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Short Communication

Seroprevalence and spatial distribution of canine leishmaniasis in an endemic region in Brazil: how has the situation changed after 10 years?

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Abstract

Introduction: Herein, we assessed the seroprevalence and spatial distribution of *Leishmania infantum* in dogs in Garanhuns, Northeastern Brazil. **Methods:** Sera samples (n = 242) were analyzed using an enzyme-linked immunosorbent assay (ELISA). The spatial distribution of dogs seropositive for anti-*Leishmania infantum* antibodies was evaluated using kernel density estimation. **Results:** A total of 2.4% (6/242) of the animals were seropositive for anti-*Leishmania infantum* antibodies. The kernel map showed their distribution to be heterogeneous over the city, with a hotspot in the northeastern region. **Conclusions:** The reported data illustrate the circulation of parasites of the genus *Leishmania* in a canine population.

Keywords: Visceral leishmaniasis. Canine visceral leishmaniais. Urban area. Spatial distribution.

Visceral leishmaniasis (VL) is an important disease that affects humans worldwide[1]. Currently, it is estimated that approximately 200,000 to 400,000 new cases occur every year[2]. This infection is caused by an intracellular obligate protozoan known as *Leishmania infantum* that parasitizes mononuclear phagocytes. It is known that several vertebrate hosts may act as a reservoir for *L. infantum*, especially wild species such as canids, marsupials, and rodents[3]. However, in urban areas, domestic dogs play an important role in the epidemiology of this zoonosis.

In endemic areas, dogs are considered the main reservoir of the parasite, with seroprevalence rates ranging from 1.9% to 35%[4]. Accordingly, it is important to monitor the infection in these animals in order to estimate and predict the number of human cases, as dogs act as a sentinel for VL.

Canine visceral leishmaniasis (CVL) is considered one of the major threats for canine health. In these animals, the clinical manifestations may vary from asymptomatic to severe disease featured by lymphadenopathy, onychogryphosis, dermatitis, alopecia, apathy, vomiting, intermittent pyrexia, diarrhea, polyuria, polydipsia, splenomegaly, and anemia[5]. The transmission of this parasite to dogs primarily occurs via insect vectors belonging to the sub-family Phlebotominae, even though alternative transmission routes of CVL have been hypothesized (e.g., ticks, fleas, and vertical transmission).

During the past few years, CVL has shown an evident spread in Brazil, from sub-urban to urban areas, due to a series of environmental, social, and demographic factors[6]. In addition, some vectors (e.g., *Lutzomyia longipalpis*), the main intermediate host of *L. infantum* in Brazil, progressively adapted to the urban environment, by sucking the blood of domestic animals[7].

Northeastern Brazil has been traditionally endemic for VL, and humans, animal reservoirs, and vectors share the same ecological niche, thus contributing to the persistence of *Leishmania* life cycle[4]. Nonetheless, in some areas, little attention has been paid to CVL over the past years.

Therefore, the aim of this study was to assess the seroprevalence and spatial distribution of *Leishmania infantum* in domiciled dogs living in a poorly studied area.

The study was conducted in the urban area of the municipality of Garanhuns (8°53′25″S and 36°29′34″W), Pernambuco, Northeastern Brazil. This region is located at a

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height of 900 m above sea level and has an average temperature of 22°C, with a maximum in January (24°C) and minimum in June (19°C). The average relative humidity is 90%.

From August to December 2016, blood samples were collected from 242 domiciled crossbreed dogs and stored at −20°C until serological processing. At this time, a detailed description of clinical history were recorded for each dog. Sampling was performed from 12 neighborhoods that constitute the study area. The minimum sample size was estimated based on the domiciled population of dogs (~13,700 animals) of the municipality of Garanhuns [95% *confidence interval* (95% CI)]. The estimated population of dogs in the study area was determined based on the study by WHO (2005)[8].

The serological analyses were performed using an enzyme-linked immunosorbent assay (ELISA) [Bio-Manguinhos/ *Fundação Oswaldo Cruz* (FIOCRUZ)]. Samples were tested in duplicate, including the blank, negative, and positive controls that were provided in the ELISA kit. The reaction was read in a spectrophotometer at a wavelength of 450nm. All procedures and determination of cut-off were performed according to the instructions provided with the ELISA kit.

Association of seropositivity with the sex of the animal was statistically analyzed using the Fisher's exact test with the software BioEstat 5.0[9]. The significance level was set at 5%.

The address of each domicile was manually geocoded using Quantum geographic information system (QGIS) version 2.8. The spatial distribution of the seropositive dogs was statistically evaluated to obtain a smooth estimate of prevalence values, applying the kernel density estimation (KDE)[10]. The KDE includes a smoothing and interpolating technique for generalizing point location for the whole study area. It consists of a simple alternative to analyze focal patterns, in which the outputs are easily readable and understood. For the study, an adaptive bandwidth was used and the adaptive kernel was chosen because the distribution of the domiciles was not homogeneous in the study area. The results were plotted using QGIS software.

Of all the animals enrolled (n = 242), 50.8% (n = 123) were females and 49.2% (n = 119) males, with ages ranging from 2 months to 18 years. A total of 19.8% (n = 48) of animals exhibited at least one clinical sign suggestive of CVL, anorexia (52.0%; 25/48), dermatitis (43.7%; 21/48), and onychogryphosis (4.1%; 2/48) being among the most commonly reported signs. In the serological analysis, 2.4% (6/242) of the animals tested positive. No statistical difference was observed in the rate of seropositivity between males and females. However, a high rate of seropositivity was observed in males less than four years of age. The numbers of positive cases according to the area of sampling are reported in **Table 1**.

The kernel map (**Figure 1**) showed a heterogeneous spatial distribution of positive dogs over the city, with a hotspot in the northeast region, more precisely in the João da Mata neighborhood. Positivity was also observed in the other 4 neighborhoods (Boa Vista, COHAB 2, COHAB 3 and Vale do Mundaú).

This study assessed the presence of anti-*Leishmania infantum* antibodies in domiciled dogs and investigated for the first time the spatial distribution of canine leishmaniasis in this poorly studied area. The overall results showed a seroprevalence of 2.4% (6/242), which was lower than that observed in a previous study performed in the same area, where 16% (25/256) of the dogs were found to be seropositive in the immunofluorescence antibody test (IFAT)[11]. This difference may be related to the features of each technique. In addition, the previous study was performed in Garanhuns[11] and utilized a cut-off of 1:40, which is low in endemic areas; hence, the possibility of false positive results could not be ruled out.

TABLE 1: Number of dogs seropositive for anti-*Leishmania infantum* antibody according to the area of the study.

Area of study	Positivity
	n/N
Boa Vista	1/21
Brasília	0/20
COHAB 1	0/15
COHAB 2	1/19
COHAB 3	1/20
Heliópolis	0/10
Indiano	0/23
João da Mata	2/22
João Maria Dourado	0/27
Magano	0/25
Severiano Moraes Filho	0/40
Quartel	0/14
Vale do Mundaú	0/21
Total	**6/242**

n: number of positives; **N:** number of animals tested.

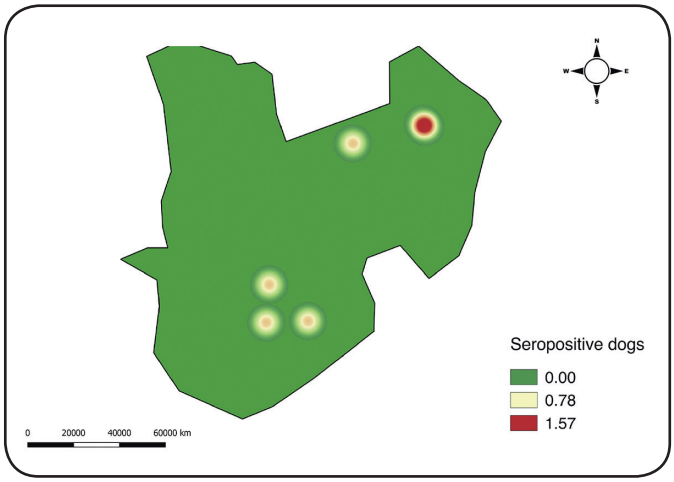

FIGURE 1: Kernel map showing the number of seropositive dogs in Garanhuns, State of Pernambuco.

Currently, the Brazilian Ministry of Health utilizes the rapid dual-path platform test (TR-DPP®, Bio-Manguinhos/FIOCRUZ) for screening, followed by an ELISA for confirmation. Although the TR-DPP® uses a combination of recombinant antigens (rK39, K26, and k9), its sensitivity appears to be low in subclinically infected animals[12]. Moreover, the specificity of both TR-DPP® and ELISA tests may be low and may compromise the efficacy of the methods used[13].

From a clinical point of view, only 24.4% (59/242) of dogs presented at least one clinical sign suggestive of CVL. The main signs reported in the present study are those frequently described in dogs infected by *Leishmania infantum*. In addition, among dogs exhibiting clinical signs, only two (3.3%; 2/59) were seropositive, demonstrating the difficulty of diagnosing CVL in asymptomatic animals. A high rate of seropositivity was observed in male dogs less than four years of age. Although this difference was not considered significant, it is believed that males have greater exposure to the vector due to their behavior. Indeed, it is true that males have greater access to outside environment whereas females are more restricted to the indoor environment[14].

Interestingly, the seropositive animals were heterogeneously distributed over the city, but with a hotspot in the João da Mata neighborhood. This area is characterized by severe environmental problems and absence of basic sanitation. In addition, it is a peripheral area close to the remnants of the Atlantic Forest, and several other animal species are found here. The other four neighborhoods where seropositive animals were detected (i.e., Boa Vista, COHAB 2, COHAB 3, and Mundaú) follow the same environmental pattern as the hotspot.

There has been no report of risk factors for CVL in Garanhuns, but the absence of basic sanitation, poor garbage-disposal practices, and presence of accumulated organic matter have been evidenced here and in other areas endemic for CVL[4,15]. Finally, to our knowledge, this study is the first to assess the spatial distribution of dogs seropositive for *Leishmania infantum* in the municipality of Garanhuns. Data reported herein are important and demonstrate the circulation of parasites of the genus *Leishmania* in the canine population. Further studies focusing on the epidemiology and identification of putative vectors in the studied area are needed.

Ethical considerations

The Ethics Committee for Animal Experimentation (ECAE) of the *Universidade Federal Rural de Pernambuco* approved the study (protocol number: 99/2016).

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Conflict of interest

The authors declare that there is no conflict of interest.

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