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Vet Pathol published online 12 March 2013
DOI: 10.1177/0300985813480192

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What is This?
Leishmania Amastigotes in Neoplastic Cells of 3 Nonhistiocytic Canine Tumors

S. Ferro¹, C. Palmieri², L. Cavicchioli¹, G. De Zan³, L. Aresu¹, and S. L. Benali¹

Abstract
Concurrent leishmaniasis and neoplasia has been reported in dogs. This study describes the presence of the protozoa within the cytoplasm of neoplastic cells in 3 different types of tumors. Leishmania amastigotes were detected by light and transmission electron microscopy and immunohistochemistry in a fibrosarcoma, a T-cell lymphoma, and an adrenocortical adenoma.

Keywords
canine neoplasms, Leishmania, immunohistochemistry, ultrastructure

Leishmaniasis is a parasitic disease of dogs, humans, and other mammals caused by obligate intracellular protozoa that replicate in macrophages and are transmitted by sandflies in Mediterranean countries and parts of Africa, India, and Central and South America. Three forms of the disease are recognized in humans: cutaneous, mucocutaneous, and visceral, the latter most often involving internal organs with or without skin lesions.⁶ In dogs, the clinical presentation usually includes cutaneous and visceral signs.⁵ The existence of Leishmania within a neoplasm is rare.¹⁴,⁷,⁸ In this study, neoplastic cells of 3 different lineages were infected with Leishmania.

Case Studies
Excisional biopsy specimens were fixed in 10% neutral buffered formalin and submitted to the diagnostic service of the University of Padova, School of Veterinary Medicine.

Dog No. 1 was a 6-year-old female Boxer dog with an approximately 5-cm diameter indolent, interscapular nodule. The dermal mass was well demarcated, unencapsulated, and extended into the subcutis. The neoplasm was composed of atypical spindloid cells arranged in regular bundles in a moderate amount of fibrous to less commonly myxoid stroma. Neoplastic cells had a moderate amount of eosinophilic cytoplasm and an oval nucleus with granular chromatin and a single nucleolus. Mild anisocytosis and anisokaryosis were present, and the mitotic index (MI) was <1. Numerous macrophages with cytoplasmic vacuoles were mixed with the neoplastic cells. The vacuoles contained an eosinophilic, 1- to 2-μm diameter body with a basophilic pole, typical of Leishmania spp amastigotes. The protozoa were also within the cytoplasm of many neoplastic cells (Fig. 1a, Suppl. Fig. S1). The diagnosis was soft tissue sarcoma with intralesional Leishmania spp. The owner declined serologic testing for Leishmania. The dog was treated with 10 mg/kg/bid allopurinol and had no recurrence of either parasitic or neoplastic disease in the following 1.5 years.

Dog No. 2 was an adult female Italian Bloodhound with unknown history. The dog had been rescued as a debilitated stray with two 8-cm diameter ulcerated masses in the skin and subcutis of the thigh and hock. Generalized hypotrichosis and scaling prompted serologic testing for leishmaniasis (enzyme-linked immunosorbent assay [ELISA] titer, 1:160). The dog died the day after surgical excision of the masses. The nodular, infiltrative mass involved the dermis and subcutis. The neoplasm was composed of atypical round cells with eosinophilic cytoplasm and a round to oval, 8- to 10-μm diameter nucleus with scattered indentations of the nuclear membrane. Nuclei had granular chromatin and 1 to 3 prominent nucleoli. There were moderate anisokaryosis and anisocytosis, and the MI was 40 (Suppl. Fig. S2). The diagnosis was small cell lymphoma. Scattered neoplastic cells contained numerous intracytoplasmic parasitophorous vacuoles with Leishmania spp bodies (Fig. 2a, Suppl. Fig. S3).

Dog No. 3 was a 7-year-old male Giant Schnauzer admitted for an enlarged right adrenal gland. The dog had polydipsia,
Figure 1. Skin, fibrosarcoma; dog No. 1. (a) Neoplastic cells form bundles and contain numerous intracytoplasmic amastigotes (arrows). Hematoxylin and eosin (HE). (b) Many KMP-11 immunolabeled *Leishmania* amastigotes are within the cytoplasm of neoplastic cells. Diaminobenzidine staining, hematoxylin counterstain. **Figure 2.** Skin, small cell lymphoma; dog No. 2. (a) Many parasitophorous vacuoles are present in the cytoplasm of neoplastic cells (arrows). HE. (b) KMP-11 immunolabeled *Leishmania* amastigotes are present within the cytoplasm of neoplastic cells. Diaminobenzidine staining, hematoxylin counterstain. **Figure 3.** Adrenal gland, adrenocortical adenoma; dog No. 3. (a) The cytoplasm of some neoplastic cells is filled with amastigotes. HE. (b) KMP-11 immunolabeled *Leishmania* amastigotes are within the cytoplasm of neoplastic cells. Diaminobenzidine staining, hematoxylin counterstain.
polyuria, truncal hypotrichosis, and proteinuric nephropathy. Based on these clinical signs, the differential diagnoses included hyperadrenocorticism and *Leishmania spp* infection. The adrenal cortex was effaced by a neoplasm composed of lobules (Fig. 4) of slightly atypical, round to polygonal cells with distinct cell borders and abundant, eosinophilic, finely vacuolated cytoplasm. Nuclei were round and central with coarse chromatin and 1 prominent nucleolus. Anisocytosis and anisokaryosis were mild. The MI was <1. Focally extensive necrosis and hemorrhage were present. Parasitophorous vacuoles, containing *Leishmania spp* amastigotes, were numerous in the cytoplasm of a few neoplastic cells (Fig. 3a) and in scattered macrophages. The diagnosis was adrenocortical adenoma with intralesional *Leishmania spp*. An ELISA test was positive for *Leishmania* (1:640). The dog was treated with allopurinol for leishmaniasis but had recurrence of the parasitic disease 1 year later and died.

**Immunohistochemistry**

In each of the 3 cases, a panel of antibodies was tested on 4-μm-thick serial paraffin sections of the tumor: anti-vimentin (clone V9, monoclonal mouse, 1:150; Dako Italia, Milano, Italy), anti–smooth muscle actin (clone 1A4, monoclonal mouse, 1:100; Dako Italia), anti-cytokeratin AE1/AE3 (monoclonal mouse, 1:100; Dako Italia), anti-CD3 antibody (clone F7.2.38, monoclonal mouse, 1:100; Dako Italia), anti-CD79acy (clone HM57, monoclonal mouse, 1:50; Dako Italia), and anti–neuron-specific enolase (clone BBS/NV/I-H14, mouse monoclonal, 1:250; Dako Italia). A monoclonal mouse anti-KMP11 antibody (dilution 1:300; Abcam, Cambridge, UK) was used to confirm that the organisms were *Leishmania*. Sections were incubated with the antibody for 24 minutes at room temperature. A canine skin biopsy specimen proven to have *Leishmania spp* amastigotes in dermal macrophages served as a positive control. Immunohistochemistry was performed with an automatic immunostainer (Ventana Benchmark XT; Roche-Diagnostics, Monza, Italy), using a secondary antibody with a horseradish peroxidase (HRP)–conjugated polymer (ultraViews Universal DAB; Ventana Medical Systems, Tucson, AZ).

In dog No. 1, the neoplastic cells had strong and diffuse reactivity for vimentin and were negative for smooth muscle actin and cytokeratin. The tumor was classified as a fibrosarcoma. KMP-11–positive *Leishmania* amastigotes were detected within the cytoplasm of neoplastic cells (Fig. 1b).

In dog No. 2, neoplastic cells had moderate and diffuse cytoplasmic immunoreactivity for CD3 and were negative for CD79acy, consistent with a diagnosis of a T-cell lymphoma. *Leishmania* amastigotes, positive for the KMP-11 antibody, were detected in the cytoplasm of neoplastic cells and macrophages (Fig. 2b).

In dog No. 3, neoplastic cells had strong, diffuse immunolabeling for vimentin and neuron-specific enolase but were negative for cytokeratin, consistent with a diagnosis of...
adrenocortical adenoma. Cytoplasmic KMP-11 immunoreactivity was evident in the neoplastic cells (Fig. 3b).

Transmission Electron Microscopy

Representative 1-mm cubes from the formalin-fixed masses were washed in Tris-buffered saline (TBS, pH 7.2) and processed for transmission electron microscopy (TEM). The samples were fixed in a 2.5% glutaraldehyde solution in 0.1M TBS, postfixed in buffered osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were coated with uranyl acetate and lead citrate.

Dog No. 1: The neoplastic cells were spindloid with an oval nucleus and moderate amounts of cytoplasm containing well-developed rough endoplasmic reticulum stacks, scattered free ribosomes, and intermediate filaments. A few round 1- to 2-µm diameter amastigotes with a nucleus and, in some cells, a kinetoplast were observed (Fig. 4).

Dog No. 2: Neoplastic lymphocytes were round, with a high nuclear/cytoplasmic ratio; a round nucleus with coarse, marginalized chromatin; and prominent nucleoli. The low to moderate amount of cytoplasm contained scattered ribosomes, rare mitochondria, and a few amastigotes that were 1 to 2 µm in diameter with well-defined nuclei and kinetoplasts (Fig. 5, Suppl. Fig. S5). Rare cells in mitosis were also parasitized (Suppl. Fig. S6). A few larger cells with ultrastructural features of macrophages contained cytoplasmic amastigotes. Dog No. 3: Neoplastic cells were round to polygonal with a round to oval nucleus, fibrillar to granular nucleoli, and abundant cytoplasm containing round vacuoles, some of which contained osmiophilic material consistent with lipid. There were moderate numbers of mitochondria and rare electron-dense lysosomes. These features are compatible with adrenal cortical cells. Numerous amastigotes, identical to those described in the other 2 neoplasms, were observed within the cytoplasm of the neoplastic cells. Occasionally, a well-defined parasitophorous vacuole was evident (Fig. 6, Suppl. Fig. S7).

Discussion

Coexistence of Leishmania and neoplasia, most frequently in lymphomas, has been reported in humans and animals.4,7 Eight reported canine cases of leishmaniasis concurrent with tumors other than lymphoma include 3 transmissible venereal tumors (TVTs), a squamous cell carcinoma, and 3 hemangiosarcomas.1,2,3,8 In 2 cases, amastigotes were identified, not only in infiltrating macrophages but also within the neoplastic cells.1,2 Because both of these tumors, a possible histiocytic origin of the neoplastic cells was considered.1 In the current study, Leishmania amastigotes were found in non-TVTS, indicating the ability of Leishmania to parasitize nonleukocytic neoplastic cells. The presence of Leishmania amastigotes within different types of cells (mesenchymal, lymphoid, and adrenocortical) suggests a nonspecific tropism of this parasite.

In human medicine, 4 associations between leishmaniasis and neoplasia are described: leishmaniasis mimicking a malignant disorder, such as lymphoma; leishmaniasis after chemotherapy for various malignancies; leishmaniasis coexisting with a neoplastic disorder in immunocompromised patients; and direct involvement of Leishmania spp in the pathogenesis of cancer, specifically in skin and mucous membranes.7 In the study described herein, the presence of Leishmania amastigotes is documented in the cytoplasm of neoplastic cells from 3 tumors of different histogenesis: fibrosarcoma, lymphoma, and adrenocortical adenoma. Although it is not possible to explain the relationship between the parasitic and neoplastic diseases, the results of this study document the ability of Leishmania to parasitize cells other than leukocytes.

Acknowledgements

The authors thank Drs S. Casarosa, A. Russo, and A. Zatelli for providing case materials and histories.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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