

Antibodies anti-trypanosomatides in domestic cats in Paraná: who is at highest risk of infection?

Anticorpos anti-tripanosossomatídeos em gatos domésticos no Paraná: quem está em maior risco de infecção?

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Abstract

The aim of this study were to detect antibodies anti-*Leishmania* spp. and anti-*Trypanosoma cruzi* in two different populations of domestic cats (*Felis catus domesticus*) from North Paraná referred for surgical castration and to determine which characteristics of the animals studied may be associated with seropositivity. Serum samples from 679 cats were analyzed using enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence antibody test (IFAT) in series. Associations between age, sex, race, year of care and animal group were verified using the simple logistic regression. Percentage of 8.5% (58/679) of cats were positive for *Leishmania* spp. and 7.6% (51/673) for *T. cruzi* by the tests ELISA and IFAT. Animals collected by non-governmental animal protection organizations presented more seropositivity for *Leishmania* spp. ($p < 0.0001$). Results shown that *Leishmania* spp. and *T. cruzi* are present in domestic cats in the northern part of the state of Paraná, as well as, owners of non-governmental animal protection organizations may be more exposed to leishmaniasis when compared to other animal owners evaluated in the present study.

Keywords: Domestic feline, American cutaneous leishmaniasis, *Trypanosoma cruzi*, zoonosis, seroepidemiology, prevalence.

Resumo

O objetivo desse estudo foi detectar a presença de anticorpos IgG anti-*Leishmania* spp. e anti-*Trypanosoma cruzi* em duas populações de gatos domésticos (*Felis catus domesticus*) do Norte do Paraná encaminhados para castração cirúrgica e determinar quais as características dos animais estudados que podem estar associadas à soropositividade. Amostras de soro de 679 gatos foram analisadas utilizando-se os testes imunoenzimático (ELISA) e a reação de imunofluorescência indireta (RIFI), em série. Associações entre idade, sexo, raça, ano de atendimento e grupo animal foram verificadas usando regressão logística simples. Um percentual de 8,5% (58/679) dos gatos apresentou positividade para *Leishmania* spp. e 7,6% (51/673) para *T. cruzi* pelos testes ELISA e RIFI. Gatos mantidos em organizações não governamentais de proteção animal apresentaram maior sororeatividade para *Leishmania* spp. ($p < 0.0001$). As sorologias reativas para *Leishmania* spp. e *Trypanosoma cruzi* mostram que esses agentes estão presentes em gatos domésticos na parte norte do estado do Paraná, bem como, os proprietários de organizações não governamentais de proteção animal podem estar mais expostos à leishmaniose quando comparados com outros proprietários de animais avaliados no presente estudo.

Palavras-chave: Felino doméstico, leishmaniose tegumentar americana, *Trypanosoma cruzi*, zoonoses, seroepidemiologia, prevalência.

Leishmania parasites can infect humans and numerous species of mammals which contract the disease through bites by *Lutzomyia* sand flies (GRAMICCIA, 2011; BRASIL, 2017). In urban areas, the

domestic dog is the main reservoir of *Leishmania* (*L.*) *infantum* (syn. *chagasi*), the etiological agent of visceral leishmaniasis (VL) in the Americas (BRASIL, 2014), while seven species act as etiological agents of american cutaneous leishmaniasis (ACL), including *L.* (*V.*) *braziliensis* and *L.* (*L.*) *amazonensis* (BRASIL, 2017). Both of these ACL parasites have been detected in dogs and humans in the state of Paraná (SILVEIRA et al., 1990, 1999; THOMAZ-SOCCOL, et al., 2009; HOFFMANN, et al., 2012).

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The increase in the number of registered cases of domestic felines infected with *Leishmania* suggests that this animal species may play an important role in the epidemiology of the disease (MANCIANTI, 2004). The first report of natural infection by *L. (L.) infantum* in cats in the Americas occurred in 2000 in Cotia, São Paulo state (SAVANI et al., 2004). Posteriorly, Brazilian studies reported the isolation of three *Leishmania* species in domestic felines: *L. (V.) braziliensis*, (SCHUBACH et al., 2004), *L. (L.) amazonensis* (SOUZA et al., 2005) and *L. (L.) infantum* (VIDES et al., 2011). The domestic feline may harbor several *Leishmania* species without clinical signs, which, according to some authors, is due to natural resistance to infection (KIRKPATRICK et al., 1984); however, infection may be symptomatic when it co-occurs with immunosuppressive diseases (SOBRINHO et al., 2012).

Another important zoonosis found in 100 species of domestic and wild animals is caused by the flagellate protozoan *Trypanosoma cruzi* (Trypanosomatidae) and is called American trypanosomiasis or Chagas disease, which is considered by the WHO as an important and formerly neglected disease (WHO, 2013). The distribution of *T. cruzi* is primarily in the Americas due to the presence of the triatomine vector (Reduviidae) on this continent (WHO, 2015).

The role of felines in the maintenance of important pathologies such as leishmaniasis and trypanosomiasis has not been fully elucidated. However, data from this study corroborate the importance of health surveillance of felines in both endemic and non-endemic areas. Da Silva et al. (2008) suggested that the common habits of domestic cats may favor infection by different species of *Leishmania*, including hunting at night, having territories that extend up to 1.5 km away from the residence, and ability to co-inhabit urban and wild environments. These factors validate the importance of felines in the epidemiology of leishmaniasis (MAIA & CAMPINO, 2011).

The aim of this study were to detect antibodies anti-*Leishmania* spp. and anti-*Trypanosoma cruzi* in two different populations of domestic cats (*Felis catus domesticus*) from North Parana referred for surgical castration and to determine which characteristics of the animals studied may be associated with seropositivity.

Between 2004 and 2014, 679 serum samples were collected from cats attended by birth control programs of the Veterinary Hospital (VH) at the Universidade Estadual de Londrina, state of Paraná, Brazil. Animals were collected from the Londrina (23°18'37" S, 51°09'46" W) and Telémaco Borba (24°19'26" S, 50°36'56" W) municipalities, which are located in the northern and midwestern regions of Paraná, respectively.

The collection of serological samples from felines was carried out under the approval of the Committee for Ethics in the Use of Animals (n° 60/08). The animals were divided into two groups: 414 from different neighborhoods of Londrina, referring to VH by their owners for surgical castration by the Birth Control Project (BCP), from 2004 to 2013; and 265 from Castration Campaign Project (CCP) of animals from non-governmental animal protection organizations of the cities of Londrina (n = 201) and Telémaco Borba (n = 64), occurred in 2014.

The serological enzyme-linked immunosorbent assay (ELISA) was adapted from Szargiki et al. (2009) to detect the presence of anti-*Leishmania* spp. and anti-*T. cruzi* IgG antibodies. The cut-off for each plate was obtained from the means of the negative sera

readings plus three standard deviations. After the calculations made per plate, the overall cut-off was estimated using the receiver operating characteristic curve (ROC curve) by the MedCalc Statistical Software 13.2.0 (SCHOONJANS et al., 1995).

Indirect immunofluorescence antibody test (IFAT) was performed as described by Oliveira et al. (2008) using promastigote forms of *L. amazonensis* and *T. cruzi* epimastigotes as antigens. The slides were read on an epifluorescence microscope (DMLB Leica®, Wetzlar, Germany). Samples with titers greater than or equal to 40 were considered positive for *Leishmania* spp., and titers greater than or equal to 20 were considered positive for *T. cruzi*.

The Epi Info program 7.1.5.2 (CDC, Atlanta, USA) was used to tabulate the variables age, sex, breed, animal group (BCP and CCP), year of surgical service and tests results. For statistical analysis were considered only the animals reactive in the two serological tests (ELISA and IFAT), for *Leishmania* spp. or for *T. cruzi*. The statistical significance of the variables was analyzed using the program R 3.3.2 (R CORE TEAM, 2016) by simple logistic regression considering a significance level of 5%. The association measure was obtained by the odds ratio (OR) calculation with a 95% confidence interval (CI).

Among the cats attended by the birth control programs at the Londrina State University, 48.0% (326/679) were female and 43.9% (298/679) were male, and for 8.1% (55/679) no information about sex was obtained. Regarding the age groups, 28.0% (190/679) were younger than one year of age, 25.5% (173/679) were aged between one and eight years, 0.1% (1/679) were older than eight years and we were unable to determined age in 46.4% (315/679). Regarding breed, 3.2% (22/679) of the cats had a defined breed, 88.4% (600/679) had no defined breed and we had no information for 8.4% (57/679).

For the anti-*Leishmania* spp. antibody tests, 15.8% (107/679) and 43.4% (295/679) seropositivities were observed by IFAT and ELISA, respectively, and anti-*T. cruzi* antibodies were detected in 30.8% (207/673) by IFAT and 23.6% (159/673) by ELISA. Considering only the samples that were positive in both techniques performed, 8.5% (58/679) of the animals were reactive for *Leishmania* spp. and 7.6% (51/673) for *T. cruzi*. Table 1 shows the seropositivity in both serological tests (IFAT; ELISA; IFAT and ELISA) for *Leishmania* spp. and *T. cruzi*. Of the animals that were seroreactive by IFAT for both pathogens, nine presented equal titers for both protozoa, 47 showed higher titers for *Leishmania* spp. and only one showed higher titers for *T. cruzi*. Of the four seroreactive animals in both IFAT and ELISA for both protozoa, three presented titers of 40 for *Leishmania* spp. and 20 for *T. cruzi* and one had titers of 160 and 40, respectively.

Table 1. Number (n) and percentage (%) of reactive domestic cats for *Leishmania* spp. and *Trypanosoma cruzi* in IFAT and ELISA serological tests.

Parasites	IFAT n (%)	ELISA n (%)	IFAT/ELISA n (%)
<i>Leishmania</i> spp. and <i>Trypanosoma cruzi</i>	57 (8.39)	78 (11.48)	4 (0.58)

Source: The authors (2017).

When analyzing the year of collection, it was verified that 2014 presented a larger number of animals with seroreactivity for *Leishmania* spp. than the other years of collection ($p < 0.0001$), and regarding *T. cruzi*, 2011 presented a larger number of animals with serum that reacted positively than the other years, except 2012 ($p = 0.0251$) (Figure 1). Furthermore, it was verified that the group BCP presented fewer animals with infection suspect by *Leishmania* spp. than the CCP group (non-governmental animal protection organizations) (Table 2). No statistically significant differences were found in the variables age, sex and breed for either *Leishmania* spp. or *T. cruzi*.

The region of the present study is endemic for human ACL and presented 15.8% and 43.4% positivity of feline samples by IFAT and ELISA, respectively. Following the same criteria adopted by the Ministry of Health for dogs, 8.5% of cats were confirmed with leishmaniasis. Many of the seroprevalence studies for *Leishmania* spp. in cats have been carried out in endemic areas, mainly for VL (VIDES et al., 2011; ALVES-MARTIN, 2013; CARDIA et al., 2013; BRAGA et al., 2014). It is noteworthy that in Brazil, there are few records of anti-*Leishmania* spp. IgG antibodies in cats, the prevalence is generally low, and there are no records of studies demonstrating the seroprevalence behavior over the years.

Figure 1 shows that the feline seroprevalence values for *Leishmania* spp. of the present study were low or that infection was absent until the year 2013, whereas in 2014 there was a significant increase. Silveira et al. (2015) described three hypotheses for the

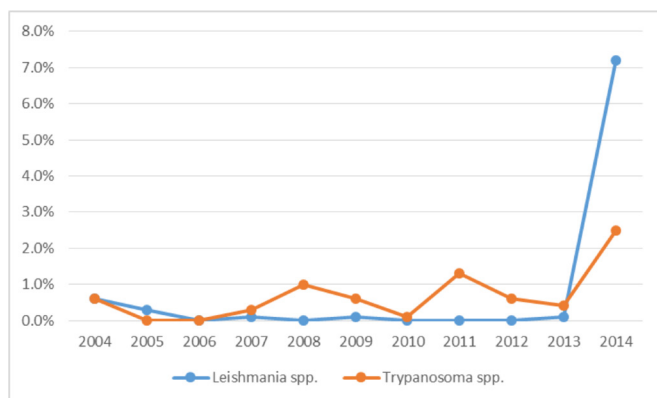


Figure 1. Percentage of seroreactive cats with *Leishmania* spp. and *Trypanosoma cruzi* as confirmed by ELISA and IFAT per year of collection. Source: The authors, 2017.

Table 2. Number (n), total of cats (Total), and percentage (%) of reactive samples for *Leishmania* spp. in both serological tests, and factor associated with cats attended by the Birth Control programs of the Veterinary Hospital, from 2004 to 2014.

<i>Leishmania</i> spp.	n/Total (%)	p-Value	OR (IC 95%)
BCP (2004-2013)	10/414 (2.4)	< 0.0001	1.00
CCP – Londrina (2014)	28/201 (13.9)		6.53 (3.10- 13.75)
CCP – Telémaco Borba (2014)	20/64 (31.2)		18.36 (8.73-38.63)

BCP-Birth Control Programs; CCP-Castration Campaign Project. Source: The authors (2017).

occurrence of higher seropositivity of *Leishmania* spp. in felines: the increase of active research in these hosts, the improvement of diagnostic techniques and an increased prevalence of leishmaniasis in felines. In a serological survey of dogs attended by the BCP from 2004 to 2012 in Londrina, Caldart et al. (2015) observed that only 1.2% (3/236) of the samples from animals were reactive by IFAT for *Leishmania* spp. In another study of dogs attended by the CCP in 2012 in the city of Telémaco Borba, positivity was observed in 6.81% (13/191) of samples by IFAT (CONSTANTINO et al., 2014). These data for dogs and cats in Londrina and Telémaco Borba led us to consider the possibility that the seroprevalence of anti-*Leishmania* spp. IgG antibodies in dogs and cats in those cities has increased over the years.

Regarding animal group, felines from Londrina CCP showed 6.53 times more chances of being positive for leishmaniasis than BCP felines (OR=6.53) and felines from Telémaco Borba CCP showed 18.36 times more chances of being positive for leishmaniasis than BCP felines (OR=18.36) ($p < 0.0001$).

Regarding trypanosomiasis, recent studies have evaluated the prevalence of Chagas disease in humans in Brazil and found that the only states with no records of this disease between 2000 and 2013 were Mato Grosso do Sul, Federal District and Parana (DIAS et al., 2016). In addition, health surveillance board of Londrina (2013) published the municipal epidemiological profile data from 2010 to 2011, which indicated that during entomological surveillance for triatomine control in rural areas, no triatomines were found to be infected with *Trypanosoma* spp.

For *T. cruzi*, a greater number of seroreactive felines were revealed by IFAT (30.8%) compared to ELISA (23.6%), and positive animals in both tests totaled 7.6%. The discrepant results between the tests were similar to those found by Alves-Martin (2013) in the state of São Paulo when evaluating felines from the protective association for animals from Ilha Solteira. The authors observed 54.9% (28/51) positivity in cats by IFAT and 39.2% (20/51) by ELISA. The authors suggested that this difference may be due to cross-reactions with *Leishmania* spp. antigens using this serological technique and that, due to the scarcity of studies using ELISA to detect *T. cruzi* in felines, the comparison with the results may have been inaccurate. Constantino et al. (2016) did not observe reactivity for *T. cruzi* for 21 dogs in the city of Curitiba. In Paraná, there are no published records of the presence of anti-*Trypanosoma* spp. in felines. Figure 1 show values for feline seroprevalence for *T. cruzi* maintained similar averages for the number of reactive animals in almost every year, except 2011 and 2012. There was no significant difference among the seroprevalence between both birth control programs (BCP, CCP).

Discussions on cross-reactivity remain controversial (ZANETTE et al., 2014) and suggest that it may occur between VL, ACL and Chagas disease (LUCIANO et al., 2009; LANGONI, 2016) because the etiological agents of these pathologies all belong to the family Trypanosomatidae and share a large number of antigens, which favors the occurrence of cross-reaction by serological methods (SOBRINHO et al., 2012). Troncarelli et al. (2009) reported that 16.5% (33/200) of canine serum samples showed reactivity for *Leishmania* spp. and *T. cruzi* in the city of Bauru, of which 30 were positive for *Leishmania* spp. by PCR; indicating the infection by *Leishmania* spp. and *T. cruzi*

cross-reactions. According to Table 1, out of the four animals that were seroreactive for both protozoa in both IFAT and ELISA, this demonstrates possible cross-reactivity and probable infection by *Leishmania* spp. However, to determine whether these are cases of cross-reactivity or co-infection, complementary molecular and/or parasitological techniques are necessary to exclude one of the hypotheses (ALVES-MARTIN, 2013).

Pet animals, such as dogs and cats, have been identified as potential sentinels for many pathologies of public health importance because these animals increasingly share the same environment and even travel with owners. Thus, pet health often reflects or is suggestive of risks to human health (SCHMIDT, 2009). The results of reactive serologies for *Leishmania* spp. and *Trypanosoma cruzi* shown that these agents are present in domestic cats in the northern part of the state of Paraná. In addition, due to the higher number of seropositivity in cats from non-governmental animal protection organizations, our study suggest that owners of such locations may be more exposed to leishmaniasis when compared to other animal owners evaluated in the present study.

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