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Geographical Variation in Clinical Signs and Prevalence of *Leishmania* sp. Infection among Dogs in Fortaleza, Ceará State, Brazil

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ABSTRACT

Background: Leishmaniosis are anthroponozoonosis caused by protozoa of the genus *Leishmania*, representing a complex of diseases with significant diversity epidemiological and clinical spectrum and can affect 40% of the canine population. Infected dogs may be asymptomatic or may develop canine leishmaniasis (CL), a severe and progressive disease associated with the appearance of clinical signs. Serological tests are frequently used for screening of dogs, however, these techniques present limitations in terms of reproducibility and specificity. In this way, information on the geographical distribution and prevalence of CL is essential to the implementation of appropriate control measures. Therefore, the aim of this study was to determine geographical variations in clinical signs and prevalence of *Leishmania* sp. infections from dogs in Fortaleza, Ceará state, Brazil.

Materials, Methods & Results: Bone marrow samples of 2829 domestic dogs were collected by puncture for parasitological diagnosis of canine leishmaniasis (CL), being considered positive by the presence of *Leishmania* sp. All dogs were examined and clinical signs were classified as score 0: no clinical signs, score 1: skin lesions, score 2: visceral signs and score 3: skin lesions + visceral signs. Kruskal-Wallis ($P < 0.05$) was used to compare the scores and parasitological diagnosis among the seven regions of Fortaleza. So, it was observed that 72% of dogs were positive and the regions I and V had the highest prevalence (78.3% and 80% respectively), no significant differences among the seven regions. Asymptomatic dogs represented 55% of infected dogs, and the main clinical sign, among symptomatic dogs (45%), was skin lesion, visualized in 43% of dogs. Furthermore, 70% of all dogs had ectoparasites.

Discussion: These results show that, despite of the campaign against the disease, there is no great progress in the control of visceral leishmaniasis in the urban areas like Fortaleza, which present an easy access in the fight against the disease. The diagnostic in dogs based in the Indirect Fluorescent Antibody Test (IFAT) associated to others factors, like irregular serological inquiry and no-treated performer personal, perhaps made possible the permanence of susceptibility animals in this area, beyond of the high number of asymptomatic and no-identified dogs. In this study, it was observed that 55% of dogs were asymptomatic, without showing evidence of risk of transmission to human population, escaping from controlling methods. From this we can imply that most of these dogs to LC are asymptomatic and the prevalence did not differ significantly in different regions of Fortaleza, requiring equal attention throughout the city. Moreover, our results demonstrate that Fortaleza city has favorable areas for the maintenance of the parasite cycle, with a high risk of transmission to human and canine population, and it requires special attention because of the large number of asymptomatic cases. Therefore, it is necessary to control the population of asymptomatic dogs with more efficient controlling methods, with the change of methodology of serological screening raising the number of detected dogs, reducing the diagnostic time and the use of diagnostic techniques with levels of specificity higher.

Keywords: dogs, leishmaniasis, clinical score, parasitological test.

INTRODUCTION

Leishmaniosis are anthroponosis caused by protozoa of the genus *Leishmania*, representing a complex of diseases with significant diversity epidemiological and clinical spectrum [10]. *Leishmania* sp. are digenetic parasites that develop in the promastigote form in the sandfly and amastigote form in vertebrate hosts, such as humans and dogs [2].

Infected dogs may be asymptomatic, either at the beginning of the infection or may develop canine leishmaniasis (CL), a severe and progressive disease associated with the appearance of clinical signs [6,3,11].

Serological tests are frequently used for screening of dogs, mainly the enzyme-linked immunosorbent assay (ELISA) and the immunofluorescence antibody test (IFAT). However, these techniques present limitations in terms of reproducibility and specificity [17]. The definitive diagnosis is obtained only with the demonstration of the parasite (parasitological test). The simplest method is the direct search of amastigotes forms in bone marrow aspirate. However, this technique is very invasive and requires trained professionals to perform [7].

Studies show that in endemic areas, leishmaniasis can affect 40% of the canine population [9]. However, it is suggested that this prevalence is underestimated [1,15].

In this way, it is indispensable to detect infected dogs and to understand the role of asymptomatic dogs as reservoir. Information on the geographical distribution and prevalence of CL is essential to the implementation of appropriate control measures [13].

The aim of this study was to determine geographical variations in clinical signs and prevalence of infection with *Leishmania* sp. from dogs in Fortaleza city, Ceará state, Brazil.

MATERIALS AND METHODS

The experimental protocol was approved by the Ethics Committee for Animal Use of the State University of Ceará (CEUA / UECE), No. 08622833-1.

Geographical area under study

Fortaleza is an urban area located between 3°43'02"S and 38°32'35", situated in the Ceará State coast in the north region of Brazil. It is a hot climate with annual average temperature around 26°C. The

population is approximately 2,393,087 inhabitants, distributed in six Regions Executive Secretary (SER I, SER II, SER III, SER IV, SER V and SER VI) and the Metropolitan Region (MR). The municipality is recovered by a type of complex vegetation of maritime area and it also has perenifolia paludosa maritime forest. The rainy season is between the months January and May, with an annual pluvial index of 1,338 mm. The dog population is estimated in 300 thousand in the municipality, divided in six regions, according to Table 1.

Table 1. Prevalence of canine *Leishmania* infection in different regions of Fortaleza city, Ceará, Brazil between March of 2005 and February of 2009.

Regions	Canine Population	Nº of dogs tested (%)	Nº of PT* positive	Prevalence (%)
SER I	45,616	272 (9.6)	213	78.3
SER II	41,416	265 (9.3)	195	73.5
SER III	46,367	810 (28.6)	518	64.0
SER IV	36,382	323 (11.4)	236	73.0
SER V	63,411	556 (19.6)	446	80.2
SER VI	65,944	272 (9.6)	198	72.8
MR	60,386	331 (11.7)	236	71.3
Total	359,522	2829 (100)	2042	72.2

*Parasitological Test.

Animals

Domestic dogs (2829) from different regions of Fortaleza city, male and female, which were 5 months to 11 years old and varying in weight, were submitted to the parasitological canine leishmaniasis diagnostic, from March 2005 to February 2009. The owners were informed about the risks of this procedure and, after the proprietor authorization, the dogs were submitted to the bone marrow samples collection through the femoral punch.

Parasitological diagnostic

After the collection, imprints from bone marrow samples were done in microscopy slides, fixed with methanol and stained with Panoptic. The visualization was done by optical microscopy under immersion oil and the samples were considered positive by the presence of amastigotes forms of *Leishmania* sp.

Survey design

All dogs were examined for poor body condition and clinical picture. The clinical signs were scored as follows: score 0: no clinical signs, score 1: skin lesions–ulcers, alopecia, desquamation, onychogryphosis; score 2: visceral signs–lymph adenopathy and score 3: skin lesions + visceral signs, according to Guimarães *et al.* [8].

Statistical analysis

The Kruskal-Wallis test was used to compare the clinical score and parasitological test among the seven regions. The relationship was considered significant when $P < 0.05$.

RESULTS

The results were expressed in prevalence to compare the different regions of the city of Fortaleza. The parasitological diagnostic is revealed in Table 2.

The highest prevalence of infected dogs with *Leishmania* sp., according to the parasitological test, was observed in SER I and V. However, no statistically significant differences between the groups were observed (Table 1).

The clinical examination showed that 15% of dogs presented a poor body condition and in 70% of the animals ticks and fleas were detected.

The test showed that 55% of dogs were asymptomatic in clinical examination and 45% were symptomatic. In these symptomatic animals, the clinical signs such as skin lesions like ulcers, alopecia, desquamation, onychogryphosis were more frequent (Table 2).

The highest values of asymptomatic dogs were observed in the regions SER III and SER V. No

statistically significant differences were observed among the seven regions (Figure 1).

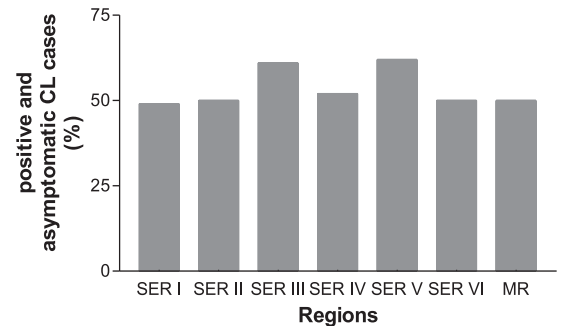


Figure 1. Prevalence of positive and asymptomatic cases of CL in different regions of Fortaleza city, Ceara, Brazil, between March of 2005 and February of 2009.

DISCUSSION

According to the Fortaleza Ministry of Health, 1041 humans were infected with *Leishmania* sp. between 2001 and 2008 years, with 100 deaths recorded in the same period, caused for this infection, being the dogs involved in the domestic transmission cycle.

Then, it is necessary the realization of an elaborate study about the epidemiology of leishmaniosis in Fortaleza, to know the prevalence of this disease in the canine population on different regions and an evaluation of the participation of different vectors in this disease transmission.

Because domestic dogs are implicated as the principal reservoir hosts for *Leishmania chagasi* in the new world, the determination of the prevalence of canine *Leishmania* infection is necessary to define

Table 2. Clinical score of dogs naturally infected by *Leishmania* sp. in different regions of the Fortaleza city, Ceara, Brazil between March of 2005 and February of 2009.

	SER I	SER II	SER III	SER IV	SER V	SER VI	MR	Total
Score 0	118 (49)	111 (50)	275 (61)	133 (52)	241 (62)	111 (50)	133 (50)	1123 (55)
Score 1	116 (48.5)	106 (47.5)	161 (36)	119 (46.5)	143 (36.5)	106 (48)	125 (47)	878 (43)
Score 2	03 (1.5)	06 (2.5)	08 (2)	05 (1.5)	05 (1)	04 (2)	05 (2)	34 (1.5)
Score 3	02 (1)	---	02 (1)	---	02 (0.5)	---	02 (1)	07 (0.5)
Total	239 (100)	223 (100)	446 (100)	257 (100)	391 (100)	221 (100)	265 (100)	2042 (100)

*The data were expressed in n(%).

control measures for zoonotic visceral leishmaniasis [18]. A simple and suitable diagnostic test is essential for a large scale screening of dog populations [12].

According to this work, in Fortaleza, it was observed, between 2005 and 2009, a high prevalence of infected dogs (72.2%) by visceral leishmaniasis, according to the parasitological test, occurring the transmission to human population too, which suggest a relation to infected humans.

These results show that, despite of the campaign against the disease, there is no great progress in the control of visceral leishmaniasis in the urban areas like Fortaleza, which present an easy access in the fight against the disease.

The diagnostic in the dogs based in the Indirect Fluorescent Antibody Test (IFAT) associated to others factors like, irregular serological inquiry and no-treated performer personal; perhaps made possible the permanence of susceptibility animals in this area [16], beyond of the high number of asymptomatic and no-identified dogs.

In this study, it was observed that 55% of dogs were asymptomatic, without showing evidence of risk of transmission to human population, escaping from controlling methods.

Then, according to the seroconversion time, 2 to 8 months, the serological inquires may be realized to 2 months each, increasing the number of positive dogs and decreasing the permanence of infected and asymptomatic dogs in human areas [16].

The proportions of symptomatic cases in infected domestic dogs and the overall prevalence of canine *Leishmania* infection were 45.0% and 72.2%, respectively. These values are higher than the values found by Mohebbali *et al.* [12]. Such a high numbers of infected dogs in lack of clinical signs may be related to development of protective

immunity, especially in older dogs, and their high exposure to *Leishmania* parasites [14].

Similar to the case of Tegumentary Leishmaniasis, a shift from rural to peri-urban and urban distribution of Visceral Leishmaniasis has occurred in North-East Brazil. This changing pattern coincides with that seems to be an adaptation of sandflies that transmit *Leishmania* to the peridomestic areas [19], putting an increased number of individuals at risk.

Also, Coutinho *et al.* [4] suggests that the vectorial capacity of ticks (*Rhipicephalus sanguineus*) for *Leishmania chagasi* should be evaluated further, opening new perspectives in the epidemiology of VL, and Coutinho e Linardi [5] showed that the possibility of oral transmission of *Leishmania chagasi* by fleas can not be proven unambiguously even though the hamsters developed infection.

In conclusion, our results demonstrate that the Fortaleza city has favorable areas for the maintenance of the parasite cycle, with a high risk of transmission to human and canine population, and it requires special attention because of the large number of asymptomatic cases. Therefore, it is necessary to control the population of asymptomatic dogs with more efficient controlling methods.

It suggests the change of methodology of serological screening, raising the number of detected dogs, reducing the diagnosis time and the use of diagnostic techniques with levels of higher specificity.

Furthermore, it suggests more studies to elucidate the levels of phlebotomine vector, the serological research in the dogs, the knowledge of the human population about the disease and the participation of other vectors in this disease transmission, in different regions of the city of Fortaleza.

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