Veterinary Parasitology 197 (2013) 665–669

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

First evidence of autochthonous cases of *Leishmania (Leishmania) infantum* in horse (*Equus caballus*) in the Americas and mixed infection of *Leishmania infantum* and *Leishmania* (*Viannia*) *braziliensis*

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ARTICLE INFO

Article history:
Received 14 December 2012
Received in revised form 28 May 2013
Accepted 12 June 2013

Keywords:
Equine
Leishmania infantum
Leishmania braziliensis
Mixed infection
Brazil

ABSTRACT

This study reports the first evidence of infection by *Leishmania infantum* in *Equus caballus* in Americas and the first mixed infection of *L. infantum*/*Leishmania braziliensis* on this mammalian species in the world. The diagnoses was based on presence of parasites in lesions and bone marrow aspirates, their identification by using specific primers for *L. infantum* and *L. braziliensis* complexes and also serological methods IFAT and ELISA. The analysis of the PCR products suggested mixed infection in three animals. Further studies involving equine leishmaniasis are carrying out in order to clarify the dynamic of *Leishmania* sp. in this mammalian specie and their role in the transmission of those parasites in urban endemic area of Belo Horizonte, Minas Gerais State, Brazil.

1. Introduction

Zoonotic leishmaniasis is present in the Old World and in the Americas, infecting humans and other mammalian species. It is a vector-borne disease, transmitted by the bites of Phlebotomine sand flies and caused by the protozoa of the genus *Leishmania* (Alvar et al., 2012).

Depending on the species, in humans, *Leishmania* can cause both tegumentary (TL) and visceral leishmaniasis (VL), producing a wide spectrum of diseases, from the localized cutaneous form, mucocutaneous, diffuse cutaneous and visceral leishmaniasis, this latest usually fatal if untreated (Silveira et al., 2004).

Canids, rodents and humans are the major affecting species, especially concerning about zoonotic and urban cycle. However a large number of mammalian species have been record as *Leishmania* hosts (Brandão-Filho et al., 2003). The records in domestic species, other than dogs, such as felines (Schubach et al., 2004; Maia and Campino, 2011) and horses (Barbosa-Santos et al., 1994; Müller et al., 2009) have been increasing wide World.

In Brazil, equine leishmaniasis have been reported at States from Ceará (Alencar, 1959), Bahia (Vexenat et al., 1986), Rio de Janeiro (Aguilar et al., 1986), Espírito Santo (Falqueto et al., 1987), São Paulo (Yoshida et al., 1988), Pernambuco (Brandão-Filho et al., 2003), Paraná (Vedovello Filho et al., 2008) and Minas Gerais (Soares et al., 2012).

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http://dx.doi.org/10.1016/j.vetpar.2013.06.014

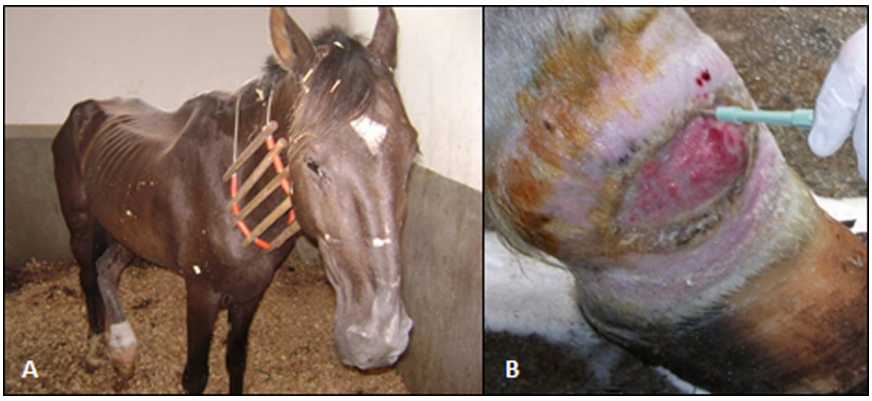

Fig. 1. Clinical presentation of animal suspicious of leishmaniasis, presenting (A) low corporal condition and (B) ulcerative and circular with well-defined, raised borders and a bed of granulation tissue.

When further investigations were possible on these Brazilian cases, the parasite identified was always *Leishmania braziliensis*.

Concerning the equine infection due *Leishmania infantum*, it was detected exclusively in Europe, causing cutaneous problems. Cases were described as autochthonous infections in Germany (Koehler et al., 2002), Spain (Solano-Gallego et al., 2003) and Portugal (Rolão et al., 2005).

In this report, we described the first autochthonous cases of naturally-infected *Equus caballus* due to *L. infantum*, in the Americas, and also the first record, in the world, of mixed infection with *L. infantum* and *L. braziliensis* in this mammalian species.

2. Materials and methods

The animals were examined in the Equine Veterinary Clinic at Veterinary School of the Federal University of Minas Gerais, Belo Horizonte, Brazil.

The patient #1, 9-year-old male mixed breed, was presented at the veterinary clinic in March 2011. The owner had noticed a large, ulcerated, granulomatous and exudative lesion, with a moderate degree of pruritus, measuring 6.5 cm × 3.5 cm. A thorough physical examination revealed no other clinical signs, a part the lesion and a low corporal condition (Fig. 1). The first clinical suspicion was equine pythiosis, which could not be confirmed due an inconclusive histologic result. It was proceeded a new biopsy, a bone marrow aspiration and a whole blood sample was extracted from the jugular vein. The samples were destined to the PCR test. In addition, serum was obtained by centrifugation of whole blood sample and frozen until use to serological tests.

The patient #2, 8-year-old female Mangalarga Marchador breed, was presented with an extensive and ulcerative lesion in the vulvar region as the major complain. The complete physical examination did not present any other disturbs. It was proceeded an incisional biopsy and the analysis diagnosed a squamous cell's carcinoma. Due to this master research that was being developed at the research group, focusing at the equine leishmaniasis, a sample was examined to *Leishmania* PCR test and to an imprint Giemsa-stained.

The animal #3, 7-year-old female mixed breed, had been included in a prior master research, focusing at locomotors problems in drafts horses. In her synovial liquid routine exam was detected a structure that resembled an amastigote. This suspicion could not be answered. One year later, this mare was presented at veterinary clinic because of gastrointestinal problems in her foal. Because of the present study, the owner authorized a bone marrow aspiration and the sample was analyzed by direct examination and PCR test. A whole blood sample was collected and serum processed to serological analysis and PCR tests.

The direct examination was conducted in the imprint of cutaneous lesions and also in the bone marrow aspirates smears, Giemsa-stained. The presence of antibodies IgG anti-*Leishmania* sp. was analyzed by the serological reactions of the immunoenzymatic assay (ELISA) and the indirect immunofluorescent antibody test (IFAT). Both tests were performed with a *L. braziliensis* (MHOM/BR/75/M2904) antigen and needed standardization to equine specie. Those were possible with a positive control that presented active lesions on left pelvic limb, which were biopsied to direct examination and PCR test, showing positive results in both analyses (Soares et al., 2012).

Briefly, in ELISA test, polystyrene 96-well microtitre plates were sensitized with 10 μg/mL antigen. Optimal concentrations of the sample sera and conjugate (Anti-Horse IgG – Peroxidase/Goat – KPL) were established at 1:400 and 1:10,000, respectively. Cut-offs were determined separately for each plate, using sera ($n = 6$) of 24–48 h old foals. The cut off was established at the mean value plus three standard deviation of negatives sera. Reactivity index (RI), the ratio between absorbance of the test serum and the cut-off, was applied like criterion diagnosis. The IRs higher than one, were considered positive.

The IFAT was carried out as described by Chiari et al. (1973), with some modifications. The promastigotes were inactivated at 55 °C per 8 min. The samples that showed reaction in dilutions equal or higher than 1:40 were considered positive, according to the experiments in humans (Pedras et al., 2003) and canines (Ferreira et al., 2007).

For molecular diagnosis, DNA was obtained using a commercial kit (NucleoSpin Tissue – Macherey-Nagel). The