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Canine Visceral leishmaniasis in Itapecerica da Serra, metropolitan region of São Paulo, SP, Brazil: new epidemiological classification

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ABSTRACT

OBJECTIVE

The Leishmaniasis are a group of anthropozoonoses diseases that are considered a major public health problem, and they represent a variety of diseases with important clinical and epidemiological diversity. Visceral leishmaniasis, a severe form of leishmaniasis, is caused by *Leishmania infantum*. It has a wide geographic distribution and an annual incidence of two million cases and 350 million people at risk, with domestic canids as the main reservoir. The objective of this study was to evaluate the presence of parasites of the genus *Leishmania* spp. in dogs from the municipalities of Itapecerica da Serra, Cotia, and Juquitiba, in metropolitan region of São Paulo, Brazil.

METHODS

The molecular, serological and parasitological diagnosis (isolation) was carried out in 153 samples of dogs from shelters.

RESULTS

Serological diagnosis showed 9.8% (15/153) positivity for anti-Leishmania antibodies and molecular diagnosis specific for *Leishmania infantum* showed 5.2% (8/153) positivity. The parasitological examination of animals previously positive in the methods used in this study was performed by fine needle puncture of the popliteal lymph node and only one animal was positive in the municipality of Itapecerica da Serra.

CONCLUSIONS

The specific molecular tests for *Leishmania infantum* corroborated the presence and circulation of the agent in the municipalities that make up the metropolitan region of São Paulo. Additionally, it draws particular attention to a new epidemiological classification of the municipality of Itapecerica de Serra, contributing to the updating of epidemiological surveillance measures for the disease in the municipality.

DESCRIPTORS

Cathepsin, Phlebotomine, Epidemiology, Neglected Diseases, *Canis familiaris*, *Leishmania infantum*.

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INTRODUCTION

Leishmaniasis are a group of zoonotic diseases that are widely distributed, characterized by insidious and chronic progression, and caused by different species of flagellated protozoa of the genus *Leishmania*[1]. The disease can be classically subdivided into tegumentary and visceral forms, the latter being responsible for more severe clinical conditions. In the American continent, visceral leishmaniasis is etiologically linked to the species *Leishmania (Leishmania) infantum*[2,3].

The mechanism of disease transmission involves insect vectors belonging to the taxonomic group of sand flies, whose females inoculate the protozoan into a possible host when feeding on its blood[4,5]. Dogs are epidemiologically considered the main reservoirs of *L. infantum*, participating in the disease cycle and being the main link in the transmission chain of visceral leishmaniasis. This finding is based on the prevalence of the disease in the canine population and that the human cases are usually preceded by canine cases. Dogs tend to have a greater number of parasites, leading to greater possibility of infecting a vector, which are very close to the ecological niches occupied by human populations[6,7]. The epidemiological condition becomes more severe as both symptomatic and oligosymptomatic (and asymptomatic) dogs are equally important sources of infection for competent vectors[8].

Despite the great importance of this disease, it remains difficult to standardize diagnostic methodologies with high predictive values. The established direct diagnostic methodologies that are usually considered as the gold standard are invasive, laborious, and have low sensitivity values[9]. Serological tests also present a series of technical limitations, such as low-specificity values because of cross-reactivity with other Trypanosomatids, low agreement levels among different serological tests, and no consensus on the nature and use of antigenic products[10,11]. Thus, the use of molecular diagnostic methodologies that positively meet the strict stages of effective validation and demonstration becomes interesting and plausible, so as to increase the accuracy of clinical diagnostic routines and epidemiological surveys[3].

Visceral leishmaniasis has been rapidly expanding in the state of São Paulo and this deserves special attention. This spatio-temporal expansion has led to an increase in the number of cities being affected and cases and deaths reported. This situation is particularly alarming as this trend moves toward the metropolitan region of São Paulo, which has one of the largest population densities in the country, thus providing a high number of susceptible hosts[12]. Therefore, a better understanding of this phenomenon in the cities affected and identification of determinants and predisposing factors can contribute to the implementation of effective surveillance and control actions, thereby preventing visceral leishmaniasis from spreading into new endemic territories or at least having its progress hampered.

Considering this scenario, the objective of the present study was to perform serological, molecular, and parasitological detection of Leishmania infantum in dogs from the municipalities of Itapecerica da Serra, Cotia, and Juquitiba, in the metropolitan region of São Paulo, SP, Brazil.

METHODS

The study was approved by the Animal Ethics Committee of Santo Amaro University (protocol no. 05/2019). The sample population included dogs from the same shelter with units in Cotia, Itapecerica da Serra, and Juquitiba, located in the southwestern subregion of the metropolitan region of São Paulo, which extensively borders the city of São Paulo, SP, Brazil.

Blood samples were collected from the jugular, radial, and cephalic veins of the dogs, and then, the total collected volume was fractionated and packed in microtubes with absolute ethanol (1:1) and in dry tubes with clotting factors for the subsequent collection of serum that was frozen.

The serum fractions obtained were used in immunochromatographic assays to detect anti-*Leishmania infantum* antibodies. The rapid diagnostic kit DPP® (Biomanguinhos/Fiocruz) was used, with antigenic substrates corresponding to K9/K26/K39 proteins.

Blood samples preserved in absolute ethanol were used for DNA extraction using the commercial PureLink Genomic DNA Mini Kit (Invitrogen®). The extracted nucleic acids were quantified using miniaturized spectrophotometry to verify extraction efficiency. The extracted samples were used for the molecular diagnosis of *L. infantum* using polymerase chain reaction (PCR) based on the specific cathepsin L-*like* marker[3], which was verified using electrophoresis (1.5% agarose gel) and subsequent visualization using ultraviolet transilluminator.

The dogs that presented positive results in serological and/or molecular tests underwent popliteal lymph node aspiration and, when possible, new peripheral whole-blood collection. To parasitological diagnosis, the samples were inoculated into culture tubes with a biphasic medium consisting of blood agar base (BAB) medium as the solid phase and liver infusion tryptose (LIT) medium as the liquid phase and maintained for 28°C and inspected from 4 days for period of 10 days.

RESULTS

A total of 153 dogs were evaluated: 42 from Cotia, 30 from Itapecerica da Serra, and 81 from Juquitiba. Of these, 9.8% (15/153) were positive for anti-*Leishmania infantum* antibodies using the immunochromatographic method and 5.2% (8/153) were positive in the molecular diagnosis using the cathepsin L-*like* marker (Table 1).

Parasitological tests were performed on the dogs that were positive in serological and/or molecular tests. However, it was not possible to perform the parasitological test on all the dogs because the shelter keepers and/or new owners refused to subject their dogs to the test. Only two dogs from Itapecerica da Serra and one from Cotia underwent fine needle popliteal lymph node aspiration, and only one dog from Itapecerica da Serra tested positive in the parasitological test.

Table 1. Frequency of positive dogs from serological and molecular tests to *Leishmania* spp in municipalities in metropolitan region of São Paulo, SP, Brazil

Municipality	Sampled individuals	Positive samples	
		DPP	CatLeish_PCR
Cotia	42	4/42 (9,52%)	1/42 (2,38%)
Itapecerica da Serra	30	4/30 (13,33%)	7/30 (3,33%)
Juquitiba	81	7/81 (8,64%)	0/81 (0%)
Total	153	15/153 (9,8%)	8/153 (5,22%)

DISCUSSION

Visceral leishmaniasis has been a public health problem in Brazil since 1934, when the first case of human visceral leishmaniasis was reported in the country. In the late 20th century, leishmaniasis forms, which seemed to be controlled, re-emerged in rural and urban areas, and appeared in areas considered free of this endemic disease due to environmental changes, human migration, chaotic urban growth, and other socioeconomic factors in large areas of the Brazilian territory[13].

Brazil is one of the countries with the highest incidence of visceral leishmaniasis in the world, and Brazilian authorities recognize it as one of the most challenging public health problems[14]. However, leishmaniasis remains as a neglected tropical

disease and the second leading cause of death from a parasitic disease worldwide[15].

Despite the great importance of this disease, it remains difficult to standardize highly predictive diagnostic tests[3,16], which explains the lack of agreement across diagnostic test results, corroborating literature reports that the available diagnostic tests have a series of technical limitations, including specificity values, because of cross-reactions with other etiologic agents[17,18,19].

In addition, immunochromatographic serological test results depend on the stage of infection in asymptomatic dogs, with increased sensitivity in the presence of clinical symptoms[17,18]. The humoral immune response in dogs infected with *Leishmania infantum* almost always involves multiple antigens that are recognized in different ways in distinct individuals and at various stages of the disease in the same host. This finding suggests that, whenever possible, it is preferable to use parallel or multiple antigen tests to identify many infected animals. Moreover, some studies show that these methods have higher specificity than other serological tests (ELISA) included in the official protocols for the confirmatory diagnosis of canine visceral leishmaniasis in Brazil[19,20].

The municipalities included in this study were close to each other, facilitating the movement of the dogs across them and to the city of São Paulo. The intense movement of the dogs is corroborated by the history of the positive animals obtained in the present study. Only one dog tested positive in all the tests. It presented clinical symptoms and has been under treatment for three years with a drug protocol established for dogs in Brazil. This dog came from the city of Vargem Grande and was adopted; this dog lives in Itapecerica da Serra and is constantly transported to the municipality of Ibiúna. Another dog who tested positive in the molecular diagnostic test came from Itapecerica da Serra and has lived in the municipality of Cotia. The third dog who tested positive in the molecular test was rescued in São Paulo, in the neighborhood of Grajaú, and was sheltered in the city of Cotia and adopted by a family from São Paulo. The other dogs that tested positive in the molecular test were born and have been living in Itapecerica da Serra.

The installation of shelters follows the guideline of the State Decree 40400/95, which restricts establishments from keeping animals within the urban perimeter, aiming to reduce odor, noise, and proliferation of rodents and harmful arthropods. These measures favor the installation and maintenance of commercial kennels and shelters in peripheral areas of São Paulo or in other municipalities of the metropolitan region that have a large remaining forest cover and consequently offer greater chances of contact with the insect vectors of the disease than in the metropolitan areas.

Itapecerica da Serra has been considered a silent, non-receptive, vulnerable municipality; in other words, there has been no confirmation of autochthonous human and canine cases of the disease as well as presence of the vector. Therefore, this is the first study to demonstrate the presence of *Leishmania infantum* in this municipality in dogs, reinforcing the need for more comprehensive surveys in the region involving both vertebrate reservoirs and insect vectors.

The present study together with other studies that demonstrate the presence of this pathogen in the metropolitan region of São Paulo have great significance because the metropolitan region of São Paulo is considered the fourth largest region in the world by population density, which has led to the high concentration of pets[20,21]. Therefore, it is essential to plan and implement public epidemiological surveillance policies that offer reasonable and feasible alternatives to prevent the geographic expansion of the disease, which would decrease the incidence and prevalence of visceral leishmaniasis.

CONCLUSION

This study showed that *Leishmania infantum* was diagnosed in dogs from Cotia, Juquitiba and Itapecerica da Serra by serological test and in Cotia and Itaperecica da Serra by molecular test. This is the first study to demonstrate the presence of *Leishmania infantum* in Itapecerica da Serra.

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