

## Atypical Tumor-Like Mass of Canine Visceral Leishmaniasis

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### Abstract

Canine visceral leishmaniasis is an important zoonosis caused by the protozoa *Leishmania infantum* (syn. *Leishmania chagasi*), whose clinical manifestations are dependent on the immune response expressed by the infected animal and the virulence of the parasite. Atypical clinical forms of canine visceral leishmaniasis have been reported. The purpose of this paper was to describe a tumor-like lesion form of canine visceral leishmaniasis and to alert clinical and pathologists veterinarians to the importance of its diagnosis. Amastigote forms were observed by cytopathological, histopathological and immunohistochemistry analysis from the tumour-like lesion and *Leishmania infantum* was isolated by culture from spleen, liver, lymph nodes and bone marrow samples. Clinical and pathologist veterinarians should include the canine visceral leishmaniasis in the differential diagnosis of tumors and chronic affections of oral mucosa, mainly in endemic regions of the disease.

**Keywords:** tumor-like lesion; *Leishmania infantum*; canine; diagnosis

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### Introduction

Visceral leishmaniasis is a chronic disease that affects humans and animals caused by protozoan parasites of the genus *Leishmania* and transmitted through the bites of infected sandflies. It is an important zoonosis in regions of southern Europe, Africa, Asia, South and Central America [1-3]. It has been considered a reemerging disease because of several factors

including the appearance of transmission cycles in peri-urban areas due to the destruction of primary forests and the establishment of human settlements in modified environments [3-6]. Importantly, dogs are described as the most important reservoirs of the disease in domestic and peridomestic environments [7]. In Brazil, visceral leishmaniasis is caused by *Leishmania infantum*, whose main vector is *Lutzomyia longipalpis* [8].

Visceral leishmaniasis has a wide range of clinical signs in both human and animal patients. In canine visceral leishmaniasis, the clinical signs are dependent on host factors (cell-mediated and humoral immune response and cytokines) and parasite virulence [9,10] and show variable degrees of severity [1,11-16]. Considering the less common clinical features, rare or atypical forms have been observed including specific skin and mucosa lesions [17-21] and disorders of the cardiovascular [13,22,23], respiratory [24,25] and musculo-skeletal systems [19,26,27].

The purpose of this paper was to describe an atypical clinical form of canine visceral leishmaniasis and to alert clinical and pathologist veterinarians to include this disease in the differential diagnosis of tumours and chronic disorders of oral mucosa, mainly in endemic regions.

## Case Report

A six-year-old male mongrel dog, weighing 22 kg, was taken to a Reference Centre for Veterinary Public Health, with a clinical and laboratorial suspicion of visceral leishmaniasis by the Municipal Health Department of Rio de Janeiro. The serum sample was tested for anti-*Leishmania* antibodies with the IFI-Leishmaniose-Visceral-Canina-Bio-Manguinhos test (Bio-Manguinhos, Rio de Janeiro, Brazil) and the dog was seroreactive with titer  $\geq 1/160$ . The clinical examination revealed loss of weight, muscle wasting, dehydration, cutaneous lesions (pressure points), localized alopecia, furfuraceous scaling, keratoconjunctivitis sicca and onychogryphosis. A swelling in the region of the right maxilla was observed (Figure 1) and upon examination was found to be caused by a gingival tumor - like lesion measuring 3cm in the longest axis. The mucosal membranes were pale and the rectal temperature was 38.8°C.



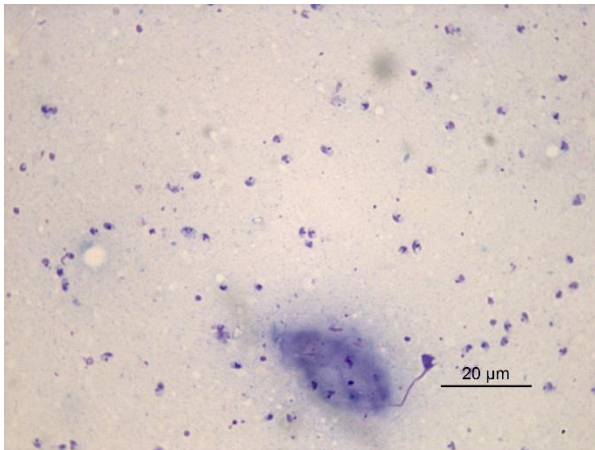
**Figure 1:** Canine presenting a tumor lesion located in the right maxilla region.

A blood sample was collected by cephalic venipuncture and placed in a tube with ethylenediaminetetraacetic acid (10 per cent) for hematological evaluation. Complete blood count (CBC) was performed with an automated cell counter (Coulter Model T-890) and the blood smears were stained by the Romanowsky method (Giemsa stain, Merck). Hematological findings included PCV 17% (reference ranges 37-55%), RBC  $3.0 \times 10^6/\mu\text{L}$  (reference ranges  $5.5-8.5 \times 10^6/\mu\text{L}$ ), hemoglobin 5.7 g/dL (reference ranges 12.0-18.0 g/dL) and total plasmatic protein 9.0 g/dL (reference ranges 5.5-8.0 g/dL).

Fine-needle aspiration of the tumour lesion was performed. Direct smears were prepared and stained by the Romanowsky method (Giemsa stain, Merck). Cytologic interpretation revealed the presence of numerous *Leishmania* organisms free in the preparation or within macrophages, characterized by a light blue cytoplasm, an oval light purple nucleus and a small, dark purple and rod-shaped kinetoplast (Figure 2).

A bone marrow aspirate was performed for cytological analysis and parasitological culture with the dog under anesthesia (anesthetic protocol not shown). Cytologic interpretation revealed the presence of numerous *Leishmania* organisms within macrophages.

Four drops of bone marrow were directly added to a biphasic culture medium (NNN supplemented Schneider's medium with 10% fetal bovine serum) and stored at 26–28°C for parasite isolation.



**Figure 2:** Fine-needle aspirate from a gingival mass located in the right maxilla. Numerous *Leishmania* organisms free in the preparation characterized by a light blue cytoplasm, an oval light purple nucleus and a small, dark purple and rod-shaped kinetoplast. Giemsa, x100 objective.

Immediately after bone marrow puncture, the dog was humanely euthanized according to standards established by Health Department (MS, 2006). Then, intact skin of the scapular region, lymph nodes and spleen fragments were collected for parasite isolation, and the oral tumour was collected for histopathological and immunohistochemistry analysis.

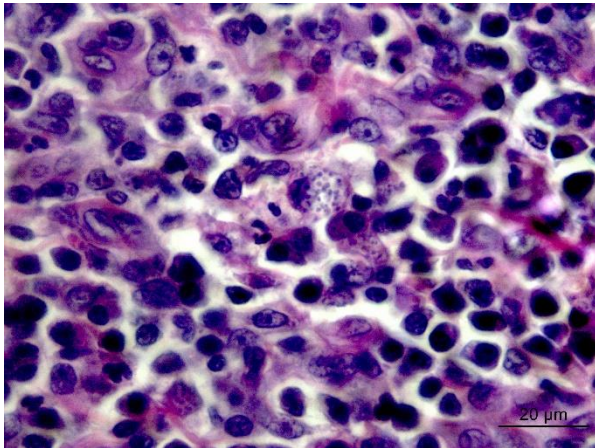
The fragments of intact skin, lymph nodes and spleen were immersed in saline containing 100 µg of 5'fluorocytocine, 1000 IU of penicillin and 200 µg of streptomycin per milliliter and stored at 4°C for 24 h. Afterwards, the fragments were transferred aseptically to a biphasic culture medium (NNN supplemented Schneider's medium with 10% fetal bovine serum) and stored at 26–28°C.

The fresh cultures were monitored weekly for thirty days. Multi-locus enzyme electrophoresis (MLEE) was adopted for characterization of isolates, in

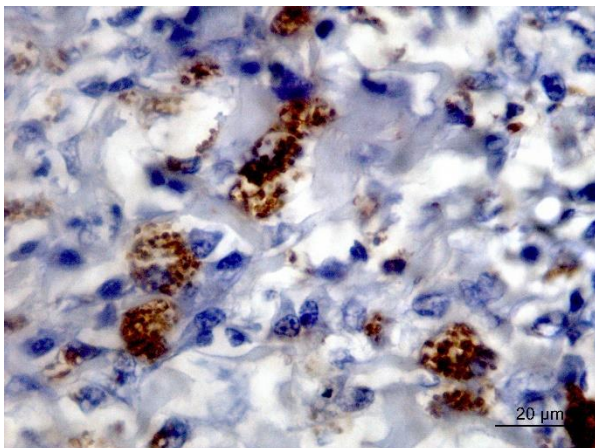
accordance with Cupolillo et al. [28]. Five enzymatic systems were employed to analyze all the isolated samples: malic enzyme (ME, E.C.1.1.1.40), nucleosidase (NH1 and NH2, E.C.3.2.2.1), glucose-6-phosphate dehydrogenase (G6PDH, E.C.1.1.1.49), glucose phosphate isomerase (GPI, E.C.5.3.1.9) and 6-phosphogluconate dehydrogenase (6PGDH, E.C.1.1.1.43). *Leishmania braziliensis* (MHOM/BR/75/M2903), *Leishmania infantum* (syn.*Leishmania chagasi*) (MHOM/BR/74/PP75) and *Leishmania amazonensis* (IFLA/BR/67/PH8) were used as reference samples. *Leishmania infantum* was isolated and identified in all samples (bone marrow, intact skin, lymph nodes and spleen).

Samples of tissue obtained from the oral tumour were fixed in 10% buffered formalin and processed in microscopic sections of 5 µ for haematoxylin and eosin (HE) stain and histopathological analysis. Additional microscopic sections were also obtained on silanated slides for anti-*Leishmania infantum* immunohistochemistry. These slides were submitted to endogenous peroxidase activity block with 30% hydrogen peroxide in 40 mL/100 mL (v/v) methanol solution, following by antigen retrieval with citrate buffer, pH 6.0, in water bath at 65.5°C for 30 minutes. Nonspecific reactions were inhibited by incubation in protein block (Ultra V Block, Thermo Scientific, CA, USA). The sections were then incubated in a moist chamber overnight at 4°C with anti-*Leishmania infantum* serum in 1.5% BSA (1:4000 dilution). The sections were then washed with Tris - buffered saline and incubated with universal biotinylated secondary antibody and the streptavidin-biotin-peroxidase complex (Dako Cytomation). The reaction was developed using diaminobenzidine (SIGMAFAST™ 3,3'-Diaminobenzidine tablets) as chromogen. A sample presenting numerous amastigote forms previously visualized by HE staining was used as positive control. Upon histopathological analysis, a

granulomatous inflammatory infiltrate was observed, located in the middle and deep portion of the lamina propria, and extending until the subjacent adipose tissue. The infiltrate was composed of plenty of plasma cells, lymphocytes and histiocytes filled with amastigotes (Figure 3).



**Figure 3:** Histologic section of the gingival mass. Dense lymphoplasmacytic and histiocytic infiltrate, spreading through collagen bundles. A large number of *Leishmania* amastigotes are observed within histiocytes. H&E, x100 objective.



**Figure 4:** Immunohistochemical stains of the gingival mass. A large number of brown-stained *Leishmania* amastigotes are observed within histiocytes. Labeled streptavidin-biotin method, haematoxylin counterstain. x400 objective.

A few neutrophils were also seen. In stromal connective tissue, there was a proliferation of fibroblasts and thickening of collagen bundles in parallel fashion. At the periphery of the lesion, a dense perivascular

infiltrate of plasma cells was observed. This infiltrate occasionally presented a perineural distribution. The immunohistochemical analysis showed plenty of brown-stained *Leishmania* amastigotes within the histiocytes (Figure 4).

## Discussion

Atypical or uncommon clinical forms of canine leishmaniasis have been related by some authors. Ferreret et al. (1990) reported two cases of atypical nodular forms, one in the interdigital space and the other in the right axilla. Font et al. [29] described four cases in which nodular lesions were present on the mucous membranes of the tongue, oral cavity, nose, penis and prepuce. The lesions were either single or multiple. Saari et al. [30] described a case of leishmaniasis mimicking oral neoplasm in a dog in Finland. Blavier et al. [19] related presence of several nodules on the tongue of a Giant Poodle and nodules on the forelimbs of a German Shepherd dog, both diagnosed with leishmaniasis. Lamothe and Poujade [31] described a case of ulcerative glossitis in a dog with leishmaniasis. Parpaglia et al. [20] showed a case of multiple nodular lesions on the tongue, as well as ocular and cutaneous lesions. In 2012, Viegas et al. [21] described one more case of tongue nodules in a 3-year-old neutered female Labrador Retriever dog with leishmaniasis.

In most cases, the diagnosis of visceral leishmaniasis was made by the observation of amastigotes on fine needle aspiration smears or tissue impression of the lesions, by the histopathological examination, by immunofluorescence antibody test or enzyme-linked immunosorbent assay and/or by detection of amastigotes in bone marrow or lymph nodes.

The present report describes a case of a dog that lived in an endemic area of visceral leishmaniasis and presented typical lesions of the disease, such as

localized alopecia, furfuraceous scaling, pressure points lesions, keratoconjunctivitis sicca and onychogryphosis. It was suggested that the lesion located in the right maxillary gingivawas compatible with a tumour lesion and thus a fine-needle aspiration was performed. The cytologic interpretation revealed the presence of numerous *Leishmania* organisms free in the preparation or within macrophages. The diagnosis of visceral leishmaniasis was confirmed by parasitological culture from the other samples (intact skin, lymph nodes, spleen and bone marrow) that isolated and identified the parasite *Leishmania infantum*.

The species identity of the amastigotes in the tumour-like lesion located in the right maxilla could not be established using the techniques available. However, considering the isolation of *Leishmania infantum* from all the other samples, we supposed it to be the same aetiological agent. In this case, it can be hypothesized that *Leishmania infantum* spread to mucous membranes of the dog, probably through haematogenous or lymphatic routes [28]. According to some authors, it is possible that parasites directly invade the mucosa through the bites or crushing of infected phlebotomine sandfly vectors [32].

Since the identification of the agent at species level was not performed in the present study, it should be stated that the coinfection with other *Leishmania* species cannot be ruled out. Considering the occurrence of tegumentary leishmaniasis in Brazil, it is important to include it as differential diagnosis of oral mucosa lesions (MS, 2007). The occurrence of mixed infection between *Leishmania (Vianna) braziliensis* and *Leishmania infantum* in dogs of suburban areas of Rio de Janeiro city indicating overlapping of tegumentary leishmaniasis and visceral leishmaniasis, emphasizes the importance of complementary research of seroreactive dogs using methods of parasitological diagnosis and identification of *Leishmania* species [33,34]. In places in which the

treatment of positive dog is performed, such potential coinfection could influence the treatment outcome. Other important differential diagnoses of oral mucosa lesions include eosinophilic granuloma, infectious and sterile granulomas, benign and malignant tumours [34-39].

In conclusion, the reported case represents an uncommon clinical presentation of canine visceral leishmaniasis, which should be included in the differential diagnosis of tumours and chronic disorders of oral cavity by clinical and pathologist veterinarians, mainly in endemic regions, since this disease constitutes a veterinary and public health problem.

#### **Conflict of interest statement**

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the content of the paper.

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