BRIEF COMMUNICATION

FIRST OCCURRENCE OF AN AUTOCHTHONOUS CANINE CASE OF *Leishmania (Leishmania)*

*infantum chagasi* IN THE MUNICIPALITY OF CAMPINAS, STATE OF SÃO PAULO, BRAZIL

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SUMMARY

An autochthonous case of visceral leishmaniasis is reported in a dog (*Canis familiaris*) as an apparently natural infection in a non-endemic area. DNA obtained from spleen and liver samples produced the expected fragment in a *Leishmania*-specific rDNA-based nested-PCR assay. The PCR product, a 490 bp fragment, was sequenced and the nucleotide sequence was identical to that of *Leishmania (Leishmania)* *infantum chagasi*. These results are surprising since no autochthonous human or canine cases of visceral leishmaniasis have ever been reported in this municipality. This case suggests that natural transmission of this disease is occurring in this area.

KEYWORDS: Visceral leishmaniasis; PCR; Epidemiology; Dogs; São Paulo, Brazil.

INTRODUCTION

Visceral leishmaniasis is a vector-borne disease mainly characterized by spleen and liver enlargement. In the Americas, this zoonosis is caused by *Leishmania (Leishmania)* *infantum chagasi* and domestic dogs are an important animal reservoir.

American visceral leishmaniasis (AVL) is showing a gradual spread throughout the State of São Paulo. The first autochthonous canine and human cases were verified at Araçatuba county region. Other areas of transmission, such as the cities of Cotia and Embu have been identified. Usually, canine visceral leishmaniasis precedes human cases, thus the detection of new areas of transmission in animals is crucial for the epidemiological surveillance of leishmaniasis and the control program of AVL in the State of São Paulo.

MATERIALS AND METHODS

In September 2009, a 9 year-old domestic female dog, called Athena, was taken to a local veterinary hospital. The dog had lost both weight and muscle mass, presented a cough, increased lymphatic ganglia, liver and spleen, onychogryphosis and skin lesions.

The dog was born and raised in the metropolitan area of Campinas, State of São Paulo, Brazil. It lived in a residential complex built in an area of environmental protection between Sousas and Joaquim Egidio sub-districts, in the eastern zone of the municipality. The region presents topography and vegetation characteristics that favor the presence of sandflies.

Athena was euthanized at the veterinary hospital, at the request of the owner, and sent to the Campinas Zoonosis Control Center for autopsy.

Spleen and liver samples and bone marrow and lymph node aspirates were sent to the São Paulo Zoonosis Control Center for parasitological and molecular tests.

Tissue samples were inoculated into blood agar base culture medium with brain heart infusion and incubated at 23 °C during one month.

DNA from every sample was extracted and submitted to a nested SSU rDNA-based PCR to detect and identify the parasite. Reactions took place in a final volume of 50 μL containing 1X PCR buffer, 2 mM MgCl₂, 0.2 mM dNTPs, 0.2 μM of each primer and 2U of *Taq* DNA polymerase (Life Technologies). The first PCR was performed using S4 and S12 primers. The 520 bp fragment produced by S4/S12 PCR was used as the template in a nested-PCR with primers S17 and S18. The 520 bp fragment produced by S4/S12 PCR was used as the template in a nested-PCR with primers S17 and S18. The reactions were performed under the same conditions as those described above.
The S4/S12 PCR product (1 μL) was denatured at 94 °C for four min and cycled 30 times; each cycle was performed at 94 °C for one min, 55 °C for one min and 72 °C for 30 sec.

The nested-PCR product, a 490 bp fragment, was purified and sequenced. Sequencing reactions were performed using ABI BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA, USA), through 25 cycles (96 °C/10 sec, 50 °C/5 sec and 60 °C/4 min). Direct sequencing of the purified amplicons was performed using an ABI 3130-XL sequencer (Applied Biosystems, Carlsbad, CA, USA). Sequences were analyzed using the BLAST program (http://www.ncbi.nlm.nih.gov/blast.html) to confirm amplification of the specific product. Sequences were imported into BioEdit® compared with the rDNA sequence of reference strains and S17 and S18 amplicons from spleen (1) and liver (2) samples from Organism identification by SSU rDNA sequence comparison. Nucleotide sequences of reference strains and S17 and S18 amplicons from spleen (1) and liver (2) samples from dog Athena were aligned and compared.

The municipality of Campinas was previously classified as silent, nonresponsive and not vulnerable to AVL. Based on the present results, a research focus was initiated in the same area where the dog Athena lived, involving blood collection from 198 dogs. This led to the identification of three other dogs with AVL and recorded the first evidence of Lutzomyia longipalpis in the city of Campinas. The Study Committee on Leishmanioses had already reclassified Campinas as a transmission area for canine AVL, although in a very circumscribed zone.

These findings indicate that entomological and canine surveillance must be maintained in Campinas to detect new cases of AVL in dogs to prevent and control this zoonosis. In this context it is also important to raise awareness in health professionals to the possible occurrence of human cases of AVL in order to indicate early treatment.

RESULTS AND DISCUSSION

The serum sample was positive at a 1:40 dilution by indirect fluorescence test for leishmaniasis at the Tecsa Laboratory.

No parasites were detected in any of the samples inoculated into the culture medium. Otherwise, liver and spleen samples and bone marrow and lymph node aspirates were positive for Leishmania when using the nested SSU-rDNA-based PCR strategy (Fig. 1). The fragments produced by S17 and S18 oligonucleotides were sequenced and identified as Leishmania (Leishmania) infantum chagasi parasite (Fig. 2).

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RESUMO

Ocorrência do primeiro caso autócctone canino por Leishmania (Leishmania) infantum chagasi no Município de Campinas, Estado de São Paulo, Brasil

Caso autócctone de leishmaniose visceral é relatado em cão (Canis familiaris), aparentemente em área não endêmica. DNA obtido a partir de amostras do baço e fígado foram submetidos a nested-PCR baseada no rDNA específico de Leishmania. Os produtos das PCR foram sequenciados e os 490 pares de base foram idênticos a Leishmania (Leishmania) infantum chagasi. Esses resultados são surpreendentes, uma vez que, nenhum caso autócctone canino ou humano de leishmaniose visceral havia sido relatado neste município. Esse caso sugere que a transmissão natural da doença está ocorrendo nesta área.

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