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Seroepidemiology of *Leishmania* spp. in dogs residing in Telêmaco Borba, Paraná, Brazil

Soroepidemiologia da *Leishmania* spp. em cães do Município de Telêmaco Borba, Paraná, Brasil

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Abstract

Leishmaniasis is an important metazoonosis caused by protozoa of the genus *Leishmania* and has a heteroxenic life cycle involving invertebrate and vertebrate hosts. Transmission occurs during the blood meal of infected phlebotomine sand flies in wild species, domestic animals, and humans. The dog is a reservoir for the parasite causing visceral leishmaniasis (VL), whereas in American tegumentary leishmaniasis (ATL), dogs are erratic hosts that are accidentally infected, as in humans. Dogs are considered an important indicator of the parasite and its vectors in the environment, thus highlighting the importance of diagnosis in these animals. This study aimed to assess the seroepidemiology of *Leishmania* spp. in dogs in the municipality of Telêmaco Borba that were part of a castration campaign. Blood samples from 191 dogs were collected, and their owners were surveyed on various epidemiological variables. Serological analysis was performed using indirect immunofluorescence (IIF) and rapid immunochromatography (DPP®). Screening by IIF identified 13 (6.81%) positive animals, none of which were positive for the DPP® test, which is specific for VL. Statistical analysis of the questionnaire responses indicated a significant association between seropositivity and the presence of stacked or composting leaves in the backyard ($p = 0.0498$), forest areas (squares, woods, parks) near the residence ($p = 0.0015$), and poorly healing ulcerated or nodular epidermal lesions in the dog ($p = 0.0138$). This study revealed the presence of anti-*Leishmania* spp. IgG antibodies in dogs residing in Telêmaco Borba, suggesting the presence of the parasite and vector in the environment. In addition, the existence of stacked or composting leaves in the backyard, forest areas near the residence, and epidermal lesions in dogs are factors associated with *Leishmania* spp. infection in pet dogs.

Key words: Leishmaniasis, pet dogs, epidemiology, indirect immunofluorescence, rapid immunochromatography test, diagnosis

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Resumo

A leishmaniose é uma importante metazoonose causada por protozoários do gênero *Leishmania,* com ciclo de vida heteroxênico que envolve hospedeiros invertebrados e vertebrados. A transmissão ocorre durante o repasto sanguíneo do flebotomíneo infectado em animais domésticos e silvestres e no homem. O cão é caracterizado como reservatório do protozoário na leishmaniose visceral, já na leishmaniose tegumentar americana é um hospedeiro errático que, assim como o homem, se infecta acidentalmente. O cão ainda pode ser considerado um importante sinalizador da presença do protozoário e dos vetores no ambiente, assinalando assim a importância do diagnóstico nestes animais. Este trabalho teve como objetivo conhecer a soroepidemiologia da *Leishmania* spp. em cães do Município de Telêmaco Borba que participaram de um mutirão de castração. Foram coletadas amostras de sangue de 191 cães e aplicado um questionário epidemiológico a seus proprietários. A análise sorológica foi realizada pelas técnicas Imunofluorescência Indireta (IFI) e Imunocromatografia rápida (DPP®). A triagem realizada pela IFI revelou 13 (6,81%) animais reagentes, destes nenhum apresentou positividade no teste DPP®, específico para leishmaniose visceral. A análise estatística das variáveis presentes no questionário epidemiológico indicou associação significativa entre a sorologia positiva e presença de folhas amontoadas/compostagem no quintal (p=0,0498), áreas de mata (praças, bosques, parques) nos arredores da residência (p=0,0015) e lesões ulceradas e/ou nodulares de difícil cicatrização na pele de animais (p=0,0138). Este estudo revelou que há anticorpos IgG anti-*Leishmania* spp. em cães do Município de Telêmaco Borba, sugerindo a presença do protozoário e do vetor no ambiente, e que a existência de folhas amontoadas/compostagem no quintal, áreas de mata nos arredores da residência e lesões na pele de animais são fatores associados à infecção por *Leishmania* spp. nestes cães.

Palavras-chave: Leishmaniose, cães domiciliados, epidemiologia, Imunofluorescência indireta, Teste de Imunocromatografia rápida, diagnóstico

Introduction

Leishmaniasis is a metazoonosis of great concern in public health that results from parasitism by protozoa of the genus *Leishmania*. This parasite has a heteroxenic life cycle involving both invertebrate hosts in the Phlebotominae (sandflies) subfamily and vertebrate mammalian hosts such as humans, wild animals, and domestic animals (ARRUDA, 2010; BRASIL, 2007). The protozoan has two distinct morphological forms: the infective promastigote form residing in the digestive tract of the sandfly and the amastigote form that infects cells of the mononuclear phagocytic system in vertebrate hosts (PESSÔA; MARTINS, 1988). The condition has various clinical forms that can compromise the skin, mucosa, and viscera, depending on the specific parasite and the host immunity (BRASIL, 2010a, 2010b).

Leishmaniasis is a neglected disease with a worldwide distribution restricted to tropical and temperate regions inhabited by the insect vectors. It primarily affects the poorest countries, especially developing countries, and is endemic in 88 countries; approximately 2 million new cases occur annually, and 350 million people worldwide are at risk of contracting the disease (WHO, 2012). Approximately 90% of visceral leishmaniasis (VL) cases occur in Brazil, Ethiopia, Bangladesh, India, Nepal, and Sudan. Brazil is also among the 12 countries with 90% of all American tegumentary leishmaniasis (ATL) cases worldwide (CDC, 2010; WHO, 2010, 2012).

Due to human activities impacting the environment, the pattern of leishmaniasis transmission has changed slightly over time. Formerly, transmission occurred primarily in rural and suburban environments, but it has recently

encroached into urban centers, semidomestic, and domestic environments (BARROS et al., 1985; BRASIL, 2006; TEODORO et al., 1993). With deforestation, road construction, exploitation of forests for extraction and leisure, climate change, and adaptation of vectors to the human environment, there has been rapprochement between the sylvatic and the urban cycles of the disease, and both wild and domestic animals such as dogs have become important in transmission (CASTRO et al., 2002; FALQUETO et al., 1986; GOMES; NEVES, 1998; GONTIJO; MELO, 2004; MARZOCHI; MARZOCHI, 1994; WHO, 2010).

The dog is a reservoir of the parasite responsible for VL (DANTAS-TORRES, 2009; DEANE, 1956 *apud* COSTA, 2012; ROMERO; BOELAERT, 2010) and is an important source of infection in sandflies. By contrast, the dog is considered an erratic host for ATL and, like humans, is accidentally infected. Studies have shown that humans who cohabitate with infected symptomatic or asymptomatic dogs were also infected by the protozoan (COUTINHO et al., 1985; FALQUETO et al., 1986; FALQUETO et al., 1991; FALQUETO, 1995; LONARDONI et al., 2006); this highlights the importance of diagnosis in dogs to improve public health and implement control measures.

The municipality of Telêmaco Borba is a favorable environment for the sandfly (vector), and the dog is an important sentinel indicating the presence of the protozoan parasite in the environment. Therefore, the present study aimed to verify the presence of anti-*Leishmania* spp. IgG antibodies in pet dogs participating in a countywide castration campaign and to determine the factors associated with *Leishmania* spp. infection in these animals through an epidemiological survey.

Material and Methods

Study area

The municipality of Telêmaco Borba is located in the east-central region of the State of Paraná, Brazil, 700 m altitude, latitude 24º 19' 26''S, and longitude 50º 36' 56''W (IPARDES, 2012). In 2010, the population was 69,872 inhabitants in a 1382.863 km^2 area (IBGE, 2012). The city has a subtropical climate with a mean temperature <18°C during the coldest month and >22°C during the hottest month. Summers are hot, frost is infrequent, and most rainfall occurs during the summer months. The mean annual relative humidity is 78.75% (TELÊMACO BORBA, 2005). The city is surrounded by a large area of reforestation (forestry), which includes the Saltinho Forest Reserve, the Samuel Klabin Ecological Park, and over 85,000 hectares of native forest connected by ecological corridors that provide significant wildlife habitat (TELÊMACO BORBA, 2005).

Sample size and sampling

Blood samples were collected from 191 dogs of both sexes, various breeds, and ages that participated in the castration campaign. The blood sample was obtained with the written informed consent of the animal's owner and was collected in September and October 2012. This study was approved by the Ethics Committee on Animal Experimentation of the State University of Londrina, protocol number 32/10.

Blood collection from dogs

Blood was collected from the cephalic or jugular vein with a disposable syringe and needle (25-G × 7), and allowed to clot. After removing the clot, the serum was separated and stored at -15°C in a polyethylene 1.5 mL microtube.

The dogs were then sedated with acepromazine (0,05 mg/kg intravenous) or anesthetized with tiletamine hydrochloride and zolazepan (0,12 mL/kg intravenous).

Indirect immunofluorescence (IIF)

IIF was performed as described by Oliveira et al. (2008) as a screening tool at the Laboratory of Protozoology, Department of Preventive Veterinary Medicine, State University of Londrina (DMVP-UEL). Promastigotes of *Leishmania (Leishmania) amazonensis*, conjugated anti-IgG (Sigma Chemical®), and canine positive and negative controls obtained from animals treated at the Veterinary Hospital of the State University of Londrina were used as antigens. Samples with titers ≥40 were considered reactive.

Rapid immunochromatography for Canine Visceral Leishmaniasis (Dual Path Platform DPP-Canine Visceral Leishmaniasis)

Samples positive on IIF were subjected to rapid immunochromatography (DPP BioManguinhos®), which is specific for VL, which was analyzed at the Laboratory of Protozoology of DMVP-UEL using the manufacturer's protocol. A 5-µl aliquot of serum was added to the first well, and 2 drops of buffer were added to the same well. After 5 min, the two blue lines corresponding to the control and test samples disappeared. Then, 4 drops of buffer were added to a second well, and the results were read in both wells 10 to 15 min later. The sample was considered positive if two red lines appeared and negative if only one red line appeared.

Questionnaire and statistical analysis

The dog owners completed a questionnaire concerning animal identification, origin, feeding, health, housing, and clinical changes in both the animals and humans. The seropositive frequency and the epidemiological variables were compared using the Chi-square (x^2) test corrected by the Yates or Fisher's exact test. The statistical software EpiInfo 3.5.3 was used. The significance level was set at 5%.

Results

Of 191 dogs evaluated, 13 (6.81%) were positive for exposure to *Leishmania* spp. by IIF. Among the reactive samples, 9 (69.23%) had titers of 40, 3 (23.07%) of 80, and 1 (7.69%) of 320. Of the 13 IIF-seropositive dogs, none was positive on rapid immunochromatography for canine VL, which is used to diagnose ATL.

The frequency of clinical findings in the population was calculated, and the relationship between the epidemiological variables and the presence of anti-*Leishmania* spp. antibodies detected by IIF were assessed. Seropositivity on IIF was associated with the presence of stacked or composting leaves in the backyard ($p = 0.0498$), forest areas (squares, woods, parks) near the residence ($p = 0.0015$), and poorly healing ulcerated or nodular epidermal lesions on the dogs ($p = 0.0138$, Table 1).

Table 1. Association between epidemiological variables and the presence of anti-*Leishmania* spp. IgG, by indirect immunofluorescence (IIF) in 191 dogs from Telêmaco Borba, Paraná, 2013.

Continua ...

Epidemiological variable	Number seropositive/Total* (%)	*p*-Value
Gender		
Male	4/63 (6.3)	0.8969
Female	9/128 (7.0)	
Race		
No defined breed	12/180 (6.7)	0.7591
Defined breed	1/11 (9.1)	
Age		
Less than 1 year	3/47 (6.4)	
Between 1 and 8 years	9/138 (6.5)	0.8409
Greater than 8 years	1/6 (16.7)	0.9451
Residence type		0.8800
Urban	13/180(7.2)	0.7591
Suburban	0/11 (0)	
Dog lived in another city >1 year previously		
Yes*	1/3 (33.3)	0.4943
No	12/188 (6.4)	
Dog travelled in the past 6 months		
Yes*	1/1 (100)	0.0855
No	12/190 (6.3)	
Diet		
Commercial product	4/81 (4.9)	0.5559
Home cooked or mixed	9/110 (8.2)	
Contact between dog and wild animals		
Yes	0/2 (0)	0.3063
No	13/189 (6.9)	
Contact between dog and domestic animals		
Yes	8/90 (8.9)	0.4289
No	5/101 (5)	
Contact between dog and rodents		
Yes	2/12 (16.7)	0.4185
No	11/179 (6.1)	
Waste disposal		
Public collection	13/190 (6.8)	0.0855
Waste pile	0/1 (0)	
Organic matter in backyard		
Yes	13/150 (8.7)	0.1090
No	0/41 (0)	
Stacked leaves or compost in backyard		
Yes	6/40 (15)	0.0498
No	7/151 (4.6)	
Debris in backyard		
Yes	1/16 (6.3)	0.6700
No	12/175 (6.9)	
Sand, dirt, grass or garden in backyard		
Yes	11/119 (9.2)	0.1548
No	2/72 (2.8)	
Vegetable garden or orchard in backyard		
Yes	2/35 (5.7)	0.9303
No	11/156 (7.1)	

... Continuação

Epidemiological variable	Number seropositive/Total* (%)	*p*-Value
Waste pile adjacent to residence		
Yes	4/64 (6.3)	0.9302
No	9/127 (7.1)	
Riparian vegetation near residence		
Yes	1/16 (6.3)	0.6700
No	12/175 (6.8)	
Native forest near residence		
Yes	1/15 (6.7)	0.6089
No	12/176 (6.8)	
Reforested area near residence		
Yes	0/8 (0)	0.9491
No	13/183 (7.1)	
Forest areas (square, woods, park) near residence		0.0015
Yes	4/12 (33.3)	
No	9/179 (5)	
Mosquitoes in residence		
Yes	10/126 (7.9)	0.5753
No	3/65 (4.6)	
Mosquito Control		
Yes	4/42 (9.5)	0.6564
No	9/149 (6.0)	
Ulcerated or nodular epidermal lesions in dog		
Yes	2/4 (50)	0.0138
No	11/187 (5.9)	
Lesion site: limbs		
Yes	1/2 (50)	0.3063
No	12/189 (6.3)	
Lesion site: tail or whole body		
Yes	1/2 (50)	0.3063
No	12/189 (6.3)	
Ulcerated or nodular epidermal lesions in resident humans		0.08553
Yes	0/1 (0)	
No	13/190 (6.8)	
Lesion site: legs of resident humans		
Yes	0/1 (0)	0.08553
No	13/190 (6.8)	
Total	**13/191 (6.81)**	

*Endemic area for canine leishmaniasis.
Source: Elaboration of the authors.

Discussion

In Brazil, the prevalence of *Leishmania* spp. infection in dogs within urban areas ranges from 4.9 to 13.2% (MONTEIRO et al., 2005; SERRA et al., 2003). In the state of Paraná, the prevalence varies from 6.7% (MEMBRIVE et al., 2012) to 56.7 %% (ZANZARINI et al., 2005) in dogs residing in the rural northern part of the state; the prevalence is 3.1 (DIAS et al., 2013) to 22.8% (PITTNER et al., 2009) in pet dogs and 26.7% in stray dogs in other regions of the state (PITTNER et al., 2009). The prevalence is associated with infection by *Leishmania* species

responsible for ATL (LONARDONI et al., 2006; MEMBRIVE et al., 2012; PITTNER et al., 2009; REIS et al., 2011; ZULPO et al., 2012) and visceral forms (DIAS et al., 2013; THOMAZ-SOCCOL et al., 2009, 2013) of the disease.

Reis et al. (2011) found 222 (45.4%) seropositive dogs by IIF among 489 examined in the municipality of Bela Vista do Paraíso, Paraná. None of these animals showed characteristic lesions, which contrasts findings by Madeira et al. (2000) in the region of Itaipu, Rio de Janeiro, where 37 (11.9%) of 310 dogs participating in a rabies vaccination campaign were positive by IIF. In the present study, 2 (50%) of 4 dogs with ATL lesions were positive by IIF; this variable was significantly associated ($p = 0.0138$) with the positive IIF result.

As shown in the present study, environmental factors, such as the presence of organic waste in the yard, have been previously associated with canine infection with *Leishmania* spp. (REIS et al., 2011) because it enables development of the immature stages of the vector (FORATTINI, 1973). Silva Filho et al. (2012) found similar results in two rural settlements in northern Paraná.

Several studies have shown that proximity to forests is conducive to infection by *Leishmania* spp. (ALMEIDA et al., 2009; MEMBRIVE et al., 2012; PITTNER et al., 2009; SILVA et al., 2005; SILVA FILHO et al., 2012). Environmental impacts and anthropogenic changes to the environment have allowed the vector to adapt to the peridomiciliary environment and spread disease. In a rural settlement in Mariluz, Paraná, Lonardoni et al. (2006) found a high number of sandflies in and around the home in areas adjacent to managed forests. In the present study, a statistically significant association was found between a positive IIF result and the presence of forest areas (squares, woods, parks) near the residence ($p = 0.0015$). We could not evaluate the association to the presence of native forest because dogs from rural areas were not included.

There was no statistically significant difference associated with the distance between the residence and forest: 25 m ($p = 0.3542$), 25–100 m ($p = 0.7739$), and >100 m ($p = 0.4065$). In Arapongas, Paraná, Membrive et al. (2012) observed that a 25 to 100 m distance between a residence and forest area, up to 25 ms between a residence and stream, and up to 10 m between a residence and banana tree orchard were risk factors for ATL in rural dogs. They also found that canine infection increased the risk of human ATL, and certain environmental factors surrounding the home increased the risk of infection in both humans and dogs, which indicates the importance of integrated environmental management measures to avoid contact between humans and sandflies. Falqueto et al. (1986), in a study conducted in Viana, Espírito Santo, also emphasized the relationship between the presence of infected dogs and the occurrence of human ATL.

There was no significant influence of sex, breed, or age on the presence of anti-*Leishmania* spp. antibodies, which is in agreement with reports by Costa (2011) and Reis et al. (2011) in the northern municipalities of Paraná, and Almeida et al. (2009; 2012) in VL endemic areas in Cuiaba, Mato Grosso. Although these other factors were not significantly associated with the presence of anti-*Leishmania* spp. antibodies by IIF, they should be considered in future studies of this type because the environmental variables affecting the ecosystem of this zoonotic disease can vary greatly.

No human cases of leishmaniasis have yet been recorded in the municipality of Telêmaco Borba according to the Notifiable Diseases Information System of the Ministry of Health (BRAZIL, 2013). However, physicians should be educated on the need to include ATL as a differential diagnosis in cases of epidermal lesions because the parasite is present in this region.

Conclusion

Given the presence of circulating anti-*Leishmania* spp. IgG in the canine population of Telêmaco Borba, Paraná State, prophylaxis and control of mucocutaneous leishmaniasis is recommended. Our results suggest the presence of *Leishmania* spp. protozoan and its vector in the environment, and indicate the potential for infection in the human population. Health education, such as recommending frequent cleaning of yards to avoid accumulation of organic matter and proper disposal of waste and sewage, should be provided to the general population. Another important preventive measure is to avoid building houses adjacent to woodlands and avoid interchange between the domestic and sylvatic environments, in order to maintain the vector at a distance from humans and animals, especially dogs, which are the link between the wild cycle and peridomiciliary leishmaniasis.

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