

Amastigota forms resembling *Leishmania* sp. on corneal ulceration in a dog: case report

[Formas amastigotas compatíveis com *Leishmania* sp. em ulceração corneal de cão: relato de caso]

F.L.C. Brito¹, L.C. Alves¹, F.C.L. Maia¹, A.R. Albuquerque¹, J.P.D. Ortiz², J.L. Laus²

¹Departamento de Medicina Veterinária, UFRPE – Recife, PE

²Faculdade de Ciências Agrárias e Veterinárias – UNESP

Via de Acesso Prof. Paulo Donato Castellane, s/n

14884-900 – Jaboticabal, SP

ABSTRACT

One dog with visceral leishmaniasis is reported presenting cutaneous lesions, cachexia, and corneal ulceration. Parasitological and serological diagnoses were performed by exam of contents of the aspirative puncture of the bone marrow and by immunofluorescence, respectively. Amastigota forms resembling *Leishmania* sp. were visualized by cytology.

Keywords: dog, ulceration corneal, *Leishmania* sp., ophthalmology

RESUMO

Relata-se o caso de um cão com leishmaniose visceral apresentando lesões cutâneas, caquexia e úlcera de córnea. Realizou-se o diagnóstico parasitológico e sorológico por meio de exame do material da medula óssea e por imunofluorescência indireta, respectivamente. À citologia da úlcera corneana, visibilizaram-se formas amastigotas compatíveis com *Leishmania* sp.

Palavras-chave: cão, úlcera de córnea, *Leishmania* sp., oftalmologia

INTRODUCTION

In Brazil, canine visceral leishmaniasis is also known as canine calazar and it is a potentially lethal zoonosis caused by a protozoan, a macrophages intracellular parasite from the *Leishmania* (*Leishmania*) *chagasi* species (Feitosa et al., 2000).

Dogs affected by visceral leishmaniasis usually develop the systemic form of the disease showing a great range of clinical signs (Noli, 1999). Ocular manifestations have been described in canine leishmaniasis affecting over 50% of the infected animals (Brito et al., 2006). Canine calazar affects mostly the anterior segment of the eye (Puchol and Gonzalez, 1989).

Corneal limited involvement is seldom observed. Keratitis associated to conjunctival and uveal

inflammation is often observed (Roze, 1986). Nevertheless, corneal exclusively pathologies, such as superficial and stromal keratitis and descemetocele may be observed in dogs with canine calazar (Molleda et al., 1993; Roze, 2002).

There are a great number of studies about canine leishmaniasis pathogeny and lesions, although very few reported in detail the ocular lesions due to this disease (Garcia-Alonso et al., 1998).

CASE REPORT

A seven year-old German shepherd male dog was referred to the veterinary hospital at the Universidade Federal Rural de Pernambuco, Brazil, with clinical suspicion of canine visceral leishmaniasis. At clinical examination, cachexia and cutaneous lesions were observed. Ophthalmic examination revealed photophobia, blepharospasm,

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E-mail: fabiobrito@click21.com.br

neovascularization and discrete corneal edema. Erosion of the corneal epithelium was observed in the right eye. The corneal ulceration was confirmed after the fluorescein test (Fig. 1). Anterior chamber, uveal tract, lens, vitreo and retina were normal in both eyes. Following the diagnosis, treatment was performed using antibiotic therapy (Tobramicina[®] eye drops¹) at regular intervals of 6 hours for 15 days and condroitin sulfato (Dunason[®] eye drops¹) at regular intervals of 8 hours over the same period. No improvement was observed following this medical treatment which demanded a reevaluation. Since the animal came from an endemic region, cytology of the wounded area was performed. Anesthetic eye drops (Anestalcon[®] eye drops¹) was instilled previously the cytology. Soon after, the cytology of the corneal area was proceeded avoiding the contact with the conjunctive. The material obtained from the ulcer was stained by the fast staining technique and evaluated using an optic microscopy at a 1000x magnification. The analyses demonstrated the presence of epithelial cells, polimorphonuclears, red blood cells and also structures resembling amastigota forms of *Leishmania* sp. (Fig. 2 and 3). Meanwhile, it was performed the aspiration of the bone marrow in which amastigota forms of *Leishmania* sp. were observed. Indirect immunofluorescence was also performed and a seropositive reaction was observed in a 1:640 titer.

DISCUSSION

Canine visceral leishmaniasis is a chronic disease associated with varied clinical signs. Due to the diversity of clinical presentation; the disease may be difficult to diagnosis. Ocular alterations have been reported in some studies, as being common in dogs infected by *Leishmania infantum* and *L. chagasi*, such as uveitis and conjunctivitis (Peña et al., 2000; Brito et al., 2006). The ophthalmic form of this disease involving the cornea is less frequent, unless other ocular structures be involved (Roze, 2002).

Despite the very few reports and the low frequency in which isolated corneal involvement occurs in animals affected by visceral leishmaniasis, similar lesions to those reported by this study were described in dogs with ocular disease secondary to leishmaniasis (Roze, 1986). Deep corneal ulceration was reported in dogs and

in one cat affected by visceral leishmaniasis (Roze, 2002; Leiva et al., 2002).

Ophthalmic reevaluation and corneal ulcer cytology in this case were due to the lack of success on previous treatment, since uncomplicated superficial clinical ulcers normally heal in a few days (Whitley and Gilger, 2003).

Corneal ulceration can be related to various factors. Fungi, bacteria and parasites may also induce corneal enzymatic degradation. Normally lesions get worse due to neutrophils quimiotatic action, which release lithic enzymes as they degranulate in the stroma (Gordon et al., 1980).

The mechanism which explains the presence of amastigota forms of *Leishmania* sp. in ocular tissues is unknown (Garcia-Alonso et al., 1996), although some admit that the parasites are carried to the cornea by antigen presenting cells (Saint-André Marchal et al., 1997). The main mononuclear cell population present at the ocular surfaces is represented by the Langerhans cells, especially at the conjuntival and corneal tissues (Wu and Zhang, 1991; Bielory, 2000). These cells have an important role at the ocular surface immunity (Gillette et al., 1982). Because macrophages, dendritic cells and Langerhans cells are the main APCs over Leishmaniasis (Ferrer et al., 1988), it is easy to understand the involvement of the immunogenic mechanisms on the ocular surface in events of CVL.

Antigenic stimulation and lymphocyte T migration leads to local antibodies production which probably achieve the cornea by diffusion. Presence of activated T lymphocyte at the cornea acts on antibody production by the secretion of the B cells growing factor. At the same time plasmocytes unchain an increase on total immunoglobulin levels. Therefore, in canine visceral leishmaniasis the response to immunoglobulins is significantly high, being deleterious instead of protective, permitting clinical signs to appear (Abbas et al., 1995; Noli, 1999). It is admitted that the lesion of the corneal epithelium in this case is connected to immune mediated events.

¹Alcon – São Paulo, Brazil

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Figure 1. Superficial ulcerative keratitis in a dog naturally infected by *Leishmania (Leishmania) chagasi*: corneal edema with erosion of the epithelium and superficial stroma stained by fluorescein.

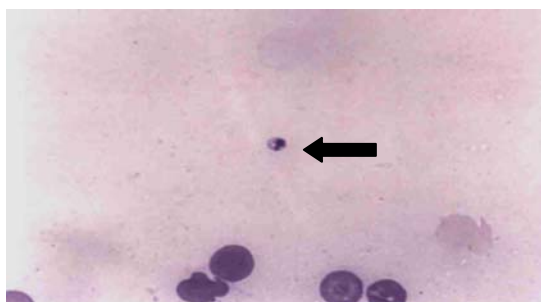


Figure 2. Photomicrography obtained by the cytology of the corneal ulceration: the presence of structures resembling amastigota forms of *Leishmania* sp. Fast staining 1000x.

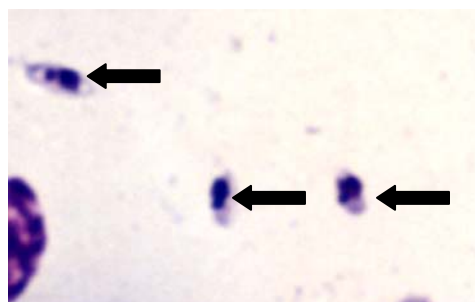


Figure 3. Photomicrography obtained by the cytology of the corneal ulceration: the presence of structures resembling amastigota forms of *Leishmania* sp. Fast staining 1400x.

In similar corneal ulceration in stromal keratitis caused by type 1 simple herpesvirus it is proposed that the response be mediated by virus-specific T lymphocytes of the delayed hypersensitivity type promoted by the Langerhans cells just like the immunogenic responses unchained by visceral leishmaniasis (McLeish et al., 1989).

The presence of *Leishmania* has been described in the conjunctival ocular of dogs naturally infected (Laugier and Verro-Baoulanger, 1992). Therefore, the care should be taken in the procedure of the corneal cytology, for also not raising lesion of the conjunctive and,

consequently, contamination of the material by conjunctival cells.

The literature consulted did not report studies showing the amastigota forms of *Leishmania* sp. at corneal cytology, except when from the nodules at cornea or limbus. Therefore, this is the first case that the authors describe the superficial ulcerative keratitis in which structures resembling amastigota forms of *Leishmania* sp. are isolated from the wounded area.

CONCLUSIONS

It is important to highlight that the ophthalmic signs in dogs are being correlated to visceral leishmaniasis, especially at the anterior segment of the eye. Therefore, in endemic areas where corneal affections non responsive to the conventional treatment is present, canine visceral leishmaniasis with ocular manifestations must be considered as a differential diagnosis. In the future, studies including a larger number of animals are necessary to elucidate the presence of the parasite in the area of the lesion.

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