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Prevalence and risk factors associated with *Leishmania* spp. and *Trypanosoma cruzi* infections in dogs presented at veterinary clinics in João Pessoa, Paraíba state, northeastern Brazil

Prevalência e fatores de risco associados às infecções por *Leishmania* spp. e *Trypanosoma cruzi* em cães atendidos em clínicas veterinárias de João Pessoa, Estado da Paraíba, Nordeste do Brasil

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Abstract

This study aimed to determine the prevalence and risk factors associated with *Leishmania* spp. and *Trypanosoma cruzi* infections in dogs presented at veterinary clinics in the city of João Pessoa, Paraíba state, northeastern Brazil. A total of 384 dogs from 34 veterinary clinics were sampled between April 2015 and May 2016. For serological diagnosis of canine visceral leishmaniasis and Chagas disease, the indirect fluorescent antibody test was used. Reactive sera were submitted to a confirmatory test using the S7 ELISA. The seroprevalence of *Leishmania* spp. was 2.8% (11/384), and that of *T. cruzi* was 1.5% (6/384). Two animals were positive for both parasites. The variable “access to the street” was identified as a risk factor for *Leishmania* spp. infection (OR = 4.81; 95% CI = 1.23–18.75). We concluded that a significant proportion of dogs presented at veterinary clinics in João Pessoa are seropositive for *Leishmania* spp. or *T. cruzi* infections, and we suggest that greater care be taken with dogs that have access to the street.

Key words: Leishmaniosis. Chagas disease. Urban environment. Domiciled dogs. Northeastern Brazil.

Resumo

O objetivo deste estudo foi determinar a prevalência e os fatores de risco associados à infecção por *Leishmania* spp. e *Trypanosoma cruzi* em cães atendidos em clínicas veterinárias na cidade de João Pessoa, Estado da Paraíba, Nordeste do Brasil. Foram utilizadas 384 cães procedentes de atendimentos

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de 34 clínicas veterinárias no período de abril de 2015 a maio de 2016. Para diagnóstico sorológico da leishmaniose visceral canina (LVC) e doença de Chagas (DC) foi empregada a reação de imunofluorescência indireta (RIFI). Os soros reagentes para ambas as infecções foram submetidos a uma prova confirmatória utilizando o ELISA S7. A soroprevalência para *Leishmania* spp. foi de 2,8% (11/384), e para *T. cruzi* foi de 1,5% (6/384). Dois animais foram positivos para ambos os parasitas. A variável acesso à rua foi identificada como fator de risco para infecção por *Leishmania* spp. (OR= 4,81; IC 95% = 1,23 -18,75). Conclui-se que os cães atendidos em clínicas veterinárias de João Pessoa estão expostos às infecções por *Leishmania* spp. e *T. cruzi*, bem como sugere-se maiores cuidados com cães que tem acesso à rua.

Palavras-chave: Leishmaniose. Doença de Chagas. Ambiente urbano. Cães domiciliados. Nordeste do Brasil.

Dogs play an important role in society as working animals, guardians, and companions. Therefore, the transmission of diseases among dogs is of great concern. In this regard, massive alterations in the environment, such as deforestation and urbanization in previously preserved areas, have altered the dynamics of canine diseases (CLEAVELAND et al., 2017). In Brazil specifically, canine visceral leishmaniasis (CVL) and Chagas disease (CD) are important endemic zoonoses. Both are also anthroponoses and are caused by *Leishmania* spp. and *Trypanosoma cruzi*, respectively. In Brazil, CVL is transmitted via the bite of hematophagous sand flies of the genus *Lutzomyia*; the disease reservoirs are wild and domestic canids (QUINNELL; COURTENAY, 2009). CD, also known as American trypanosomiasis, is mainly transmitted via contact between susceptible individuals and the feces of hematophagous insects of the Reduviidae family after the latter have ingested a blood meal. However, the disease can also be transmitted congenitally and via the ingestion of contaminated food. The reservoirs of *T. cruzi* comprise about 200 domestic and wild mammals (GALVÃO, 2014).

In the 1990s, *Leishmania* spp. and *Trypanosoma cruzi* were restricted to the rural environment. Recently however, due to increasing urbanization, particularly the inhabitation of peri-urban areas, the pathogens have proliferated in the urban environment as well. As a consequence, the number of infected people and domestic animals has risen (WHO, 2015; FERNANDES et al., 2016). According to the

National System for Disease Notification (SINAN) in Brazil, approximately 50,000 cases of visceral leishmaniasis were diagnosed in humans from 2001 to 2015, with most cases reported in the northeastern region. Furthermore, it is estimated that about 1 million people in Brazil are infected with CD, with the greatest occurrence in the northern region (WHO, 2015).

Epidemiological investigations into leishmaniasis and CD provide an overview of the distribution, monitoring, and mapping of these diseases. Research involving dogs is particularly important, as these animals are the main reservoirs of these zoonoses. Thus, the objective of the present study was to determine the prevalence and risk factors of *Leishmania* spp. and *T. cruzi* infections in dogs presented at veterinary clinics in João Pessoa, Paraíba state, northeastern Brazil.

The survey was conducted in the municipality of João Pessoa, which is the capital city of Paraíba state and has about 720,000 inhabitants distributed among 59 boroughs. The city has an area of preserved Atlantic Forest in the center and is bordered by mangroves. Its climate is humid tropical, with annual average temperatures of 23°C and an annual rainfall of above 1,906 mm.

The veterinary clinics were selected based on data from the Regional Council of Veterinary Medicine (CRMV-PB), which listed 40 registered clinics. However, on-the-spot verification revealed that four establishments had closed and that the owners of two others preferred not to participate. Thus, 34

veterinary clinics were included, distributed among the four zones of the city (North, South, East, and West).

The minimum number of animals was determined using the following formula for simple random sampling (THRUSFIELD, 2004):

$$n = \frac{Z^2 \times P (1 - P)}{d^2}$$

Where n = number of sampled animals, Z = normal distribution for a confidence level of 95%, P = expected prevalence of 50% (sampling maximization), and d = error of 5%.

In total, 384 apparently healthy dogs were selected. All were either unvaccinated against leptospirosis or had been vaccinated more than 6 months prior to recruitment in the study. Females in the periparturient or lactation periods were excluded from the study. Data were collected between April 2015 and May 2016. Blood samples were taken by venipuncture of the cephalic or jugular vein using 5-mL vacuum tubes, and the sera were placed into microtubes and frozen until the serological tests were performed.

The indirect fluorescent antibody test (IFAT) was used to diagnose both diseases, with a cut-off point of 1:40. Specifically, the reagent samples were titrated at dilutions of 1:40, 1:80, 1:160, 1:320, and 1:640 (CAMARGO, 1966). For CVL diagnosis, the slides were coated with antigen prepared from *L. major*-like promastigotes (strain MHOM/SU/73/5-ASKH; Fiocruz IOC/L0581), while the antigen for CD diagnosis was prepared from *T. cruzi* (strain Y) epimastigotes. Both cultures were maintained in liver infusion triptose and Neal, Novy, Nicolle culture media. The conjugates were produced by the immunology sector of the São Paulo Zoonosis Control Center at a dilution of 1:120. To wash the slides, a buffered saline solution of pH 7.2 was used. The slides were read using a ZEISS biological microscope (AXIO Scope A1, 40x), with a fluorescence filter for fluorescein isothiocyanate

(green). Positive and negative control sera, for both parasites, were provided by the Núcleo de Pesquisa em Zoonoses (NUPEZO), Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Botucatu, São Paulo state. The final antibody titer was determined to correspond to the highest dilution of the sera whereby the membranes of at least 50% of the promastigotes (CVL) and epimastigotes (CD) emitted readable fluorescence with a cutoff of 40 or higher (FERNANDES et al., 2016).

The samples that reacted positively both to *Leishmania* spp. and *T. cruzi* were submitted to a confirmatory test using the ELISA S7 kit (Biogene Indústria & Comércio Ltda ME, Recife-PE, Brazil) to diagnose CVL or CD, respectively. The test was carried out as specified by the manufacturer. Briefly, dogs were considered positive for *Leishmania* spp. or *T. cruzi* when they were both positive in the IFAT at a minimum dilution of 1:40 and reactive in the ELISA S7 test. The samples that reacted positively for both agents were identified as co-infections.

Epidemiological questionnaires were delivered to dog owners to obtain data for the risk factor analysis. The analyzed variables and their respective categories were: educational level of the owner (illiterate, incomplete primary school, complete primary school, incomplete high school, complete high school, incomplete university degree, university degree), gender (female, male), dog breed (pure-bred, crossbred), dog age (up to 48 months, 49–72 months, > 72 months), access to street (no, yes), feed (commercial food, homemade food, food scraps, raw meat), access to treated water (no, yes), reason for keeping dog (company, guard, other), contact with other dogs (no, yes), contact with cows (no, yes), contact with horses (no, yes), contact with wild animals (no, yes), contact with cats (no, yes), contact with goats (no, yes), contact with sheep (no, yes), contact with swine (no, yes), type of housing (masonry, shack, stilt house, mud hut), where the animal is kept (soil, cement, soil/cement), environment where the animal lives (rural or urban), environment near woodlands

or agricultural areas (no, yes), regular garbage collection (no, yes), cleaning and disinfection of the environment (daily, weekly/fortnightly, monthly), vaccination (no, yes), contact with flooded areas (no, yes), presence of rodents (no, yes), history of abortion (no, yes), occurrence of stillbirth (no, yes), and presence of ticks (no, yes).

A univariable exploratory analysis of the data was carried out to identify variables with a P -value ≤ 0.20 using the chi-square or Fisher's exact test. The identified variables were then subjected to a multivariable analysis using multiple logistic regression with a significance level of 5% (HOSMER; LEMESHOW, 2000). Collinearity between independent variables was verified using correlation analysis. When variables had strong collinearity (correlation coefficient > 0.9), one of the two was excluded from the multiple analysis depending on biological plausibility (DOHOO et al., 1997). We evaluated confounding by monitoring alterations of more than 20% in the model's parameters when adding new variables. Data analyses were carried out using SPSS 23.0 for Windows software.

For *Leishmania* spp., 21 of the 384 evaluated samples (5.4%) were positive by IFAT, with the following titer frequency: 1:40 (22.2%), 1:80 (33.3%), 1:160 (33.3%), and 1:640 (10.2%). However, only 11 (2.8%) samples were positive by ELISA S7. Six (1.5%) of the tested samples were positive for *T. cruzi*. The antibody titers and respective frequencies were as follows: 1:40 (25%), 1:80 (50%), and 1:320 (25%). Two samples were positive for both agents, both with titers of 1:40 for *Leishmania* spp. and 1:80 for *T. cruzi*. A map of the João Pessoa municipality along with the spatial distribution per residence of the dogs positive and negative for *Leishmania* spp. and *T. cruzi* are presented in Figures 1A and 1B, respectively. In the univariable analysis of the risk factors for *Leishmania* spp., the following variables were identified as having a P -value ≤ 0.20 : age of the animals, access to the street, contact with flooded areas, and occurrence of stillbirths (Table 1). In the multivariable analysis, access to the street was identified as risk factor (odds ratio = 4.81; 95% CI = 1.23–18.75; $P = 0.024$). No risk factors for CD were not identified.

Table 1. Univariate analysis of potential risk factors for canine leishmaniasis in the municipality of João Pessoa ($P \leq 0.2$).

Variable	Categories	Total No. of animals	Nº of seropositive animals (%)	P -value
Age	Up to 48 months	219	5 (2.3)	0.191
	49–72 months	63	4 (6.3)	
	Above 72 months	102	2 (2.0)	
Access to street	No	238	3 (1.3)	0.024
	Yes	146	8 (5.5)	
Contact with flooded areas	No	356	9 (2.5)	0.188
	Yes	28	2 (7.1)	
Occurrence of stillbirths	No	377	10 (2.7)	0.185
	Yes	7	1 (14.3)	

Domestic dogs are reservoirs of leishmaniasis and play an important role in disease transmission in endemic areas such as northeastern Brazil. Recent studies into CVL in domiciled dogs in this region presented seroprevalences of 7.8%, using IFAT and ELISA as diagnostic methods (FERNANDES et al., 2016) and 10.3% (BARBOSA et al. 2015). The prevalence value in the present study was lower, possibly because the animals in the present study had better living conditions all were domiciled and attended veterinary clinics, suggesting that their owners take greater care of their health and hygiene. One concerning issue is that none of the animals seropositive for CVL in the present study presented clinical signs of the disease. According to Lachaud et al. (2002) more than 60% of leishmaniasis-positive animals are asymptomatic, and the presence of asymptomatic seropositive individuals is a strong indicator that the parasite is endemic in the municipality.

As shown in Figure 1, CVL was geographically distributed across all regions of the João Pessoa municipality. Many of these areas suffered rapid urbanization characterized by the deforestation of preserved areas to clear space for residential sectors. Santos et al. (2016), using information from patients' records at the University Hospital of the Federal University of Paraíba, stated that, between 2007 and 2012, 50 people contracted visceral leishmaniosis in the municipality of João Pessoa.

CD is also endemic in domiciled dogs in Northeastern Brazil, with prevalence ranging from 4.08% to 38% (MENDES et al., 2013; FERNANDES et al., 2016). Importantly, CD is an anthroponosis that occurs in predominantly rural areas; indeed, all the studies mentioned above involved animals from such areas. This may explain the low prevalence of CD in the present study, in which all dogs lived in an urban environment. Despite the low prevalence of infection in this study, the occurrence of CD-positive dogs in João Pessoa should alert authorities to improve control strategies the disease.

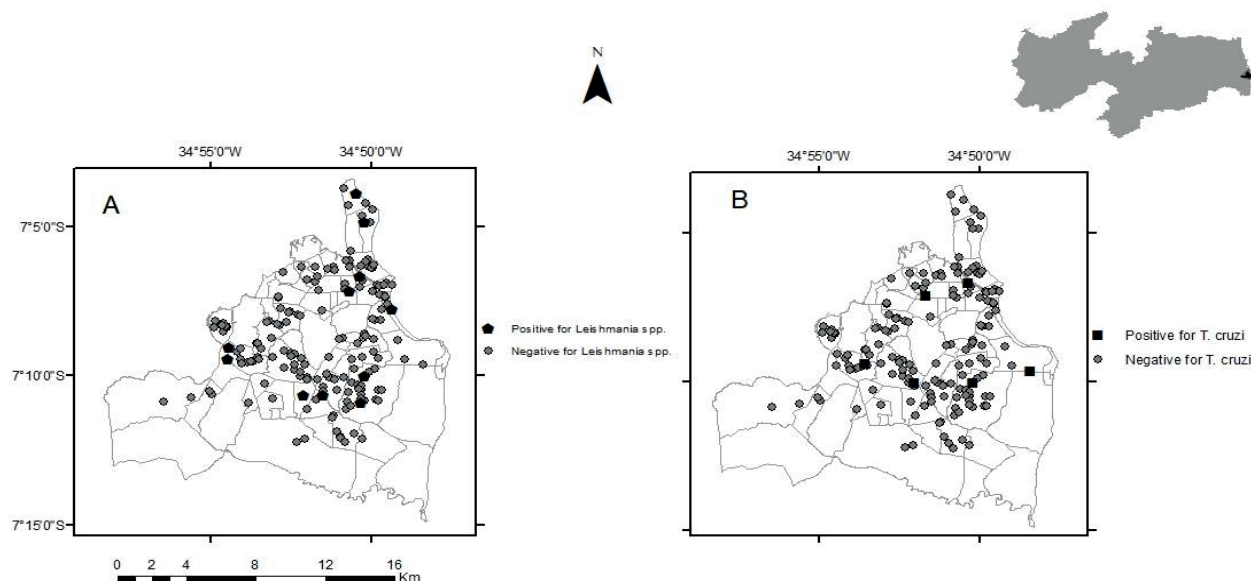
Two animals were positive for both *Leishmania* spp. and *T. cruzi*, with antibody titer levels of 1:40 and 1:80, respectively, indicating no greater discrepancies between titers. The state of Paraíba is endemic for CVL and presents confirmed cases of CD in dogs (BRITO et al., 2016; MENDES et al., 2013). Luciano et al. (2009), who used IFAT to evaluate cross reactions between *Leishmania* spp. and *T. cruzi* in dogs, concluded that cross reactions are common, and that cross reactions can be identified by analyzing the titers.

Access to street was a risk factor for *Leishmania* spp. infection in dogs in João Pessoa, perhaps because this increases the risk of exposure to disease vectors. Furthermore, in several districts of the city, there are areas with accumulations of organic matter, favoring the reproduction of the transmitting insects.

Visceral leishmaniosis and CD in dogs and humans are becoming more frequent in urban centers, mainly due to environmental and socioeconomic imbalances. The municipality of João Pessoa has undergone rapid urbanization in the past few decades, and the results of the present study should alert the city authorities that more complex, multidisciplinary, and multisystemic approaches are needed as advocated by the "One Health" initiative particularly in the case of zoonotic diseases with a strong environmental component (CLEAVELAND et al., 2017).

In conclusion, a significant proportion of dogs presented at veterinary clinics in João Pessoa are seropositive for *Leishmania* spp. and *T. cruzi*, which should raise public health concerns, as both organisms are important zoonoses with an environmental component. Based on the risk factors, we suggest that greater care should be taken with dogs that have access to the streets.

Figure 1. Distribution of dogs seropositive for *Leishmania* spp. (A) and *Trypanosoma cruzi* (B) presented at veterinary clinics in the city of João Pessoa between April 2015 and May 2016.



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