

## Occurrence of gastrointestinal protozoans in cats from Londrina, Paraná, Brazil

## Ocorrência de protozoários gastrointestinais em gatos de Londrina, Paraná, Brasil

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### Abstract

Protozoans are widely distributed, and several species may parasitize the digestive tracts of cats (*Felis catus*), and can be transmitted to humans. The present study aimed to evaluate the species and occurrence of gastrointestinal protozoans in cats in the city of Londrina, Paraná, Brazil. A total of 206 cat fecal samples were tested, of which 141 were from shelter animals, and 65 were from pets owned by local people. Samples were processed by parasitological techniques. Coproparasitological techniques (Willis, Faust and Ziehl-Neelsen) were performed for detection of protozoan parasites. Subsequently, all samples were processed by PCR protocols specific to *Toxoplasma gondii*, *Giardia* spp., and *Cryptosporidium* spp. PCR products from positive samples were selected for sequencing. No samples were found to be positive for *Cryptosporidium* spp. using the Ziehl-Neelsen technique. Using specific PCR protocols, 1/206 (0.48%) samples tested positive for *Cryptosporidium* spp. After purification, this one positive sample was sequenced, and it demonstrated a 100% identity match to *Cryptosporidium muris*. Using specific PCR protocols, 13/206 (9.22%) cat fecal samples tested, including 2/65 (3.08%) pet cat fecal samples, were positive for *T. gondii*. PCR analysis revealed that 37/206 (17.96%) of cat fecal samples were positive for *Giardia* spp., including 27/141 (19.15%) of shelter cat fecal samples, and 10/65 (15.38%) pet cat fecal samples ( $p = 0.5124$ ). When sequenced, these positive samples showed a 100% identity match with *Giardia duodenalis*. This study demonstrated that infections with *Cryptosporidium* spp., *Toxoplasma gondii*, and *Giardia duodenalis* are present in the population of both pet cats and shelter cats in the city of Londrina. This poses a risk to public health, because these parasites have a high zoonotic potential.

**Key words:** PCR. Feline. Shelter. *Cryptosporidium muris*. *Toxoplasma gondii*. *Giardia duodenalis*.

### Resumo

Os protozoários são amplamente distribuídos, e várias espécies podem parasitar o trato digestivo de gatos (*Felis catus*) e podem ser transmitidas para humanos. O presente estudo teve como objetivo avaliar as espécies e a ocorrência de protozoários gastrintestinais em gatos na cidade de Londrina, Paraná, Brasil.

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Um total de 206 amostras de fezes de gato foram testadas, das quais 141 eram de animais de abrigo, e 65 eram de animais de propriedade da população local. As amostras foram processadas por técnicas parasitológicas. Willis e esfregaços fecais foram confeccionados e corados pela técnica de Ziehl-Neelsen modificada para detecção de oocistos de *Cryptosporidium* spp. Posteriormente, todas as amostras foram processadas por protocolos de PCR específicos para *Toxoplasma gondii*, *Giardia* spp. e *Cryptosporidium* spp. Os produtos de PCR de amostras positivas foram selecionados para sequenciamento. Nenhuma amostra foi considerada positiva para *Cryptosporidium* spp. usando a técnica de Ziehl-Neelsen. Utilizando protocolos específicos de PCR, 1/206 (0,48%) amostras apresentaram resultados positivos para *Cryptosporidium* spp. Após a purificação, essa amostra positiva foi sequenciada e demonstrou 100% de equivalência de identidade com *Cryptosporidium muris*. Utilizando protocolos específicos de PCR, 13/206 (9,22%) amostras fecais de gatos testadas, incluindo 2/65 (3,08%) amostras fecais de gatos de estimação, foram positivas para *T. gondii*. A análise de PCR revelou que 37/206 (17,96%) das amostras de fezes de gatos foram positivas para *Giardia* spp., incluindo 27/141 (19,15%) de amostras fecais de gatos de abrigo, e 10/65 (15,38%) amostras fecais de gatos de estimação ( $p = 0,5124$ ). Quando sequenciadas, essas amostras positivas apresentaram 100% de equivalência de identidade com *Giardia duodenalis*. Este estudo demonstrou que as infecções por *Cryptosporidium* spp., *Toxoplasma gondii* e *Giardia duodenalis* estão presentes na população de gatos de estimação e gatos de abrigo na cidade de Londrina. Isso representa um risco para a saúde pública, pois estes parasitas têm um alto potencial zoonótico.

**Palavras-chave:** PCR. Felinos. Abrigo. *Cryptosporidium muris*. *Toxoplasma gondii*. *Giardia duodenalis*.

## Introduction

Pets, especially dogs and cats, are beneficial to people and society, as they contribute to the physical, social, and emotional development of children, and the well-being of their owners. However, close contact between animals and humans may involuntarily represent a risk to human health. Therefore, adequate control of parasites is essential to reduce environmental contamination with infectious forms of parasites, and, consequently, minimize infection risks for humans and animals (Dantas-Torres & Otranto, 2014; Robertson, Irwin, Lymbery, & Thompson, 2000).

*Cryptosporidium* is a genus of protozoans, and its transmission occurs by the oral fecal route, with outbreaks reported around the world (Kourenti, Karanis, & Smith, 2007) with the UK accounting for 24% of outbreaks, worldwide. *Giardia duodenalis* and *Cryptosporidium parvum* account for the majority of outbreaks (132; 40.6% and 165; 50.8%, respectively). Currently, 29 *Cryptosporidium* species have been described, and 17 of these are considered to have zoonotic potential. However, the *Cryptosporidium* species can only be differentiated

using molecular techniques (Zahedi, Papparini, Jian, Robertson, & Ryan, 2016). The worldwide prevalence of *Cryptosporidium* species in cats has been described as ranging from 0.6 to 15.4% (Bowman & Lucio-Forster, 2010). Regarding *Cryptosporidium* species in cats, *C. felis* has been the most frequently identified, with *C. muris* found at a lower frequency, although both have zoonotic potential (Santin, Trout, Vecino, Dubey, & Fayer, 2006; Yang, Ying, Monis, & Ryan, 2015).

*Toxoplasma gondii* is a protozoan parasite that can infect a wide range of animal species, including humans. Felids are important in the life cycle of *T. gondii*, since they are definitive hosts, and therefore able to shed oocysts through feces, contaminating the environment (Hill & Dubey, 2002). The importance of toxoplasmosis in public health lies mainly in outbreaks of toxoplasmosis, including ocular toxoplasmosis, and the severity of congenital toxoplasmosis infection and its effects on fetuses (Hill & Dubey, 2002; Nissen et al., 2017). However, the pathogenesis of the disease is dependent on the infectious dose, the strain, and the immunologic host status (Ajzenberg, 2011). Reports of *T. gondii*

in cat feces from Brazil are scarce. Nevertheless, it is well known that *T. gondii* isolates from Brazil show higher diversity when compared with isolates from the northern hemisphere (Dubey et al., 2004; Pena, Soares, Amaku, Dubey, & Gennari, 2006; Shwab et al., 2014).

*Giardia duodenalis* is one of the most common parasitic infections in dogs and cats worldwide, and infected animals may show moderate to severe clinical illness or become asymptomatic (Ballweber, Xiao, Bowman, Kahn, & Cama, 2010). According with molecular analysis, there are seven genotype groups of *G. duodenalis*, and although cats are primarily infected with Assemblage F, they can carry Assemblage A and B, which have zoonotic potential, making cats as important source of infection for humans (Xiao & Fayer, 2008).

Many studies using shelter cats in several countries found that the cats were shedding a large number of protozoan species, such as *Isospora* spp., *Giardia* spp., *Sarcocystis* spp., and different helminths species (Bissett et al., 2009; Christie, Dubey, & Pappas, 1976; Gracenea, Gómez, & Torres, 2012; Guterbock & Levine, 1977; Lucio-Forster & Bowman, 2011; Robben et al., 2004; Sabshin et al., 2012).

This study aimed to detect gastrointestinal protozoans in cats from two distinct habitats in Brazil.

## Material and Methods

### *Local sampling and cats*

Two hundred and six stool samples from cats were collected between September 2012 and May 2013 in Londrina city, Paraná state, Brazil, including 141 from cats in shelters (referred to hereafter as 'shelter cats'), and 65 from local households (referred to hereafter as 'pet cats').

Samples were collected immediately after cats defecated, stored in appropriately labelled containers

in thermal boxes and processed in the Laboratory of Veterinary Parasitology of Londrina State University. All animal procedures were approved by the Ethics Committee for Animal Use of Londrina State University (CEUA n° n° 17396.2012.48), and by the cat owners in an authorization form.

### *Parasitological exams*

Fecal samples were processed using the techniques of Willis, Faust, and Ziehl-Neelsen, with modifications for the detection of cysts and protozoan oocysts (Faust et al., 1939; Ortolani, 2000; Willis, 1921). The parts of the fecal samples not used for this analysis were stored at -20 °C for DNA extraction and PCR.

### *DNA extraction and PCR*

DNA was extracted from all fecal samples using a commercial kit (Macherey-Nagel NucleoSpin Tissue Kit, Germany), according to the manufacturer's instructions.

PCRs for *T. gondii* were performed by amplification of a specific fragment of 529 bp that repeats 200 to 300 times, according to previously described methodology (Homan, Vercammen, De Braekeleer, & Verschuere, 2000). For *Cryptosporidium* spp., a nested PCR that amplifies an 18S rRNA gene fragment, was used, as previously described (Xiao et al., 1999). For *Giardia* spp., a nested-PCR targeting an 18S rRNA gene fragment was performed, as previously described (Appelbee, Frederick, Heitman, & Olson, 2003; Hopkins et al., 1997).

PCR products were submitted to a 1.5% agarose gel electrophoresis, stained with SYBR® Safe (DNA Gel Stain; Invitrogen, Brazil), and visualized under UV light. A 100 bp DNA ladder (100 bp DNA Ladder, Invitrogen, Brazil), and positive and negative controls were included in all agarose gels.

### PCR-RFLP

In fecal samples in which *Toxoplasma*-like oocysts were observed and confirmed by PCR, DNA samples were used to perform genetic characterization by means of PCR-RFLP using 11 genetic markers (SAG1, SAG2, alt. SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and APICO) according to previously described methodology (Su, Shwab, Zhou, Zhu, & Dubey, 2010; Su, Zhang, & Dubey, 2006). DNA from reference samples (GT1, PTG, CTG, TgCgCa1, MAS, TgCatBr5, TgCatBr64, and TgRsCr1) and ultrapure water were included in all reactions, and used as positive and negative controls, respectively. The obtained results were compared, identified, and classified in accordance with the genotypes present in ToxoDB at <http://toxodb.org/toxo>. Phylogenetic analysis comparing the isolates with the reference strains was performed using the Split Tree software version 4.13 (Huson & Bryant, 2006).

### Sequencing

Samples that were PCR-positive for *Cryptosporidium* spp. and *Giardia* spp. were purified using PureLink Quick Gel Extraction Kit (Invitrogen Carlsbad, California, USA), following the manufacturer's recommendations. Sequencing was performed using Big Dye terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), and an ABI300 genetic analyzer sequencer (Applied Biosystems, Foster city, CA, USA).

The nucleotide sequences obtained were compared to standard sequences deposited in GenBank using the BLAST sequence analysis tool.

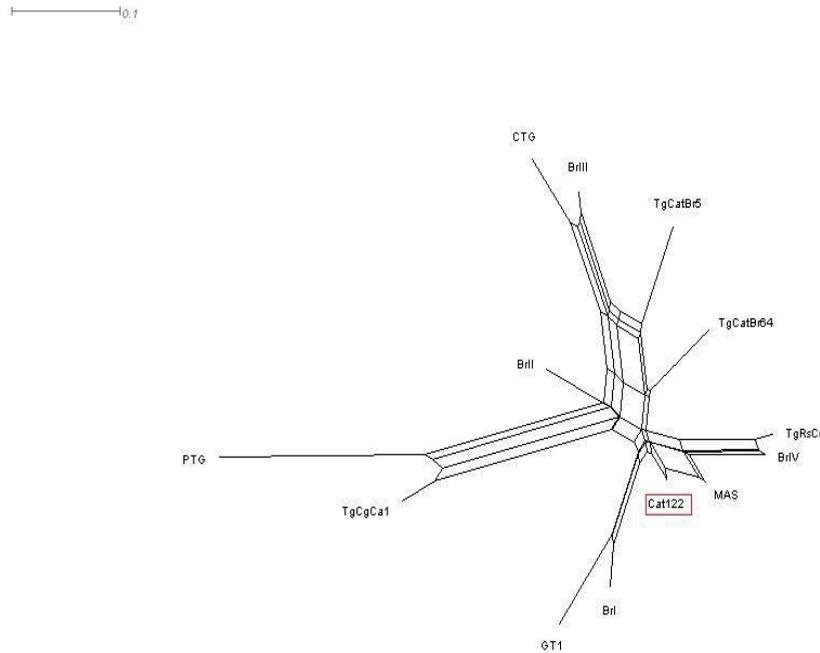
### Statistical analysis

To analyze the results, the Wilson score interval was used to estimate confidence intervals, and Yates corrected Chi-square test was used to measure statistical association. All statistical analyses were performed with the Epitools software and epidemiological calculators (Sergeant, 2017).

### Results

Regarding the copro-parasitological exams, 36/206 (17.48%) of the samples were positive for any of the protozoans (Table 1). We observed mixed infections in only one (0.48%) cat, and the protozoans observed were *Toxoplasma*-like and *Cystoisospora* spp.

Regarding the PCR analysis, 15/206 (7.28%; 95% CI: 4.46 – 11.67) samples were positive for *T. gondii*, including 13/141 (9.22%; 95% CI: 5.47 – 15.14) samples from shelter cats, and 2/65 (3.08%; 95% CI: 0.85 – 10.54) samples from pet cats ( $p = 0.115$ ). When comparing the samples that were positive for *Toxoplasma*-like in the coproparasitological analysis with samples that were positive for *T. gondii* in the PCR analysis, only one sample tested positive using both techniques, which was the only sample genotyped (Table 2). According to ToxoDB, the sample was characterized as genotype #65, an atypical strain not related with clonal strains (Figure 1). It was not possible to genotype the other samples because they did not amplify in all markers, probably due to low DNA concentrations.



**Figure 1.** Phylogenetic tree of the *Toxoplasma gondii* isolate obtained (red box) from cats from Londrina, Paraná state, Brazil compared with the reference strains GT1, PTG, CTG, TgCgCa1, MAS, TgCatBr5, TgCatBr64, TgRsCr1, BrI, BrII, BrIII, BrIV.

Regarding *Cryptosporidium*, all of the 206 fecal samples analyzed using the Ziehl-Neelsen method were negative. However, one sample (from a shelter cat, 0.48%; 95% CI: 0.01 – 2.70) was positive when tested with the 18S rRNA PCR. The sequencing result showed 100% identity match with *Cryptosporidium muris* (access no. KY483984). Regarding *Giardia duodenalis*, 37/206 (17.96%;

95% CI: 13.32 – 23.77) samples tested positive using the nested PCR, which included 27/141 (19.15%; 95% CI: 13.51 – 26.43) samples from shelter cats, and 10/65 (15.38%; 95% CI: 8.58 – 26.06) samples from pet cats ( $p = 0.5124$ ). Four samples were randomly selected and sequenced, and all of these showed 100% identity match with *Giardia duodenalis* (access no. KX384156).

**Table 1**  
**Frequency of intestinal protozoans observed in different coproparasitological exams (Willis; Faust and Ziehl-Neelsen) of shelter and pet cats from Londrina, Brazil**

	Shelter (n=141)		Pet (n=65)		Total (n=206)	
	Positive (%)	95% IC	Positive (%)	95% IC	Positive (%)	95% IC
<i>Cystoisospora</i> spp.	11 (7.80)	4.41-13.43	9 (13.85)	7.46-24.27	20 (9.71)	6.37-14.52
<i>Giardia</i> spp.	13 (9.93)	5.47-15.14	0 (-)	0.01-5.58	13 (6.31)	3.72-10.50
<i>Sarcocystis</i> spp.	0 (-)	0.01-2.65	1 (1.54)	0.27-8.21	1 (0.49)	0.01-2.70
<i>Toxoplasma</i> -like	3 (2.13)	0.73-6.07	0 (-)	0.00-5.58	3 (1.46)	0.50-4.19
<i>Cryptosporidium</i> spp.	0 (-)	0.01-2.65	0 (-)	0.01-5.58	0 (-)	0.00-1.83

**Table 2**  
**PCR-RFLP genotypic profiles from *Toxoplasma gondii* obtained from cats from Londrina, Paraná state, Brazil**

Isolate		Markers											Genotype ToxoDB	
		SAG1	SAG2	alt. SAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico		
GT1	Reference	I	I	I	I	I	I	I	I	I	I	I	I	#10
PTG	Reference	II/III	II	II	II	II	II	II	II	II	II	II	II	#1
CTG	Reference	II/III	III	III	III	III	III	III	III	III	III	III	III	#2
TgCgCa1	Reference	I	II	II	III	II	II	II	u-1	I	u-2	I	#66	
MAS	Reference	u-1	I	II	III	III	III	u-1	I	I	III	I	#17	
TgCatBr5	Reference	I	III	III	III	III	III	I	I	I	u-1	I	#19	
TgCatBr64	Reference	I	I	u-1	III	III	III	u-1	I	III	III	I	#111	
TgRsCr1	Reference	u-1	I	II	III	I	III	u-2	I	I	III	I	#52	
Cat122	This study	I	I	II	III	III	III	u-1	I	I	III	I	#65	

## Discussion

Shelter cats are known to have a higher seroprevalence of *T. gondii* when compared to pet cats (Bolais et al., 2017), although, in our dataset, there were no statistically significant differences between *T. gondii* infection rates of shelter cats and pet cats. This result may have been influenced by sampling, environmental, and behavioral factors.

Depending on the environments inhabited by cats, which may allow exploration of backyards and surrounding areas, shelter cats and pet cats can prey on infected birds and rodents. In Londrina, 22.3% of eared doves (*Zenaidia auriculata*) were found to be seropositive for *T. gondii* (Barros et al., 2014), while 8.8% of rodents tested positive for *T. gondii* antibodies (Ruffolo et al., 2016), indicating that this parasite can infect animals that can be naturally preyed on by cats.

Most cats that hunt frequently are re-exposed to *T. gondii*, maintaining their immunity against the parasite. This may explain why, in a previous study, *T. gondii* oocysts were detected in less than 1% of cat fecal samples (Dubey, 2010). Considering this, our results were unexpected, since we found that 7.28% of the cat fecal samples were PCR-positive for *T. gondii*.

Cats from Costa Rica also showed high shedding rates for *T. gondii*, with 23.2% of cats testing positive for *T. gondii* (Ruiz & Frenkel, 1980). These results contrast with another study that analyzed 237 cat fecal samples from 15 municipalities of São Paulo, Brazil, and identified only three positive samples (Pena et al., 2006). An environment with high contamination of *T. gondii* oocysts is of extreme importance in the maintenance of the protozoan, as this environment serves as a source of infection for both humans and animals (Moura, Osaki, Zulpo, & Marana, 2007).

Genotyping by means of PCR-RFLP found one *T. gondii* genotype classified as ToxoDB#65. This genotype has been previously described in cats in Brazil (Pena, Gennari, Dubey, & Su, 2008; Pena et al., 2006). Additionally, a genotype of *T. gondii* found in chickens, humans, eared doves, and pigs in Brazil (Barros et al., 2014; Dubey et al., 2008; Ferreira et al., 2011; Samico-Fernandes et al., 2015) was characterized as atypical, which is consistent with a previous study that found a high genetic diversity in *T. gondii* isolates from South America (Shwab et al., 2014). The fact that the same genotype was recorded in different animal species indicates that *T. gondii* is widely spread around Brazil, infecting different animal species from different regions.

The frequency of *Cryptosporidium* in cats living in shelters has been described as 100% in the United States, and 13% in Bogotá, Colombia (Fayer, Santin, Trout, & Dubey, 2006; Santin et al., 2006). This is in contrast to our results, as we found low prevalence of *Cryptosporidium* (1/206 samples), and these previous studies report a much higher frequency. In Brazil, in a study conducted on shelter cats from Nova Iguaçu, nine of 30 cats tested positive for *Cryptosporidium* spp. by microscopic examination. Using RFLP analysis, three of these nine *Cryptosporidium* spp. positive samples were characterized as *C. felis*, a zoonotic species that is commonly found in cats (Huber, Silva, Bomfim, Teixeira, & Bello, 2007).

In the present study, we found that one sample from a shelter cat contained *C. muris*. Since this species is known to infect rodents, and considering that rodents are a prey species for cats, it is not abnormal to find this species of *Cryptosporidium* in cats. *C. muris* has a zoonotic potential, and was described as a cause of chronic infection in healthy humans (Chappell et al., 2015).

Infection by *Cystoisospora* spp. in shelter cats was described in the Americas, Europe, and Oceania, with infection rates ranging from 1% to 21% (Bissett et al., 2009; Christie et al., 1976; Lucio-Forster & Bowman, 2011; Robben et al., 2004). Few studies have been conducted on this parasite in cats in Brazil. In one study, using shelter animals from Rio de Janeiro, 2.2% (2/91) of cats tested positive for *Cystoisospora* spp. (Pereira et al., 2017). This is in contrast with our findings, as we found higher rates of *Cystoisospora* spp. in shelter cats and pet cats.

Another intestinal protozoan commonly found in cats is *Giardia* spp., and some assemblages of *Giardia* have zoonotic potential. It has been shown that shelter cats are disseminators of this protozoan in the environment, with shedding rates ranging from 1% to 28% of cats (Bissett et al., 2009; Bowman &

Lucio-Forster, 2010; Gracenea et al., 2012; Lucio-Forster & Bowman, 2011; Robben et al., 2004; Sabshin et al., 2012; Yang et al., 2015). Our results emphasize the potential risk of cats spreading *Giardia duodenalis* cysts in the environment. Cats may, as such, play a role as a source of infection both for humans and other animals. No statistical difference was observed between pet cats and shelter cats. It is likely that the shelter environment does not result in a high prevalence of *Giardia* when compared with the environment of pet cats, and so both populations could be equally exposed (Bissett et al., 2009).

One neglected protozoan of the domestic cats is *Sarcocystis* spp. Different studies have found this protozoan in shelter cats in the United States (Christie et al., 1976; Lucio-Forster & Bowman, 2011). Similar to our study, *Sarcocystis* spp. was found in pet cats from Rio de Janeiro, with 0.8% (1/131) of cats testing positive (Serra, Uchôa, & Coimbra, 2003). In another study, conducted in a veterinary hospital in São Paulo city, 1.4% (7/502) of cats were found to be shedding this parasite (Gennari, Ferreira, Pena, Labruna, & Azevedo, 2016).

## Conclusions

In conclusion, cats in the urban area of Londrina were found to shed the following protozoans: *Cryptosporidium muris*, *T. gondii*, and *Giardia duodenalis*. *T. gondii* was found more frequently in shelter cats than in pet cats, although this was not statistically significant. Based on zoonotic importance, the situation in shelters regarding parasite shedding appears to be of greater importance than that of pet cats owned by local families.

To the authors' knowledge, this is the first study on *Giardia duodenalis*, *Toxoplasma gondii*, and *Cryptosporidium muris* in shelter cats in Brazil.

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## Competing Interests

All the authors declare that they have no competing interests.

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