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Case report

Factors associated with positivity for canine visceral leishmaniosis in an endemic area in Brazil

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ABSTRACT

Visceral leishmaniosis (VL) is a public health problem and its occurrence depends primarily on the presence of the vector and susceptible hosts; in the urban environment, the dog is the main reservoir. This study aimed to analyze the distribution of canine visceral leishmaniosis (CVL) and factors associated with it in an urban area endemic for VL. Analysis of the variables was based on 2755 epidemiological records of dogs positive and negative for CVL over a three-year period (2009, 2010 and 2011). A dog was considered positive when it presented amastigotes in the cytological examination of lymph node biopsy and/or was seropositive by immunoenzymatic and indirect immunofluorescence assays. CVL positive dogs were observed throughout the town, but significant differences were observed between the sectors analyzed ($P < 0.0001$), with two sectors showing higher positivity. CVL prevalence was 35.9% and was significantly associated with age and breed ($P < 0.0001$). Concerning symptoms, 44.3% of symptomatic dogs were positive for LV ($P < 0.0001$) in an urban area endemic for this zoonosis.

1. Introduction

Visceral leishmaniosis (VL) is a zoonosis caused by protozoa of the genus *Leishmania* (Kinetoplastida, Trypanosomatidae) and *Leishmania infantum chagasi* is the etiological agent in Brazil. The promastigote form of the parasite develops in the gut of the vector, while in mammalian hosts, the disease occurs due to the presence of the rounded amastigote, with no free flagella, and multiplies within macrophages (Ready, 2014).

VL is considered endemic in the Indian subcontinent, East Africa and South America. Every year 200–400 thousand new cases of VL are registered worldwide, with over 90% of these cases occurring in six countries, Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan (WHO, 2015).

The urbanization process of VL in Brazil began in the 1980s and was multifocal, beginning in the cities of Teresina, PI, and São Luís, MA. In less than a year, there were epidemics in urban areas of at least five states (Harhay et al., 2011).

The transmission of the parasite to humans and mammals occurs through the bite of the female sand fly of the Phlebotominae family. The species of greatest importance in Brazil is *Lutzomyia longipalpis*, although *L. cruzi* has also been described as a vector species (Desjeux, 2004; Ready, 2014). Other arthropods, such as ticks and fleas, have been studied and presented viable forms of *Leishmania*. However, their role as vectors has not yet been elucidated (Dantas-Torres et al., 2010).

The occurrence of VL in a particular area depends on the presence of the vector and susceptible hosts, and in urban areas, the dog is the main reservoir (Gontijo and Melo, 2004). In some endemic regions, analyses of spatiotemporal studies conclude that the human cases tend to be concentrated in areas with higher incidences of canine visceral leishmaniosis (CVL) (Camargo-Neves et al., 2001; Teixeira-Neto et al., 2014; Werneck, 2014).

CVL has a variable incubation period of 1 month to 4 years, it is multisystemic and the parasite can be found in different tissues and organs, causing immunological reactions and changes in tissue characteristics (Alvar et al., 2004). The most common clinical manifestations include skin lesions, generalized lymphadenopathy, progressive weight loss, muscle atrophy, exercise intolerance, decreased appetite, lethargy, splenomegaly, eye damage, onychogryphosis and lameness (Solano-Gallego et al., 2009).

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To reduce the transmission rate and the mortality associated with VL, the Brazilian Ministry of Health established the Visceral Leishmaniosis Surveillance and Control Program (*Programa de Vigilância e Controle da Leishmaniose Visceral*, PVCLV) (Brasil, 2006), which is based on three strategies: early diagnosis and treatment of human patients; vector control through environmental management and the use of insecticides; and the identification and euthanasia of the canine reservoir. The last strategy is the subject of considerable controversy, despite its aim to reduce the source of infection for humans (Gontijo and Melo, 2004; Brasil, 2014).

Actions aimed at controlling the vector and the canine reservoir are based on the fact that human incidence is directly related to the number of infectious dogs and vector competence (Werneck, 2014). Thus, knowledge of disease distribution in endemic areas and possible associations between the disease and risk factors assist in the development of control strategies (Frehse et al., 2010, Figueiredo et al., 2014). From this perspective, we sought to determine the distribution and factors associated with CVL positivity in an urban area endemic for this zoonosis.

2. Materials and methods

2.1. Study area

The town of Pereira Barreto is located in the northwestern part of the state of São Paulo (20°38′18″S, 51°06′33″W), has flat topography, a total area of 979.960 km^2 and is situated at an altitude of 347 m above sea level. The climate is tropical, with rainfall in the summer and dry in the winter. The municipal area is divided into five (5) urban sectors and one rural sector defined by the Epidemiological Surveillance Service of the municipality. The urban population was estimated at 25,755 inhabitants in 2014 (IBGE, 2010) and the canine population was estimated at 5224 dogs (São Paulo, 2014). Most of the population lived in single-story buildings and, when residents had pets; most dogs had access to the streets in the period when the data was recorded. The presence of the vector *Lutzomyia longipalpis* in Pereira Barreto has been confirmed, together with autochthonous cases of human and canine VL, thus VL was classified as sporadic transmission (< 2.4 human cases/year) by the time data was collected (Rangel et al., 2013).

2.2. Data collection

As part of the PVCLV in the state of São Paulo, the Pereira Barreto Zoonosis Control Center (ZCC) holds an annual canine serological census survey to identify cases of dogs seropositive for CVL and subsequently euthanize this reservoir (Brasil, 2014). To prepare the dataset containing the variables of interest (sex, age, breed, clinical signs and town sector), the epidemiological surveys of dogs positive and negative for CVL of Pereira Barreto conducted by the ZCC in 2009, 2010 and 2011 were used.

This dataset was organized using Microsoft Excel 2000®. A dog was considered positive when it presented amastigotes in the cytological examination of lymph node aspiration biopsy (PBA) and/or was positive by immunoenzymatic assay (ELISA), followed by confirmation by indirect immunofluorescence assay (IFA), as recommended by the PVCLV-SP at the time (Brasil, 2006).

2.3. Statistical analysis

To verify the association between positivity and the variables (sex, age, breed, clinical signs and sector), the Chi square test was used with the BioEstat program (5th edition). A significance level of 5% was adopted.

Table 1
Number (n) and percentage (%) of dogs negative and positive for CVL in the municipality of Pereira Barreto, SP, Brazil, in the period of 2009–2011, according to the sectors analyzed.

Sector	Total		Pos		Neg	
	n	%	n	%	n	%
Rural	284	100.0	39	13.7	245	86.3
Sector 1	907	100.0	291	32.1	616	67.9
Sector 2	224	100.0	167	74.6	57	25.4
Sector 3	239	100.0	152	63.6	87	36.4
Sector 4	285	100.0	145	50.9	140	49.1
Sector 5	816	100.0	196	24.0	620	76.0
Total	2755	100.0	990	35.9	1765	64.1

Chi square test ($p < 0{,}0001$)

3. Results

A total of 2.755 dogs were tested for CVL during the canine serological survey in the period of 2009 to 2011 in the municipality of Pereira Barreto; 990 of these dogs were positive for CVL resulting in a prevalence of 35.9% (Table 1). Positivity was evidenced in peripheral and non-peripheral areas; all sectors have peripheral areas near small fragments of riparian vegetation close to streams, rivers, and wetlands. In sectors two and three, we observed a higher number of houses with chickens and shaded backyards. A significant difference ($P < 0.0001$) in CVL was observed among all sectors, however, sector two showed the highest CVL positivity (74.6%), followed by sector three (63.6%) (Table 1).

The percentage of positive dogs was significantly different ($P < 0.0001$) for most of the variables analyzed, except for sex ($P = 0.3504$) (Table 2). The age group in which CVL positive dogs were most frequently identified was 37–60 months old 48.2% (163/338). Regarding breed distribution, roughly 43.1% (361/838) of pure breed dogs were positive, while mixed breeds presented 32.8% (629/1917) positivity. The most frequent breeds were poodles 6.9% (68/990), followed by pinschers 6.6% (65/990) and pit bulls 6.4% (63/990).

Of the 2755 dogs, 81.1% (2234/2755) were classified as asymptomatic and 18.9% (521/2755) as symptomatic. Among symptomatic dogs, 44.3% (231/521) were positive, while among asymptomatic dogs 34.0% (759/2234) were positive, showing a statistically significant difference ($P < 0.0001$) between these conditions.

Among symptomatic dogs, the most frequent clinical sign observed during physical examination was onychogryphosis 22.5% (117/521),

Table 2
Number (n) and percentage (%) of dogs negative and positive for CVL in the municipality of Pereira Barreto, SP, Brazil, according to the variable analyzed.

Variable	Total		Pos		Neg		P[a]
	n	%	n	%	n	%	
Sex							
Male	1374	100.0	506	36.8	868	63.2	0.3504
Female	1381	100.0	484	35.0	897	65.0	
Age months							
0–12 s	973	100.0	253	26.0	720	74.0	< 0.0001
13–36	1088	100.0	434	39.9	654	60.1	
37–60	338	100.0	163	48.2	175	51.8	
over 60	356	100.0	140	39.3	216	60.7	
Breed							
Pure	838	100.0	361	43.1	477	56.9	< 0.0001
Mixed	1917	100.0	629	32.8	1288	67.2	
Clinical status							
Asymptomatic	2234	100.0	759	34.0	1475	66.0	< 0.0001
Symptomatic	521	100.0	231	44.3	290	55.7	

[a] Chi square test.