

Canine leishmaniasis in the semi-arid region of Pernambuco, northeastern Brazil: epidemiology, factors associated with seropositivity and spatial analysis

Leishmaniose visceral canina na região semiárida de Pernambuco, nordeste do Brasil: epidemiologia, fatores associados à soropositividade e análise espacial

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Abstract

This study aimed to determine the seroprevalence, factors associated with seropositivity to *Leishmania* infection in dogs and spatial analysis in six municipalities in the semi-arid region of Pernambuco, Brazil. Blood samples were collected from 462 dogs, 77 in each municipality, and used for serological analysis [dual path platform (DPP®) and enzyme-linked immunosorbent assay (ELISA)]. Clinical signs of dogs were evaluated and associated factors for *Leishmania* infection were analyzed using robust Poisson regression model. A seroprevalence of 42.8% (198/462, IC: 95% = 38.6%-47.6%) was detected in dogs that tested positive in both tests, ranging from 29.8% to 55.8%, with higher prevalence in the municipality of Cabrobó (55.8%; P = 0.006). About 67% (132/198) of the seropositive dogs showed one or more clinical signs suggestive of canine leishmaniasis (CanL), such as lymphadenomegaly, skin lesions and conjunctivitis, which were associated with seropositivity. High seroprevalence levels were identified in urban and rural areas in all the municipalities, and the buffer for sand flies around cases covered almost these entire areas. Spatial analysis revealed a significant cluster, showing a relative risk of 1.88 in the urban area of Cabrobó. The higher density of seropositive dogs in urban areas indicates the need effective control measures against CanL to prevent the emergence of canine and human diseases.

Keywords: *Leishmania*, diagnosis, serology, dogs, geoprocessing.

Resumo

Este estudo objetivou determinar a soroprevalência, fatores associados com a soropositividade da infecção por *Leishmania* em cães e análise espacial em seis municípios do semiárido de Pernambuco. Amostras de sangue foram coletadas de 462 cães, 77 em cada município, e utilizadas para análise sorológica [teste rápido imunocromatográfico (DPP®) e ensaio de imunoabsorção enzimática (ELISA)]. Os sinais clínicos dos cães foram avaliados, e os fatores associados à infecção por *Leishmania* foram analisados, usando-se modelo robusto de regressão de Poisson. Foi detectada uma soroprevalência de 42,8% (198/462, IC: 95% = 38,6%-47,6%) em cães com resultados positivos em ambos os testes, variando de 29,8% a 55,8%, com maior prevalência no município de Cabrobó (55,8%; P = 0,006). Cerca de 67% (132/198) dos cães apresentaram um ou mais sinais clínicos sugestivos de CanL, como linfadenomegalia, lesões de pele e conjuntivite, os quais foram associados à soropositividade. Altos níveis de soroprevalência foram identificados nas áreas urbanas e rurais de todos os municípios, e os *buffers* da área de

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vão do flebotomíneo em torno dos casos cobriam quase todas as áreas dos municípios. A análise espacial revelou um cluster significativo, mostrando um risco relativo de 1,88 na área urbana de Cabrobó. A maior densidade de casos em áreas urbanas indica a necessidade de medidas efetivas de controle contra o CanL, para evitar o surgimento da doença canina e humana.

Palavras-chave: *Leishmania*, diagnóstico, sorologia, cães, geoprocessamento.

Introduction

Visceral leishmaniasis (VL), a neglected tropical disease of worldwide distribution, is considered a public health problem (WHO, 2017). Every year, 200 to 400 thousand new cases of VL are reported around the world (Alvar et al., 2012; WHO, 2015). VL is present in 18 countries of Americas, and 96% (57,582) of human cases are reported in Brazil (PAHO, 2019). Dogs are the main domestic reservoir hosts of *Leishmania infantum*, which is transmitted mainly by the female phlebotomine sand fly *Lutzomyia longipalpis*, a vector species (Otranto & Dantas-Torres, 2013; Marcili et al., 2014; Brazil et al., 2015).

The incidence of leishmaniasis is high due to its expansion into urban areas adjacent to residual forests, thereby increasing the number of confirmed cases (Werneck, 2008; Conti et al., 2016). These changes in spatial patterns of transmission provide favorable conditions for the spread of phlebotomine species to peridomiliary environments, as does the presence of domestic dogs, thus increasing the number of confirmed human cases of leishmaniasis (Conti et al., 2016; Sevá et al., 2017).

Geoprocessing is very useful in epidemiological studies to shed light on the dynamics of VL transmission, because it enables the analysis of spatial correlations between canine (CanL) and human (VL) leishmaniasis, vector distribution, and identification of areas with high incidence levels (Oliveira et al., 2001; Werneck et al., 2007; Teixeira-Neto et al., 2014). The analysis of these issues can contribute toward targeted prevention and control strategies (Marcondes et al., 2011).

Human and canine cases of VL are frequently reported in northeastern Brazil (Dantas-Torres, 2006; Dantas-Torres et al., 2006; Harhay et al., 2011; Belo et al., 2013; Fernandes et al., 2016). From 2007 to 2018, 21,703 human cases were reported in this region, and the state of Pernambuco was particularly affected during this period, with 1,186 human cases reported (SINAN, 2019). Moreover, during this period, there was a wide geographic expansion of the disease in this region (Dantas-Torres, 2006; Pimentel et al., 2015).

The state of Pernambuco is divided into four mesoregions, one of which, located in the semiarid part of the state, is the São Francisco region. High prevalence levels and distribution of CanL have been recorded in this area (Cesse et al., 2001; Dantas-Torres, 2006; Araujo et al., 2016a). The wide distribution of this disease causes the frequent emergence of new cases of human and canine leishmaniasis, particularly in municipalities situated in endemic regions (Araujo et al., 2016b).

Although leishmaniasis is described as an endemic disease in the semiarid region of Pernambuco, and dogs are considered the main reservoirs and crucial for its dissemination, no study has so far focused on the investigation of seroprevalence in dogs in the municipalities of this region, enabling the unperceived perpetuation of the disease. Therefore, this study aimed perform a serological survey, observing spatial distribution and other factors associated with leishmaniasis among dogs in areas endemic for this parasite in the municipalities of this study.

Materials and Methods

Ethical considerations

This study was approved by the Research and Ethics Committee of the Federal University of Vale do São Francisco, under Protocol No. 0032/260911.

Study area

The study was conducted in six municipalities located in the São Francisco region of Pernambuco, in northeastern Brazil (Figure 1). These municipalities, which are served by the Petrolina Regional Public Health Service (VIII GERES) of Pernambuco, are Afrânio (8°30'54" S, 41°00'18" W), Cabrobó (8°30'51" S, 39°18'36" W), Dormentes (8°26'49" S, 40°46'15" W), Lagoa Grande (8°59'49" S, 40°16'19" W), Orocó (8°37'12" S, 39°36'7" W) and Santa Maria da Boa Vista

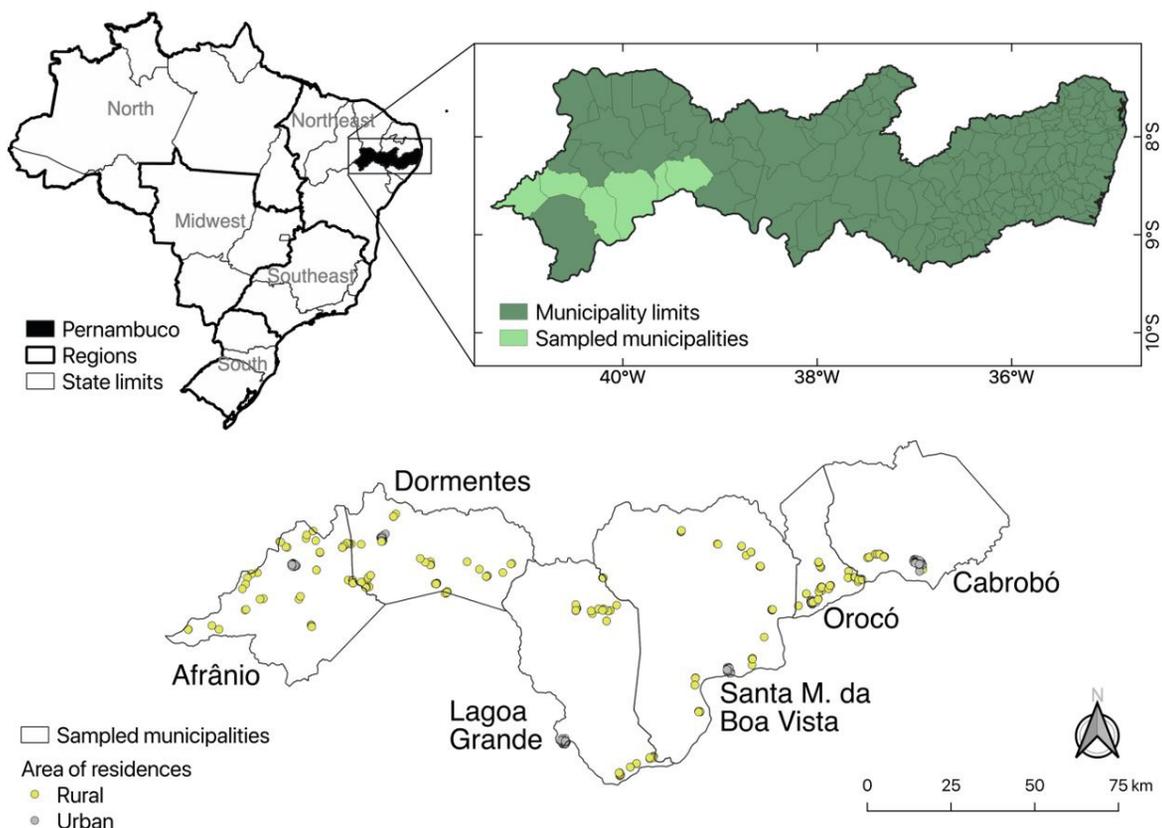


Figure 1. Residences in which the biological samples of dogs were collected in the municipalities (Afrânio, Dormentes, Cabrobó, Lagoa Grande, Orocó and Santa Maria da Boa Vista), in urban and rural areas, located in state of Pernambuco, northeastern Brazil.

(8°48'32" S, 39°49'30" W). The area is situated in the semi-arid region, characterized mainly by Caatinga biome with stretches of hyperxerophile deciduous forest (IBGE, 2011). An annual average of nine human cases of VL were reported in these municipalities between 2007 and 2017 (SINAN, 2018).

Sampling procedures and clinical examination

The study was conducted from June 2016 to December 2017. The sample size was determined using Epi Info version 7.1 software, with a 95% confidence interval, 2% margin of error, and an estimated prevalence of 11.2% (Araujo et al., 2016b). Considering the calculation for an infinite population, the sample size required for our study was approximately 462 dogs, comprising 77 animals per municipality (Table 1), divided between urban and rural areas (UA and RA) (Figure 1).

Table 1. Number of samples to be collected according to the human population in each urban and rural area of the municipalities.

Municipalities	Urban Pop.	%	Samples	Rural Pop.	%	Samples	Total Pop.
Afrânio	5,859	33.3	26	11,729	66.7	51	17,588
Cabrobó	19,811	64.1	49	11,072	35.9	28	30,883
Dormentes	6,004	35.5	27	10,911	64.5	50	16,915
L. Grande	10,369	45.6	35	12,350	54.4	42	22,719
Orocó	4,614	35.0	27	8,562	65.0	50	13,176
S.M.B.V	14,861	37.6	29	24,612	62.4	48	39,473
Total	61,518		193	79,236		269	140,754

S.M.B.V = Santa Maria da Boa Vista, L = Lagoa, Pop = Population.

The number of blood samples to be collected in each municipality was determined based on the human population living in each neighborhood of the municipality, according to data provided by the Brazilian Institute of Geography and Statistics (IBGE). Sampling points were selected randomly, and blood samples were collected only from dogs 6 months or older that had not been vaccinated against leishmaniasis.

Approximately 4 mL of blood were collected by cephalic venipuncture from each animal. The blood serum was obtained by centrifugation and was stored at -20°C for subsequent serological analysis.

Information was obtained about the sex, age, breed, dog pelage and size of each animal. Each dog was physically examined by a veterinarian to identify the main clinical signs of CanL, as emaciation, epistaxis, icterus, pale mucous membranes (ocular and oral), lymphadenomegaly (evaluation of the main popliteal, prescapular and submandibular lymph nodes), hepatosplenomegaly (by abdominal palpation), cutaneous alterations (alopecia, dermatitis, ulcers, lesions on the ears, face and limbs), cachexia, onychogryphosis and conjunctivitis (Amusatogui et al., 2003; Alvar et al., 2004).

Serological diagnosis

Serum samples were tested by two different methods to detect the presence of anti-*Leishmania* IgG antibodies. The first was qualitative screening by the Dual Path Platform (DPP[®]) rapid immunochromatographic test, produced by Fiocruz (Bio-Manguinhos Unit, Rio de Janeiro, Brazil), which contains a multi-epitope, recombinant chimeric protein (rK28) derived from the fusion of *L. infantum* gene k9, single repeat units of k39 and k26 (Boarino et al., 2005). All the procedures were performed as recommended by the manufacturer. The second method was the confirmatory quantitative test using a commercial ELISA kit (IMUNOTESTE[®] *Leishmania*; Imunodot, Jaboticabal, São Paulo, Brazil), also performed as recommended by the manufacturer. The cut-off value was determined as the mean absorbance value of the negative controls (provided with the kit) multiplied by 2.5, with all the readings above the cut-off value considered positive. Absorbance was read at 405 nm on an automated EL 800G ELISA microplate reader (Bio-Tek Instruments, Winooski, VT, USA).

Factors associated with seropositivity

The head of each household was asked to answer a comprehensive questionnaire to provide information about independent variables that could be associated with seroreactivity to *L. infantum* (dependent variables), to describe the general and individual characteristics of the canine population and their environment, and to determine the factors associated with seropositivity for CanL.

The following variables were considered: breed (mongrel or purebred), sex (male or female), age (up to 12, 13 to 84, or > 84 months), dog pelage (light or dark), size (small, medium or large), presence of green area/trees (yes/no), urban area (yes/no), rural area (yes/no), street access (yes/no), contact with other animals (yes/no), veterinary care (yes/no), contact with forest/Caatinga (yes/no), interaction with wildlife (yes/no), presence of a chicken coop (yes/no), and human cases of leishmaniasis (in the household and/or neighborhood).

To conduct the analysis of factors associated with seroprevalence, univariable analysis was initially performed, in which each independent variable underwent an association analysis in relation to the dependent variable (positivity in serological tests). Variables with P -value ≤ 0.2 in the Chi-square test or Fisher's exact test were selected for multivariable analysis using robust Poisson regression. Collinearity between independent variables was verified by a correlation analysis; for those variables with a strong collinearity (correlation coefficient > 0.9), one of the two variables was excluded from the multiple analysis according to the biological plausibility (Dohoo et al., 2003). To assess how well the model fits the Person Chi-square was used, and the significance of the model was verified with Omnibus test. The significance level adopted in the multiple analysis was 5%, and the software used was SPSS for Windows version 20.0.

Spatial analysis

The geographical coordinates of sampled households were determined by GPS (global positioning satellite). The data were combined using QGIS[®] v. 2.18 software to create spatial maps.

Kernel density mapping was employed to visually identify hotspots in the municipalities, indicating the occurrence of an event concentration and the agglomeration of positive cases in a spatial distribution (Pfeiffer et al., 2008).

A spatial cluster analysis was done to detect areas with significant incidence levels, using the local cluster evaluation method developed by Kulldorff & Nagarwalla (1995) and SaTScan 9.5 version software.

Given that the average flight radius of the vector of VL is 250 m (Oliveira et al., 2013), buffer zones of 250 m were simulated around seropositive dogs to identify areas of greater risk for the presence of infected vectors, and therefore for the disease in dogs and humans.

Results

The serological tests to detect the presence of antibodies to *L. infantum* revealed positivity levels of 58.8% (272/462) by DPP® (25.1% in the urban area – UA and 33.7% in the rural area – RA) and 43.9% (203/462) by ELISA (21.2% in the UA and 22.7% in the RA). Prevalence data were calculated based on the number of dogs seropositive by both DPP® and ELISA, showing an overall seroprevalence of 42.8% (198/462, IC: 95% = 38.6%-47.6%). Seroprevalence was significantly higher ($P = 0.003$) in UA than in RA, i.e., 51% (98/192 IC: 95% = 44.19%-58.3%) as opposed to 37% (100/270 IC: 95% = 31.8%-43.4%). Seropositivity levels among dogs in the municipalities were as follows: Cabrobó 55.8% (43/77), Lagoa Grande 46.7% (36/77), Orocó 45.4% (35/77), Santa Maria da Boa Vista 46.7% (36/77), Dormentes 32.4% (25/77), and Afrânio 29.8% (23/77) (Table 2).

Table 2. Prevalence of anti-*Leishmania* spp. antibodies in dogs in urban and rural areas from six municipalities, State of Pernambuco, Brazil. Prevalence % (seropositive dogs/tested dogs).

Municipalities	Serological tests								
	DPP® UA	DPP® RA	DPP® Total	ELISA UA	ELISA RA	ELISA Total	DPP® + ELISA UA	DPP® + ELISA RA	DPP® + ELISA Total
Afrânio	23.1 (6/26)	51 (26/51)	41.5 (32/77)	19.2 (5/26)	43.1 (22/51)	35.1 (27/77)	19.2 (5/26)	35.3 (18/51)	29.8 (23/77)
Cabrobó	89.8 (44/49)	53.6 (15/28)	76.6 (59/77)	71.4 (35/49)	28.6 (8/28)	56 (43/77)	71.4 (35/49)	28.6 (8/28)	55.8 (43/77)
Dormentes	37 (10/27)	62 (31/50)	53.2 (41/77)	37 (10/27)	32 (16/50)	33.8 (26/77)	37 (10/27)	30 (15/50)	32.4 (25/77)
L. Grande	48.7 (17/35)	69 (29/42)	59.7 (46/77)	48.7 (17/35)	45.2 (19/42)	46.8 (36/77)	48.6 (17/35)	45.2 (19/42)	46.7 (36/77)
Orocó	70.4 (19/27)	58 (29/50)	62.3 (48/77)	66.6 (18/27)	34 (17/50)	45.5 (35/77)	67 (18/27)	34 (17/50)	45.4 (35/77)
S. M. B. V	69 (20/29)	54.2 (26/48)	59.7 (46/77)	44.8 (13/29)	48 (23/48)	46.8 (36/77)	45 (13/29)	48 (23/48)	46.7 (36/77)

DPP® = Dual Path Platform, UA = Urban Area, RA = Rural Area, L. = Lagoa, S.M.B.V = Santa Maria da Boa Vista.

With regard to possible associations between independent variables and seropositivity among dogs (Table 3), the variables municipalities, age (months), breed, presence of green areas/trees, area, and dog pelage were selected for multivariate analysis ($P \leq 0.20$).

Clinical signs compatible with CanL were detected in 64.9% (300/462) of the sampled dogs. Approximately 67% (132/198) of the seropositive animals presented clinical signs. Table 4 describes the most frequent clinical signs shown by seropositive dogs. These signs, which included lymphadenomegaly in prescapular lymph nodes ($P = 0.097$), popliteal lymph nodes ($P = 0.181$), submandibular lymph nodes ($P = 0.130$), ocular lesions ($P = 0.192$), conjunctivitis ($P = 0.102$), lesions around the ears ($P = 0.143$), face ($P = 0.102$) and limbs ($P = 0.102$), selected for multivariate analysis ($P \leq 0.20$).

The multiple analysis of independent variables described in Table 5 enabled the identification of factors associated with seropositivity for CanL. These factors were significantly associated with the prevalence of *L. infantum* antibodies ($P \leq 0.05$) among 13 to 84-month-old dogs ($P = 0.010$), light pelage ($P = 0.007$), living in the municipality of Cabrobó ($P = 0.006$) and Lagoa Grande ($P = 0.017$) or in rural area ($P = 0.039$). The model presented good fit (Pearson Chi-square: value = 266.10; degrees of freedom - df = 499; value/df = 0.593) and statistical significance (Omnibus test: likelihood ratio Chi-square = 22.40; df = 12; $P = 0.033$).

The location of households with positive and negative dogs indicated that they were widely distributed in the six municipalities of this study (Figure 2).

The kernel map shows the spatial density of seropositive cases, revealing a higher concentration of seropositive dogs in the urban areas of the municipalities (Figure 3).

Overall, the local spatial analysis showed only one significant cluster of high incidence of seropositivity with a p-value of 0.01 ($P \leq 0.05$) (Table 6) located in the municipality of Cabrobó (Figure 4).

Based on the buffer zones, significant agglomerations of positive dogs were identified in each municipality, mostly in urban areas (Figure 5).

Table 3. Univariate analysis with the distribution of the variables associated with *Leishmania* spp. in dogs from Afrânio, Cabrobó, Dormentes, Lagoa Grande, Orocó and Santa Maria da Boa Vista municipalities, State of Pernambuco, Brazil.

Variable	Category	Total number of dogs	Positives (%)	P-value
Municipalities	Afrânio	77	23 (29.8)	0.010*
	Cabrobó	77	43 (55.8)	
	Dormentes	77	25 (34.4)	
	L. Grande	77	36 (46.7)	
	Orocó	77	35 (45.4)	
	S. M. B. V	77	36 (46.7)	
Age (months)	up to 12	123	41 (33.3)	0.032*
	13 to 84	297	140 (47.1)	
	> 84	42	17 (40.4)	
Breed	mongreal	425	177 (41.6)	0.108*
	purebreed	37	21 (56.7)	
Presence of green area/trees	no	105	51 (48.5)	0.178*
	yes	357	147 (41.1)	
Area	rural	270	100 (37)	0.004*
	urban	192	98 (51)	
Dog pelage	light	284	135 (47.5)	0.014*
	dark	178	63 (35.4)	

*Variables selected for multivariate analysis ($P \leq 0.2$). L = Lagoa, S.M.B.V = Santa Maria da Boa Vista.

Table 4. The recorded clinical signs in the total sample and in the dogs that were seropositivity for anti-*Leishmania infantum* antibodies.

Clinical signs	Total number of dogs presenting the sign	Total number of seropositive dogs presenting the sign	Seroprevalence (%)	P-value
Lymph node Prescapular				
No	311	125	40.1	0.097*
Yes	151	73	48.3	
Lymph node Popliteal				
No	273	110	40.2	0.181*
Yes	189	88	46.5	
Lymph node submandibular				
No	385	159	41.2	0.130*
Yes	77	39	50.6	
Ocular Lesions				
No	458	195	42.5	0.192*
Yes	4	3	75	
Conjunctivitis				
No	460	196	42.6	0.102*
Yes	2	2	100	
Lesion on the ears				
No	421	176	41.8	0.143*
Yes	41	22	53.6	
Face Lesion				
No	460	196	42.6	0.102*
Yes	2	2	100	
Limb Lesion				
No	457	194	42.4	0.102*
Yes	5	4	80	

* Variables selected for multivariate analysis ($P \leq 0.2$).

Table 5. Multivariate analysis (robust Poisson regression) with the factors associated with *Leishmania* spp. seropositivity in dogs from Afrânio, Cabrobó, Dormentes, Lagoa Grande, Orocó and Santa Maria da Boa Vista municipalities, State of Pernambuco, Brazil.

Variable category	Coefficient estimates	Standard error	Wald Chi-square	Prevalence ratio	95% confidence interval	P-value
Rural area	0.236	0.115	4.239	1.27	1.01; 1.59	0.039
Light pelage	0.332	0.123	7.282	1.39	1.10; 1.78	0.007
Age of 13 to 84 months	0.366	0.143	6.581	1.44	1.09; 1.91	0.010
Municipality of Cabrobó	0.555	0.201	7.616	1.74	1.18; 2.58	0.006
Municipality of Lagoa Grande	0.520	0.218	5.701	1.68	1.10; 2.58	0.017

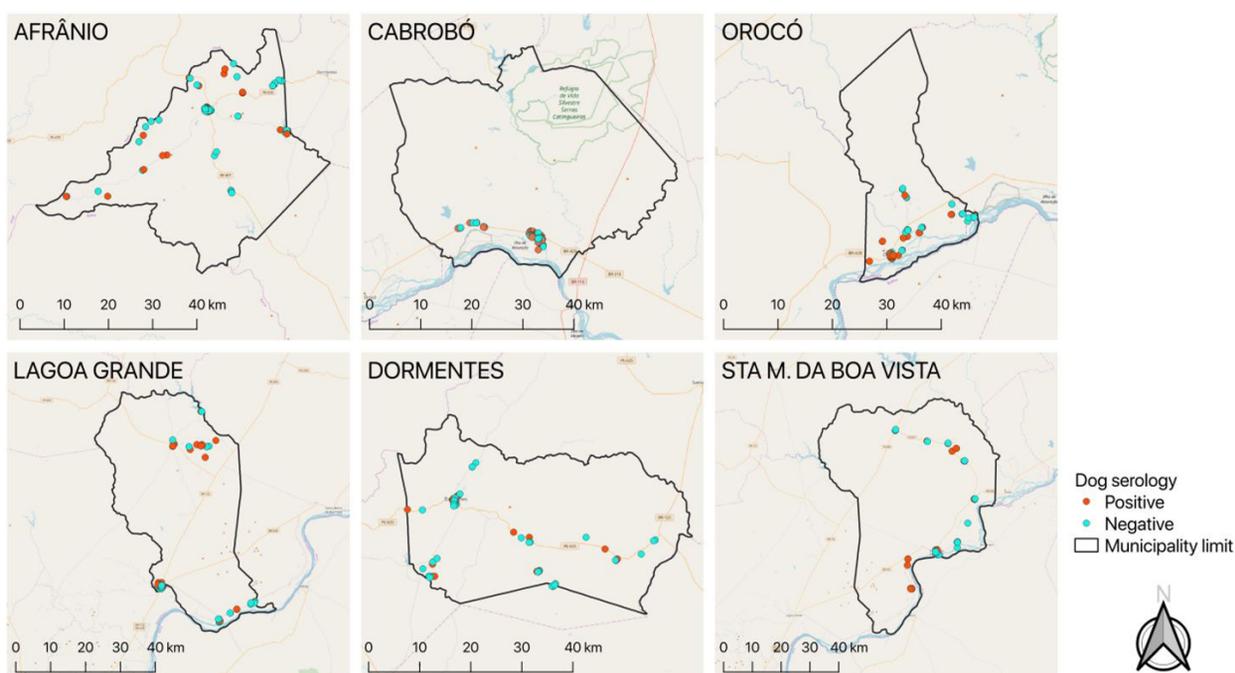


Figure 2. Distribution of dogs that were seropositive for the presence of anti-*Leishmania* spp. antibodies in the municipalities of Afrânio, Cabrobó, Dormentes, Lagoa Grande, Orocó and Santa Maria da Boa Vista, state of Pernambuco, northeastern Brazil.

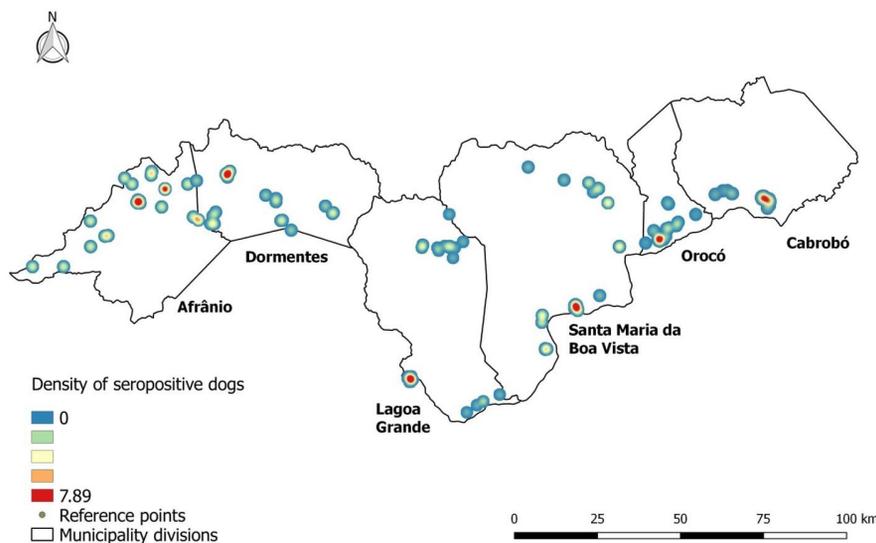


Figure 3. Kernel density mapping for canine leishmania infection in the municipalities of Afrânio, Dormentes, Lagoa Grande, Orocó and Santa Maria da Boa Vista, state of Pernambuco, northeastern Brazil.

Table 6. Local cluster analysis results using spatial scan statistics.

	Cabrobó		Orocó		L. Grande		S.M.B.V	
	DC	OCA	DC	OCA	DC	OCA	DC	OCA
Population	43	34	27	50	5	72	4	73
Positives	32	11	21	14	5	31	4	32
Prevalence (%)	74.4	32.3	77.8	28	46.7	43	46.7	43.8
RR*	1.88		1.91		2.37		2.36	
p-value	0.010*		0.105		0.934		0.999	
Radius (Km)	1.62		2.27		0.35		0.29	

*($P \leq 0.05$);cRR: Relative Risk, DC= Detected Cluster, OCA= Out of Cluster Area, L. = Lagoa, S.M.B.V= Santa Maria da Boa Vista.



Figure 4. Location of the cluster and intensity cases of positive animals in the municipality of Cabrobó, state of Pernambuco, northeastern Brazil.

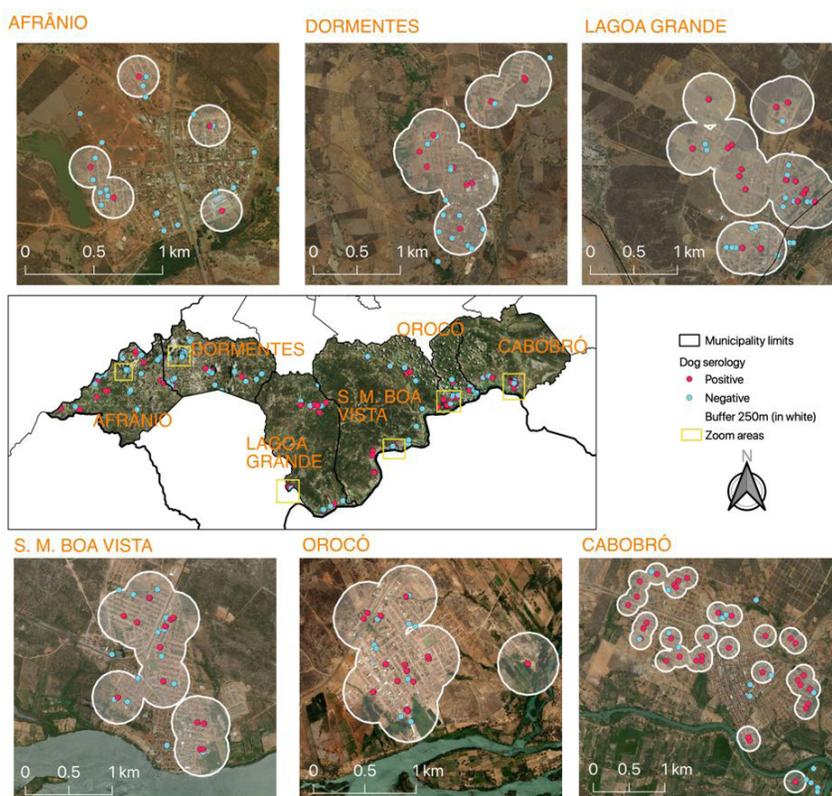


Figure 5. Buffer with a 250 m radius generated around seropositives dogs for *Leishmania* spp. in the municipalities of Afrânio, Cabrobó, Dormentes, Lagoa Grande, Orocó and Santa Maria da Boa Vista, state of Pernambuco, northeastern Brazil. Clusters of cases were observed in urban area of main districts of each municipality.

Discussion

The study area, which presented high VL incidence and prevalence levels, is considered an endemic area of intense transmission due to a combination of environmental and socioeconomic factors (Cesse et al., 2001; Maia et al., 2014).

Reported CanL prevalence levels vary depending on which serological test is used, on the canine population, and on the region under study (Julião et al., 2007; Silva et al., 2016; Belo et al., 2017; Silva et al., 2017). This has been demonstrated in various studies carried out in Brazil's northeast, south and central west regions (Santis et al., 2013; Leça et al., 2015; Belo et al., 2017).

Domestic dogs are the main urban reservoirs of *L. infantum* in zoonotic VL transmission (Belo et al., 2013). Therefore, knowledge about the epidemiological profile of CanL in important endemic regions such as northeastern Brazil and about risk factors associated with the infection in dogs is crucial, contributing toward control of the disease and the development of strategies for serological surveys in the region, focusing specifically on this silent disease (Dantas-Torres, 2006; Dantas-Torres et al., 2006; Marcondes et al., 2011; Laurenti et al., 2013).

This study is the first CanL serosurvey conducted in various municipalities of the São Francisco mesoregion (Afrânio, Cabrobó, Dormentes, Lagoa Grande, Orocó and Santa Maria da Boa Vista) located in the semi-arid region of northeastern Brazil. The mean prevalence of anti-*L. infantum* IgG antibodies was 42.8%, ranging from 29.8% to 55.8%. These values are higher than those reported in other endemic areas in northeast Brazil (Lira et al., 2006; Queiroz et al., 2010; Pimentel et al., 2015; Mendonça et al., 2017; Silva et al., 2017) and in Petrolina, in the state of Pernambuco, where a prevalence of 11.2% was reported (Araujo et al., 2016b). In Petrolina, situated in the same mesoregion as this study, the reported number of seropositive dogs was low compared to that of our study, possibly because the indirect fluorescent antibody test (IFAT) was used, which is more specific (Laurenti et al., 2014), and more sensitive (Adams et al., 2012; Silva et al., 2013b) than the DPP method.

Although ELISA is widely used in this country, Brazil's Ministry of Health recommends the use of a protocol that combines two sensitive tests (DPP® and ELISA) (Travi et al., 2018). This may negatively influence canine serological diagnosis, resulting in numerous false positives and thus overestimating prevalence levels (Laurenti et al., 2014; Arruda et al., 2016). However, the Ministry of Health continues to favor the use of these tests, which are extensively used because they are fast and easy to perform, enabling the analysis of numerous samples in a short time (Grimaldi et al., 2012; Silva et al., 2016). The detection of clinical signs in infected dogs is important for the early diagnosis of suspected cases of the CanL, and the presence of these signs pertains mainly to the immune-mediated nature of this disease, causing the parasite to multiply in the host's tissues (Dantas-Torres, 2007; Queiroz et al., 2010; Michelin et al., 2018). The researchers Almeida et al. (2005), Baneth et al. (2008) and Silva et al. (2017) reported that, in their studies, most of the dogs seropositivity for CanL were clinically affected. This finding is similar to that of our study, in which 66.6% (132/198) of the dogs were clinically affected, with lymphadenomegaly and skin lesions being the most prevalent clinical signs. Conjunctivitis and ocular lesions, which were clinical signs commonly exhibited by infected dogs in our study, were also reported by Cordeiro et al. (2016).

The univariate analysis revealed an association between mongrel dogs and seropositivity for CanL. Among the factors that make these animals more vulnerable to infection are unhindered access to the street and to contact with the sand fly vector, as well as immunosuppression due to a high degree of malnutrition (Cortes et al., 2012; Silva et al., 2013a, 2016).

The multivariate analysis indicated that 13 to 84-month-old dogs were more predisposed to become infected with CanL. This finding corroborates studies by Paltrinieri et al. (2010) and Rondon et al. (2008), who associated the higher susceptibility of this age group to their immature immune system, making them more susceptible to infection. In addition, Alvar et al. (2004) state that 8-year-old animals may be serologically positive due to prolonged immune response.

The analysis of risk factors in this study showed a direct association between seropositivity levels and living in rural areas, coinciding with the findings of Silva-Abreu et al. (2008), Silva et al. (2013a) and Leça et al. (2015). Although the change in the spatial transmission pattern of the disease began in the 1990s (Harhay et al., 2011), in rural area the presence adjacent to residual forests, accumulation of organic matter and the diversity animals, favors the presence of phlebotomine sand flies and reservoirs of *Leishmania* spp. in the environment (Camargo-Neves et al., 2001).

Living in Cabrobó and Lagoa Grande was also a risk factor showed. This suggests the need for the adoption of more effective leishmaniasis control and prevention measures to reduce the impact of environmental and

peridomestic factors, such as intense population pressure on the environment and the ruralization of some urban areas, lack of garbage collection, poor sanitation, presence of the vector, and the canine population (Dantas-Torres, 2007; Santos et al., 2010; Michel et al., 2011; Belo et al., 2013).

Spatial analysis tools used in research into the prevalence and incidence of VL are essential to garner epidemiological data from a specific area, in order to identify sites where cases of the disease are concentrated and thereby contribute toward the design of effective measures of control and prevention (Borges et al., 2014; Teixeira-Neto et al., 2014; Oliveira et al., 2015).

The high concentration of cases in the urban areas of the six municipalities of this study, revealed by kernel density maps, can be attributed to the higher concentration of reservoir hosts, such as dogs. Kernel mapping enables the implementation of epidemiological surveillance, thus contributing to control and prevent the disease in these areas (Dujardin, 2006; Araujo et al., 2016b).

An analysis of the clusters of CanL cases revealed a significant cluster in the municipality of Cabrobó, where dogs are at 1.88-fold higher risk of infection than in other locations. This finding indicates that the probability of the emergence of new cases of seropositive dogs is greater in this area (Carvalho & Souza-Santos, 2005; Dantas-Torres, 2006). Given the positive correlation between canine and human cases of VL, it is reasonable to postulate that this high risk of canine infection in Cabrobó may also represent a risk for transmission of the disease to humans (Oliveira et al., 2001; Souza et al., 2012).

The 250m buffer zones created around CanL cases enable one to identify significant clusters of positive dogs and to pinpoint areas where the infecting vector may be present more frequently. The UAs were almost entirely covered by buffer zones in all the municipalities except Afrânio. This analysis shows where public health actions are needed most urgently to prevent the emergence of new cases. It also indicates the need for entomological research to prevent the perpetuation of the vector population, given the favorable conditions of these areas for its maintenance (Abrantes et al., 2018).

In conclusion, our findings suggest a high prevalence of *Leishmania* spp. in the six municipalities of the semi-arid mesoregion of Pernambuco. The most common clinical signs found in seropositive dogs were lymphadenomegaly, skin lesions and conjunctivitis, and the risk factors most strongly correlated with the presence of positive dogs were the 13 to 84-month-old age group and living in rural areas and/or in the municipality of Cabrobó.

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