Leishmania (*Viannia*) *braziliensis* in dogs in Brazil: epidemiology, co-infection, and clinical aspects

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ABSTRACT. Leishmaniasis is an endemic disease present in 98 countries. In Brazil, the northeast region accounts for approximately half of the cases in humans, and has experienced an increased number of positive cases in dogs. In this study, we investigated the epidemiology of canine leishmaniasis in the city of Ilhéus, Bahia, using serological and molecular techniques and evaluated the possible environmental risk factors and associated clinical signs. Blood samples were collected from 560 dogs in urban and peri-urban areas in Ilhéus, northeastern Brazil. Genomic DNA was extracted from the selected animals and

subjected to molecular analysis using *Leishmania* species-specific primers and diagnosis of *Trypanosoma cruzi*. A total of 54.72% of dogs were positive for *Leishmania braziliensis*, and animals positive for both *Leishmania infantum* and *T. cruzi* were not identified. Hematologic variables were not statistically associated with cases of *L. braziliensis*. However, the positive animal group showed lower red blood cell and platelet counts and higher levels of urea and serum creatinine. Few dogs presented clinical signs compatible with the presence of *Leishmania*. Age of more than 2 years and specific hair colors were associated with positive results for *L. braziliensis*. The geoclimatic characteristics of the region may improve parasite survival, reproduction, and vectors. This may explain the higher rate of dogs identified as positive in this study.

Key words: Canine; Leishmaniasis; Polymerase chain reaction; Serology; Tropical region

INTRODUCTION

Leishmaniasis is a zooanthroponosis with worldwide distribution, which is caused by protozoa of the genus *Leishmania* and is transmitted to animals and humans by hematophagous flies in the Phlebotomidae family. Based on its clinical manifestations, the disease can be classified in 2 groups, visceral (LV) and tegumentary (LT). Brazil has the highest number of recorded cases in the American continent, with an estimated 3000 human cases of LV and 22,000 cases of LT americana (LTA) annually (WHO, 2013). The northeast region accounts for approximately 47% of the human cases of LV and 35.16% of the LT cases, while the State of Bahia accounts for 52.43% of these cases (Brazil, 2013).

Thousands of dogs are affected by canine leishmaniasis (LCan) in Brazil and worldwide (Werneck et al., 2006; Baneth et al., 2008). In Brazil, the main agents of canine visceral leishmaniasis (LVCan) are *Leishmania* (*Leishmania*) *infantum* and those of cutaneous canine leishmaniasis (LTCan) are *Leishmania* (*Viannia*) *braziliensis*. In the northeastern region of Brazil, 24% of dogs are estimated to be infected with LCan (Rondon et al., 2008). A similar prevalence has been observed in dogs in the cities of Jequié (23.5%) and Camaçari (21.7%) in the State of Bahia (Paranhos-Silva et al., 1996; Julião et al., 2007).

Dogs play distinct roles in the cycles of *L. infantum* and *L. braziliensis*, and they represent a potential risk of LV infection to humans (Alvar et al., 2004; Dantas-Torres, 2007). However, the role of dogs as a possible reservoir of LT has not been clearly defined. Previous studies have regarded dogs as accidental hosts (Alvar et al., 2004; Dantas-Torres, 2007; Cavalcanti et al., 2012), whereas others regard them as a reservoir (Gomes et al., 2008).

Only 5-10% of the dogs positive for the protozoan show clinical changes suggestive of LVCan (Solano-Gallego et al., 2009). There is little clinical evidence for cases of LTCan (Afonso-Júnior et al., 2010), and long periods of time may pass before dogs show any clinical signs of the disease, regardless of whether the manifestations are subclinical or chronic (Baneth et al., 2008).

The clinical signs of LCan are similar to those of the human disease with systemic impairment in LVCan (Brito et al., 2010) as well as skin lesions and a few systemic changes in LTCan. Renal changes are also commonly present in dogs positive for LCan, although the

signs of kidney failure can only be identified when much of the organ is compromised (Costa et al., 2003; Solano-Gallego et al., 2009).

The objective of this study was to investigate the epidemiology of canine leishmaniasis in the city of Ilhéus, northeastern Brazil, using serological and molecular techniques and to evaluate possible environmental risk factors and associated clinical signs.

MATERIAL AND METHODS

Study area

The municipality of Ilhéus is located at a latitude of 14°47'S and a longitude of 39°02'W, in the south of Bahia, in the Atlantic Forest biome, and is classified as the Cocoa Region of Bahia covering an area of 1760 km² (Figure 1). The average annual temperature is 28°C with an annual rainfall of 1500-2000 mm. The area has an average elevation of 52 m and a population of 184,616. According to the municipal government, the canine population in 2012 was estimated to be approximately 20,000.
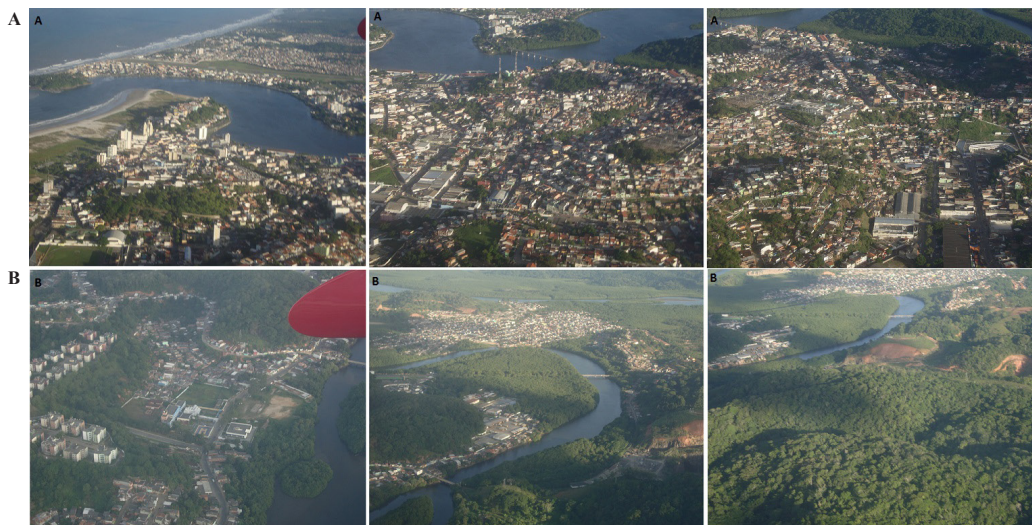

Figure 1. View area of the Ilhéus city presenting urban (**A**) and suburban (**B**) regions.

Animals

For sample calculation, a sampling error of 5% was expected, with an expected prevalence of 30% at the 99% confidence level obtained from a minimum number of 543 animals. The expected prevalence was based on the results of the seroprevalence of LCan in the State of Bahia (Rondon et al., 2008). The sample included 560 animals distributed throughout the town. The areas were selected based on greater territory coverage, including the peripheral or peri-urban areas and central and urbanized areas.

Collection stations were designated for sample collection in each neighborhood. Prior notice was given in the area of the collection for the date and times through various

media outlets, such as the internet, local radio stations, and on posters between March and August 2013.

This study followed the guidelines pertaining to the use of animals in experimentation and was approved by the Ethics Committee on the use of animals - CEUA/UESC under Protocol No. 12/018. All procedures were in accordance with the guidelines established by the Brazilian College of Animal Experimentation (COBEA) under the direction of Federal Law No. 11.794.

Blood and tissue collection

After registration, authorization, and physical inspection of the dogs, the animals were physically restrained with a muzzle for the collection of approximately 5-10 mL blood through puncture of the jugular or cephalic vein. Blood was collected in tubes with and without EDTA as an anticoagulant. In animals that displayed skin alterations, skin scrapings were obtained. All material was maintained at 4°-10°C until use.

Hematology and biochemistry

The complete blood count and total protein quantification calculations were performed using the ABX Vetpack kit (ABCVET™ Animal Blood Counter, Horiba Instruments, Irvine, CA, USA), and specific blood cells were read on stained glass slides using the Quick Panótico® kit and analyzed using an optical microscope (Zeiss Star-Cousin, Jena, Germany) at a magnification of 100X.

The serum obtained from a 10-min centrifugation at 1300 *g* was used to quantify the serum enzymes alanine aminotransferase, urea, and creatinine using Labtest® kits (Labtest Diagnóstica S.A., Brazil) following the manufacturer recommendations. The reading was performed using a Bio2000 apparatus (Bioplus Ltda., Brazil).

Serology

Serology was performed using an enzyme-linked immunosorbent assay (ELISA) and EIE-LVC equipment 12PEL017Z, with antigens from *L. major* from Bio-Manguinhos (Rio de Janeiro, RJ, Brazil) following the manufacturer recommendations and using analyzing plates with the TP-ELISA Reader (Thermo Scientific, Waltham, MA, USA).

Molecular analysis

For molecular diagnosis, only samples that were reagents for the serological test were used, and those with hematologic changes, such as anemia and/or thrombocytopenia, or dogs with cutaneous changes were evaluated.

DNA extraction

To extract genomic DNA from the samples, the pulp from the leukocytes obtained by centrifugation at 1300 *g* for 10 min was used. The samples were lysed with extraction buffer (20 mM Tris, 50 mM EDTA, 5 µg/mL proteinase K, and 1% SDS) and maintained at

60°C for 80 min. To recover the DNA, 25:24:1 phenol:chloroform:isoamyl alcohol was used (Invitrogen, Carlsbad, CA, USA) and then centrifuged at 16,000 *g* for 10 min. DNA was precipitated using 100% ethanol and 5 M ammonium acetate, eluted with ultra-pure water, and stored at -20°C. The DNA from each sample was quantified using a NanoDrop2000 (Thermo Scientific).

Polymerase chain reaction (PCR)

Specific primers were used to differentiate between *L. braziliensis* (de Bruijn and Barker, 1992) and *L. infantum* (Lachaud et al., 2002) as well as for the differential diagnosis of *T. cruzi* (Ávila et al., 1990). Similar conditions were used for *L. infantum* (2 mM $MgCl_2$ and 1.25 U Taq DNA polymerase) and for *T. cruzi* (1.5 mM $MgCl_2$ and 1.5 U Taq DNA polymerase). The thermocycler conditions were as follows: 94°C for 1 min, and then 65°, 59°, or 61°C for 1 min for the primer annealing of *L. braziliensis*, *L. infantum*, and *T. cruzi*, respectively, and a final extension for 1 min at 72°C for a total of 35 cycles. The amplicons were visualized using a 2% agarose gel and stained with ethidium bromide. Control samples for *L. braziliensis*, *L. infantum*, and *T. cruzi* were provided by the Gonçalves Muniz Research Centre (FIOCRUZ, Bahia).

Statistical analysis

Statistical evaluation was performed on the results of dogs selected for molecular diagnosis. Parametric variables were subjected to analysis of variance, *t*-test, and Pearson correlation coefficient using the SAS 9.1.3 program (SAS Institute, Inc., Cary, NC, USA). Risk factors and clinical, hematological, and biochemical changes associated with dogs testing positive for *L. braziliensis* were analyzed using the chi-squared statistical test with Yates correction using the EpiInfo 7 program (CDC, Atlanta, GA, USA). For variables with $P < 0.25$ in the univariate analysis, multivariate analyses were also performed by logistic regression using EpiInfo 7. The kappa test was performed to verify the agreement between the results of the ELISA and PCR tests. A significance level of 5% was considered to be significant.

RESULTS

ELISA results revealed the presence of 234 (41.78%) animals with antibodies against *Leishmania*. Of the 560 dogs, 179 (31.96%) had some cutaneous signs and/or hematology suggestive of LCan. Changes in the skin resulted from alopecia, erythema, desquamation, itching, sores, and hyperkeratosis. Hematological triage parameters were adopted during the screening for anemia, thrombocytopenia, and hyperproteinemia.

The skin scrapings revealed the presence of 10 (1.8%) dogs with mange (*Demodex canis*) and 29 (5.2%) with fungal spores in their coats, as well as 5 dogs with signs of fungal spores and scabies at the same time. Scabies and/or fungus were present in half of the animals that tested positive for *L. braziliensis*.

A total of 413 dogs were selected for molecular analysis, and 226 animals (54.72%) were identified as PCR-positive for *L*. (*V.*) *braziliensis* (Figure 2). No animals tested positive for *L*. (*L.*) *infantum* or *T. cruzi*.

A **B1** **B2**

Figure 2. Dogs present cutaneous lesions and clinical changes suggestive of *Leishmania* spp. **A.** Dog parasitized by *Demodex canis* PCR-negative for *Leishmania* spp, **B1.** and **B2**. Dog presents ulcer in ears and PCR-positive for *Leishmania braziliensis*.

The kappa test between the ELISA and PCR results revealed a low level of agreement with K = 0.107 and P = 0.029.

A total of 215 dogs from peri-urban areas and 198 dogs from urban areas were analyzed; of these, 129 (60.0%) and 97 (48.9%) were PCR-positive, respectively. Dogs from peri-urban areas were more likely to have had contact with the parasite (P = 0.03) and were 1.56-fold more likely to be infected.

Significant differences were not identified in the hematological and biochemical data between the evaluated groups of dogs, although the PCR-positive animals showed lower values of erythrocytes and hematocrit and increases in urea and serum creatinine values (Table 1).

Table 1. Hematological and biochemical profile of the dogs (N = 560).

Parameters	G1 (N = 147)		G2 (N = 187)		G3 (N = 226)		Reference
	Means ± SD	Minimum-maximum	Means ± SD	Minimum-maximum	Means ± SD	Minimum-maximum	
Erythrocytes	6.56 ± 0.74[a]	4.58-8.42	5.80 ± 1.24	2.26-8.60	5.66 ± 1.37	1.63-8.53	5.5-8.5 (x 10^6/μL)
Hematocrit	45.49 ± 5.06	36-60	39.10 ± 8.93	15.5-59.6	37.87 ± 9.66	11.20-58.60	37-55 (%)
Hemoglobin	15.32 ± 1.75	12-21.1	13.25 ± 3.03	5-19.8	13.02 ± 3.30	3.6-20.8	12-18 (g/dL)
VGM	69.46 ± 5.50	49-98	67.59 ± 5.38	55-103	67.16 ± 7.61	51-141	60-77 (fL)
Leukocytes	8.67 ± 3.02	3.64-21.3	12.89 ± 5.16	1.4-37	12.15 ± 5.45	3.6-36.4	6.0-17.0 (x 10^3/μL)
Platelets	2.92 ± 1.05	1.59-8.62	1.80 ± 1.26	0.24-6.92	1.79 ± 1.32	0.21-7.03	1.6-5.0 (x 10^3/μL)
Total Protein	7.77 ± 0.87	6-12	8.14 ± 1.15	5.8-12.6	8.10 ± 1.09	5.8-12.0	5.3-7.8 (g/dL)
ALT	51.05 ± 33.84	17-250	48.42 ± 38.34	14-268	44.90 ± 29.41	10-211	10-88 (IU/L)
Urea	38.98 ± 15.73	10-92	35.45 ± 17.70	10-141	39.20 ± 27.82	10-267	10-40 (mg/dL)
Creatinine	0.98 ± 0.31	0.4-1.8	0.97 ± 0.26	0.3-1.8	1.01 ± 0.31	0.4-2.4	0.5-1.5 (mg/dL)

G1 = dogs without clinical and negative changes; G2 = dogs with clinical and negative changes; G3 = dogs with clinical and positive changes.

In the risk factor assessment, only the variables of age and hair color were statistically associated with cases of *L. braziliensis*. Animals aged 2 years and older and those with light hair were more susceptible to the parasite (P < 0.01; Tables 2 and 3).

Only 32 animals were identified to have clinical and cutaneous changes characteristic of *Leishmania*. Nine animals were PCR-positive and had ulcers, particularly on the ears, muzzle, feet, and testicles. Three other animals with ulcerative lesions were PCR-negative. Changes associated with cutaneous signs in animals positive for *L. braziliensis* were also not statistically significant (P > 0.05). The results were confirmed by logistic regression analysis.

Table 2. Univariate analysis of the risk factors associated with positivity for *Leishmania* (*V.*) *braziliensis* in the group of animals selected for molecular tests (N = 413).

Variable	Number	Prevalence	OR	95%CI	P value
Sex					
Male	235	56.9%	1.145	0.774-1.693	0.562
Female	178	43.1%			
Breed					
Crossbreed	248	60.0%	1.029	0.693-1.529	0.966
Breed	165	40.0%			
Age					
<2 years	158	38.26%			
>2 years	255	61.74%	1.98	1.324-2.962	0.010
By size					
Curt	343	83.05%	0.979	0.584-1.640	0.959
Long	70	16.95%			
Hair color					
Light	206	49.9%	0.547	0.370-0.810	0.010
Dark	207	50.1%			
Yard					
Yes	336	81.4%	1.00	0.613-0.658	0.926
No	77	18.6%			
Environment					
Urban	198	47.94%			
Peri-urban	215	52.06%	1.562	1.058-2.307	0.031
Access street					
Yes	129	31.3%			
No	284	68.7%	1.214	0.797-1.849	0.474
Ticks					
Yes	53	12.8%			
No	360	87.2%	1.091	0.610-1.952	0.883
Access veterinary					
Yes	209	50.6%	1.153	0.782-1.698	0.536
No	204	49.4%			
Traveled to another city					
Yes	56	13.6%			
No	357	86.4%	1.120	0.634-1.978	0.805

OR = odds ratio; 95%CI = confidence interval.

Table 3. Logistic regression of the risk factors associated with positivity for *Leishmania* (*V.*) *braziliensis* (N = 413).

Variable	OR	95%CI	P value
Hair color			
Light	0.564	0.379-0.839	0.0047
Dark			
Age			
<2 years			
>2 years	1.929	1.284-2.896	0.0015

OR = odds ratio; 95%CI = confidence interval.

DISCUSSION

Dogs in the city of Ilhéus, northeastern Brazil, showed a high prevalence of *L. braziliensis*, although few animals showed clinical signs of the disease.

There was no correlation between the serological and molecular test results. Similar responses were identified in animals affected by the parasite for *L. infantum*, which was

diagnosed by ELISA and PCR (Dias et al., 2011). The results obtained from the kappa test indicate the differences in the characteristics of sensitivity and specificity of the techniques. The possibility of cross-reactions in serological tests to detect antibodies because of the low levels of immunoglobulins generated by the parasite during subclinical manifestation of the disease or high sensitivity of DNA-based diagnostics were considered. Alternatively, the dogs were affected by other species of *Leishmania* that favor antibody detection by ELISA. *Leishmania* (*Leishmania*) *amazonensis* was previously detected in the northeastern region of Brazil (Dias et al., 2011).

The isolated use of ELISA for LCan diagnosis does not determine the presence of the parasite or disease in the animal, supporting the recommendation of the combined use of serological and/or molecular techniques, particularly in clinically healthy dogs and in non-endemic regions (Solano-Gallego et al., 2009). Therefore, ELISA was used to sort and select suspected animals.

Molecular diagnosis of LCan by PCR from the kDNA region is effective and specific for direct parasite detection (Gomes et al., 2008), although the sensitivity can vary according to analysis or because of variations in primer design (Miró et al., 2008; Maia et al., 2009). The use of PCR for whole blood specimens is a practical and only mildly invasive technique that can be used in epidemiological studies involving large numbers of animals, although different tissues and secretions can be used (Silva et al., 2010; de Almeida Ferreira et al., 2012).

Finally, molecular diagnosis was used to enable differential diagnosis and identify the species of *Leishmania* present in the studied dogs. The absence of animals testing positive for *L. infantum* and *T. cruzi* is significant, indicating that the studied canine population was free of these parasites.

Worldwide, and in Brazil, the number of dogs infected with *L. infantum* varies from 20 to 90% (Coura-Vital et al., 2011; Bigeli et al., 2012); however, in the studied region, there was no record of this parasite affecting humans or animals. There is also no evidence of the presence of *Lutzomyia longipalpis* in the municipality of Ilhéus (Carvalho et al., 2010). This may explain the results of this study.

The differential diagnosis for *T. cruzi* was also necessary because this is an important zoonotic disease that affects dogs (Barros et al., 2012) and produces cross-reactivity during serological examinations for leishmaniasis (Porrozzi et al., 2007). Dogs testing positive for *T. cruzi* (Leça-Júnior et al., 2012) were found in a rural area in this region.

In Brazil, few studies have examined the prevalence and risk factors associated with LTCan cases. A previous study was conducted in Paraná and Pernambuco. Initially, using tissue samples and blood, 11.88 and 6.99% LTCan-positive dogs were identified from different species (Velasquez et al., 2006). In the second study in rural areas, 48.78% of dogs were identified as infected by *L. braziliensis*, while 5% were co-infected with *L. infantum* based on blood sample analyses (Dantas-Torres et al., 2010).

The prevalence of dogs with *L. braziliensis* in Pernambuco is consistent with the results of this study and indicates the involvement of dogs in parasite cycles in the northeastern region of Brazil (Dantas-Torres et al., 2010).

In the municipality of Ilhéus, dogs from peri-urban areas showed a higher prevalence of *L. braziliensis* (P = 0.031), which is consistent with the data for LCan in other areas of the country with peri-urban and rural profiles (Amóra et al., 2006; Velasquez et al., 2006; Dantas-Torres et al., 2010).

Lower levels of infrastructure were present in the peri-urban areas studied, and these areas are close to the Atlantic Forest, biological reserves, and farms in the cocoa-cabruca system. These factors may facilitate vector contact and wild reservoirs of the parasite. At distances of 25-100 m between residences and in areas with forests and streams, important risk factors for human cases and canine leishmaniasis exist and offer greater contact with vectors and wild reservoirs of the parasite (Membrive et al., 2012). The invasion of humans and dogs in forest areas and the presence of rodents and synanthropic animals in peri-domestic environments are the main forms of contact with the LTCan parasite (Alvar et al., 2004; Dantas-Torres, 2007; Lara-Silva et al., 2014).

The anthropic action in the wild environment can alter the balance between the flora and fauna and favors greater numbers of *Leishmania* vector contact with humans and dogs.

In the region studied, some urbanized areas also showed a high incidence of dogs positive for LTCan. These areas were close to woodlands, streams, and rivers and were susceptible to vectors and synanthropic animals, which may explain our results.

The Phlebotominae fauna of Ilhéus consists of approximately 13 species present in both domiciliary and peri-domestic environments (Azevedo et al., 1996), with a greater frequency of the *Lutzomyia whitmani* and *L. fischeri* flies in rural areas and of *L. cortelezzii* in urban areas. However, *L. longipalpis* flies were not identified (Carvalho et al., 2010). Examples of *L. whitmani* infected by *L. braziliensis* have been found in different regions of Brazil (Carvalho et al., 2010), and the predominance of these vectors in the region may be a vector for LTA (Azevedo et al., 1996). *L. whitmani* may have adapted to the domestic environment, and dogs may have incorporated this organism into their life cycles (Azevedo et al., 1996). The geo-climatic characteristics in the municipality of Ilhéus are ideal for the survival and reproduction of several of these vectors, which can foster greater contact with dogs and humans.

Despite the presence of vectors and the high prevalence of dogs positive for *L. braziliensis* identified in the peri-urban areas of Ilhéus, one area showed the lowest rates of positive animals. The characteristics of this area are similar to those of other peri-urban areas, with woodlands and rivers, favoring vector contact and contact with the wild hosts of the parasite. Additionally, northern region houses are in the municipality's industrial center, with factories that process cocoa, rubber, coffee, and citrus juices. These companies, particularly the cocoa factories, emit large volumes of smoke that spread throughout neighboring areas, which may limit the vectors and wild animals in these areas. A second possibility is the presence of chemical constituents with repellent effects in the emitted smoke, which may limit vectors in the region, leading to a reduced number of infected animals. These hypotheses require further analysis.

Lower rates of human LTA cases were also found in the northern region of the municipality. The central and southern areas of the municipality of Ilhéus showed the highest number of human LTA cases, and thus a higher risk of contamination in the population. These data demonstrate the transmission dynamics of LTA in the municipality and are complementary to the results of this study (Campos-Júnior, 2007).

Only 7.7% of the dogs presented clinical evidence of disease, and the vast majority appeared to be healthy. Dermatological lesions on some dogs were related to scabies and fungal spores, which were identified in the skin scrapings. Dogs positive for *L. braziliensis* were also identified as having mites and fungi, which may mask or make the diagnosis of LCan more difficult. These characteristics of LTCan in this canine population may have neutralized the protective effect of infrequent access to a veterinarian.

Particularly, for *L. braziliensis*, the lower frequency of dogs with clinical signs

reinforces the subclinical and asymptomatic LCan profile, which is consistent with the results of previous studies (Solano-Gallego et al., 2009; Figueredo et al., 2012) that reported few canines with any clinical evidence of the disease. Furthermore, most dogs were resistant and without clinical or pathological signs throughout their lives. The results must be interpreted cautiously for healthy dogs showing PCR-positive and PCR-negative results for *Leishmania* serologies, and the animal must show clinical and serological evidence of the disease (Solano-Gallego et al., 2009; Silva et al., 2010).

In the analysis of hematologic data of the dog groups, there were variations for all parameters studied, but no changes indicated a possible LCan association. PCR-positive animals showed reduced numbers of erythrocytes and hematocrit. Some studies in endemic areas of LVCan revealed small reductions in the number of cells in the erythrogram, indicating anemia, as well as thrombocytopenia and hyperproteinemia in animals with positive serology (Da Silva et al., 2011). Similar changes were observed in dogs positive for *L. braziliensis* (Figueredo et al., 2012).

In the biochemical examinations of the liver and kidney functions, no significant changes were observed, despite slightly increased urea and creatine levels in the positive animals. There was a strong association between kidney disease and LCan, and often this was the only clinical evidence of infection, although detection of these changes only occurred in advanced cases of renal tissue impairment (Costa et al., 2003).

Animals older than 2 years were more likely to have had contact with *Leishmania*, which is consistent with the data in dogs from the Mediterranean (Cortes et al., 2012) and other regions of Brazil (Rondon et al., 2008). Animals under the age of 2 years should remain in the home environment longer to reduce the possibility of vector contact.

Evaluation based on size and hair color showed differing results, and animals with lighter coat colors were statistically more prone to *Leishmania*, which was inconsistent with the results of previous studies, indicating that animals with short and dark hair were more susceptible to LVCan (Rondon et al., 2008; Cortes et al., 2012). Coat size was found to be a risk factor in this study, which is consistent with previously published data (Coura-Vital et al., 2013). It is thought that the discrepancies between these results may have resulted because most dogs in this study (83.05%) had short hair. Animals with lighter hair may attract *Leishmania* vectors more easily by reflecting ambient light with a greater intensity, making them more visible to the parasite, but this requires further analysis. Vectors of the genus *Lutzomyia* are drawn to brightness (Andrade, 2010). However, further research is necessary to better define the role of these variables as risk factors for LCan.

The large number of dogs positive for *L. braziliensis* identified in the studied region requires attention and clinical monitoring using serological and molecular techniques to identify sick animals. Physical, biochemical, and hematological evidence may not be available in the animals, making a positive clinical diagnosis of affected canines difficult. Future studies should determine the clinical pattern of dogs positive for LTCan and may aid in diagnosis.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOLWEDGMENTS

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