Autochthonous case of Canine Visceral Leishmaniasis in a non-endemic area in Minas Gerais, Brazil

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ABSTRACT.- Faria M.T., Barbosa F.S., Teixeira-Neto R.G., Pinheiro G.R.G., Manhani M.N., Marcelino A.P., Barbosa J.R. & Fontes G. 2017. Autochthonous case of Canine Visceral Leishmaniasis in a non-endemic area in Minas Gerais, Brazil. Pesquisa Veterinária Brasileira 37(12):1505-1508. Campus Centro Oeste, Universidade Federal de São João del Rei, Rua Sebastião Gonçalves Coelho 400, Bairro Chanadour, Divinópolis, MG 35501-296, Brazil. E-mail: gilberto.fontes@pq.cnpq.br

Visceral Leishmaniasis by Leishmania infantum chagasi is an endemic zoonosis present in many areas of Brazil. This parasite needs reservoirs for maintenance of the infection and the presence of dogs in urban areas is a key factor for the spread of canine visceral leishmaniasis (CVL). The aim of this study was to report the first autochthonous case of CVL in the municipality of Iguatama, in west central region of Minas Gerais State. Dog infection by Leishmania infantum chagasi was confirmed in the municipality, previously considered as non-endemic area to CVL. The canine infection by Leishmania was confirmed by three immunological tests for antibodies: indirect immunofluorescence assay (IFA), rapid Dual Path Platform (DPP®) CVL immunochromatographic test, enzyme-linked immunosorbent assay (ELISA), and microscopic demonstration of Leishmania amastigotes in imprints of spleen and bone marrow stained by Giemsa. The species Leishmania infantum chagasi was confirmed by molecular diagnosis (PCR). Studies are being carried out, aiming to describe the importance and the prevalence of this disease in the region and factors associated with its transmission.

INDEX TERMS: Leishmania infantum chagasi, canine visceral leishmaniasis, dogs, zoonosis.

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RESUMO.- [Caso autóctone de Leishmaniose Visceral Canina em área indene em Minas Gerais.] Leishmaniose visceral causada por Leishmania infantum chagasi é uma zoonose endêmica em algumas regiões do Brasil. Este parásito necessita de reservatórios para a manutenção da infecção e a presença de cães em áreas urbanas é um fator importante para a manutenção e expansão da leishmaniose visceral canina (LVC). O objetivo deste estudo foi relatar o primeiro caso autóctone de LVC no município de Iguatama, na região Centro Oeste de Minas Gerais, cidade onde a LVC era tida como não existente. A infecção canina por Leishmania foi confirmada por três testes imunológicos para pesquisa de anticorpos: reação de imunofluorescência indireta (RIFI), teste rápido de imunocromatografia com plataforma dupla (DPP® LVC) e ensaio imunoenzimático (ELISA), e demonstração microscópica de amastigotas de Leishmania a partir de aposições de amostras de baço e de medula óssea corados pelo Giemsa. A espécie Leishmania infantum chagasi foi confirmada por diagnóstico molecular (PCR). Estudos estão sendo realizados com o objetivo de descrever a importância e a prevalência desta parasitose na região e os fatores associados com a transmissão.

TERMOS DE INDEXAÇÃO: Leishmania infantum chagasi, leishmaniose visceral canina, caninos, zoonose.
INTRODUCTION

Human visceral leishmaniasis (HVL) is an endemic zoonosis in many areas of Brazil, caused by *Leishmania (Leishmania) infantum* (syn. *Leishmania chagasi*) (Mauricio et al. 2000). Recently, Marcili et al. (2014) by phylogenetic studies on trypanosomatid validates *Leishmania infantum chagasi* from Brazil differentiating it from *L. infantum* for the first time. HVL represents a problem of public health due to its high morbidity and wide geographic distribution (Brasil 2006). The dog has an essential role in the maintenance of this parasitosis and as a result of its proximity to the humans it is considered the main reservoir of the aethiologic agent of visceral leishmaniasis (VL) in urban areas (Gontijo & Melo 2004, Teixeira-Neto et al. 2014). The canine infection has been pointed out as an essential factor in every registered outbreak of HVL, and no HVL cases are reported without the presence of infected dogs (Oliveira et al. 2008, Teixeira-Neto et al. 2014). The increasing of VL cases and disease’s morbidity rates in the state of Minas Gerais, associated to the role of the dog in the biological cycle of the parasite, makes the study of canine visceral leishmaniasis (CVL) important to understand clearly the epidemiology of the disease (Gontijo & Melo 2004, Dantas-Torres & Brandão-Filho 2006, Oliveira et al. 2008, Teixeira-Neto et al. 2014).

The city of Iguatama, located on the west central region of the state of Minas Gerais it is not considered, up to the moment, an endemic area of the disease since there are no reports of HVL or CVL cases or epidemiological studies about leishmaniasis in the municipality. This paper describes the first autochthonous case of CVL reported in Iguatama.

MATERIALS AND METHODS

An about two-year-old mixed-breed male dog, born and raised indoors in Iguatama urban area, was found presenting suggestive signs of infection by *Leishmania*. Physical examination was carried out to evaluate the animal. However, clinical diagnosis of CVL is hard to be determined due to the great variability of clinical alterations that *Leishmania*-infected dogs may present including non-specific signs commonly observed in dogs with other diseases (Brasil 2006). Thus, the laboratorial diagnosis of canine leishmaniasis has been confirmed using serological exams to avoid clinical misdiagnosis.

Three tests were performed to confirm CVL by antibodies detection: Rapid immunochromatographic test based in dual path platform (DPP), the immunofluorescent-antibody test (IFA), and the enzyme-linked immunosorbent assay (ELISA), using the respective TR DPP® CVL, EIE-LVC and IFI-LVC serological kits, all of them produced by Instituto de Tecnologia em Imunobiológicos, Bio-Manguinhos, Fiocruz, Brazil) according to the instructions provided by the manufacturer. IFA was considered positive at serum dilution >1:40.

DPP® and ELISA are currently recommended by the Ministry of Health for CVL diagnosis. IFA was used until 2012 to confirm CVL diagnosis in ELISA positive dogs. Serodiagnosis were carried out at the Laboratório de Parasitologia da Universidade Federal de São João del Rei - Campus Centro-Oeste, in Divinópolis, Minas Gerais, accredited by the Ministry of Health of Brazil and Ezequiel Dias Foundation (FUNED) to carry out serological tests of CVL.

According to the Ministry of Health, it is recommended that dogs identified as seropositives by DPP® and ELISA as a confirmatory test must be euthanized, using the protocol regulated by the Federal Board of Veterinary Medicine (CFMV) (Brasil 2006).

Parasitological examinations were used to confirm infection by *Leishmania*. Imprinting of biological samples were collected, stained by Giemsa and examined under optical microscopy for identification the amastigotes forms of the parasite. Part of the same material was used to identify the *Leishmania species* involved in the dog’s infection using DNA-based techniques. In the present study PCR-RFLP was used having as target *HSP70* gene (1,300 bp), followed by digestion with *HaeIII* restriction enzyme for parasite identification.

RESULTS

The tested dog serum sample was positive for canine leishmaniasis (CL) by three methods, DPP®, ELISA and IFA (serum dilution = 1:80).

The main clinical signs observed in the infected dog were: severe weight loss, dermatitis, alopecia, opaque bristles, keratoconjunctivitis, corneal opacity, blindness of one of the eyes, ulcerative lesions and paw edema (Fig.1A-C).

The dog was in debilitated conditions and as the three serological tests indicated *Leishmania* infection (CL), the owner authorized euthanasia of the animal for further parasitological confirmation.

After the euthanasia procedure imprints of the spleen, lymph nodes and bone marrow revealed the presence of *Leishmania* amastigotes using optical microscopy (Fig.1D).

The spleen sample tested by PCR-RFLP presented a restriction enzyme pattern comparable to the reference strain of *Leishmania infantum chagasi* (MHOM/BR/74/PP75), proving the dog was infected with the causative agent of CVL (Fig.2).

![Fig.1. Clinical signs of the first dog associated with canine leishmaniasis in Iguatama municipality.](image-url)
etologic agents, hosts, and vectors (WHO 2013). Further investigations are being carried out to identify the vectors of the studied area considering sand fly identification and vector control is essential to prevent leishmaniasis spread. Other control measures to reduce or interrupt transmission of the disease are: migration control of dogs from endemic areas, mandatory serological diagnosis and reporting system for CVL, and educational measures targeting dog owners, veterinarians and civil society are important measures to prevent disease dissemination in Brazil (Figueiredo et al. 2012).

In addition to their proven roles as reservoirs of the aethiologic agent of visceral leishmaniasis (VL), dogs are also suspected by researchers to be reservoirs of cutaneous leishmaniasis. Therefore, when the first autochthonous case of CVL in a municipality is diagnosed through serological tests, identification of the parasite must be required in order to identify the Leishmania specie involved (Montalvo et al. 2010, Silva et al. 2010). Various techniques can be used to characterize the species responsible for the infection, such as molecular biology and immunohistochemical methods (Brasil 2006). PCR-RFLP method allows the discrimination of the majority of Leishmania species of significant medical and veterinary importance on the Old and New World, including the main species found in Brazil: Leishmania infantum, L. amazonensis, L. braziliensis, L. guyanensis (Quaresma et al. 2009).

In a study carried out on domestic dogs of Belo Horizonte PCR was used to detect Leishmania in biological material from naturally infected dogs (Quaresma et al. 2009). In this study, the PCR positive samples were further submitted to RFLP and two species were found parasitizing the dogs, L. infantum and L. braziliensis, being the last uncommon in the region (Quaresma et al. 2009). Thus, identifying the species causing CVL is essential to epidemiological understanding of the disease.

The main challenges for the VL surveillance on the country are related to the lack of available resources, especially in country towns, and also the absence of epidemiological research of the distribution of VL.

Studies to determine the distribution of CVL, especially in regions with no previous reports, are extremely important to identify new areas of transmission of the parasite (Teixeira-Neto et al. 2014). Thus, controls measures can be taken in order to break the chain of and avoid the spread of VL.

The results of this study confirm the first case of a dog naturally infected with Leishmania infantum chagasi in the municipality of Iguatama, on the west central region of the state of Minas Gerais. Further studies are necessary to evaluate the epidemiological situation in the area, in order to stop the expansion of the canine disease and consequently the emergence of human cases.

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