Hindawi Publishing Corporation
Case Reports in Veterinary Medicine
Volume 2014, Article ID 603852, 4 pages
http://dx.doi.org/10.1155/2014/603852

Case Report

Leishmania sp. Amastigotes Identification in Canine Transmissible Venereal Tumor

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Received 30 April 2014; Revised 9 July 2014; Accepted 16 July 2014; Published 24 August 2014

Academic Editor: Renato L. Santos

Leishmaniasis is a vector-borne disease with *Leishmania chagasi* being the etiological agent of canine visceral leishmaniasis in South America. Canine venereal tumor is a transplantable round cell tumor of histiocytic origin which is mostly observed in sexually active male and female intact dogs. It has been shown that *Leishmania* amastigotes have higher tropism for the canine male genital tract tissues and venereal leishmaniasis transmission has been documented in dogs but, to date, a canine venereal tumor-dependent transmission route has not been fully demonstrated. In this report, a 10-year-old, mixed breed, intact female dog presented a vaginal venereal transmissible tumor but no other clinical abnormalities otherwise. Unexpectedly, tumor tissue imprint smears examination revealed *Leishmania* sp. amastigotes within infiltrating macrophages. In addition to the cytological direct identification, the protozoan was confirmed within the neoplastic tissue by means of immunohistochemistry and polymerase chain reaction. This report illustrates an asymptomatic *Leishmania* sp. infection that may have started on or from the canine venereal tumor tissue, the latter option further supporting previous evidence of such an alternative vector-independent route of transmission for canine visceral leishmaniasis in areas where these diseases coexist.

1. Introduction

Leishmaniasis is an anthropozoonotic disease with wide geographic distribution, affecting humans, dogs, and several wildlife species, and is caused by the obligate intracellular protozoa belonging to the genus *Leishmania*. Among *Leishmania* species, *Leishmania chagasi* and *L. braziliensis* are the documented species found in Araçatuba, northwest state of São Paulo, Brazil [1]. Depending on the infecting *Leishmania* species and the immunocompetence of the host, the infection can result in visceral, cutaneous, or mucocutaneous disease. Visceral leishmaniasis in dogs (CanVL) is

associated with variable clinical manifestations ranging from unapparent subclinical infections to a systemic disease characterized by progressive weight loss, hepatosplenomegaly, lymphadenopathy, and a range of ocular and dermatological manifestations [2]. Canine transmissible venereal tumor (CTVT) is a unique neoplastic entity that is sexually transmissible and regarded as the oldest known mammalian somatic cell neoplasm in continuous propagation [3]. CTVT has worldwide distribution, with higher incidence in tropical areas, and has been mostly reported in dogs (*Canis familiaris*) and foxes (*Vulpes* sp.). Clinically, CTVT lesions are red to tan, friable, verrucous to multilobulated masses, predominantly affecting genital organs, and are usually ulcerated and inflamed [4]. Metastasis may occur, with lymphatic or visceral dissemination generally associated with underlying immunological impairment [5]. In addition, CTVT extragenital lesions, such as cutaneous, are relatively common and have been reported even in the absence of primary genital lesions [5, 6]. CTVT transmission occurs by means of direct neoplastic cell implantation, and successful CTVT implantation has been linked to an ineffective immune response. Conversely, an efficient postimplantation adaptive immune response is believed to be associated with the mechanism of natural regression that commonly occurs with this tumor [5]. CTVT and CanVL can overlap epidemiologically particularly in regard to their geographical distribution, as in the case presented herein. *Leishmania* sp. reportedly has tropism for the canine male genital tract [7] although the same was not observed in the female genital tract [8]. Venereal transmission via semen has been demonstrated [9]. However, it remains to be experimentally demonstrated whether *Leishmania*-laden CTVT cells can successfully transmit the protozoan to another host. In veterinary medicine, CanVL has been previously identified concurrently with canine transmissible venereal tumor as well as *Leishmania* sp. amastigotes within CTVT neoplastic cells per se [10–12]. Interestingly, in a recent retrospective study, 5 out of 19 dogs affected by both leishmaniasis and CTVT also had detectable amastigotes within the CTVT neoplastic tissue [12].

In regard to the synergistic nature of systemic parasitism and neoplasia, it has been proposed that concurrent diseases may occur secondary to the CanVL-driven immune impairment or, alternatively, neoplastic diseases could hamper the immune system, thus triggering the onset of clinical leishmaniasis in an already infected but asymptomatic dog [12, 13]. The coexistence in the same lesion of CTVT and *Leishmania* has been previously attributed to the systemic dissemination of the latter [10], where amastigote-laden macrophages get recruited into the CTVT tissue. It has been suggested that the histiocytic immunophenotype ofv CTVT [14] may play an active role in the parasitic invasion of the CTVT neoplastic cells [10]. Furthermore, the fact that *Leishmania* amastigotes can be identified within CTVT neoplastic cells supports the hypothesis of a monocyte/macrophage lineage histogenesis of this tumor [6]. Finally, it has been suggested that *Leishmania* amastigotes-laden neoplastic CTVT cells can represent an alternative mode of transmission of canine leishmaniasis in areas where these diseases coexist [11, 12, 15].

2. Case Report

A 10-year-old, mixed breed intact female dog was submitted to the Veterinary Teaching Hospital with a 3-month history of a 4.0 × 3.0 cm vaginal vestibule mass (Figure 1) without any other significant systemic clinical signs. Examination of tumor imprint smears revealed abundant typical CTVT cells admixed with degenerate neutrophils. Hematological findings were within normal limits. At the time of CTVT diagnosis, *Leishmania* sp. serology as performed by Lima et al. [16] was negative. Therefore, standard chemotherapy protocol using intravenous vincristine sulphate 0.5 mg/m^2 was administered once weekly. After the fourth chemotherapy session, and with a reminiscent 3.0 × 2.0 cm mass, additional cytology sampling and biopsy were performed. Cytology smears consisted predominantly of mature keratinized epithelial cells admixed with neutrophils, lymphocytes, and plasma cells, as well as moderate numbers of mixed-morphology bacteria. Few 2-3 μm oval *Leishmania* amastigotes with characteristic perinuclear rod-shaped kinetoplast could be observed within macrophages (Figure 2) as well as free in the smear. At this point, no remaining CTVT neoplastic cells could be identified cytologically. Histopathology findings consisted of extensive inflammatory infiltrate within the subepithelial vaginal stroma composed of lymphocytes and plasma cells, moderate amounts of neutrophils, and abundant macrophages, several of which were loaded with amastigote organisms (Figures 3 and 4), as well as rare reminiscent CTVT neoplastic cells within reactive newly formed collagen (fibrosis). Immunohistochemistry performed as described by Nogueira et al. [17] labeled myriads of *Leishmania* sp. amastigotes within the cytoplasm of histiocytoid cells (Figure 5), interpreted as macrophages, and dispersed through the neoplastic tissue. Furthermore, polymerase chain reaction (PCR) as performed by Moreira et al. [18] confirmed the presence of *Leishmania* sp. DNA in the tissue. Apart from the vaginal mass, the dog was healthy, presenting no overt clinical signs of canine leishmaniasis. This dog continued to have negative serology for *Leishmania* and was devoid of clinical disease up to one year following the last biopsy of the reminiscent CTVT lesion, but after this time it was lost to follow-up.

3. Discussion

Low cost and near 100% specificity make cytology the most accessible method for diagnosing CTVT and canine leishmaniasis (CanVL) provided that the examiner is familiar with the unique cellular morphology of the CTVT cells and the identification of *Leishmania* amastigotes. Identification of *Leishmania* amastigotes within hematoxylin and eosin-stained tissue sections is challenging and depends on the affected tissue, stage of infection, severity of the secondary inflammatory response, and number of organisms [18]. However, as in this case, such diagnostic challenges may be circumvented by using ancillary techniques such as intissue antibody-based identification of amastigotes via immunohistochemistry [18, 19]. CTVT diagnosis is routinely done based on its characteristic cytological findings. Tumors that receive chemotherapy and have concurrent regression response

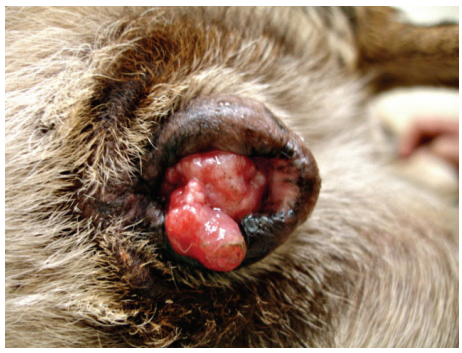

Figure 1: Dog. Vaginal vestibule, red verrucous, and lobulated mass protruding from the vagina.

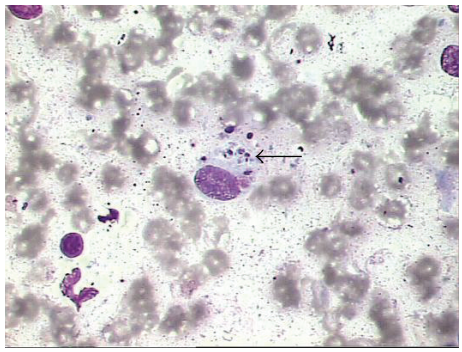

Figure 2: Dog. Photomicrograph of cytology imprint from the vaginal mass with a histiocyte-like cell with three intracytoplasmic protozoa amastigotes (arrow) (Diff-Quick, 100x).

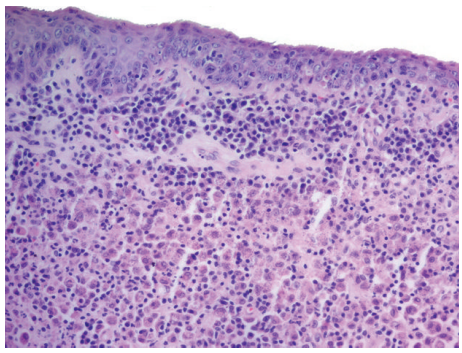

Figure 3: Dog. Photomicrograph of vaginal mucosa with the subepithelial stroma effaced by canine transmissible venereal tumor cells, mixed with macrophages and with a mild diffuse lymphoplasmacytic infiltrate (H&E, 20x).

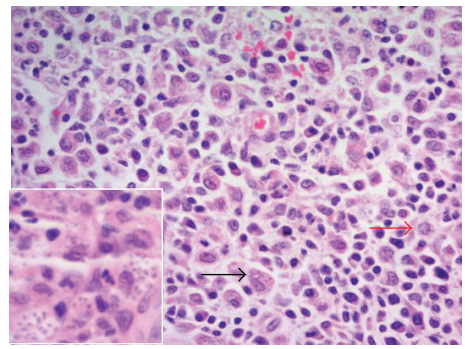

Figure 4: Dog. Photomicrograph of vaginal mucosa and canine venereal tumor cells (red arrow); accompanying inflammatory infiltrate within the subepithelial stroma was composed of lymphocytes, plasma cells, few neutrophils, and amastigote-laden macrophages (black arrow) which are shown in higher magnification in the inset (H&E, 40x and 63x).

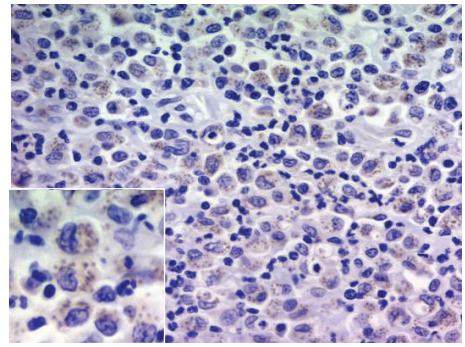

Figure 5: Dog. Photomicrograph of vaginal mucosa and immunohistochemistry staining highlighting *Leishmania* sp. amastigotes (DAB hematoxylin-counterstained, 40x and 63x).

are histologically characterized by decreased neoplastic cell population, with predominantly degenerate remnant cells amidst extensive stromal remodeling and lymphoplasmacytic inflammatory infiltrate [20]. These changes are in agreement with our findings of absent typical CTVT cells in the second cytology sample as well as the predominantly inflammatory and fibrosing morphology of the biopsy sample. Furthermore, the occurrence of *Leishmania* infection within the tissue possibly resulted in added granulation tissue formation that grossly resembled a neoplasm that was responding to chemotherapy unsatisfactorily.

The coexistence of CTVT and leishmaniasis in this dog is corroborated by the endemic nature of both diseases in Araçatuba (northwest state of São Paulo, Brazil), particularly among roaming dogs [1]. This dog had CTVT genital lesions which could have provided a feeding site to the female sand fly vector; the fact that *Leishmania* amastigotes could have transplanted with CTVT cells to this new host, thereby circumventing the vector in the classic transmission route of canine leishmaniasis, is also a tempting possibility [12, 15].

4. Conclusions

This report illustrates an asymptomatic *Leishmania* sp. infection concurrent with a transmissible venereal tumor. We speculate that the *Leishmania* sp. infection may have started on or from the CTVT, the latter option further supporting previous evidence of such an alternative vector-independent route of transmission for CanVL in areas where these diseases coexist. While it was not possible to determine whether this

dog had been previously infected with *Leishmania* prior to CTVT, negative serology for *Leishmania* at the time of CTVT diagnosis could indicate an early stage of infection favoring the preexistence of the CTVT. Nonetheless, a negative serology result due to a CanVL-driven impaired adaptive immune system cannot be discarded. Experimental evidence of amastigotes uptake by and viability within CTVT cells, as well as amastigote-laden CTVT cells transmission capabilities, is critically lacking. Thoroughly screening CTVT-affected dogs for leishmaniasis in areas where these diseases coexist is highly warranted. The present case underscores the diagnostic challenges, complex nature, and the potential epidemiological consequences of overlapping infectious diseases.

Conflict of Interests

The authors declared no potential conflict of interests with respect to the research, authorship, and/or publication of this paper.

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