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Original Article

Factors associated with *Leishmania* infection in dogs and geospatial analysis in the Sertão of Paraíba, Northeast Brazil

Fatores associados à infecção por *Leishmania* em cães e análise espacial no Sertão da Paraíba, Nordeste do Brasil

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Abstract

Visceral leishmaniasis (VL) is a zoonosis with a worldwide distribution that has a major impact on public health. The aim of this study was to verify the prevalence of canine infection by *Leishmania infantum*, the factors associated with the infection and its spatial distribution in the municipality of Mãe D'Água, in the Sertão region of Paraíba State, Northeast Brazil. Blood samples were collected from 150 dogs for diagnosis by the DPP®, ELISA-S7®, ELISA-EIE® and qPCR assays. The prevalence was calculated considering the positivity in at least two tests. SaTScan® was used for spatial analysis. The prevalence of canine infection with *Leishmania* was 18.6% (28/150), with the rural area being identified as a risk factor (Odds Ratio (OR) = 2.93). The permanence of the dog loose during the night (OR = 0.33) and deworming (OR = 0.30) were identified as protective factors. A risk cluster was formed in the northern region of the urban area. Mãe D'Água showed a pattern of active transmission in the rural area, but VL control measures also need to be carried out in the urban area to prevent human cases and the spread of the disease in the risk zone.

Keywords: *Cluster*, georeferencing, leishmaniasis, real-time PCR, serology.

Resumo

A leishmaniose visceral (LV) é uma zoonose com distribuição mundial de grande impacto na saúde pública. O objetivo deste estudo foi verificar a prevalência da infecção canina por *Leishmania infantum*, os fatores associados à infecção e sua distribuição espacial no município de Mãe D'Água, na região do Sertão da Paraíba, Nordeste do Brasil. Amostras de sangue foram coletadas de 150 cães para diagnóstico pelos ensaios DPP®, ELISA-S7®, ELISA-EIE® e qPCR. A prevalência foi calculada considerando-se a positividade em pelo menos dois testes. O SaTScan® foi utilizado para a análise espacial. A prevalência da infecção canina com *Leishmania* foi de 18,6% (28/150), sendo a zona rural identificada como fator de risco (Odds Ratio (OR) = 2,93). A permanência do cão solto durante a noite (OR = 0,33) e a vermifugação (OR = 0,30) foram classificadas como fatores de proteção. Um *cluster* de risco foi formado na região Norte da área urbana. Mãe D'Água apresentou um padrão de transmissão ativa na área rural, porém medidas de controle da LVC também precisam ser realizadas na área urbana para evitar casos humanos e a dispersão da doença na zona de risco.

Palavras-chave: *Cluster*, georreferenciamento, leishmaniose, PCR em Tempo Real, sorologia.

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Introduction

Visceral leishmaniasis (VL) is distributed worldwide and has a great impact on public health. The World Health Organization (2018) estimated that the global VL case count in 2018 increased from 50,000 to 90,000, with most of cases in Brazil, Eastern Africa, and Southeast Asia.

In Brazil, the disease is known as “kala-azar” and, currently, occurs in the five regions of the country, encompassing 19 states, in which 10,063 cases were reported from 2015 to 2017. According to Sousa et al. (2018), most of the cases were reported in the Northeast region, especially in the states of Maranhão and Ceará.

In Brazil, the transmission occurs mainly through the blood repast of infected females of the *Lutzomyia longipalpis* phlebotomine (Lainson & Rangel, 2005; Missawa & Lima, 2006) and, rarely by means of either blood transfusion (Cohen et al., 1991) or via the trans-placental route. In the wild, coyotes and foxes (*Dusicyon vetulus* and *Cerdocyon thous)* act as reservoirs, and in the domestic environment, dogs preserve the transmission cycle (World Health Organization, 2014), thereby acting as important sources of infection for the disease vectors (Ashford, 1996); humans are accidental hosts of the parasite.

The Brasil (2011) proposes the use of the DPP® rapid test and ELISA EIE-LVC® as screening and confirmatory tests, respectively, for the diagnosis *Leishmania* infection in dogs. When the animal is positive, treatment is only possible after obtaining the responsible guardian’s permission and with drugs that are not used in humans, such as Milteforan, a miltefosine-based medicine, registered with the Ministry of Agriculture, Livestock and Supply (MAPA). However, the use of Milteforan is an individual protection measure for the dog and is not used as a public health measure to control the disease. (Brasil, 2016). In Brazil, based on an analysis of the local epidemiological situation, the Visceral Leishmaniasis Control Program (PCLV) suggests that preventive and control measures should be adopted to tackle this zoonosis. These measures consist of entomological surveillance, treatment of human cases, confirmation, and the culling of positive dogs (Brasil, 2006); however, it is questioned whether the latter measure can spread of the infection (Werneck, 2014).

The prevalence of *Leishmania* infection in dogs is itself a risk indicator for its occurrence in humans, and its study indicates to the local surveillance agencies the control actions to be implemented. Although the Ministry of Health indicates the serological survey as a surveillance measure, over the last decades the action has not been carried out by health surveillance services in the states and municipalities. Numerous studies have been conducted by the scientific community in the states of the Northeast region, such as Rio Grande do Norte (Amóra et al., 2006), Maranhão (Barbosa et al., 2010), Piauí (Figueiredo et al., 2017), Ceará (Rodrigues et al., 2017), and Paraíba (Brito et al., 2016; Fernandes et al., 2018; Silva et al., 2018); these studies have emphasized the prevalence of infection with *Leishmania*.

Three cases of human visceral leishmaniasis (HVL) were reported between 2014 and 2018 in the municipality of Mãe D’Água in the state of Paraíba (Sinan, 2019); a new case was reported in December 2019, but it has still not been logged in the national disease notification system (SINAN). As a result, a serological survey was conducted in the municipality on 55 stray dogs by the endemic disease control agents and tested at the Central Laboratory of Public Health of Paraíba (LACEN), as recommended by the Ministry of Health, and the occurrence of positive animals was 10.9% (6/55).

Mãe D’Água is situated in the Sertão mesoregion of Paraíba state, in the metropolitan region of the municipality of Patos. The preserved xerophytic caatinga vegetation is set in a relief denominated “Sertanejo Plain,” which constitutes an extensive pediplain surface. This surface is formed by a set of elongated and aligned mountains over the regional geological structure, which extends through the municipalities of Manaíra to Teixeira. Located in this region is the Jabre Peak, with an altitude of 1,197m, which constitutes the highest point of the state (Beltrão et al., 2005). According to Silva et al. (2017a), the Caatinga is a well-recognized ecological region that lies in the semiarid hinterland of northeastern Brazil and that covers 912,529 km^2 and refers mostly to a seasonally dry tropical forest (SDTF) that presents a mosaic of different physiognomies. This type of biome favors the occurrence of vector (*Lutzomyia longipalpis*) throughout the year as well as maintenance of the disease cycle.

In view of this, the aim of this study was to analyze the prevalence of canine infection by *Leishmania* in the municipality of Mãe D’Água, to study the epidemiology of the zoonosis by analyzing the associated factors, and to verify the spatial distribution of the disease using georeferencing. The results will allow us to identify priority areas and disease patterns, so that adequate prevention and control measures are implemented in the future.

Material and Methods

Study design and setting

The study was conducted in the urban and rural areas of the municipality of Mãe D'Água (7°15'10"S,37°25'58'' W), located in the semi-arid region of Paraíba State, in the Sertão mesoregion (Figure 1). The area has a hot and dry climate and high temperatures throughout the year, with annual variations between 23 °C and 30°C, with the average rainfall in 2018 of 746.9 mm (AESA, 2019) (Köppen climatic classification: BSh). The estimated population is 4,019 inhabitants spread over 243.75 km^2, 1,569 and 2,450 of whom live in the urban and rural areas, respectively (IBGE, 2020). The municipality does not have a territorial division of neighborhoods, and the central neighborhood is considered the urban area.

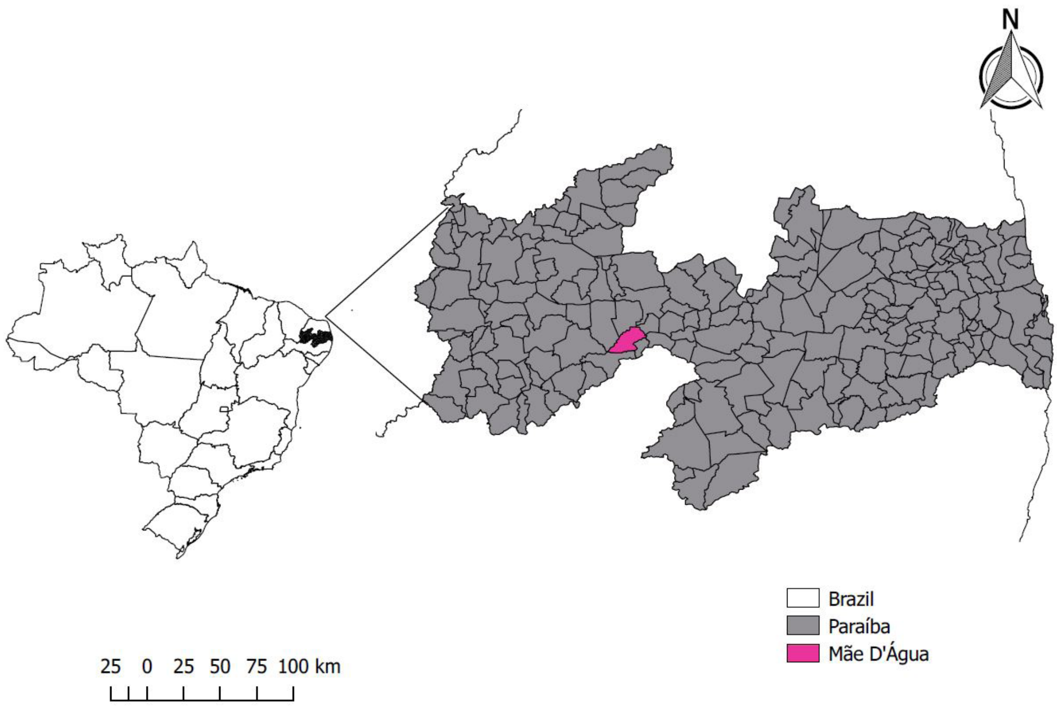

Figure 1. Map of the municipality of Mãe D'Água, Semi-arid region of Paraíba State, Brazil, 2018.

Ethical considerations

The work was approved by the Ethics Committee of the Health and Rural Technology Center (CSTR) of the Federal University of Campina Grande (UFCG), under protocol n. 156 059/2018.

Sample size

Based on methodology of Savani et al. (2003) and take into consideration that there is one dog for every seven inhabitants, the estimated canine population of the municipality was 574 animals. Using the simple random sampling formula, with an expected prevalence of 50%, a 99% significance level, and a sampling error of 5% (Thrusfield, 2007), the minimum estimated sample size was 146 animals.

Sample and data collection

The samples were collected from May to June 2018, in the urban and rural locations, and in points chosen using the municipality sketch. Home visits were carried out with the support of healthcare and endemic disease agents of the Municipal Healthcare Department. Blood samples were collected from 150 male and female domestic dogs (75 each from the urban and rural areas) over 6 months old. The samples were collected by jugular, cephalic, or saphenous venipuncture, using 5-mL sterile syringes and 25 ×8mm needles, added to tubes containing 4% sodium citrate, refrigerated in a thermal box, and forwarded to the Semi-arid Molecular Biology Laboratory (BiolMol/UFCG), Patos Campus, Paraíba. Plasma and whole blood samples were used for serological and molecular diagnosis, respectively. The samples were aliquoted in 1.5 ml microtubes, identified and stored at -20 ° C until the exams were performed.

The guardians answered an epidemiological survey containing 31 variables related to the guardian, the animal, and the environment where the animal lives, aiming to identify epidemiological indicators associated with the occurrence of the disease.

Serological tests

The prevalence of canine infection by *Leishmania* was estimated by the immunochromatographic Dual Path Platform DPP® (Rapid Test Canine Visceral Leishmaniasis, Bio-Manguinhos, Rio de Janeiro, Brasil), and two enzyme-linked immunosorbent assays (ELISA-EIE®, Canine Visceral Leishmaniasis, Bio-Manguinhos, Rio de Janeiro, Brazil; ELISA-S7® kit, Biogene Industry and Commerce Ltda, Recife/PE, Brazil) and the analyses were carried out by the team of the Municipal Laboratory of Patos/PB and of the Semi-arid Molecular Biology Laboratory (UFCG), respectively. The protocol followed the manufacturers' recommendations. The samples that reacted in the DPP® were sent to the Public Health Central Laboratory of Paraíba (LACEN), which is responsible for conducting the ELISA- EIE® test. As proposed by the Ministry of Health, only the positive samples in the DPP® were tested in the ELISA kit EIE-CVL® (Canine Visceral Leishmaniasis).

Real-time quantitative polymerase chain reaction (qPCR)

The DNA of the blood samples were extracted using U-Trinzol®, following the manufacturer's recommendations and qPCR was performed as previously described (Fernandes et al., 2018). *Leishmania infantum* culture was used as a positive control and ultra-pure water as a negative control. The primers used for the amplification of minicircle DNA sequences from the kinetoplast (kDNA) of *L. infantum* were Leish RV1 (5'-CTT TTCTGGTCCCGCGGGTAGG-3') and Leish RV2 (5'-CCACCCGGCCTATTTTACACCA-3'). Thermocycling was conducted using a Bioer Technology® thermocycler. The prevalence was determined taking into consideration the positivity in at least two serological techniques or only in PCR.

Statistical analysis

The analysis of the risk factors associated with infection was calculated in two stages using univariate and multiple analysis, and the independent variables were categorized and codified. The categories income, level of schooling, cleaning frequency, and types of vaccine were aggregated to facilitate the statistical analysis due to the small sample size. Either chi-squared or Fisher's exact tests (Zar, 1999) were used in the univariate analysis; the variables with $p \leq 0.20$ multiple analysis were carried out by multiple logistic regression (Bruce & Bruce, 2017; Fávero et al., 2009; James et al., 2014; Hosmer & Lemeshow, 2000). The significance level adopted in the multiple analysis was 5%. The analysis was carried out with IBM SPSS® Statistics for Windows, version 22.0 (IBM Corp., Armonk, USA).

Georeferencing

The geographical coordinates of each animal's residence were obtained using the Garmin eTrex 30 receiver global positioning system (GPS) during the application of the questionnaire with the guardians of the dogs. Using the obtained geographic coordinates, the cases were inserted into the cartographic base of the municipality (Figure 3), using a geographic information system (GIS) and Google Earth 7.3.0; the maps were created in quantum GIS (QGIS). A heatmap or quadratic Kernel estimation was constructed using QGIS 2.18.0 software to observe the disease clusters. SaTScan® software was used to verify the areas with cases exceeding the expected number, by means of scan statistics for the detection of clusters, using a Bernoulli model (Gatrell et al., 1996; Levine, 2017).

Results

As proposed by the Ministry of Health, only the positive samples in the DPP® (19.3%; 29/150) were tested in the ELISA kit EIE-CVL®, resulting in 12.6% (19/29) of *Leishmania* infection.

Of the 150 serum samples analyzed, 19.3% (29/150), 41.3% (62/150), and 3.3% (5/150) tested positive in the DPP® screening test, ELISA-S7®, and qPCR, respectively.

Considering as positive criteria animals that were reactive in at least two serological tests or only in qPCR, the prevalence of *Leishmania* infection in dogs in the municipality of Mãe D'Água was 18.6% (28/150) with 10.6% (8/75) and 26.6% (20/75) from urban and rural areas, respectively. In the univariate analysis, the variables with a p-value ≤ 0.2 (Table 1) were: origin, income, gender, breed, type of rearing, feeding, contact with wild animals, contact with felines and birds, rearing environment, cleanliness, frequency of cleaning, deworming, whether the dog was adopted, hunting activity, where the dog sleeps, and how does if spend the night (loose or fastened on a leash).

Table 1. Univariate analysis of the risk factors associated with canine visceral leishmaniasis in the Municipality of Mãe D'Água, Semi-arid region of Paraíba State, between May and July 2018. Significant variables in the univariate analysis (p ≤ 0.2).

Variable	Category	N. of animals	Positives	*Odds ratio*	Confidence interval	P-value
Origin	Urban	75	8(10.6%)			
	Rural	75	20(26.6%)	3.04	1.28 – 7.84	0.01*
Level of schooling of the owner	Illiterate	12	3(25%)			
	Any form of literacy	138	25(18.1%)	0.66	0.18 – 3.14	0.55
Income of the owner	Less than 2MS	91	14 (15.3%)			
	2 or >2 MS	59	14(23.7%)	1.71	0.74 – 3.94	0.20*
Gender of the animal	Male	105	25(23.8%)			
	Female	45	3(6.6%)	0.22	0.05 – 0.70	0.02*
Age of the animal	6 months - 2 years	17	4 (23.5%)			
	>2 Years	133	24(18%)	0.71	0.22 – 2.71	0.58
Breed	Mixed breed	132	27(20.4%)			
	Pure breed	18	1(5.5%)	0.22	0.01 – 1.19	0.16*
Type of rearing	Domiciliary	70	7(10%)			
	Semi-domiciliary	62	17(27.4%)	3.4	1.34 – 9.42	0.01*
	Free	18	4(22.2%)	2.57	1.60 – 9.79	0.17*
Type of feed	Commercial feed	13	1(7.7%)			
	Homemade food	125	26(20.8%)	3.15	0.57 – 58.71	0.28*
	Both	12	1(8.3%)			
Contact with other animals	Yes	125	26(20.8%)	3.02	0.81 – 19.57	0.15*
	No	25	2(8%)			
Contact with equines	Yes	12	3(25%)	1.50	0.31 – 5.47	0.55
	No	138	25(18.1%)			
Contact with wild animals	Yes	20	2(10%)	0.44	0.06 –1.67	0.29*
	No	130	26(20%)			
Contact with felines	Yes	38	10(26.3%)	1.86	0.75 – 4.4	0.16*
	No	112	18(16%)			
Contact with dogs	Yes	85	17(20%)	1.22	0.53 – 2.90	0.63
	No	65	11(17%)			
Contact with swines	Yes	17	2(11.7%)	0.54	0.08 – 2.11	0.44
	No	133	26(19.5%)			
Contact with small ruminants	Yes	15	3(20%)	1.1	0.23 – 3.78	0.88
	No	135	25(18.5%)			
Contact with bovines	Yes	15	4(26.6%)	1.68	0.43 – 5.40	0.40
	No	135	24(17.7%)			
Contact with birds	Yes	84	21(25%)	2.80	1.15 – 7.57	0.02*
	No	66	7(10.6%)			
In which environment is reared	Soil	93	21(22.5%)	0.20	0.17 – 0.46	0.12*
	Cement	57	7(12.2%)			
Execution of the cleansing of the environment	Yes	89	12(13.4%)	0.43	0.18 –1.00	0.05*
	No	61	16(26.2%)			
Variable	Category	N. of animals	Positives	*Odds ratio*	Confidence interval	P-value

Table 1. Continued...

Variable	Category	N. of animals	Positives	*Odds ratio*	Confidence interval	P-value
Cleaning of the environment frequency	Does not perform	61	16(26.2%)			
	Daily	63	8(12.6%)	0.40	0.15 –1.01	0.06*
	Weekly/Fortnightly/monthly	26	4(15.3%)	0.51	0.13 –1.59	0.27*
Vaccinated animal (Anti-rabies, viruses, CVL)	Yes	126	22(17.4%)			
	No	24	6(25%)	0.33	0.12 –0.79	0.38
Vermifugated animal	Yes	70	7(10%)	0.31	0.11 –0.75	0.01*
	No	80	21(26.2%)			
Presence of ticks	Yes	115	21(18.2%)	0.89	0.35– 2.46	0.81
	No	35	7(20%)			
Has Always lived with the same owner	Yes	98	18(18.3%)	0.94	0.40 –2.29	0.89
	No	52	10(2%)			
Adopted	No	86	14(16.2%)			
	From the streets	15	2(13.3%)	0.79	0.11 – 3.30	0.77
	Other owner	49	12(24.4%)	1.66	1.69 – 3.97	0.24*
Has Always lived in the same municipality	Yes	125	25(20%)	1.83	0.57 – 8.17	0.35
	No	25	3 (12%)			
The animal hunts	Yes	18	6(33.3%)	2.5	0.79 – 7.19	0.09*
	No	132	22(16.6%)			
Where does it sleep	Indoors	42	5(11.9%)			
	Peridomicile	98	21(21.4%)	2.01	0.75 – 6.41	0.19*
	Street	10	2(20%)	1.85	0.23 – 10.46	0.50
How spends the night	Free/without collar	90	11(12.2%)	0.39	0.21 –0.68	0.01*
	Tied	60	17(28.3%)			
Travels	Yes	12	2(16.6%)	0.86	0.12 – 3.52	0.86
	No	138	26(18.8%)			
Repellent collar	Yes	1	-	7.51	0,00 – 0,00	0.99
	No	149	28(18.8%)			

The categories that remained at the end of the multiple logistic regression model were the following: animals that stay loose at night without restraint by leashes (OD = 0.33) and deworming (the animal has been dewormed at least once) (OD = 0.30). These categories were identified as protection factors for the occurrence of CVL and rural origin was identified as a risk factor (OD = 2.93) (Table 2). Dogs from the rural area were 2.93 times more likely to contract the disease. Dogs that were loose (not fastened on a leash at night) and dewormed were 67% and 70% more protected, respectively, than the others.

Table 2. Multiple analysis of the risk and protection factors associated to canine visceral leishmaniasis in the municipality of Mãe D'Água, Semi-arid region of Paraíba State, between May and July 2018. Significant variables in the multiple analysis ($p \leq 0.05$).

	Variable	*Odds ratio* CI (95%)	P-value
Risk factor	Origin (rural)	2.93 (1.18 – 7.92)	0.02
Protection factor	Vermifugated animal (yes)	0.30 (0.10 – 0.77)	0.01
	How the animal spends the night (Free)	0.33 (0.13 – 0.80)	0.01

The Kernel density estimator allowed us to determine the location for greater risk for the disease occurrence, and the areas with a higher concentration of cases are highlighted in red. These areas are situated in the North, Northwest, Northeast, central or urban area, and South of the municipality, with densities varying from 0.55 to 2.21 (Figure 2). The settlements situated in the Southeast region were not analyzed because access to these locations was not possible during the study period., while the endemic disease agents stated that there was an insignificant number of dogs in this area.

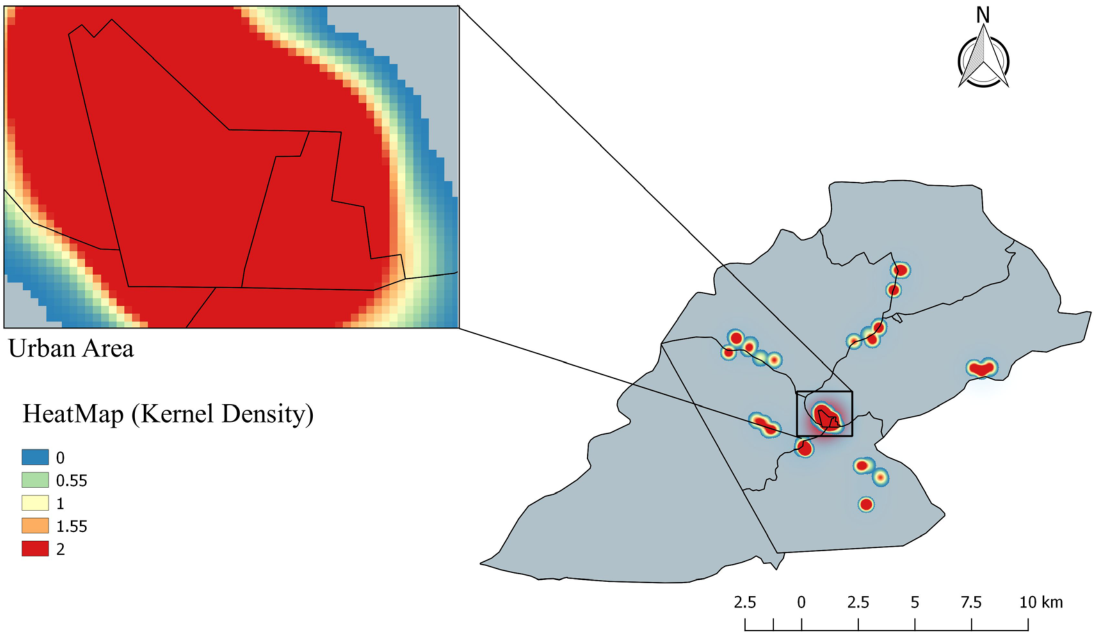

Figure 2. Kernel map showing areas with the greater distribution of canine visceral leishmaniasis cases in the municipality of Mãe D'Água, Semi-arid region of Paraíba State, 2018.

Using SaTScan, the clusters were calculated based on the relative risk. A primary cluster of significant risk (p = 0.010) was in the northern area of the urban zone (Figure 3), where dogs were 17.45 times more likely to become infected than those in other areas of the municipality. This cluster expanded to areas of vegetation without residencies in the limits of the municipality, except for a small portion in the Southeast extremity.

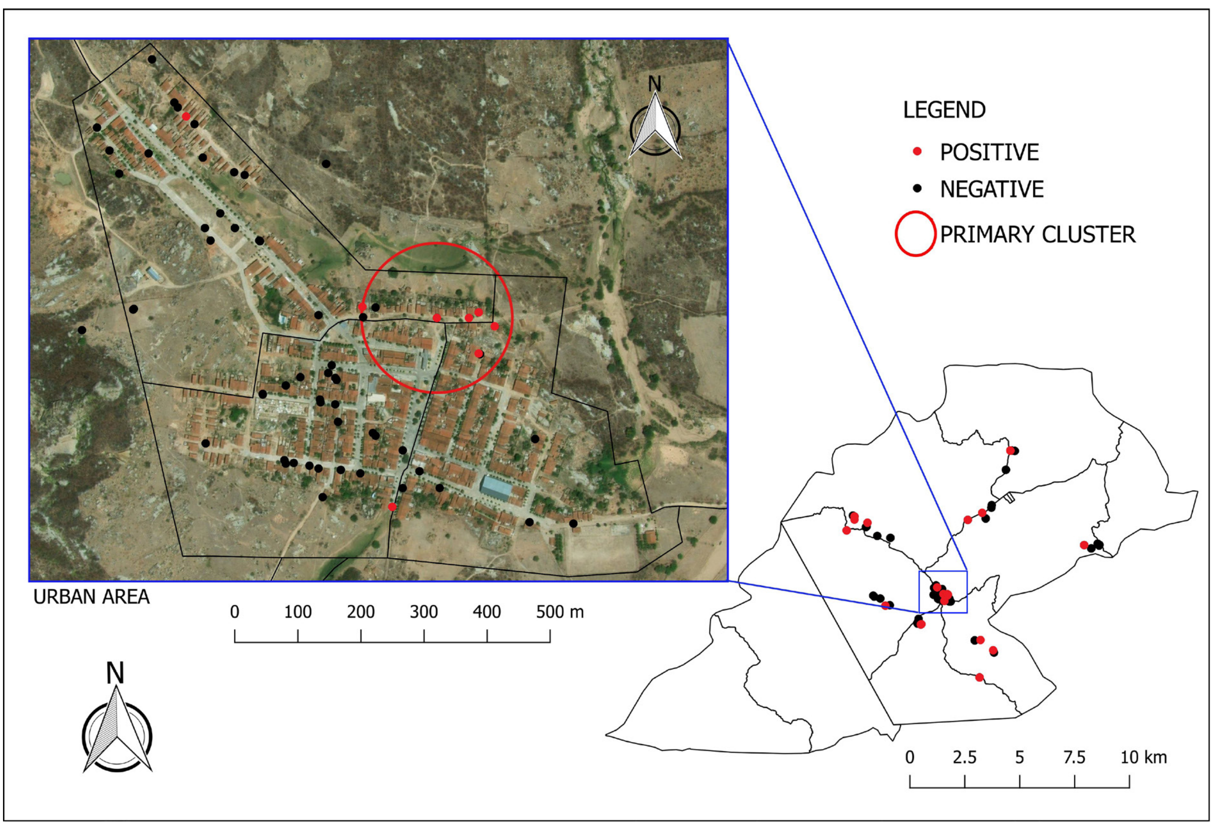

Figure 3. Satellite image showing the risk cluster for the occurrence of canine visceral leishmaniasis in the urban area of the municipality of Mãe D'Água, Paraíba, Brazil, 2018.Source: Google Earth.

Discussion

Although the methodologies differ, the prevalence of canine *Leishmania* detected in this study is similar to that of several international studies conducted, e.g., in Iran (15.4%) (Mahshid et al., 2014), Turkey (10.9%) (Bakirci et al., 2016), and Brazil in Niterói-Rio de Janeiro (21.66%) (Abrantes et al., 2018) as well as in other municipalities of the Sertão region of the Paraíba State, such as Cajazeiras (20%), Uiraúna (16.3%), Sousa (10.5%), and Patos (7.2%) (Silva et al., 2018). The highest or lowest prevalence may be attributed to the study region, the population characteristics, the statistical analysis used, and the sample size (Felipe et al., 2011; Wang et al., 2011; Brito et al., 2016; Silva et al., 2017b; Costa et al., 2018).

In the present study, the prevalence of canine *Leishmania* infection was higher in the rural area (26.6%), and this variable was considered a risk factor for the occurrence of the disease, reinforcing the old dynamics of endemicity of visceral leishmaniasis in rural areas (Amóra et al., 2006). Although the disease has expanded to urbanized areas since 1950 (Figueiredo et al., 2012; Guimarães et al., 2017), this factor does not apply to the municipality studied, as the area is small and there is a close contact between the urban and rural areas.

The predominant vegetation in the municipality of Mãe D'Água is the Caatinga, still quite dense and preserved, which is a favorable environment for the survival of the disease vector, *Lutzomyia longipalpis*. It is important to mention that buildings in rural areas and the proximity to woodlands facilitate the contact of dogs and humans with the disease vectors in their natural habitat, thereby enabling the maintenance of the transmission cycle and increasing the chances of these animals becoming infected (Abrantes et al., 2018; Barbosa et al., 2010).

The occurrence of CVL is also related to social issues such as migration and disorderly construction of houses on the outskirts of cities, precarious basic sanitation and climatic changes resulting from deforestation, which allow the vector to reproduce and survive (Camargo et al., 2007; Gontijo & Melo, 2004). The locations visited presented propitious environmental and social characteristics for the maintenance of the transmission cycle, such as the rearing of animals in the peridomicile and precarious sanitary conditions resulting from the organic matter produced.

In Mãe D'Água, 60% of the guardians let their animals free during the night (loose, not fastened on a leash), and the category "free during the night/without a collar" was considered a protection factor, as these dogs were 67% more protected against the infection than those fastened on a leash at night during the study period. On the contrary, the prevalence of the zoonosis was higher in semi-domesticated dogs and those that spent the nights contained (Amóra et al., 2006). Our results showed that the dog's permanence in the peridomicile was an important risk factor for infection and was associated with higher contact incidences with vectors (Almeida et al., 2009).

It is believed that animals that spend the night fastened are restricted in a certain area, thus face a greater risk of being bitten by a vector. In contrast, an animal that is permitted to roam free during the night has higher mobility and can walk and sleep in different places, thereby decreasing its chances of becoming infected. Borges et al. (2009) observed that humans are infected more when the dogs are maintained indoors at night, which is the period that vector feed on hosts (from evening until dawn) (Felipe et al., 2011; Galati et al., 1996; Wang et al., 2011). The night also represents the time when most people and animals are in a restricted area inside their houses.

According to the Ministry of Health, prophylactic measures, such as vaccination and deworming, are essential to guarantee that animals are protected against diseases. In the present study, only 10% (7/70) of the dewormed dogs ($p= 0.01$) were seroreactant, which was indicative of protection against *L. infantum* infection, and may be associated with a better immune status of the evaluated dogs, as a result of greater care and wellbeing provided by the guardian (Brasil, 2003).

Spatial analysis is a tool that has been used in CVL epidemiological studies, with the intention of identifying priority areas and disease patterns as well as helping identify control measures for the zoonosis (Margonari et al., 2006). Using SaTScan®, a risk cluster ($p = 0.010$) was identified in the northern region of the urban area, with a light expansion to uninhabited areas and to a region of preserved woodland in the urban perimeter.

Most (96.4%) of the urban residencies in the municipality of Mãe D'Água were located in public streets with afforestation, and only 15.2% were in areas with adequate urban features, such as drains, sidewalks, and paving (IBGE, 2020). The houses in the urban zone of the municipality were close to secondary vegetation with frequent accumulation of organic matter.

Rural and peripheral urban areas tend to have higher occurrence of VL when in proximity to abundant vegetation (Cerbino Neto et al., 2009), which is proven to be the natural habitat of phlebotomine vectors, whose presence is indispensable for the maintenance of the transmission cycle; therefore, entomological monitoring is recommended in localities with active transmission (Borges et al., 2009).

In this study, the disease was more prevalent in the rural area, but was dispersed all over the municipality, a fact that, according to Mestre et al. (2011) and Paulan et al. (2012), is related to the adaptation of the vector to the peridomicile conditions. Such localities are often characterized by a lack of infrastructure or anthropogenic activities, or by the migration of dogs and infected individuals.

The pattern of active transmission of *Leishmania* in Mãe D'Água was more prevalent in the rural area. As human VL cases were recently reported, the adoption of immediate control measures, such as the use of repellent collars, control of vectors in household environments and in the peridomicile with insecticides, removal of organic matter from the peridomicile, and refrainment of rearing domestic animals close to the domicile, are recommended. Additionally, these measures need to be implemented in the urban area to prevent the expansion of the disease and the occurrence of human cases in the risk zone.

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