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Presence of antibodies against *Leishmania* spp. in domestic dogs from Toledo, Paraná, Brazil

Ocorrência de anticorpos anti-*Leishmania* spp. em cães domiciliados da região de Toledo, Paraná, Brasil

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Abstract

Leishmaniases are a group of diseases caused by *Leishmania* protozoa affecting various vertebrate hosts, including humans and dogs. Dogs represent the most important domestic reservoir of leishmaniasis. For this reason, the aim of this study was to detect the presence of antibodies against *Leishmania* spp. in dogs being treated at the veterinary hospital of our institution. Samples were obtained from 175 animals by jugular or cephalic venipuncture, and serum was subsequently separated. Anti-*Leishmania* spp. IgG antibodies were detected in these specimens using indirect immunofluorescence. This revealed that of the 175 serum samples, those of four (2.28%) animals reacted positively and the antibodies title was 40. These data are consistent with the transmission of *Leishmania* spp. in the region, suggesting that dogs represent a reservoir of American cutaneous leishmaniasis. This is the first description of circulating antibodies against *Leishmania* spp. in the West region of Paraná State.
Key words: Serological reaction. Leishmaniasis. Canine population. Toledo-PR.

Resumo

As leishmanioses são doenças causadas pelo protozoário *Leishmania* e acometem vários hospedeiros vertebrados, incluindo humanos e cães. Cães representam o reservatório doméstico mais importante da leishmaniose. O objetivo do presente trabalho foi detectar a presença de anticorpos contra *Leishmania* spp. em cães atendidos na rotina de animais de companhia do Hospital Veterinário desta instituição de ensino. Amostras de sangue de 175 animais foram obtidas por venopunção jugular ou cefálica com posterior obtenção dos soros. Estas amostras foram submetidas à reação de imunofluorescência indireta para detecção de anticorpos da classe IgG anti-*Leishmania*. A reação sorológica dos 175 soros realizados revelou quatro (2,28%) animais reagentes com título 40. Estes dados revelam a presença de espécies de *Leishmania* spp. circulando na região, sugerindo o papel de cães como reservatórios para leishmaniose cutânea americana. Esta é a primeira descrição da circulação de anticorpos contra *Leishmania* spp. na região Oeste do Paraná.
Palavras-chave: Reação sorológica. Leishmaniose. População canina. Toledo-PR.

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Introduction

Leishmaniasis is a disease caused by obligate intracellular protozoa of the genus *Leishmania* transmitted by insect vectors, consisting of *Lutzomyia* (New World) and *Phlebotomus* (Old World) sand flies (HERWALDT, 1999; KAYE; SCOTT, 2011). According to its clinical presentation, this condition is classified as canine visceral leishmaniasis (CVL) or canine cutaneous leishmaniasis (CCL) (SOCCOL et al., 2009a). In Brazil, the latter is caused principally by the species *Leishmania braziliensis*, *Leishmania amazonensis*, and *Leishmania guyanensis* (BASANO; CAMARGO, 2004; SILVEIRA et al., 2004), while the former results from *Leishmania infantum* infection (MAURICIO at al., 2000).

The cycle of this disease depends on the presence of the vector and the mammalian host, with humans and canines being the main parasite reservoirs (PACE, 2014). Domestic dogs are the most important reservoir of *L. infantum*, and play a key role in maintaining the transmission of CVL between humans and animals (DANTAS-TORRES, 2007). Dogs can harbor high levels of parasitism, favoring infection of sand flies (DINIZ et al., 2008). The role of canines in the CCL chain of infection remains an active area of research; however, it has been observed that in areas endemic for the human form of this disease, dogs are also infected with protozoa (FALQUETO et al., 1986; PITTNER et al., 2009).

The euthanasia of dogs diagnosed with CVL is advocated by the World Health Organization (WHO) as a control measure for both human and canine VL. However, this approach has been questioned because in endemic areas, cases of CVL have increased despite continued efforts to control epidemics. Regarding CL, canine euthanasia is not recommended, owing to a lack of studies concerning the actual role of dogs as a reservoir of the disease (WHO, 2010)

Clinical manifestations of VL are similar in dogs and humans, and include non-specific signs, such as irregular fever over long periods, anemia, progressive weight loss, and cachexia in its final stages (PUMAROLA et al., 1991). As the infection and disease progress, many dogs exhibit clinical signs including alopecia, scaling and ulcers, weight loss, increased serum liver enzyme activity, and elevated urea and creatinine levels (CIARAMELLA et al., 1997). However, it is recommended that diagnosis be made based on clinical signs, epidemiological conditions in the region, and in particular, laboratory tests (IKEDA-GARCIA; MARCONDES, 2007).

In the state of Paraná, especially in the north and northwest, both human and canine CL are endemic (SILVEIRA et al., 1996). Autochthonous cases of human VL have been reported in Altônia, and the sand fly vector has been observed in Foz do Iguaçu (CAT et al., 1973/1974; AYALA et al., 1980; SANTOS et al., 2012). To date, there have been no reports of autochthonous cases in dogs; however, alien cases have been recorded in the state (SOCCOL et al., 2009b). Although these municipalities are close to the city of Toledo, in Parana state, we have no information regarding the occurrence of *Leishmania* spp. in the region. Thus, this study aimed to verify the presence of anti-*Leishmania* spp. antibodies in domesticated dogs being treated in the clinical care unit of our institution.

Methods and Materials

This study was approved by the Ethics Committee on Animal Use of the Pontifical Catholic University of Parana (protocol No. 649/11).

Area of study

The city of Toledo (24°43'11.12"S/53°44'35.86"W) is located in the western region of the state of Paraná, at 560 m above sea level. The climate is subtropical, with an average annual temperature above 16 °C, and rainfall distributed throughout the year, especially

in summer. The estimated human population is 130,295 (IBGE, 2014).

Animal sampling

Between February 2012 and December 2013, 175 dogs were selected for this study. Selection was random, and independent of the main complaint, breed, sex, or age of the animal. Owners agreed to the procedure by signing a consent form for animal use.

Biological samples

Whole blood was collected from all animals included in the study by jugular or cephalic venipuncture, from which a serum sample was obtained after centrifugation in the Veterinary Parasitology Laboratory. Sera were stored in microtubes and kept frozen until processing.

Serologic Testing

For serologic diagnosis, all 175 samples were subjected to an indirect immunofluorescence assay (IFAT), as described by Marzochi et al. (1980). To perform this technique, slides were prepared with crude extracts of *L. amazonensis* (strain PH8) promastigotes, and samples were considered positive when reactive at titers greater than or equal to 40. FITC-conjugated anti-canine IgG (Sigma-Aldrich, St. Louis, MO, USA) was standardized for each reaction. Samples considered positive were two-fold serially diluted until they gave a negative result. Positive and negative controls were also included in all tests.

Results and Discussion

Of the 175 dog serum samples evaluated, four (2.28%) were found to be positive for *Leishmania* spp. according to IFAT, with reaction titers of 40. No positive animal presented with lesions compatible with either cutaneous or visceral forms of the disease at the time of consultation.

The importance of dogs as a reservoir for the causative agent of CCL is not yet understood (DANTAS-TORRES, 2007; CASTRO et al., 2007). However, Santos et al. (2005) highlighted the fact that the presence of clinical or subclinical forms of this disease in the canine population has been associated with human infection, thus suggesting the involvement of these animals as a reservoir.

In Brazil, since the 1980s, the occurrence of human CL has been increasing in Paraná, which has the highest number of cases among the southern states (SOCCOL et al., 2009a). In Santa Catarina and Rio Grande do Sul, there have been few reports of the cutaneous form of the disease. In a study of CCL, De Jesus et al. (2006) used serum samples from 200 dogs from a focal point of human CL in Porto Alegre, finding that 3.5% of these animals were seropositive based on IFAT. Heusser Júnior et al. (2010) tested for anti-*Leishmania* spp. antibodies in 275 dogs in Balneário Camboriú, reporting a prevalence of 8.75%. Although the observed seropositivity rates are relatively low, together with our data, these results highlight the possibility that parasite transmission is occurring in the region. More detailed molecular studies are needed to determine which *Leishmania* species, whether those causing cutaneous or even visceral forms of the disease, are present.

On the other hand, several studies have indicated high leishmaniasis occurrence in Paraná, especially in the North and Northwest regions of the state, which are known to be endemic areas for human CL. Large numbers of dogs seropositive for *Leishmania* spp. have been documented in this state, with a rate of 38.73% (67/173) recorded in the municipality of Umuarama (GERÔNIMO et al., 2014), 18.2% (24/132) in Jussara and Terra Boa (SILVEIRA et al., 1996), 19% (24/126) in Mariluz (LONARDONI et al., 2006), 6.6% (11/169) in Cianorte (CERINO et al., 2010), 45.4% (222/489) in Bela Vista do

Paraíso (REIS et al., 2011), and 55.2% (37/67) (ZANZARINI et al., 2005) and 24% (12/50) (PITTNER et al., 2009) in the Maringá region. Such high estimates stress that elevated seropositivity is due to the region's ecosystem and its status as an area endemic for American CL in humans.

In an investigation of CCL in two rural settlements in the cities of Arapongas and Alvorada do Sul, both in the Northern region of Paraná state, Silva Filho et al. (2012) found 8.2% (14/169) of dogs to be seropositive. In Londrina, Costa (2010) determined that 9.6% (13/135) of dogs found in recycling sites and adjacent woods had antibodies against *Leishmania* spp. Furthermore, in a study of the canine population of a veterinary hospital in Londrina, Zulpo et al. (2012) reported an 11.6% (13/112) seropositivity rate for *Leishmania* spp., also using the IFAT technique. Employing the same method, Constantino et al. (2014) established that 6.81% (13/191) of serum samples from dogs in Telêmaco Borba, Paraná, were positive for *Leishmania* spp. Of the positive samples, all tested negative for *L. infantum* using the rapid immunochromatographic method. These studies, which recorded lower rates of seropositivity in animals, are similar to the present investigation in their use of random sampling in non-endemic areas and cities.

Hoffmann et al. (2012) reported the clinical form of VL in a dog from the city of Cambé, Paraná, detecting the presence of *L. amazonensis* using the multilocus enzyme electrophoresis technique. The animal showed clinical signs consistent with the visceral form of the disease, such as polyarthritis and progressive weight loss. However, *L. amazonensis* is considered to cause the cutaneous form of leishmaniasis, and thus its identification may affect the differential diagnosis of CVL. In addition, Tolezano et al. (2007) identified *Leishmania* (*Leishmania*) *amazonensis* in two domestic dogs in Araçatuba, São Paulo state, an area endemic for the visceral form of this disease. They were initially diagnosed with VL, however, *L.* (*L.*) *amazonensis* was detected in these two animals using lymph node aspirates and PCR employing oligonucleotide S8, which is specific for this protozoan species. The dogs presented with lethargy, emaciation, onychogryphosis, splenomegaly, skin lesions, and conjunctivitis, clinical signs of the visceral form, but were conclusively shown to be infected with a species associated with CL.

Conclusion

Our results provide important indications of the movement of CL-related species within the Toledo region, which, according to the Brazilian Ministry of Health, has had no human cases of this disease since 2007 (BRASIL, 2015). However, more studies are needed to identify vectors and wild animals in this region, and elucidate the true epidemiological role of the host-parasite-vector triad in the city of Toledo, Paraná, Brazil.

Here, we identified animals with serum reactive against *Leishmania* spp. Therefore, the protozoa and mosquito vectors may be disseminated throughout the city. However, further research should be conducted in this locality focusing on the vector, as well as on animals and humans, in order to elucidate the protozoan species implicated and gain a better understanding of leishmaniasis epidemiology.

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