



Detection of *Leishmania infantum* in dogs (*Canis familiaris*) from São Paulo municipality, Brazil

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ABSTRACT

OBJECTIVE

Visceral leishmaniasis is caused by *Leishmania infantum*. In the state of São Paulo, this zoonosis is expanding to urban areas and the incidence of human disease is related to canine infection. Herein, we performed a serological and molecular diagnosis on 152 dogs (*Canis familiaris*) from the Grajaú and Ilha do Bororé neighborhoods, in the extreme south of the São Paulo municipality, Brazil.

METHODS

We observe the prevalence of *L. infantum* in these animals that are identified as the main reservoirs of the etiologic agent of visceral leishmaniasis.

RESULTS

Serological tests showed a low positivity rate, while the molecular tests showed positive animals at 3.94% and 0.65% for the *Leishmania infantum* L-like cathepsin and ITS of ribosomal gene, respectively.

CONCLUSIONS

Molecular diagnosis of 3.94% positivity suggesting the introduction of *L. infantum* in this locality of São Paulo, which still preserves 80% of the Atlantic Forest. Our data point to the need for effective control of infected dogs to prevent the spread of visceral leishmaniasis in humans.

DESCRIPTORS

Leishmania infantum. Dogs. Serology. Molecular diagnosis. São Paulo. Brazil.

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INTRODUCTION

Visceral leishmaniasis is caused by the protozoan *Leishmania infantum*¹, and is a zoonotic parasitosis is classified by the World Health Organization as one of the most important neglected tropical diseases. The disease affects about 80 countries in Asia, Africa and Latin America². Of the total amount of records, 90% are concentrated in Bangladesh, India, Nepal, South Sudan and Brazil. Brazil contributes with 90% of the cases documented in the American continent³.

In the interval between 2013 and 2015, the transmission of visceral leishmaniasis was reported in 1339 municipalities in 21 of the 27 Brazilian states; in 2015 the incidence coefficient reached an annual average of 1.6 cases/100,000 inhabitants. In São Paulo state, data from epidemiological series as well as statistical projection models show that the disease is in a clear dispersion, with a diffusion route towards the metropolitan region, with the largest demographic contingent in the country⁴. The São Paulo municipality is classified as a vulnerable non-receptive silencer, and only the municipalities of Cotia and Embu, which belong to the metropolitan region of São Paulo, are considered with canine transmission.

In the epidemiology of the disease, dogs are considered the main reservoirs of the pathogen, so that the main clinical implications acquired as a function of the infection are: biochemical changes in the urine, onychogribose, progressive thinning, formation of alopecia areas followed by desquamation, presence of lesions pustular, exfoliative or ulcerative disorders, adenitis, spleen and liver involvement, leukocytosis and neutrophilia, lymphopenia and thrombocytopenia⁵. Studying the behavior of the leishmaniasis in dogs can help in our understanding of the disease as a whole and of how it integrates within human health. However, despite the clinical and epidemiological impact of the disease, data from leishmaniasis surveys in canine populations in the São Paulo municipality of are rather scarce and insipient.

In this work, we investigated the occurrence of *Leishmania infantum*, by serological and molecular diagnosis, in dogs from the Grajaú and Ilha do Bororé neighborhoods located in the extreme south of the São Paulo municipality, Brazil. Our data point to the need for effective control measures for infected dogs to prevent the transmission of visceral leishmaniasis in the human population.

METHODS

Study area and sample collection

This study was conducted in Grajaú and Ilha do Bororé, are neighborhoods located in the extreme south of São Paulo municipality. Particularly the Ilha do Bororé surrounded by the Billings Dam. The neighborhood has about 80% of its useful area covered by the vegetation of the Atlantic forest, preserved in private sites and properties. Some points of irregular occupation are noted in the São Paulo neighborhood, and there are large quantities of dogs in the residence and/or abandoned on the streets.

Sampling included dogs of both sexes, older than three months and of mixed breeds. At the time of collection the animals underwent a physical evaluation that included observation of the oral mucosa, search for dermatological lesions, attribution of body score and palpation of pre-scapular and popliteal lymph nodes. A total of 152 animals were restrained for physical examination and collection of biological material. Two aliquots of blood were collected, from the cephalic vein or, if this was not possible, jugular vein was collected from each animal, one was packed in sterile tubes and then centrifuged for physical separation of plasma and red blood

cells and a second aliquot of blood was transferred to sterile ethanol-containing tubes for PCR analysis. All procedures were performed in accordance with the Ethics Committee on Animal Use (05/2016).

Serological analysis

The serum of these animals was used to perform different serological techniques: a) Rapid test of DPP® canine leishmaniasis (Biomanguinhos / FIOCRUZ); b) ELISA immunoenzymatic test - (Canine Visceral Canine Leishmaniasis Kit, Biomanguinhos/FIOCRUZ) and c) Indirect immunofluorescence reaction (IFAT) with *Leishmania infantum* promastigote forms antigen obtained from a dog from the State of Maranhão-Brazil and deposited in the Brazilian Collection of Trypanosomatids of the Department of Veterinary Preventive and Animal Science of FMVZ-USP. Cutoff point for determination of reagent result was the 1:80 dilution. For all tests the manufacturer's instructions were followed and samples of animals known to be infected and uninfected were used as a control.

Extraction and amplification of DNA from dogs blood

Blood samples stored in absolute ethanol had their DNA extracted using the Purelink Genomic DNA Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The extracted DNA was quantified in a spectrophotometer and used in the conventional PCR reactions using as markers the genes of and ribosomal internal transcribed spacer (ITS SSUrDNA)⁶ and cathepsin L-like⁷. As a positive control of the reaction, DNA from cultures of *L. infantum* was used.

RESULTS

A total of 152 dog samples were collected in the neighborhoods of Grajaú and Ilha do Bororé. Among these, thirteen animals presented some dermatological lesions, six had hypercorated mucous membranes, two had cachexia and no animals had altered lymph node size.

The immunochromatographic test revealed only one positive animal (0.65%) whereas the ELISA immunoenzymatic assay did not show positivity for any tested dog. The test based on the RIFI technique demonstrated positivity for the same animal that was also positive in the immunochromatographic test (Table 1).

The molecular assay revealed six positive animals for the cathepsin L-like gene (3.94%) and one positive animal for the ITS gene (0.65%), and this positive animal in the ITS gene was equally positive for the gene of cathepsin L-like. The positive animal serologically by ELISA and immunochromatographic method was positive for the molecular test based on the cathepsin L-like gene (Table 1).

Table 1 - Percentage of positivity of samples of dogs tested by diagnostic methods of visceral leishmaniasis.

Tested dogs	Diagnostic Methodology				
	Serological Testing			Molecular Assay	
	DPP®	ELISA	RIFI	Cathepsin	ITS
Reagent Animals (%)	01 (0,65%)	00 (0%)	01 (0,65%)	06 (3,94%)	01 (0,65%)
Non-reactive animals (%)	151 (99,35%)	152 (100%)	151 (99,35%)	146 (96,06%)	151 (99,35%)

DISCUSSION

In the state of São Paulo, the first identification of the presence of vectors of visceral leishmaniasis occurred in Salto de

Pirapora city, this epidemiological finding was the first one to call attention to an eventual possibility of transmission of the disease in the state⁸. However, the disease acquired intense transmission status only in the 1990s, with the emergence in the northwest region of the state. These findings were initially concentrated in the Araçatuba municipality in which the vector was found in 1997, and only two years later the first autochthonous cases were recorded in humans⁹.

During the following decade the spread of the disease was accentuated; in 2011, the disease was already accumulating notifications in 73 cities in the western region of São Paulo¹⁰.

The main factors that explain this wide dispersion are the increase in the vector densities of the arthropod vectors and the migration of susceptible and infected hosts, both caused by anthropic interventions in the natural space, among them the expansion of sugarcane monoculture, the interconnection with the Mato Grosso do Sul via the Marechal Rondon highway, the Noroeste road and the Brazil-Bolivia gas pipeline works¹⁰⁻¹².

The retrospective data of the epidemiological series and the analysis of risk factors allowed the inference of predictive models that indicate a route of dispersion of the disease that leaves from this region of the northwest of São Paulo towards the eastern region of the state⁴. In order to represent a threat to the metropolitan region and consequently to the city of São Paulo that have a high population.

The results of this work corroborate a hypothesis of dispersion of the disease by showing that there are dogs infected by *L. infantum* in São Paulo municipality, contributing to the increase of data referring to this important public health zoonosis in the largest city in Latin America and corroborate the data of parasitological presence of *L. infantum* in metropolitan region of São Paulo¹³.

Considering the diagnostic protocol recommended by Brazilian health surveillance authorities, no animal in the study can be serologically classified as positive for canine visceral leishmaniasis, since only positive screening tests (ELISA) are tested in the immunochromatographic test.

The incidence of infected reservoirs plus the presence of potential vectors for the protozoan in the metropolitan region indicates a high risk of increased prevalence of visceral leishmaniasis in these urban cycles¹⁴. Therefore, this context of disease expansion indicates that the measures widely used since the second half of the 20th century and purely based on the application of insecticides and euthanasia of seropositive dogs are insufficient, and it is necessary to adopt integrated prevention and control strategies that work in all points of the environmental dynamics of transmission.

ACKNOWLEDGMENTS

We would like to thank the support provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

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