Rev Soc Bras Med Trop 51(5):683-688, Sep-Oct, 2018
doi: 10.1590/0037-8682-0429-2017

Short Communication

Seroprevalence and risk factors associated with canine visceral leishmaniasis in the State of Paraíba, Brazil

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Abstract

Introduction: The aim of this study was to determine the seroprevalence and risk factors associated with canine visceral leishmaniasis in Paraíba State. **Methods:** Blood samples were collected from 411 dogs in four municipalities of Paraíba State. The seroprevalence was assessed by using ELISA. **Results:** The seroprevalence ranged from 7.2% to 20%. The risk factors that were associated with the disease were the presence of ticks and contact with other animals. **Conclusions:** The seroprevalence of canine visceral leishmaniasis is high in Paraíba, indicating the need for health authorities to resume visceral leishmaniasis control activities. The ruralization of suburban areas in the region tends to homogenize the risk factors between suburban and rural areas.

Keywords: Dogs. ELISA. *Leishmania* sp. Prevalence.

Visceral leishmaniasis (VL) is a zoonotic disease that is endemic in many tropical and subtropical countries. In Brazil, the disease is caused by *Leishmania infantum* (syn. *Leishmania chagasi*). The main vector is the sandfly *Lutzomyia longipalpis,* and the dog is the main domestic reservoir[1].

According to the Brazilian Information System for Notifiable Diseases [*Sistema de Informação de Agravos de Notificação* (SINAN)], 433 cases of human visceral leishmaniasis (HVL) were reported in the Paraíba State from 2001 to 2013. Several factors, which are still controversial, have been associated with CVL; these are the breed, fur length, age, the presence of other animals in the household or peridomestic environments, resting place, and the function of the dog[2-6].

Little is known about the actual prevalence of canine visceral leishmaniasis (CVL) in Paraíba counties and the associated factors with CVL. The perception that there has been an increase in the prevalence of CVL is based on the following observations: a) there is an increased frequency of symptomatic dogs in veterinary clinics; b) there is an increased serological diagnosis of infection in animals referred for vaccination against leishmaniasis, and c) there is an increased frequency of stray dogs with suggestive lesions. Thus, the aim of this study was to determine the seroprevalence and the risk factors associated with CVL in pet dogs in counties of the Paraíba State.

The dogs were from the counties of Patos, Sousa, Cajazeiras, and Uiraúna, all of which are located in the semi-arid mesoregion of the Paraíba State in northeastern Brazil, which is endemic area for CVL (**Figure 1**). The climate of this region is semi-arid, hot and dry. The region is subject to prolonged droughts, with an annual rainfall below 500mm and an annual average temperature exceeding 26°C[7].

The sample size was calculated assuming a simple random sample would be obtained, with an expected prevalence of 50%, a minimum confidence of 95%, and a statistical error of 5%[8]. These parameters provided a sample size of 385 animals; however, blood samples were collected from 411 dogs in four counties. Dogs older than six months that were residing in the urban, peri-urban, and rural areas were included in this study. In the urban and peri-urban areas, sample collection was performed at collection points, one in each neighborhood, after an extensive campaign to increase the knowledge of the population through a local radio. In the rural area, sample collection was carried out at sites pre-selected by lottery; the sampling frame for the lottery comprised all houses and all dogs in the selected localities. Sampling was performed in 2011. A blood sample was obtained through a cephalic venipuncture using a 5-mL syringe and a 25 × 8mm needle. A total of 5mL of blood was immediately placed in a vacuum tube containing 4% sodium citrate as an anticoagulant. The samples were processed at the

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Received 1 November 2017
Accepted 4 April 2018

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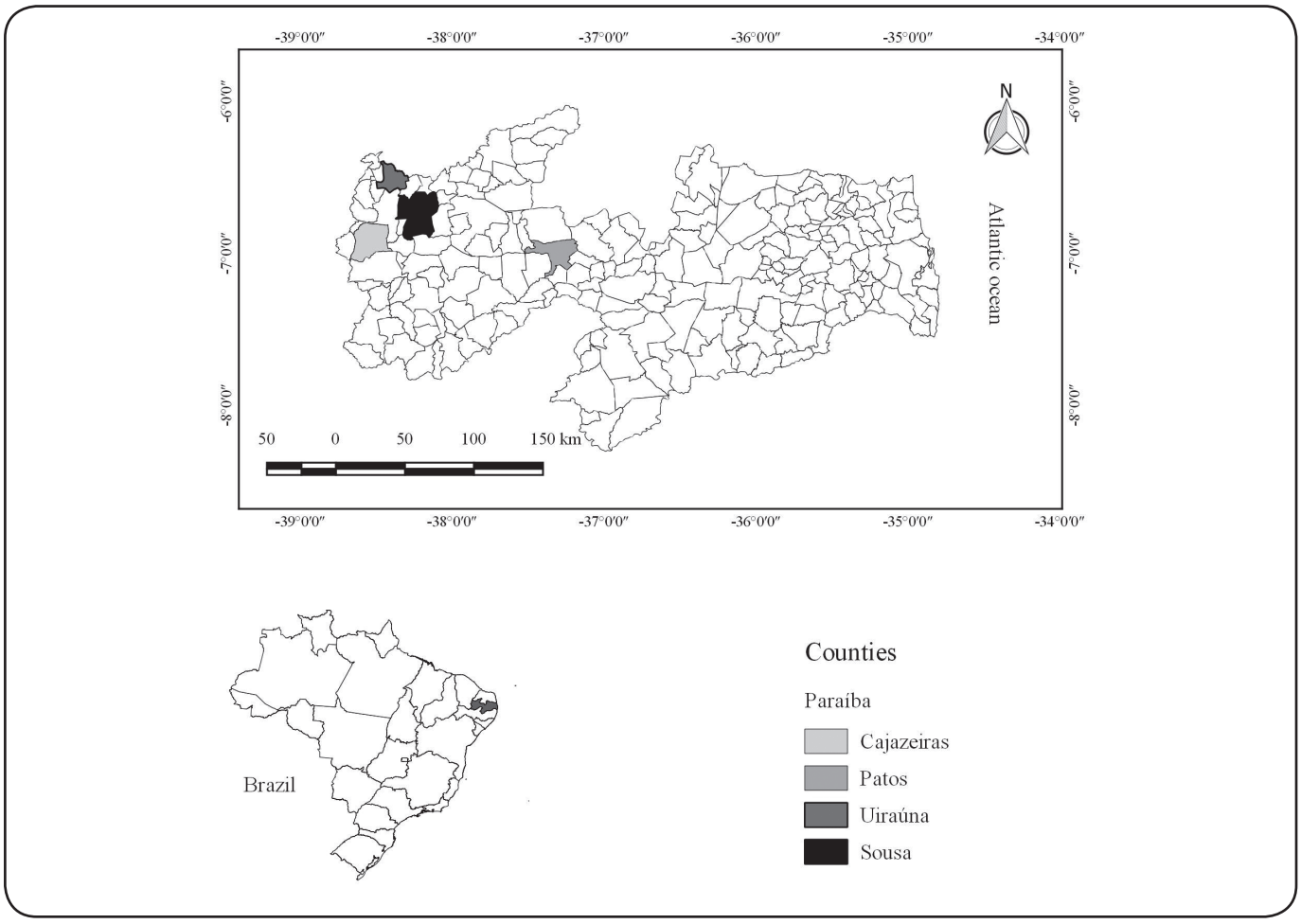

FIGURE 1: Map of the State of Paraíba, Brazil. The studied area comprised the counties of Patos, Sousa, Uiraúna, and Cajazeiras.

Laboratory of Molecular Biology at the Center for Health and Rural Technology [*Centro de Saúde e Tecnologia Rural* (CSTR)] of the Federal University of Campina Grande [*Universidade Federal de Campina Grande* (UFCG)] in the Paraíba State. Blood samples were centrifuged at 2,000*g* for 10 minutes, and plasma was transferred to 1.5mL microcentrifuge tube and stored at -20°C until analysis.

The dogs' tutors answered questions in an epidemiological questionnaire that was developed with the objective of identifying whether standard management practices were used by the tutors and identifying other conditions that could be associated with the development of canine visceral leishmaniasis. The variables considered in the questionnaire were family income and education level; presence of other species of animals (domestic or wild) either in or around the house; environmental conditions of the rearing place; dog's characteristics (sex, age, breed, type of rearing, vaccination history, deworming history, and infestation with ticks).

An enzyme-linked immunosorbent assay (ELISA) kit (S7®, *Biogene Indústria e Comércio* Ltda., Recife-PE, Brazil) for CVL, registered at the Brazilian Ministry of Agriculture, Livestock and Supply, was used to diagnose the infection. The kit contains a recombinant antigen composed of the *L. infatum* heat shock protein (HSP70) carboxy-terminal moiety (the kit has a sensitivity of 96% and specificity of 90%). The protocol was performed according to the manufacturer´s instructions.

Statistical calculations were performed using Statistical Package for the Social Sciences (SPSS) 20.0 for Windows software at a 5% significance level. To identify the factors associated with seropositivity, univariate analysis was performed using either the Pearson´s chi-square or Fisher's exact test. Each independent variable was compared with the dependent variable (serological result), and independent variables that had a $p \leq 0.20$[9] were selected for multivariate logistic regression analysis[10]. A descriptive statistical analysis of the relative and absolute frequencies of ELISA (sero) positive dogs was performed.

Blood samples were collected from a total of 411 dogs in the counties of Patos, Cajazeiras, Sousa and Uiraúna. The prevalence of anti-*Leishmania* antibodies was 12.9% [95% confidence interval (95% CI)] = 9.0-14.9%) (53 dogs were tested positive). The seroprevalence was 20% (95% CI = 12.6-27.4%), 16.3% (95% CI = 10.9-21.7%), 10.5% (95% CI = 8.1-14.0%), and 7.2% (95% CI = 6.4-7.6%) for Cajazeiras, Uiraúna, Sousa, and Patos counties, respectively.

Epidemiological information was obtained using questionnaires. Mongrel dogs comprised 76.2% of the canine population, and 58.6% of the dogs were males. Dogs aged between 2 and 4 years had the highest (18.1%) prevalence of CVL, but there was no significant statistical difference with the other age groups. Most (70%) dogs were kept within the house. There was an increase in the seroprevalence that was inversely related to confinement: the prevalence in dogs strictly kept within houses (resident) was 11.8%; in semi-resident dogs, 15.1%; and in free-roaming dogs, 16.2%. However, there was no significant statistical difference between these categories. The tutor´s family income and educational level were not associated with CVL.

The variables that were significant in the univariate analysis were contact with wild animals, contact with other domestic species, kennel cleaning frequency, and tick infestation (**Table 1**). The results of the multiple logistic regression analysis confirmed tick infestation [odds ratio (OR) = 3.89] and contact with other domestic species (OR = 3.44) as factors associated with CVL (**Table 2**).

TABLE 1: Univariate analysis of possible risk factors of canine visceral leishmaniasis in the studied area.

Variable	Total number of animals	Positive animals (%)	P-value
Level of education			
illiterate	51	8 (15.7)	0.733
1st grade incomplete	152	18 (11.8)	
1st grade full	58	8 (13.8)	
2nd grade incomplete	48	8 (16.7)	
2nd grade full	69	8 (11.6)	
3rd grade incomplete	9	2 (22.1)	
3rd grade full	24	1 (4.2)	
Family income (minimum wages)			
< 2	276	33 (12.0)	0.633
2-4	109	17 (15.6)	
5-6	15	1 (6.7)	
> 6	11	2 (18.2)	
Sex			
male	241	31 (12.9)	1.0
female	170	22 (12.9)	
Age			
6-12 months	96	10 (10.4)	0.259
12-24 months	89	7 (7.9)	
24-48 months	94	17 (18.1)	
4-6 years	76	10 (13.2)	
> 6 years	56	9 (16.1)	
Race			
undefined race	313	42 (13.4)	0.694
Known race	98	11 (11.2)	
Type of confinement			
resident dogs	288	34 (11.8)	0.593
semi-resident dogs	86	13 (15.1)	
free-roaming dogs	37	6 (16.2)	
Food			
commercial food	45	4 (8.9)	0.587
homemade food	252	32 (12.7)	
both	114	17 (14.9)	
Contact with animals			
not	84	9 (10.7)	0.627
yes	327	44 (13.5)	

Continue...

TABLE 1: Continuation.

Variable	Total number of animals	Positive animals (%)	P-value
Contact with horses			
not	410	53 (12.9)	1.0
yes	1	0 (0.0)	
contact with wild animals			
not	373	44 (11.8)	0.070*
yes	38	9 (23.7)	
Contact with cats			
not	244	33 (13.5)	0.756
yes	167	20 (12.0)	
Contact with dogs			
not	205	27 (13.2)	0.985
yes	206	26 (12.6)	
Contact with pigs			
not	400	52 (13.0)	1.0
yes	11	1 (9.1)	
Contact with other species			
not	380	45 (11.8)	0.045*
yes	31	8 (25.8)	
Forms of contact			
contactless	58	5 (8.6)	0.605
pets at home	163	21 (12.9)	
neighbors has animals	87	14 (16.1)	
going to a pet shop	6	0 (0.0)	
frequent traveling	20	4 (20.0)	
more than one form	77	9 (11.7)	
Environment where the dog is created			
land	141	20 (14.2)	0.602
concrete	170	23 (13.5)	
land and concrete	100	10 (10.0)	
Cleanliness of the place			
not	64	10 (15.6)	0.613
yes	347	43 (12.4)	
Cleaning frequency			
not	64	10 (15.6)	0.073*
daily	312	36 (11.5)	
weekly	31	6 (19.4)	
fortnightly	1	1 (100.0)	
monthly	2	0 (0.0)	
Vaccination			
not	104	12 (11.5)	0.758
yes	307	41 (13.4)	
Deworming			
not	240	28 (11.7)	0.465
yes	171	25 (14.6)	
Tick infestation			
not	157	9 (5.7)	0.001*
yes	254	44 (17.3)	

MW: minimum wages.* Variables used in the multiple logistic regression.

TABLE 2: Risk factors of canine visceral leishmaniasis in the studied area, as determined using multiple regression analysis.

Risk factors	*Odds ratio*	CI 95%	P-value
Tick infestation	3.89	1.80 – 8.38	0.001
Contact with other animals	3.44	1.38 – 8.57	0.008

CI 95%: confidence interval 95%.

Contact with other animals in the nearby environment was reported by 79.6% (327) of tutors, and 31 dogs had contact with other animal species; the risk of infection of CVL was increased by 3.44 times in such dogs.

The seroprevalences found in this study are similar to those previously reported for endemic counties and regions[11,12]. The prevalence varies greatly between different locations in the same county; generally, the prevalence is higher in rural than in urban areas[2,11]. As noted by Belo et al.[5] and Azevedo et al.[12], prevalence estimates can be influenced by many factors, such as the study area, the diagnostic method, and the sampling method. The prevalence estimate can be safely used to assess the risk of CVL in the region and may help public health authorities to plan for control programs against human visceral leishmaniasis.

There is no consensus among experts with regard to the factors associated with infection (as it with regard the prevalence); there factors vary between the Brazilian regions and between countries. All animals were domiciled, and both female and male dogs had similar roaming patterns. There was no significant statistical difference between genders with respect to the prevalence. Moreover, Curi et al.[6], in a study carried out in preserved areas of the Atlantic Forest, have suggested that sedentary animals and those that remain in restricted spaces have a greater risk of infection because they are easier targets for the sandfly. Based on this suggestion, we speculate that the lack of correlation between the prevalence and the mobility of the animals (although all animals were domiciled) points to the role of other risk elements in the epidemiological chain.

According to Cesse et al.[13], the *ruralization* of the urban outskirts in semi-arid landscapes causes a significant increase in the number of potential breeding sites for *Lutzomyia longipalpis*; this process (ruralization) has been observed repeatedly in the counties studied in Paraiba and may constitute the most relevant risk factor for both CVL and human visceral leishmaniasis.

Both low family income and low education level are considered to be risk factors for human visceral leishmaniasis[5,13]. In relation to CVL, Coura-Vital et al.[4] also pointed out that low income, as well as limited tutor´s knowledge about the vector, is a risk factor for CVL. The difference between the results cited above and those obtained in our study may arise from the difference in the distribution of social strata in large cities compared to that in the Paraíba counties; further work is required to confirm this hypothesis

The seropositivity index was 3.89 times higher in dogs infested with *R. sanguineus*. There have been reports of infection of ticks with *L. infantum* and *L. donovani*, but there is still controversy whether these ticks transmit the disease. Coutinho et al.[14] demonstrated that it is possible to infect hamsters orally by feeding them with infected macerated *R. sanguineus,* and Dantas-Torres et al.[15] demonstrated experimental transovarian transmission of *L. infantum* in infected *R. sanguineus*. However, the latter authors were unable to show any association between seropositivity and dog infestation with *Amblyomma ovale* and *R. sanguineus*. Taking into account that the environment around the house is an important factor and that the presence of unpaved yards and livestock offer breeding sites and blood meals, respectively, for both ticks and sand flies, we advance the hypothesis that ticks are not directly involved, but they serve as an indicator of the environmental conditions that favor the presence of sand flies and of breeding sites.

As described by Azevedo et al.[12], other animals (hens, swine, and horses) serve as an additional food source to phlebotomines; hence, the former can facilitate maintenance of the latter and allow development of high vector densities around houses. Moreira Jr. et al.[3] have also reported that other animals, especially pigs, but not chickens, significantly increase the risk of CVL. In our study, pig rearing was not associated with CVL, but other livestock may be involved, as suggested by Cesse et al.[13]. Although other vertebrates can serve as food for the sandfly and favor its maintenance in areas around houses, the former can also reduce the number of infectious bites for the dogs.

The current seroprevalence of canine visceral leishmaniasis in semi-arid Paraíba hinterland is high, suggesting the need for more effective control measures.

Although the risk factors for CVL in Brazil are controversial, our study suggested that the environment around the house is an important element with respect to the risk of disease.

The *ruralization* of suburban areas in the region tends to homogenize the risk factors between suburban and rural areas.

Ethical considerations

The project was approved by the Ethics Committee on Animal Use of the Universidade Federal de Campina Grande (Protocol CEP nº 59-2011).

Conflict of interest

The authors declare that there are no conflict of interest.

REFERENCES

1. Gontijo CMF, Melo MN. Leishmaniose visceral no Brasil: quadro atual, desafios e perspectivas. Rev Bras Epidemiol. 2004;7(3): 338-49.

2. França-Silva JC, Costa RT, Siqueira AM, Machado-Coelho GLL, Costa CA, Mayrink W, et al. Epidemiolgy of canine visceral leishmaniasis in the endemic área of Montes Claros Municipality, Minas Gerais state, Brazil. Vet Parasitol. 2003;111(2-3):161-73.

3. Moreira Jr ED, de Souza VMM, Sreenivasan M, Lopes NL, Barreto RB, de Carvalho LP. Peridomestic risk factors for canine leishmaniasis in urban dwellings: new findings from a prospective study in Brazil. Am J Trop Med Hyg. 2003;69(4):393-7.

4. Coura-Vital W, Marques MJ, Veloso VM, Roatt BM, Aguiar-Soares RDO, Reis LES, et al. Prevalence and factors associated with *Leishmania infantum* Infection of dogs from an urban area of Brazil as identified by molecular methods. PLoS Neglected Trop Dis. 2011;5(8):1-10.

5. Belo VS, Struchiner CJ, Werneck GL, Barbosa DS, Oliveira RB, Teixeira Neto RG, et al. A systematic review and meta-analysis of the factors associated with *Leishmania infantum* infection in dogs in Brazil. Vet Parasitol. 2013;195(1-2):1-13.

6. Curi NHA, Paschoal AMO, Massara RL, Marcelino AP, Ribeiro AA. Factors associated with the seroprevalence of leishmaniasis in dogs living around Atlantic Forest fragments. PLoS One. 2014;9(8):1-11.

7. Pereira MCA, Melo DF, Meló RGC, Silva RFB, Farias SAR. Mesorregiões da Paraíba e suas necessidades de captação de água de chuva [Internet]. Campina Grande (BR): Universidade Federal de Campina Grande; 2012. Citado: 15 out 2013. Disponível em: http://www.bibliotekevirtual.org/simposios/8SBCMAC/8sbcmac-a054.pdf.

8. Thrusfield M. Epidemiologia Veterinária. 2nd edition. São Paulo: Roca; 2004. 556p.

9. Zar JH. Biostatistical analysis. 4th edition. New Jersey: Prentice-Hall; 1999. 663p.

10. Hosmer DW, Lemesho WS. Applied Logistic Regression. 2nd edition. New York: John Wiley and Sons; 2000. 375p.

11. Amóra SSA, Santos MJP, Alves ND, Costa SCG, Calabrese KS, Monteiro AJ, et al. Fatores relacionados com a positividade de cães para leishmaniose visceral em área endêmica do Estado do Rio Grande do Norte, Brasil. Cienc Rural. 2006;36(6):1854-9.

12. Azevedo MAA, Dias AKK, Paula HB, Perri SHV, Nunes CM. Avaliação da leishmaniose visceral canina em Poxoréo, Estado do Mato Grosso, Brasil. Rev Bras Parasitol Vet. 2008;17(3):123-7.

13. Cesse EAP, Carvalho EF, Andrade PP, Ramalho WM, Luna L. Organização do espaço urbano e expansão do calazar. Rev Bras Saude Mater Infant. 2001;1(2):167-76.

14. Coutinho MTZ, Bueno LL, Sterzik A, Fujiwara RT, Botelho JR, Maria M, et al. Participation of *Rhipicephalus sanguineus* (Acari: Ixodidae) in the epidemiology of canine visceral leishmaniasis. Vet Parasitol. 2005;128(1-2):149-55.

15. Dantas-Torres F, Martins TF, Paiva-Cavalcante M, Figueredo LA, Lima BS, Brandão-Filho SP. Transovarial passage of *Leishmania infantum* kDNA in artificially infected *Rhipicephalus sanguineus*. Exp Parasitol. 2010;125(2):184-5.