Seroprevalence and Spatial Analysis of Canine Visceral *Leishmaniasis* in the Pantanal Region, Mato Grosso State, Brazil

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Abstract

Introduction: Visceral Leishmaniasis (VL) is a zoonotic disease of public health importance, whose main reservoir is the domestic dog. The purpose of this study was to evaluate the prevalence and spatial distribution of Canine Visceral Leishmaniasis (CVL), as well as factors associated with the disease, in the municipality of Barão de Melgaço.

Methods: Serum samples from 402 dogs in urban and rural areas were subjected to an immunochromatographic rapid test (DPP, TR DPP® LVC–Bio-Manguinhos/FIOCRUZ) and the enzyme-linked immunosorbent assay (ELISA, EIE® LVC–Bio-Manguinhos/FIOCRUZ). An epidemiological questionnaire was used to identify factors associated for CVL using a logistic regression model. The presence of spatial autocorrelation was performed using global and local Moran's indices.

Results: Seventeen dogs were reactive to both diagnostic tests, indicating a prevalence rate of 4.2% in the municipality, and a statistically significant association with hyperkeratosis and splenomegaly (P<0.05). Through the global Moran index, a density map was built for show the spatial distribution of CVL cases, showing a positive correlation among all the evaluated areas (I<0.25). Local Moran index was used to create a cluster map, identifying areas with high, low and intermediate priority for control of CVL.

Conclusions: The prevalence of CVL in Barão de Melgaço was of 4.2%, being characterized as area with moderate transmission and receptive to the occurrence of human cases. In this study, the use of spatial analysis stood out as a strategic tool in the control of CVL, enabling different action strategies to be applied in each region.

Keywords: Dog; Epidemiology; Georeferencing; *Leishmania chagasi*; Zoonotic disease

Introduction

Brazil is among the six countries most affected by Visceral Leishmaniasis (VL) [1]. It is estimated that about 90% of VL cases in the New World occur in Brazil, where it is rapidly spreading to the country's five regions [2,3]. In the New World, this zoonotic disease is caused by the protozoan *Leishmania chagasi* (syn. *Leishmania infantum*) transmitted to vertebrate hosts through the bite of infected dipterans [1,4].

Dogs, which are considered the main hosts and reservoirs of the disease in urban environments, are highly susceptible to infection, characterized by intense cutaneous parasitism, which favors infection of the vector and, hence, transmission to humans [5,6].

The proximity of Barão de Melgaço to regions endemic for CVL, such as Cuiabá [7], Rondonópolis [8], Poxoréo [9], Jaciara and Várzea Grande [10], allied to the region's climatic and physiographic characteristics, which favour the presence of the vectors *Lutzomyia longipalpis* and *L. cruzi* already reported in the municipality [11], can contribute to the persistence and dissemination of this disease. This means the region represents a risk to the local population and to tourists from different parts of the world, which come in search of the natural attractions of North Pantanal fauna and flora.

Given the importance of dogs in the epidemiological cycle of VL and the tourist potential of Barão de Melgaço, this study aimed to evaluate the seroprevalence and spatial distribution of CVL in Barão de Melgaço, associating the disease to environmental and individual factors.

Methods

Study design

A descriptive cross-sectional study was conducted in the city of Barão de Melgaço, located in the Pantanal region of the state of Mato Grosso, Brazil (coordinates: latitude 16°11'40''S and 55°58'03''W). The minimum sample size was estimated at 283 dogs, considering a dog to human ratio of 7:1, at the estimated population of 7,526 in 2015 [12], using a confidence level of 95%, acceptable error of 5% and prevalence of 50% [3]. Samples were collected from dogs in all the urban neighbourhoods, and from dogs in 11 rural communities.

Samples and serological testing

Blood samples were drawn from dogs of both sexes, different breeds, and ages equal to or older than six months, which had been previously examined and classified as symptomatic or asymptomatic [13]. At each residence, the dog's owner was asked to fill out an epidemiological questionnaire containing information about the dog's breed, sex, age, fur length, function, the place it occupies in the house, its access to the street, proximity of the house to forest and rivers, location of the house, the presence of other animals and/or chicken coops or pig sties, vegetation around the house, and garbage collection.

The dogs were serologically diagnosed using the tests recommended by the Ministry of Health (MS): DPP for screening and ELISA for confirmation. Both tests were performed according to the instructions of the manufacturer, Bio-Manguinhos/FIOCRUZ.

The DPP kit for rapid detection of K26/K39-specific antibodies was performed as a screening tool at the Laboratory of Leishmaniasis, Department of Clinic Veterinary Medicine, The Federal University of Mato Grosso (CLIMEV-UFMT). The sample was considered positive if two red lines appeared and negative if only one red line appeared. Subsequently, the samples positive on DPP were subjected to ELISA, which employs a soluble antigen from promastigote forms of L. major-like parasites. This serology was performed in the Laboratory of Leishmaniasis of the CLIMEV-UFMT. The sample was considered positive when the ELISA reading was higher than the cut-off point. The results were obtained by a combination of the two tests.

All of the laboratorial procedures were executed according to the Principles of Good Laboratory Practices [3] for the protection of the manipulator, sample and environment.

Data analysis

Two-step logistic regression, i.e., univariate and multivariate analysis, was employed to correlate the seroprevalence of anti-*L. chagasi* antibodies in dogs and the independent variables. Variables presenting a chi-square $P \leq 0.20$ in the univariate analysis were selected for the multivariate analysis, and $P \leq 0.05$ were considered significant. The correlation between the two diagnostic tests was established based on the Kappa test [14]. The tests were performed in R 3.3.0 using the R Commander package [15].

Spatial analysis

In the spatial analysis, the global Moran index was employed as a measure of spatial autocorrelation in the various census tracts of Barão de Melgaço, this index provides a single value, potentially varying from -1 to 1. As for the local Moran index, the tracts were classified into four quadrants based on the Moran scatter plot, where: quadrant HH (+/+) and quadrant LL (-/-) are areas with a positive spatial association, while quadrant HL (+/-) and quadrant LH (-/+) are areas with a negative spatial association. Three areas were defined with different CVL control priorities: High priority–quadrant HH (+/+), low priority–quadrant LL (-/-), and intermediate priority–quadrants HL (+/-) and LH (-/+). A representative map was used to examine the areas with statistically significant spatial autocorrelations ($P \leq 0.01$) [16]. The analyses were performed in R 3.3.0 using the R Commander package [15].

Ethics

This study was conducted in accordance with the ethical principles approved by the Ethics Committee on Animal Use (CEUA)–UFMT under protocol number 23108.014950/11-5. The dog owners were informed of the research objectives and were required to sign an informed consent form before sample and data collection.

Results

Of the 402 evaluated dogs (248 from urban and 154 from rural areas), assuming parallel results, 94 samples tested positive in at least one of the serological assays, with a seroprevalence of 23.4% (95% CI: 19.39 to 27.89). In an individual analysis, the DPP screening test showed a seroprevalence of 19.4% (95% CI: 15.72 to 23.68, 78/402), while the confirmatory test, ELISA, showed a seroprevalence of 8.2% (95% CI: 5.8 to 11.45, 33/402).

Table 1: Univariate analyses for variables considered for the study of risk factors associated with canine visceral leishmaniasis in 402 dogs from Barão de Melgaço, State of Mato Grosso, Brazil.

| Variables | DPP and ELISA | | P-value | OR (95% CI)* |
| | Positive | Negative | | |
	n (%)	n (%)		
Breed[a]				
CRD	1 (2.5)	39 (97.5)	1	**
SRD	16 (4.4)	346 (95.6)		
Sex				
Male	11 (4.8)	218 (95.2)	0.51	1.4 (0.46-4.72)
Female	6 (3.5)	167 (96.5)		1
Age groups				
Indefinite age	4 (6.25)	60 (93.75)	0.671	**
≤1	1 (3.2)	30 (96.8)		
≥1-3	4 (3.6)	106 (96.4)		
≥3-5	3 (2.7)	110 (97.3)		
≥5	5 (6.0)	79 (94.0)		
Fur length				
Short	16 (4.4)	345 (95.6)	1	**
Long	1 (2.4)	40 (97.6)		
Clinical status				
Asymptomatics	3 (1.9)	154 (98.1)	0.064	1
Symptomatic	14 (5.7)	231 (94.3)		3.1 (0.84-17.12)
House role				
Guard	6 (3.9)	148 (96.1)	0.95	**
Company	8 (4.4)	172 (95.6)		
Both	2 (4.1)	47 (95.9)		
Hunt	1 (5.3)	18 (94.7)		
Dog habitat				
Intradomestic	-	10 (100.0)	0.609	**
Peridomiciliar	17 (4.8)	336 (95.2)		
Both	-	39 (100.0)		
Proximity to				
Forest	6 (4.4)	131 (95.6)	0.952	**
Wasteland	1 (92.6)	37 (97.4)		
River	3 (3.3)	89 (96.7)		
Any combination	5 (5.2)	91 (94.8)		
Neither	2 (5.1)	37 (94.9)		
Residence type				
Urban	10 (4.0)	238 (96.0)	0.803	1
Rural	7 (4.5)	147 (95.5)		1.13 (0.35-3.38)

*Odds Ratio; 95% CI: 95% Confidence interval; **Has not been determined due to lack of categories, null or very low frequencies; [a]CRD–With defined race; SRD–Without defined race

Table 2: Univariate and multivariate analyses to detect risk factors associated with the DPP and ELISA positivity of canine visceral leishmaniasis in dogs from Barão de Melgaço, State of Mato Grosso, Brazil.

| Variables	Dogs		Univariate analysis		Multivariate analysis	
	n	Positive (%)	X2	P	P	OR (95% CI)*
Splenomegaly						
Yes	63	6 (9.5)	5.29	0.017	0.014	4.05 (1.32-12.43)
No	355	11 (3.1)				
Lymphadenopathy						
Yes	164	8 (4.9)	0.288	0.591	-	-
No	238	9 (3.8)				
Onychogryphosis						
Yes	32	3 (9.4)	2.273	0.131	0.997	1.0 (0.19-5.09)
No	170	14 (3.8)				
Ocular disturbances						
Yes	37	4 (10.8)	4.359	0.036	0.486	1.63 (0.41-6.50)
No	365	13 (3.6)				
Periocular alopecia Yes						
No	1	1 (100.0)	22.70	< 0.01	0.991	**
	401	16 (4.0)				
Hyperkeratosis						
Yes	4	2 (50.0)	20.89	<0.01	0.033	16.19 (1.24-210.6)
No	398	15 (3.8)				
Domestic animals						
Yes	395	11 (3.7)	0.684	0.408	-	-
No	107	6 (5.6)				
Animal husbandry						
Yes	227	10 (4.0)	0.04	0.841	-	-
No	175	7 (4.0)				
Access to the street						
Yes	297	12 (4.0)	0.099	0.752	-	-
No	105	5 (4.8)				
Vegetation						
Yes	363	15 (4.1)	0.086	0.769	-	-
No	39	2 (5.1)				
Garbage collection						
Yes	244	10 (4.1)	0.026	0.871	-	-
No	158	7 (4.4)				
*Odds ratio; 95% CI: 95% Confidence interval. **Has not been determined due to lack of categories, null or very low frequencies						

Based on the combined results and serial protocol stipulated by the MS, 17 samples were positive in both tests, with a seroprevalence of 4.2% (95% CI: 2.56 to 6.82). Rural dogs (4.5%) showed a 0.5% higher seroprevalence than urban dogs (P=0.803). The Kappa test of the DPP and ELISA results showed a slight agreement of 21% (95% CI: 0.12 to 0.30).

In general, this study contained predominantly male dogs (57%, 229/402), mixed breed dogs (90%, 362/402), adults (92.8%, 371/402), unneutered (96%, 373/402), and short-haired dogs (89.8%, 361/402), and no statistical association was found between their characteristics and the prevalence of infection (P>0.05) **(Table 1).** In the clinical examination, 14 (82.35%) seropositive dogs showed clinical signs suggestive of CVL (P=0.064) **(Table 1),** the most common ones being lymphadenopathy (47.1%) and skin lesions (41.2%), followed by splenomegaly and onychogryphosis (35.3%), ophthalmopathy and alopecia (23.5%). Scaling and hyperkeratosis (11.8%), ear tip ulcers and periocular alopecia (5.9%) were less frequent.

In the univariate analysis for anti-*L. chagasi* antibodies, the variables of splenomegaly, ophthalmopathy, periocular alopecia and hyperkeratosis showed values of P<0.20 and were selected for multivariate analysis. This analysis indicated that the main factors associated with canine infection were the presence of hyperkeratosis and splenomegaly, which were, respectively, 16.19 and 4.05 times more likely to be present in dogs seropositive for CVL than in those without these clinical signs **(Table 2)**.

A choropleth map **(Figure 1a)** was created to ascertain the incidence rates of CVL in the census tracts of Barão de Melgaço. On this map, dark areas indicated a higher concentration of cases appearing in the north-south direction of the municipality, while light areas in the central and western part indicated the absence or a lower concentration of cases. To identify the spatial correlation structure that best describes the data, a Moran correlogram was built using the global Moran index **(Figure 1b),** which revealed a positive correlation for all the evaluated tracts, since the value was above zero, although the correlation was small or zero (I<0.25). A cluster map based on the Moran scatter plot **(Figure 2a)** showed that only one tract presented high priority–HH (+/+) and three presented low priority–LL (-/-), indicating points of positive spatial association, i.e., tracts with neighbors showing similar values. As for the other tracts, four showed intermediate priority–HL (+/-), LH (-/+), indicating points of negative spatial association, i.e., contiguous census tracts with different values. The local Moran index **(Figure 2b)** indicated that there was no significant local correlation.
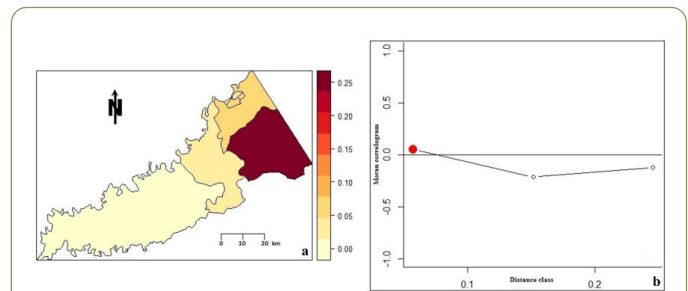
Figure 1: Incidence rate of dogs reactive to CVL, based on Brazil's Ministry of Health protocol (a). Global Moran index, indicating spatial correlation for CVL rates in the municipality of Barão de Melgaço, based on the Ministry of Health protocol (b).
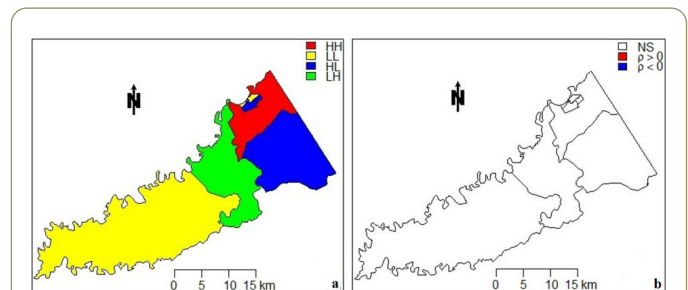
Figure 2: Moran scatter plot: blue tracts represent regions with high CVL rates with transition to lower rates (yellow). Red tracts represent high CVL rates, and green tracts represent low rates with transition to high rates of the disease (red). Yellow tracts represent low CVL rates (a). Local Moran Index indicating no significant local correlation among CVL rates in the municipality of Barão de Melgaço, based on the Ministry of Health protocol (b).

Discussion

This epidemiological survey of canine leishmaniasis, which revealed a 4.2% prevalence of anti-*L. chagasi* antibodies, was motivated by the proximity of Barão de Melgaço to cities endemic for CVL. Similar results were found in studies conducted in different regions of Brazil. CVL prevalence rates of 3.4% were reported in the municipality of Cuiabá-MT [17], of 4.63% in Divinópolis-MG [18], and of 4.5% in Presidente Prudente-SP [19]. Despite these similar results, the literature describes prevalence rates ranging from 3.4-67% [17,20] in different regions of the country.

Serological tests are important tools in the diagnosis of CVL, and are often used in epidemiological surveys on canines.

Therefore, agreement between the two tests recommended by the MS is very important, in order to ensure that animals testing false-negative in the DPP be subjected to ELISA, which was not observed in this study and was also described by Fonseca et al. [21] and in the meta-analysis of Peixoto et al. [22]. According to the literature, low concordance between tests may be caused by factors such as areas where transmission is still incipient, CVL prevalence rates, and the fact that concordance between diagnostic tests tend to be low [22]. Moreover, dogs infected with *L. chagasi* present different humoral immune responses to various antibodies at different stages of the disease [23,24]. This means that serological tests may present different degrees of sensitivity, depending on the clinical course of the infection, genetic variability of the host, and specific immune responses [25,26].

Other factors also interfere in the serological diagnosis, such as the type of antigen used for the diagnosis of antibodies [23,27]. In this context, the DPP assay uses recombinant proteins, and despite the technique's high sensitivity (98%) and specificity (96%) in symptomatic animals, it shows low sensitivity (47%) in asymptomatic dogs [25]. On the other hand, the ELISA, which uses *L. major*-like crude antigens, provides varying rates of sensitivity (8-100%) and specificity (60-100%) in the diagnosis of CVL due to antigenic similarities between *Leishmania* species [22,28]. Both subject to cross-reactions and false positive results in overlapping areas of trypanosomatids [28,29].

In this study, symptomatic dogs accounted for 82.35% of the seropositive dogs. This high percentage corroborates results reported in the literature that associate the positivity of serological tests with the severity of the disease [25]. Nevertheless, among animals infected with *L. chagasi*, asymptomatic animals are usually more prevalent in epidemiological studies [30]. Therefore, further research should be encouraged to devise techniques that are more sensitive to asymptomatic animals. The clinical signs observed in this study were the same as those described in other epidemiological studies of CVL [17], and the univariate and multivariate analysis revealed a positive correlation between hyperkeratosis and splenomegaly and the presence of the disease. This is an important finding in the clinical analysis of animals suspected of having the disease, in view of the large number of its clinical symptoms.

Spatial analysis tools have been increasingly used in studies of VL, and have been the focus of research in different regions of Brazil [18,31]. Thus, epidemiological studies that include spatial analysis can be used in VL surveillance and control programs to identify epidemiologically important areas and to prioritize municipalities that require the adoption of control measures [17,31].

In this context, the incidence of CVL was distributed unevenly in the various tracts of the municipality, with some tracts showing a high concentration of cases and others showing no CVL or a lower concentration of the disease. Only one census tract was identified as high priority for control of the disease. This variation in prevalence rates in the municipality may be ascribed to the different characteristics of each area in Barão de Melgaço, such as the population's socioeconomic status, agglomerations of animals and humans, poor basic sanitation, and houses usually located near rivers (humid environments) and forests. It should be noted that the only census tract considered as high priority contains both urban and rural areas, and because the municipality is located in the Pantanal wetlands, it is subjected to the annual flood cycle, which means that 98% of its territory is flooded during the rainy season from January to March. As a result, the population tends to migrate from the rural to the urban area, thus favoring the spread of the disease to the urban environment, since sick animals coming from rural areas can contribute to urbanize the disease. Moreover, the decreasing areas of dry land may lead to greater contact between wildlife reservoirs of the disease and the population, and the accumulation of organic matter favours increased vector density. Thus, the municipality may be in the process of urbanization of the disease, because unlike other Brazilian municipalities with larger urban populations, a large part of the population of Barão de Melgaço is rural, which justifies this hypothesis.

Conclusion

The results of this study revealed a CVL prevalence rate of 4.2% in urban and rural areas of the municipality of Barão de Melgaço, being characterized as area with moderate transmission and receptive to the occurrence of human cases. Hyperkeratosis and splenomegaly were identified as factors associated with CVL. The spatial analysis methodology allowed determining priority control areas for CVL, which should be the focus of prevention and control measures.

Acknowledgements

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