (< one year, ≥ one year), presence or absence of diarrhea and use of anthelmintics were available, but not for all the cat samples. Out of this total, it was known that 262 of the cats were male and 228 were female; 105 were young (< one year) and 340 were adults (≥ one year); 137 belonged to a breed and 349 did not belong to any defined breed; 170 presented diarrhea and 98 did not have diarrhea; and 148 had had anthelmintics administered previously and 87 had never used anthelmintics.

Diagnostic techniques

The routine diagnostic procedures used at the parasitological service consist of three methods: centrifugal sedimentation in water-ether (FERREIRA et al., 1962), flotation in sucrose solution (d = 1.203 g/cm³) (OGASSAWARA & BENASSI, 1980) and flotation in saturated NaCl solution. *Dipylidium caninum* was diagnosed based on the presence of proglottids in feces. All the methods were qualitative, and the results were expressed as presence of parasites.

Statistical analyses

The frequency of positive cats and respective binomial 95% confidence intervals (CI) were calculated for each parasite. The associations between the frequencies of each parasite and the variables of age, breed, gender, occurrence of diarrhea and anthelmintic treatment were investigated using the chi-square or Fisher's exact tests (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 the analyses were performed using the BioEstat software (AYRES et al., 2007), version 5.03.

Results

A total of 502 cats were examined, and the overall infection occurrence rate was 18.1% [CI_{95%}: 15.0-21.7%; 91/502]. Mixed infection was presented by 4.4% [CI_{95%}: 2.1-5.4%; 17/502] of the animals examined, i.e. 18.7% (17/91) of the positive samples. The most frequent associations of infection were *Giardia* spp. and *Cryptosporidium* spp. (3/502) and *Giardia* spp., *Cryptosporidium* spp. and *Cystoisospora* spp. (3/502).

The protozoa *Cryptosporidium* spp., *Giardia* spp. and *Cystoisospora* spp. were the most common parasitic agents infecting the cats, with occurrence rates (with 95% CI in brackets) of 5.9% [4.2; 8.4%], 5.2% [3.4; 7.5%] and 4.6% [3.1; 6.8%], respectively. The nematodes with highest frequencies were *Toxocara cati* and *Ancylostoma* spp., respectively with 2.2% [1.2; 3.9%] and 1.4% [0.7; 2.9%]. At lower positivity rates, protozoa of the genera *Sarcocystis* spp (7/502) and *Chilomastix* spp. (1/502) and the helminths *Platynosomum* spp. (4/502), *Toxascaris leonina* (2/502), and *Dipylidium caninum* (2/502) were also found. Table 1 presents the frequencies of cats positive for each parasite infection.

Table 2 presents the associations between each parasite and the variables analyzed. Cats aged less than one year and cats with diarrhea were associated with parasite infection (p < 0.05).

Table 1. Frequency of occurrence (with 95% CI) of gastrointestinal infection in fecal samples from 502 cats in the metropolitan region of São Paulo, according to parasite genus and species.

Parasites	Positive cats		
	Number	Occurrence (%)	CI _{95%} [%]
Helminths			
<i>Toxocara cati</i>	11	2.2	[1.2; 3.9]
<i>Ancylostoma</i> spp.	7	1.4	[0.7; 2.9]
<i>Platynosomum</i> spp.	4	0.8	[0.3; 2.0]
<i>Toxascaris leonina</i>	2	0.4	[0.1; 1.4]
<i>Dipylidium caninum</i>	2	0.4	[0.1; 1.4]
Protozoa			
<i>Cryptosporidium</i> spp.	30	5.9	[4.2; 8.4]
<i>Giardia</i> spp.	26	5.2	[3.4; 7.5]
<i>Cystoisospora</i> spp.	23	4.6	[3.1; 6.8]
<i>Sarcocystis</i> spp.	7	1.4	[0.7; 2.9]
<i>Chilomastix</i> spp.	1	0.2	[0.04; 1.1]

When the parasites were analyzed individually, *Cryptosporidium* spp. was associated with age, such that there was higher frequency in young cats ($p < 0.05$), and *Sarcocystis* spp. with presence of diarrhea ($p < 0.05$).

Table 3 shows the results regarding the most common nematodes and protozoa (*T. cati*, *Ancylostoma* spp., *Giardia* spp., *Cryptosporidium* spp. and *Cystoisospora* spp.) found in the present study and comparisons with values previously published by the same laboratory service (same techniques and cats also attended by HOVET), during different periods (1991-1995, 1996-1999 and 2000-2004). For *T. cati*, *Cryptosporidium* spp. and *Cystoisospora* spp., the frequency rates found for the latest period (2005-2014) were lower ($p < 0.05$) than values observed in the previous periods. For *Ancylostoma* spp. and *Giardia* spp., the frequencies were similar ($p > 0.05$) to those of the preceding period (2000-2004) and lower ($p < 0.05$) than the values found in the other periods analyzed (1991-1995 and 1996-1999).

Discussion

The cats used in the present study were client-owned cats under care at a university veterinary hospital. Therefore, the results represent this category of household cats, and comparisons with shelter or stray cat surveys should be made with caution. Previous studies on household, stray and shelter cats found significant lower occurrence in the group of owned animals (REMBIESA & RICHARDSON, 2003; SERRA et al., 2003; MCGLADE et al., 2003; JOFFE et al., 2011). However, in spite of having an owner and a home, some of those cats were free-roaming. They were allowed to go outdoors and thus became more exposed to sources of infection. They therefore represent a reservoir of parasites that are potentially zoonotic to humans and other companion animals.

In this study, the most frequent parasites were *Cryptosporidium* spp. (30/502), *Giardia* spp. (26/502) and *Cystoisospora* spp. (23/502).

Table 2. Frequency (%) of helminths and protozoan parasites in feces from 502 cats according to age, breed, sex, presence of diarrhea and use of anthelmintics. São Paulo, 2005-2014.

Parasites	Age		Breed		Sex		Diarrhea		Anthelmintic treatment	
	< 1 year	≥ 1 year	Pure	Mixed	Male	Female	No	Yes	No	Yes
	No. positive (%)									
Total	33 (31.4)*	43 (12.6)*	19 (13.9)	68 (19.5)	47 (17.9)	42 (18.4)	45 (14.5)*	44 (25.9)*	62 (18.2)	27 (18.2)
Mixed infection	4 (3.8)	8 (2.4)	3 (2.2)	12 (3.4)	8 (3.1)	8 (3.5)	9 (2.9)	8 (4.7)	12 (3.5)	5 (3.4)
Helminths										
<i>Toxocara cati</i>	4 (3.8)	4 (1.2)	1 (0.7)	9 (2.6)	4 (1.5)	6 (2.6)	6 (1.9)	4 (2.4)	6 (1.8)	5 (3.4)
<i>Ancylostoma</i> spp.	3 (2.9)	3 (0.9)	3 (2.2)	4 (1.1)	3 (1.1)	4 (1.8)	6 (1.9)	0 (0)	7 (2.1)	0 (0)
<i>Toxascaris leonina</i>	0 (0)	1 (0.3)	1 (0.7)	0 (0)	2 (0.8)	0 (0)	2 (0.6)	0 (0)	2 (0.6)	0 (0)
<i>Platynosomum</i> spp.	0 (0)	2 (0.6)	1 (0.7)	2 (0.6)	1 (0.4)	3 (1.3)	2 (0.6)	2 (1.2)	2 (0.6)	2 (1.4)
<i>Dipylidium</i> sp.	0 (0)	1 (0.3)	0 (0)	1 (0.3)	1 (0.4)	0 (0)	1 (0.3)	1 (0.6)	1 (0.3)	1 (0.7)
Protozoa										
<i>Cryptosporidium</i> spp.	14 (13.3)*	10 (2.9)*	6 (4.4)	22 (6.3)	13 (5)	16 (7)	14 (4.5)	16 (9.4)	20 (5.9)	10 (6.8)
<i>Giardia</i> spp.	9 (8.6)	13 (3.8)	3 (2.2)	23 (6.6)	15 (5.7)	11 (4.8)	12 (3.9)	14 (8.2)	18 (5.3)	7 (4.7)
<i>Cystoisospora</i> spp.	7 (6.7)	14 (4.1)	5 (3.6)	18 (5.2)	12 (4.6)	11 (4.8)	11 (3.5)	12 (7.1)	15 (4.4)	8 (5.4)
<i>Sarcocystis</i> spp.	0 (0)	7 (2.1)	2 (1.5)	5 (1.4)	5 (1.9)	2 (0.9)	1 (0.3)*	6 (3.5)*	6 (1.8)	0 (0)
<i>Chilomastix</i> spp.	0 (0)	1 (0.3)	0 (0)	1 (0.3)	0 (0)	1 (0.4)	0 (0)	1 (0.6)	1 (0.3)	0 (0)

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

Table 3. Frequency of helminths and protozoa in fecal samples from cats seen at the Veterinary Hospital, University of São Paulo Veterinary School, São Paulo, SP, Brazil, from 1991 to 2014.

Parasites	Period Positive (%)			
	1991-1995 ¹ (n = 187)	1996-1999 ² (n = 303)	2000-2004 ³ (n = 372)	2005-2014 ⁴ (n = 502)
Helminths				
<i>Toxocara cati</i>	64 (34.2) ^a	31 (10.2) ^b	20 (6.1) ^b	11 (2.2) ^c
<i>Ancylostoma</i> spp.	25 (13.4) ^a	12 (3.9) ^b	7 (2.1) ^{bc}	7 (1.4) ^c
Protozoa				
<i>Giardia</i> spp.	30 (16.0) ^a	44 (14.5) ^a	27 (8.3) ^b	26 (5.2) ^b
<i>Cryptosporidium</i> spp.	27 (14.4) ^a	31 (10.2) ^a	37 (11.3) ^a	30 (5.9) ^b
<i>Cystoisospora</i> spp.	72 (38.5) ^a	40 (13.2) ^b	27 (8.3) ^c	23 (4.6) ^d

Different letters in the same row indicate significant differences by the chi-square partitioned test ($p < 0.05$). ¹Gennari et al. (1999), ²Gennari et al. (2001), ³Funada et al. (2007), ⁴Present study, n = number of cats examined.

After these three protozoa, the nematodes *T. cati* (11/502) and *Ancylostoma* spp. (7/502) were the next most frequent. With the exception of the results observed during the first period analyzed (1991-1995), in which *T. cati* was among the three most common parasites, over the last 20 years the protozoa *Cryptosporidium* spp., *Giardia* spp. and *Cystoisospora* spp. have presented the highest frequencies in cats seen at HOVET.

Infections with more than one parasite were not very frequent, accounting for only 4.4% of all the samples examined, and the most common multiple infections were also between protozoa (*Giardia* spp. and *Cryptosporidium* spp.; and *Giardia* spp., *Cryptosporidium* spp. and *Cystoisospora* spp., each in 3/502). This result deserves special attention from veterinarians, who often use anthelmintics as a preventive control measure, without fecal examination. Several commercial anthelmintics did not show any effectiveness against protozoan parasites.

Unlike what has been observed with ascarids in dogs, *T. cati* presents a low level of resistance to reinfection in older cats and can be found throughout the animals' lives (OVERGAAUW & NEDERLAND, 1997). *T. cati* is often reported to be the most prevalent nematode in cats around the world, as observed in surveys conducted in the USA (NOLAN & SMITH, 1995), (REMBIESA & RICHARDSON, 2003), Mexico (MARTÍNEZ-BARBABOSA et al., 2003), Chile (LÓPEZ et al., 2006), Iran (SHARIF et al., 2007), Australia (PALMER et al., 2008), Canada (JOFFE et al., 2011), Germany (BARUTZKI & SCHAPER, 2011), Egypt (KHALAFALLA, 2011), Italy (ZANZANI et al., 2014) and China (YANG & LIANG, 2015). Also, in Brazil, *T. cati* was found to be the most prevalent helminth in cats in Rio Grande do Sul (LORENZINI et al., 2007; PIVOTO et al., 2013), Paraná (TESSEROLLI et al., 2005), Santa Catarina (STALLIVIERE et al., 2009) and the metropolitan region of São Paulo (GENNARI et al., 2001; RAGOZO et al., 2002; FUNADA et al., 2007).

Although parasites of the genus *Uncinaria* have already been observed in stray cats in the city of Rio de Janeiro (UCHÔA et al., 1998), there is no description of this hookworm in dogs or cats from São Paulo, and, in this study, all diagnosed hookworm eggs were classified as *Ancylostoma* spp.

In the present study, the second most frequent nematode was *Ancylostoma* spp., which is considered to be the most prevalent nematode in dogs in Brazil (reviewed by LABRUNA et al., 2006), and also the one with the highest prevalence in cats in some Brazilian regions (SERRA et al., 2003; LABARTHE et al., 2004; COELHO et al., 2009; RAMOS et al., 2013; MONTEIRO et al., 2016). However, in all surveys on cats in the metropolitan region of São Paulo (OGASSAWARA et al., 1986; GENNARI et al., 1999, 2001; RAGOZO et al., 2002; FUNADA et al., 2007) since 1986, *Ancylostoma* spp. has been the second most prevalent nematode, and in four of the five studies, *T. cati* occurrence was around three times higher than that of *Ancylostoma* spp., in both owned and shelter cats.

Platynosomum fastosum is a trematode that inhabits the hepatobiliary system of domestic and wild felids in tropical and subtropical regions (HEADLEY et al., 2011). In a retrospective study on cats seen at the University of Londrina Veterinary Hospital, in Paraná, Brazil, the most common pathological alterations associated

with parasitism by *P. fastosum* were bile duct hypertrophy and adenomatous epithelial hyperplasia (HEADLEY et al., 2011).

In Brazil, pathological alterations due *P. fastosum* have also been described in cats in Bahia (SAMPAIO et al., 2006), São Paulo (XAVIER et al., 2007; CARREIRA et al., 2008) and Minas Gerais (VIEIRA et al., 2009). Compared with previous studies, in which this trematode was present with occurrence rates of 1.07% (GENNARI et al., 1999) and 0.3% (FUNADA et al., 2007), the rate of 0.8% found in the present period is almost unchanged.

Dipylidium caninum has been found to be very prevalent in all studies in which the animals were necropsied, because the whole proglottid, i. e. the diagnostic stage, is seldom seen in feces. By means of fecal examination, Martinez-Carrasco et al. (2007) found 1% of the dogs examined were positive, whereas necropsies on the same population showed that this tapeworm was present in 47% of the dogs. Thus, the findings from the present study, which were obtained through fecal examination, are certainly underestimates.

The protozoa *Cryptosporidium* spp., *Giardia* spp. and *Cystoisospora* spp. presented very similar occurrence rates in this study and were the most common parasites found in these cats. Similar results were observed in the two previous periods compared: 1991-1999 (GENNARI et al., 1999, 2001) and 2000-2004 (FUNADA et al., 2007). However, the values found in the present period were lower ($p < 0.05$) than those of the previous periods for *Cryptosporidium* spp. and *Cystoisospora* spp. For *Giardia* spp., although the occurrence rate was lower, this difference was not statistically significant. Ogassawara et al. (1986) and Ragozo et al. (2002), who both studied shelter cats in the metropolitan region of São Paulo, and Coelho et al. (2009), who studied shelter cats in Andradina, in the interior of the state of São Paulo, found that *Cystoisospora* spp. and *Cryptosporidium* spp. were the most frequent protozoan parasites. Their results thus confirm the present findings that cats in the state of São Paulo are frequently infected by these protozoa.

Cystoisospora spp. and *Giardia* spp. are also very frequent in cats in other regions of Brazil, with descriptions in Rio de Janeiro, Rio Grande do Sul, Paraná and Pernambuco (SERRA et al., 2003; TESSEROLLI et al., 2005; LORENZINI et al., 2007; MONTEIRO et al., 2016). According to Barutzki & Schaper (2011), *Giardia* spp. is one of the most prevalent parasites of cats in some regions of Europe.

Although *Cryptosporidium* spp. showed an association with age, such that it was more frequently found in young cats (< one year of age), *Cystoisospora* spp. and *Giardia* spp. were present in cats of all ages, with similar occurrence. This result indicates that routine fecal examinations are also important for older cats, and that preventive use of anthelmintics, without previous stool examination, may be very useful for helminth control. However, these treatments may not be effective for protozoan parasites, since several formulations show efficacy only in relation to helminths. The cause of reduction in the occurrence of many of these parasites in recent years, especially helminths, is difficult to explain based only on data that is sent along with fecal samples. There can be many causes, from the more constant use of anthelmintics for all life of animals, lower contact of cats with sources of infections, keeping

them with less access to the streets, to the greater consciousness of the owners of health care, among others.

Although infections by *Cryptosporidium* spp. usually cause diarrhea, especially in young animals, in this study there was no association between the presence of diarrhea and *Cryptosporidium* spp. infection. The same result was previously described by Lucas & Hagiwara (1997) among cats in the metropolitan region of São Paulo.

According to Dubey et al. (2016), cats are definitive hosts for numerous *Sarcocystis* species. In the present study, 3.5% of the fecal samples presented *Sarcocystis* spp. sporocysts, and it was the only parasite in this study that presented an association with diarrhea ($p < 0.05$), such that 0.3% and 3.5% of the cats without and with diarrhea, respectively, were positive for these species.

Information about the use of anthelmintics was available for 235 cats. Out of this total, 148 (63%) had been administered anthelmintics previously and 87 (37%) had never used anthelmintics. At least one third of the owners were uninformed about the importance of anthelmintic treatment and control of endoparasites in their animals.

The cats in the present study were infected by potentially zoonotic parasites such as *Giardia* spp., *Cryptosporidium* spp., *T. cati* and *Ancylostoma* spp. Cats have an important role in the homes on which they live, often sharing with their owners the same locations in the house. This study provides information for veterinarians to be able to implement control measures, thereby not only acting on pets, but also reducing the risk of parasite transmission.

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