

Spatial distribution of canine *Leishmania infantum* infection in a municipality with endemic human leishmaniasis in Eastern Bahia, Brazil

Distribuição espacial de infecção canina por *Leishmania infantum* em um município com leishmaniose humana endêmica no leste da Bahia, Brasil

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Abstract

Efforts to control a zoonotic disease such as visceral leishmaniasis (VL) caused by *Leishmania infantum* can be successful if they rely on comprehensive data on animal infection. In Bahia state, Brazil, human VL is endemic, yet some areas have no epidemiological data on canine *L. infantum* infection and canine leishmaniasis (CanL) to date. We aimed to perform an epidemiological study describing the spatial distribution and characterizing canine *L. infantum* infection in two districts of the municipality of Muritiba, where human cases have occurred. Brazilian official serodiagnostic protocol (ELISA and immunochromatographic tests), PCR and clinical examination were performed in 351 owned dogs. A seroprevalence of 15.7% (55/351) was found, and *L. infantum* identified in 88.8% (32/36) of PCR tested samples. Spatial distribution of positive dogs indicated infection in both urban and rural districts. There was no association between seropositivity and sex or breed, but dogs older than 2 years were 3.8 times more likely to be seropositive (95% CI 1.57 - 9.18) than younger dogs. Among seropositive dogs, 80% (44/55) had clinical manifestations of CanL: 75% (33/44) presented dermatopathy, 50% (22/44) emaciation, and 29.5% (13/44) ophthalmopathy. This is the first report on canine seroprevalence and natural *L. infantum* infection in Muritiba, Bahia.

Keywords: Dog, georeferencing, zoonotic leishmaniasis, One Health, seroepidemiology, zoonosis.

Resumo

O sucesso dos esforços para controlar uma doença zoonótica como a leishmaniose visceral (LV), causada por *Leishmania infantum*, depende de dados abrangentes sobre a infecção animal. Na Bahia, Brasil, embora a LV humana seja endêmica, diversos municípios, como Muritiba, ainda não dispõem de dados epidemiológicos sobre infecção por *L. infantum* e leishmaniose canina (LCan). Objetivou-se realizar um estudo epidemiológico para descrever a distribuição espacial e caracterizar a infecção canina por *L. infantum* em dois distritos de Muritiba, onde notificam-se casos humanos. Foi aplicado o protocolo sorodiagnostics oficial brasileiro (ELISA e imunocromatografia), PCR e exame clínico em 351 cães domiciliados. Encontrou-se uma soroprevalência de 15,7% (55/351) e positividade de 88,8% (32/36) na PCR para *L. infantum*. A distribuição espacial dos cães positivos

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indicou infecção em ambos os distritos, urbano e rural. Não houve associação entre soropositividade e sexo ou raça, mas cães com idade acima de 2 anos foram 3,8 vezes mais soropositivos (IC 95% 1,57 - 9,18). Dentre os cães soropositivos, 80% (44/55) apresentavam manifestações clínicas de LCan: 75% (33/44) apresentavam dermatopatia; 50% (22/44) emagrecimento e 29,5% (13/44) oftalmopatia. Este é o primeiro estudo a determinar a soroprevalência e confirmar a circulação natural de *L. infantum* e LCan em cães de Muritiba.

Palavras-chave: Cão, Georreferenciamento, leishmaniose zoonótica, Saúde Única, soroepidemiologia, zoonose.

Introduction

Leishmaniasis is a spectrum of neglected vector-borne diseases caused by different species of protozoan parasites of the genus *Leishmania*. Leishmaniasis continues to spread worldwide, and as of January 2020, the WHO Global Leishmaniasis program declared 56 countries as endemic for human visceral leishmaniasis (HVL), which is caused by *Leishmania infantum* in America and Europe and *Leishmania donovani* in Africa and Asia. More than 90% of these cases occur in developing countries such as Brazil, Ethiopia, India, Kenya, Somalia, South Sudan and Sudan (Alvar et al., 2012). In Brazil, *L. infantum* is transmitted mainly by the sand fly *Lutzomyia longipalpis*, causing a zoonotic infection that affects humans, companion dogs and other domestic and wild mammals (Quinnell & Courtenay, 2009; Trüeb et al. 2018). In urban areas, the domestic dog has been considered the main reservoir of the parasite and a key element in the epidemiological cycle (Campos et al., 2017). Thus, monitoring canine *L. infantum* infection and canine leishmaniasis (CanL) cases is crucial to provide reliable data to promote and support One Health programs.

Data on human and canine infections show that the northeastern states of Brazil have the highest incidence and prevalence of zoonotic leishmaniasis when compared to other regions. In 2019, Bahia State ranked third with the highest number of cases of HVL in the Northeast Region, with an estimated incidence of 1 case/100,000 inhabitants (Brasil, 2020; IBGE, 2020a). The number of new cases changes over time and is often underestimated because of underreporting of human cases and the lack of adequate surveillance systems for canine disease.

Studies in different Brazilian regions have performed spatial analyses demonstrating the association between CanL and human VL occurrence (Bevilacqua et al., 2001, Bruhn et al., 2018; Lana et al., 2018; Arruda et al., 2019). Bavia et al. (2005), using remote sensing and geographic information systems classified, between 1990 and 1998, municipalities of the state of Bahia correlating environmental factors to human VL risk. Data on the spatial distribution of canine *L. infantum* infection are important to delimit the main risk areas for the human disease (Campos et al., 2017; Lins et al., 2018). However, there is still lack of information regarding the geographical distribution of CanL in many municipalities of Bahia, which hampers the comprehension of factors associated with the disease's spreading in the state.

The municipality of Muritiba, in the Recôncavo Baiano region of Bahia, is an example of a district where HVL cases have occurred (Bahia, 2018), but no epidemiological studies covering canine *L. infantum* infection prevalence have ever been conducted. The absence of data on zoonotic infections hinders efforts to put strategic disease control practices into place in an area where the unstable basic public services of sanitation, health care or education, coupled with a favorable natural environment for the vectors, foster parasite dissemination (Costa et al., 2005).

In this context, the present study aimed to perform a serology-based epidemiological survey using official serological tests as determined by the Health Ministry of Brazil, then a molecular and clinical characterization of CanL in the municipality of Muritiba, Bahia state, and to provide the spatial distribution of canine *L. infantum* infection cases.

Materials and Methods

Ethics approval

The study was conducted in accordance with the ethical principles for animal experimentation adopted by the National Council for Animal Experimentation Control (CONCEA), and approved by the Committee for Ethics in the Use of Animals (CEUA) of the School of Veterinary Medicine and Zootechny of the Federal University of Bahia (EMEVZ-UFBA) under the protocol No. 013/2017. The dogs' owners were informed about the objectives of the study and signed an informed consent form.

Study location and design

This cross-sectional study of the canine population took place from March 2018 to July 2019 in the municipality of Muritiba, Bahia State. This area was selected due to its classification as an area of periodic transmission of human VL by *L. infantum*, whose last notification was recorded in 2018 (Costa, 2019). In addition, there are no official records on the epidemiological situation of canine infection by *L. infantum* or CanL in the region.

The municipality of Muritiba is located in the Recôncavo Baiano region (12° 37'35" south latitude, 38° 59'24" west longitude) and is characterized by having an Atlantic forest biome. The municipality is divided into two districts, one urban (Centro) and one rural (São José do Itaporã), and covers a territorial area of 86.311 km². Muritiba had an estimated population of 29,410 inhabitants in 2020 (IBGE, 2020b). In this municipality, a census performed in 2010 determined a human development index (HDI) of 0.660, indicating low purchasing power and education indices, while the control of water supply and sewage treatment benefited only 14.6% of the population (IBGE, 2020b).

The sample size in this study was calculated using the following criteria: estimated canine population as 11% of the human population (Junqueira & Galera, 2019), an expected prevalence of CanL of 50%, with a 95% confidence interval, and a 5% margin of error. Thus, the calculated sample size was 343 dogs. The inclusion criteria included the following: dogs should be older than 6 months of age regardless of sex and clinical condition, owner's consent, and the area is accessible. Dogs from the two districts (urban and rural) were examined and the sample was accessed by convenience, based on the agreement of the owners to participate in the study and the accessibility of the area. Locales that presented difficult access or that were dangerous due to criminal activity were not visited.

Clinical evaluation and disease staging

Dogs underwent a clinical examination according to the previously determined criteria in the specialized literature for the clinical presentation of CanL (Solano-Gallego et al., 2011). The assessment of disease severity was based on clinical signs and changes observed at the physical examination and followed the criteria established by Silva et al. (2017) with adaptations. Each clinical sign of disease received a value of 1 (one) if present or zero if absent. If the dog exhibited a total score up to 3, it was categorized as having mild disease, a total score between 4 and 9 was categorized as moderate disease, and if the dog had total score of 10 and above, it was categorized as having severe disease.

Sampling and serological evaluation

After the physical examination, samples of peripheral blood were collected from all dogs for serological diagnosis. Serum samples were tested using two serological tests, the Dual Path Platform TR-DPP® immunochromatographic screening test (Biomanguinhos, Fiocruz, BR) and the ELISA-based Canine Visceral Leishmaniasis test (EIE-LVC, Biomanguinhos, Fiocruz, BR), following the manufacturer's recommendations. The diagnostic conclusion was based on the official determination from the Brazilian Ministry of Health (Brasil, 2011), which establishes that a confirmed infection case is when a dog presents positive results in both TR-DPP® and EIE-LVC tests. Accordingly, a second sample collection was carried out in the dogs that were double-positive in the official serotests, to detect *Leishmania infantum* DNA thus identifying it as the species that was causing the infection. Approximately 500 µL of bone marrow aspirates were collected from the sternum under sedation with acepromazine (0.05 mg/kg) and local anesthesia with lidocaine (2-5 mg/kg) (Paparcone et al., 2013) for evaluation of *L. infantum* infection by PCR.

Leishmania infantum DNA detection by PCR

Total DNA from bone marrow samples was extracted using the commercial Wizard® Genomic DNA Purification Kit (Promega®, USA), according to the manufacturer's recommendations. Conventional multiplex PCR was performed for simultaneous detection of the target region of the *L. infantum* DNA and of the endogenous control region of canine glyceraldehyde-3-phosphate (GAPDH). The primers used for the amplification of the specific region of the endogenous glyceraldehyde-3-phosphate (GAPDH) control were: 5' CCAAAGTTGTCATGGATGACC-3' (F) and 5'-CCTTCATTGACCTCAACTACAT-3' (R) (Birkenheuer et al., 2003). Primers used for the amplification of *L. infantum* DNA were: 5'-CTTTCTGGTCCCGCGGGTAGG-3' (RV1) and 5'CCACCTGGCCTATTTACACCA-3' (RV2) (Lachaud et al., 2002). The PCR conditions were: 13.3 µL ultrapure water free of RNase and DNase; 2.5 µL of 10x buffer (Invitrogen®, USA); 1 mM MgCl₂; 200 µM dNTP (Invitrogen®, USA); 10 pmol of initiators; 1.2 U of Taq Platinum Polymerase (Invitrogen®, USA); and 1 µL (80 to 100 ng) of DNA, in a final reaction volume of 20 µL. The thermal cycler (Applied

Biosystems 2720 Thermal Cycler®) was adjusted to the following settings: 94°C for 2 min; 94°C for 30 sec; 61°C for 30 sec; 72°C for 30 sec; 72°C for 5 min for a total of 35 cycles. The amplification products were stained with Syber Gold (Invitrogen®, USA) and visualized on a 1.5% agarose gel, using the gel documentation system Biometra® (BioDocAnalyze, Germany). Products with approximately 450 and 145 base pairs were considered positive for endogenous control (GAPDH) and *L. infantum*, respectively.

Georeferencing

During the evaluation of the dogs at their homes, the address was georeferenced in geographic coordinates (SIRGAS2000, UTM 24 South) using Garmin Etrex 10 GPS equipment (Garmin, USA). The coordinates were plotted on the cartographic basis of the municipal network and census sectors using data from the Brazilian Institute of Geography and Statistics (IBGE, 2020b) for the Muritiba municipality. Thematic maps were constructed to demonstrate the spatial distribution of the seropositive dogs for *L. infantum* infection and their homes. Households with at least one seropositive dog were considered positive. Spatial distribution was performed using the QGIS program (version 3.12).

Statistical analysis

An analysis of the association between sex, breed, age and domiciliation (to what extent the dogs lived within the household of the owner or also had access to the streets) of the dog versus seropositivity for canine *L. infantum* infection was performed by calculating the odds ratio and applying the chi-square test (χ^2) (Martin & Austin, 1991; CDC, 2019) with a significance level of 0.05. Additionally, a descriptive analysis of the clinical signs found in dogs was performed to demonstrate their frequencies.

Results and Discussion

The study was carried out in Muritiba (Figure 1A) because of the demand for veterinary assistance from owners of pet dogs that became sick and eventually had a confirmed diagnosis of *L. infantum* infection associated with CanL in private veterinary clinics. The seroprevalence of canine *L. infantum* infection in the municipality of Muritiba, Bahia, Brazil, was determined by an epidemiological survey based on the active search for and examination of 351 dogs using a convenience sampling assessment, according to the inclusion criteria. Among those dogs, 258 (73.5%) were housed in the urban area, while 93 (26.5%) lived in a rural area within the municipality. The clinical

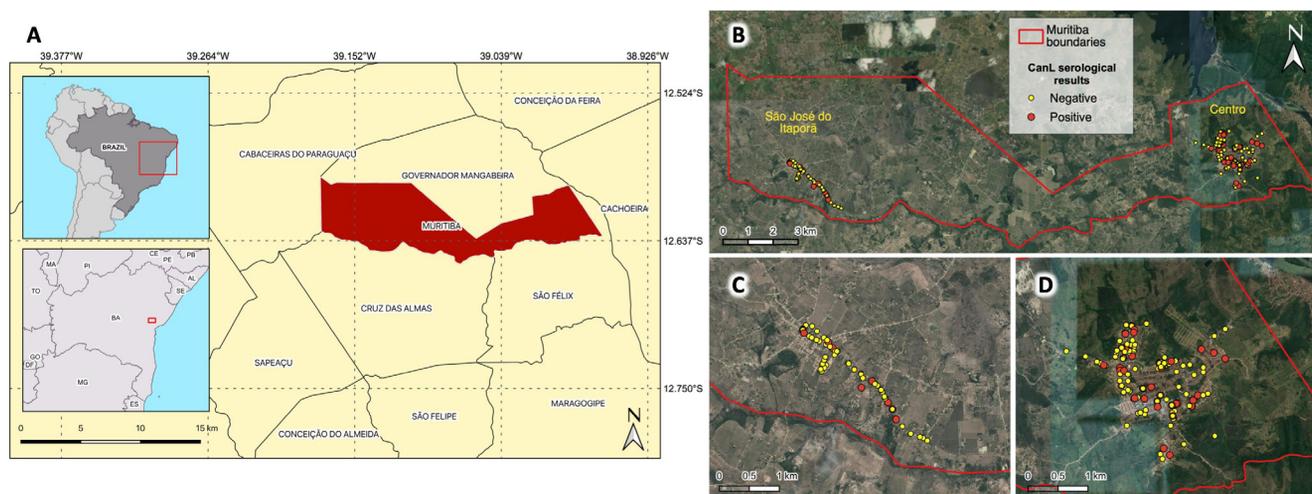


Figure 1. Spatial distribution of seropositive dogs for *Leishmania infantum* infection in Muritiba, Bahia, Brazil. Seropositivity criterion was the dog being positive for anti-*Leishmania* antibodies in both, the Dual Path Platform immunochromatographic screening test (TR-DPP®) and the ELISA-based Canine Leishmaniasis test (EIE-LVC) as determined officially by the Brazilian Ministry of Health (NT 01/2011 – CGDT – CGLAB/DEVIT/SVS/MS) (Brasil, 2011). Geographical location of the municipality of Muritiba, in the Recôncavo Baiano, Bahia State, Brazil (A) Distribution of cases of canine *L. infantum* seropositivity within the municipality of Muritiba (B), Brazil, in the localities of São José do Itaporã (C) and Centro (D). Yellow dots indicate seronegative dogs, red dots indicate seropositive dogs. Source IBGE (2020b), Datum: SIRGAS2000 (IBGE, 2005), Geographic coordinates.

characteristics of the evaluated canine population are detailed in Table 1, where the majority was characterized by males (50.6%), mongrels (63.5%), adults or old (71.5%) and strictly domiciled (57.8%) dogs.

Canine sera were initially screened using the TR-DPP® immunochromatographic test, which detected 98 out of 351 (27.9%) dogs as positive. Among these seropositive dogs in the TR-DPP® test, 56.1% (55/98) were confirmed as positive by the EIE-LVC® test. These results represent a prevalence of positive cases of 15.7% (55/351), in accordance with the Brazilian Ministry of Health criteria, which defines a dog that tests positive for both, the TR-DPP® immunochromatographic test and the EIE-LVC® test, as a confirmed case of canine *L. infantum* infection (Brasil, 2011). Among the 55 dogs confirmed as seropositive cases, 45 (17.4%) dogs were housed in urban area and 10 (10.7%) in rural area. No statistically significant association was found in the analysis between areas (Table 1).

Table 1. Association between seropositivity in both tests for canine leishmaniasis, the TR-DPP® immunochromatographic test and the ELISA-based EIE-LVC® test, and the characteristics of the studied population of 351 dogs from the municipality of Muritiba, Bahia state, Brazil, evaluated from March 2018 to July 2019.

Parameters	Total number of dogs (%)	Serology Result number of dogs (%)		OR ^a (CI ^b 95%)	p-value*
		Positive	Negative		
Sex					
Female	174 (49.6)	28 (50.9)	146 (49.3)	0.9386 (0.52- 1.67)	0.83
Male	177 (50.5)	27 (49.1)	150 (50.7)		
Breed					
Mongrel	223 (63.5)	38 (69.1)	185 (62.5)	1.34 (0.72 – 2.49)	0.35
Pure breed	128 (36.5)	17 (30.9)	111 (37.5)		
Age					
Adult (> 2 years)	251 (71.5)	49 (89.1)	202 (68.2)	3.80 (1.57 -9.18)	0.0017
Young (< 2 years)	100 (28.5)	6 (10.9)	94 (31.8)		
Domiciliation					
Semi-domiciled	148 (42.2)	26 (47.3)	122 (41.2)	1.28 (0.72-2.28)	0.4043
Domiciled	203 (57.8)	29 (52.7)	174 (58.8)		
Muritiba´s district					
Centro (urban)	258 (73.5)	45 (17.4)	213 (82.6)	1.75 (0.84-3.64)	0.1282
São José do Itaporã	93 (26.5)	10 (10.7)	83 (89.3)		

*Chi-square test. ^aOR = Odds ratio; ^bCI = Confidence interval.

Among the 55 dogs that were positive in the serological methods, 36 (65.5%) had bone marrow samples tested by PCR, which confirmed that *L. infantum* was the species responsible for the infection. The remaining 19 dogs were not tested because it was not possible to collect bone marrow samples. Some seropositive dogs died between the first and second sample collection, or the owners decided not to consent to the second procedure. The results of the PCR assays were positive for the detection of *L. infantum* DNA in 88.8% (32/36) of the samples.

The spatial distribution of the seropositive dogs showed the presence of canine *L. infantum* infection and CanL in both urban and rural districts (Figure 1B). Although we were able to examine an adequate number of dogs in relation to the local demography, the distribution of the canine population sample was taken by convenience. Furthermore, an attempt to perform cluster Kernel's analysis to identify hot spots for *L. infantum* infection would not be precise enough, due to the lack of official information on the canine population density in the two districts. However, this shows the need for more studies in the area. Spatial distribution studies provide a diagnosis that can be used to indicate the risks to which the population is exposed, monitor the spread of the disease, provide subsidies for causal explanations, define intervention priorities and assess the impact of interventions (Hino et al., 2006).

In 2006, a study conducted in another municipality, also located in the Recôncavo Baiano region, showed a lower prevalence of CanL, which was calculated as 10% of the tested populations (Souza et al. 2006). It is possible that the frequency of infected dogs in the region has increased with time considering the time lapse between the two studies. Interestingly, we did not find any significant difference in the frequency of seropositive dogs between the two areas ($X^2=2.315$; $p=0.1282$), with 17.4% (45/258) and 10.8% (10/93) positive cases in urban and rural areas of Muritiba, respectively. This finding reinforces the idea that the urbanization of zoonotic leishmaniasis has taken place even in small urban areas. This process can be attributed to socioeconomic and environmental aspects of the study area, which is characterized by having a population that is low income and has less education, while living in areas that are poorly serviced by public services concerning sanitation and infrastructure. Such factors favor the accumulation of waste and the availability of organic material, providing plenty of conditions for vector adaptation, as previously described (Costa et al., 2005).

Among the 55 dogs confirmed as seropositive cases, 28 (50.9%) were female, 38 (69.1%) were mongrel, and 29 (52.7%) were strictly domiciled (Table 1). No statistically significant association was found in the analysis of these variables. Currently, there is no consensus about which phenotypical characteristics are associated with a higher susceptibility to develop infection by *L. infantum* and CanL manifestations. However, the dog's genetic immunological susceptibility to *Leishmania*, environmental factors and socioeconomic condition of the human population are the major risk factors associated with a higher canine *L. infantum* prevalence (Belo et al., 2013). Our studied population reflects the general characteristics of dogs in the more deprived Brazilian environments, having more mongrels than bred dogs, with half of them having free access to the streets. Indeed, dogs that were strictly domiciled were not more protected than dogs that roamed free, which is expected for a flying vector-borne disease.

In the present study, dogs older than 2 years were 3.8 times more likely to be seropositive for *L. infantum* infection (95% CI 1.57 - 9.18) than younger dogs (Table 1). As described previously, younger dogs (<1 year old) and older dogs (> 8 years old) are more prone to *Leishmania* spp. infection with fatal disease progression, probably due to their immature or less functional immune response, which might render them more susceptible to higher parasite loads and/or to comorbidities (Solano-Gallego et al., 2011). In this regard, we suppose that the more susceptible dogs might have died more often and thus were less represented in the canine population of the studied endemic area, while adult dogs survived long enough to develop an insidious chronic disease and tested positive in a survey.

Most of the dogs assessed in this study presented a poor clinical condition, regardless of canine *L. infantum* seropositivity, with high frequencies of nonspecific clinical signs such as weight loss, dehydration and skin lesions. Only 11 out of 55 (20%) of the double-seropositive dogs had no clinical signs of disease, while the other 44 (80%) had clinical manifestations of CanL. The poor clinical condition found in the dogs can be attributed to the lower indices of human development of the owners, hampering the supply of adequate nutrition to their dogs, as well as veterinary care that results in the lack of preventive care and, ultimately, the diagnosis of a life-threatening disease such as CanL. This finding should be seen as an important indication that the area requires public attention considering the principles of One Health.

Regarding the frequency of cutaneous manifestations found in the seropositive population, 75% (33/44) of the dogs had skin ulcers, alopecia and onychogryphosis. Among the other clinical signs of CanL, the most frequently observed were weight loss, present in 50% (22/44) of the dogs, lymph node enlargement in 43.2% (19/44), spleen enlargement in 34.1% (15/44), ocular alterations in 29.5% (13/44) and pale mucous membranes in 25% (11/44). Among the positive dogs with clinical signs, 45.4% (20/44) had CanL classified as mild, 43.2% (19/44) as moderate and 11.4% as severe (5/44) (Figure 2). Other diseases, such as ehrlichiosis, anaplasmosis and babesiosis, can coexist in the same endemic areas as CanL, causing coinfections that lead to an aggravation of the dogs' clinical condition (Krawczak et al., 2015).

In the context of One Health, epidemiological studies should monitor canine *Leishmania* infection prevalence by determining the dog's clinical status to estimate the risk of transmission of the pathogen between mammalian hosts and vectors. Multiple authors have demonstrated a significantly positive correlation between CanL clinical staging, parasite load and infectivity to the vector. In line with this finding, Courtenay et al. (2002) had already demonstrated that sick dogs transmitted the *L. infantum* parasite to the vector three times more than dogs with subclinical infections.

Concerning the PCR positivity for *L. infantum* DNA, it is interesting to note that among the 36 double-seropositive dogs that had their bone marrow tested by PCR, the only four PCR-negative dogs also had no clinical alterations, although they were seropositive in both serological tests. There is a possibility that some seropositive results can occur because of antibody cross-reaction with hemoparasite infections that affect dogs, as has already been



Figure 2. Clinical manifestations in dogs naturally infected with *Leishmania infantum* in Muritiba, Bahia, Brazil. Dogs were positive in both serological tests, the Dual Path Platform immunochromatographic screening test (TR-DPP®) and the ELISA-based Canine Leishmaniasis test (EIE-LVC) as determined officially by the Brazilian Ministry of Health (NT 01/2011 - CGDT - CGLAB/DEVIT/SVS/MS), and in the molecular test PCR for *L. infantum*. (A) Infected dog, apparently healthy; (B) Dog with mild CanL showing ulcers on the tips of the ears; (C) Dog with moderate CanL showing emaciation and dermatological lesions on the face and limbs characterized by desquamation and ulceration; (D) Dog with severe CanL showing cachexia, pale membrane mucous, and generalized dermatological lesions characterized by alopecia, desquamation and ulceration.

reported (Alves et al., 2012). Another possibility is that the seropositivity of these dogs might have indeed resulted from exposure to the parasite, but they possibly had a lower parasite load in the bone marrow than that of the PCR-positive dogs at the time of sample collection. As this was a cross-sectional study, we could not determine the stage of the infection. However, in veterinary clinical settings, the data found in the present study strongly recommends a confirmatory test using a more specific diagnostic method, such as PCR, to support a seropositive result in dogs.

The total number of households assessed in Muritiba was 226, with 161 households in the urban district and 65 in the rural district. In the urban district, there was at least one seropositive dog for *Leishmania* infection in 22.4% (36/161) of the households, while in the rural district, 13.8% (9/65) of the households had at least one seropositive dog. The occurrence of canine seropositivity in the two districts of the studied municipality might be related to the anthropic occupation in the Recôncavo Baiano region, leading to the deforestation of the Atlantic forest and ecological imbalance. In addition to the environmental impact, the two districts lack adequate sanitation conditions.

These factors contribute to *Leishmania* vector adaptation in households, which makes humans and dogs more susceptible to sandfly bites and *Leishmania* infection.

The most recent official notification of a case of human VL caused by *L. infantum* in Muritiba occurred in 2018. According to the criteria of the Brazilian Ministry of Health, Muritiba would be close to being considered a municipality free of VL transmission. However, no human epidemiological survey has been reported for the area since then. On the other hand, here we report for the first time the occurrence of canine infection by *L. infantum* in the municipality, showing the circulation of the parasite among canine hosts. Parasite transmission to humans can occur at any time, especially if control strategies for human VL are not implemented.

The present study provides evidence of the natural circulation of *L. infantum* in Muritiba as an etiological agent of spontaneous canine disease that confirms the characteristics described for CanL in other endemic areas of Brazil (Nery et al., 2017) and in Europe (Solano-Gallego et al., 2011). Studies with more robust experimental designs are needed to determine the endemicity of canine *L. infantum* infection and CanL in the whole area of Recôncavo Bahiano, including Muritiba and other municipalities. Likewise, entomological surveillance is also important to identify the vector species involved in the epidemiological cycle of *L. infantum* in the region.

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