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***Leishmania spp.* amastigotes in the peripheral blood of dogs**

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Abstract

Visceral Leishmaniasis is a systemic disease caused in Brazil, by the protozoan parasite *Leishmania chagasi*, which is transmitted by the bite of haematophagous phlebotomine sandflies. Dogs are the major urban reservoir host for human visceral leishmaniosis. The parasitologic diagnosis is performed through the demonstration of the parasite in biopsy specimens or samples from bone marrow, lymph nodes, spleen and liver. The aim of this article is to report the presence of *Leishmania spp.* amastigotes in the peripheral blood smears of dogs during the hematological analysis. Accurate diagnosis of canine infection by *Leishmania* is essential in veterinary practice in order to control the disease dissemination.

Keywords: Visceral Leishmaniasis, peripheral blood, dogs, amastigotes, monocytes.

Amastigotas de *Leishmania* sp. no sangue periférico de cães

Resumo

Leishmaniose Visceral Canina é uma doença sistêmica causada, no Brasil, pelo protozoário *Leishmania chagasi*. É transmitida por mosquitos hematófagos flebotomíneos e os cães são os principais reservatórios da doença para os humanos. O diagnóstico parasitológico é feito através da demonstração do parasito em material de biopsia ou punção aspirativa de baço, fígado, medula óssea e linfonodos. O objetivo desse trabalho é relatar a presença de amastigotas de *Leishmania* sp. no esfregaço de sangue periférico de cães durante a realização do hemograma. O diagnóstico preciso da leishmaniose no cão é essencial na prática veterinária para o controle da disseminação da doença.

Palavras-chave: Leishmaniose Visceral, sangue periférico, cães, amastigotas, monócitos.

Visceral Leishmaniasis (VL) is an antroponosis caused by a protozoan parasite, that belongs to the *Leishmania* genus and to the *Donovani* complex, comprising *Leishmania* (*Leishmania*) *donovani*, *Leishmania* (*Leishmania*) *infantum* and *Leishmania* (*Leishmania*) *chagasi*. The latter is the one found in Brazil (SANTA ROSA et al., 1997; FEITOSA et al., 2000; RIBEIRO et al., 2001). Transmission among vertebrate hosts occurs through the bite of hematophagous phlebotomine sandflies, which comprise several species of *Lutzomyia*, including *L. longipalpis*, the main vector in Brazil. These sandflies live in varied habitats, but the immature forms develop in moist, rich soil, with low incidence of light, what makes its control very difficult. Until not so long ago, VL was considered a disease of rural and wild areas. Nowadays, it is already present in urban and periurban areas, reaching the North, Northeast, Middle west and Southeast regions of Brazil. Campo Grande, in Mato Grosso do Sul state, has an increasing and

worrying number of canine and human cases. The disease is in process of dissemination throughout the country (GONTIJO & MELO, 2004; IKEDA et al, 2005). The prevalence rates range from 1,9 to 35% in dogs in endemic areas (NUNES et al., 1991; FRANÇA-SILVA et al., 2003). Dogs usually present the chronic form of the disease. They may be asymptomatic or may show hyporexia, weigh loss, fever, local or generalized lymph nodes enlargement, onicogriphosis, skin and ocular disease, epistaxis, lameness, anemia, diarrhea, renal, liver, lung, heart and neurologic disease (SANTA ROSA et al., 1997; FEITOSA et al., 2000; RIBEIRO et al., 2001; IKEDA et al., 2003). Parasitologic diagnosis is made through the demonstration of the parasite in biopsy specimens or spleen, liver, bone marrow and lymph nodes aspiration, besides the detection of amastigotes in the peripheral blood, isolation in cultive means, sorological tests (indirect immunofluorescence assay - IFA and ELISA), molecular methods, such as Polymerase Chain Reaction - PCR, that can be performed in many tissues, including whole blood (ASSIS et al., 2008) and also intraperitoneal inoculation in hamsters (FEITOSA et al., 2000; GONTIJO & MELO, 2004). Amastigotes have also been observed in the thyroid (CORTESE et al., 1999) and urine and semen (RIERA & VALLADARES, 1996). FEITOSA et al. (2000) and IKEDA et al. (2003) found that one of the most important hematologic alterations is anemia. One may also observe leucopenia or leucocytosis, lymphopenia or lymphocytosis and monocytosis, with activated monocytes. An increase in total plasmatic protein concentrations is frequently found. However, the detection of *Leishmania spp.* amastigotes in the peripheral blood is considered rare. It was observed for the first time inside a monocyte by RUIZ DE GOPEGUI & ESPADA in 1998. Previously, SCHALM (1979) detected a single inclusion of *Leishmania donovani* inside a neutrophil. IKEDA et al. (2003) have occasionally found amastigotes while performing hematologic examinations of *Leishmania* infected dogs. Also, MANZILLO et al. (2005) found a significant number of *Leishmania* amastigotes

inside and outside neutrophils in a dog showing severe clinical signs of VL. The amastigotes can be easily recognized by their spheric to ovoid shape, from 2 to 5 μm with a round nucleus and a long kinetoplast (FEITOSA et al., 2000). Neutrophils are more capable of destroying the parasite if compared to eosinophils and monocytes (MANZILLO et al., 2005).

This article reports the detection of *Leishmania* spp. amastigotes in the peripheral blood cells of dogs, with or without VL typical clinical signs.

Six *Leishmania* spp. naturally infected dogs had been hematologically evaluated, because of diverse symptoms. They were three males and three females, one mongrel, one Poodle, one Rottweiler, one Cocker Spaniel, one Fila Brasileiro and one Pinscher, from two to nine years of age. None of them had a previous diagnosis of VL.

The exams were performed in a specialized veterinary laboratory in Campo Grande, Mato Grosso do Sul, in a fourteen-month period. Blood smears were Diff-Quick stained.

Later on, the animals were submitted to IFA, being all positive to anti-*Leishmania* spp. antibodies.

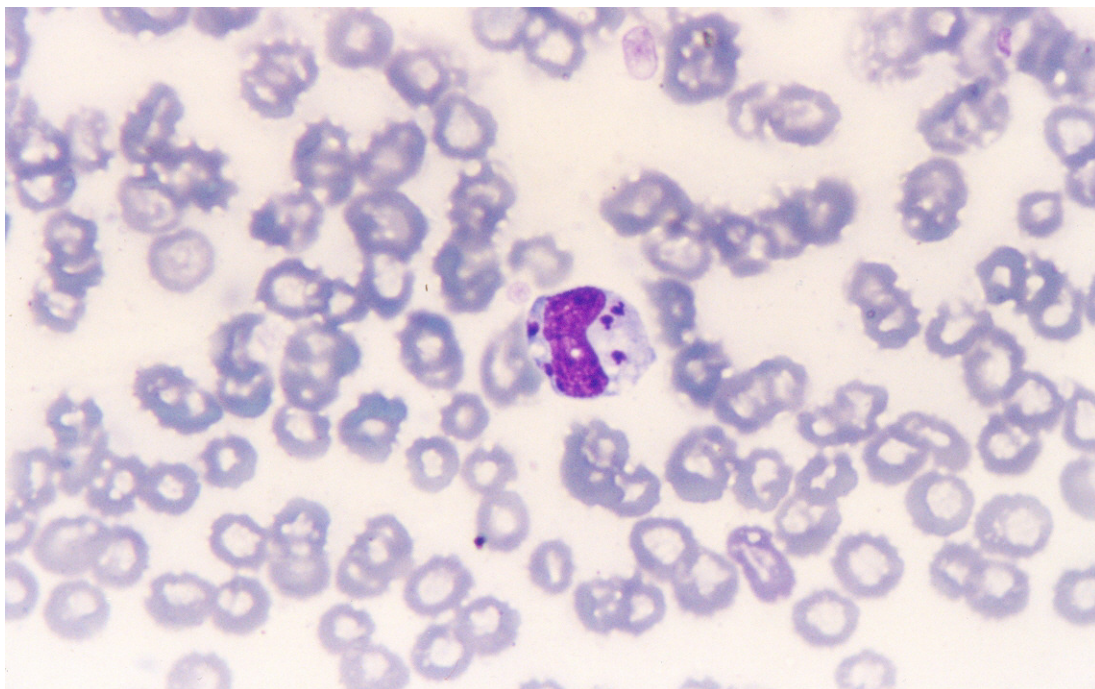


Figure 1. *Leishmania* spp. amastigotes in a monocyte in peripheral blood of a dog (Diff-Quick, immersion)

Amastigotes of *Leishmania* spp. have been detected inside neutrophils and monocytes (Figure 1) in the peripheral blood, while performing hematological examinations for any reasons. Most dogs showed non specific signs such as anorexia, weigh loss and depression, besides dark colored diarrhea, joint pain and swelling, followed or not by discomfort to walk, which comprise clinical manifestations already reported by FEITOSA et al. (2000) as possible signs of VL. The most important hematologic changes were: normocytic normochromic anemia, with globular volume ranging from 12 to 35% in five animals, as reported by IKEDA et al. (2003). The increase in total plasmatic protein concentrations (from 8,6 to 9,3 g/dl), as well as the increase in fibrinogen (600 mg/dl) have been observed in three animals. White blood cell counts were within normal ranges in five dogs, but one of these showed mild leukopenia. Four animals had absolute lymphopenia and one dog showed absolute neutrophilia and

monocytosis, as previously reported by MANZILLO et al. (2005). Four animals had a great amount of activated monocytes. POMPEU et al. (1991) demonstrated that the polymorphonuclear cells predominated during the early phase of infection in mice infected with *L. amazonensis*, and suggested an important parasitocidal role for these cells, at least in the early phase of leishmanial infection.

In the present study, we have been able to detect the presence of amastigotes inside neutrophils and monocytes of peripheral blood of six dogs, in a period of fourteen months. Despite also living in an endemic area, MANZILLO et al. (2005) found several amastigotes in only one leishmaniotic dog.

The fact that VL has become a serious public health problem in Brazil and other American countries, besides Europe and Asia, brings to the veterinarian clinician an important role in the control of the disease.

Hematologic evaluation of dogs suspected of VL is a helpful diagnostic tool. It may provide information regarding the systemic response of the animal, besides the values of red and white blood cells. Moreover, when thoroughly examined, the blood smear might lead to the conclusive diagnosis of VL, if we find *Leishmania* amastigotes inside neutrophils and/or monocytes, mainly in dogs that live in enzootic regions, such as the studied area. Although we could not confirm the species of *Leishmania*, we may assume it should be *Leishmania chagasi* once it is the species involved in canine leishmaniasis in Campo Grande, Mato Grosso do Sul.

However, we cannot forget that the definite diagnostic method of VL is based on the findings of amastigotes in bone marrow or lymph nodes biopsy specimen and on the results of ELISA and IFA tests, besides Polymerase Chain Reaction (PCR).

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