Canine cutaneous leishmaniasis

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Abstract Canine cutaneous leishmaniasis (CCL) is a significant veterinary problem. Infected dogs also serve as parasite reservoirs and contribute to human transmission of cutaneous leishmaniasis. Histologically, the lesions were nodular to diffuse interstitial granulomatous dermatitis with histiocytic pseudorosettes together with numerous amastigotes within macrophages and occasionally within the interstitium. Organisms were often contained within clear and intracellular vacuoles. The other inflammatory cells, which were present in the biopsies of the *Leishmania*-infected dog, were lymphocytes and plasma cells. The histopathology results emphasized the role of dog, particularly asymptomatic dog, as reservoirs for CCL because of the high cutaneous parasite loads. These results may help to explain the maintenance of high transmission rates and numbers of CCL cases in endemic urban regions.

Keywords Cutaneous leishmaniasis · Dog · Histopathology · Inflammatory cells · Dermatitis

Introduction

Leishmaniasis is a worldwide disease caused by protozoa of the genus *Leishmania* which infect wild and domestic mammals, including humans (Gradoni 1999). The spectrum

of clinical forms of leishmaniasis can vary from focal cutaneous to disseminated visceral disease (Slappendel and Ferrer 1998). Cutaneous Leishmaniasis (CL) is a zoonotic disease transmitted via the bite of female sand flies belonging to the genera *Phlebotomus* in the Old World and *Lutzomyia* in the New World (Koutinas et al. 1993). CL and visceral leishmaniasis (VL) are endemic in large areas of the tropics, subtropics, and the Mediterranean basin. The disease in dogs is characterized by local, self-healing ulcerative lesions on the ears, scrotum, feet, nipples, and muzzle (Madeira et al. 2006). However, canine cutaneous leishmaniasis is a neglected disease, mainly because most of the cases occur far away from the cities, that is, far from veterinary services. Dogs have been identified to be an important host for the parasite. Infected dogs normally develop both visceral and cutaneous lesions, whereas restriction to the skin occurs in some forms of human leishmaniasis (oriental sore).

Histopathology and immunohistochemistry are frequently used in the current routine for the histological diagnosis of *Leishmania* in dogs, but these methods have limited accuracy and do not allow species identification (Mehregan et al. 1999; Perez-Molina et al. 1999). The identification of species of *Leishmania* is currently only possible by parasitological culture followed by multilocus enzyme electrophoresis (MLEE), which is the reference method, and by PCR (Ayllon et al. 2008). The clinical signs are caused by inflammatory processes and immune-mediated lesions, which are associated with the multiplication of amastigotes inside macrophages and other cells of the mononuclear phagocytic system. Hence, alternative histological methods are necessary to improve the accuracy of diagnosing infection of dogs with *Leishmania*. The aim of this study was to evaluate lesions histopathology in cutaneous leishmaniasis.

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Methods

The dog was examined and sampled with routine procedures. The clinical examinations did not affect animal welfare. Sacrifice of animal did not occur. Suspicious signs or lesions included dermatitis, alopecia, cutaneous ulcerations, weight loss, ocular or nasal lesions. For the collection of samples, one 3-mm punch biopsy specimen was obtained from the intact skin over the scapula after disinfection with 70 % alcohol and local anesthesia with 2 % lidocaine. Each specimen obtained was divided into two samples. One of them was immersed in sterile saline with antimicrobials (Ramos-Santos et al. 2000) and submitted for parasitological culture.

Skin samples collected in saline were seeded in the biphasic culture medium NNN (Novy, MacNeal and Nicolle)-Schneider's insect medium (Sigma-Aldrich Co., St. Louis, MO) containing 10 % fetal bovine serum and were incubated at 26–28 °C. The other fragment was fixed in 10 % neutral buffered formalin and processed for routine paraffin embedding (Tafari et al. 2004). For histopathology, serial sections of 5 µm were stained by hematoxylin-eosin.

Results

The cutaneous lesions of *leishmania* that were observed in this case included periorbital and snout the multiple foci pseudo-nodular large and small, white to grayish and or reddish (Fig. 1).

The lesions consisted of nodular to diffuse accumulations of macrophages throughout the dermis with scattered neutrophils, lymphocytes, plasma cells, and mast cells. There were accumulations of lymphocytes, plasma cells, and fewer eosinophils around blood vessels (chronic granulomatous dermatitis). The histopathologic evaluation

of the skin biopsies revealed that in the *Leishmania*-infected dogs, the predominant inflammatory cells were macrophages. They were usually more abundant in the superficial dermis and characterized by a moderate amount of finely vacuolated and lightly eosinophilic cytoplasm (epithelioid cells). Within the cytoplasm of macrophages, variable numbers of amastigote forms of *Leishmania* were identified as round to oval organisms with a round nucleus and a kinetoplast surrounded by a clear halo. The other inflammatory cells, which were present in the biopsies of the *Leishmania*-infected dogs, were lymphocytes and plasma cells. Organisms were often contained within round, clear, intracellular parasitophorous vacuoles, many of which contained more than a dozen amastigotes at their periphery (Fig. 2).

Discussion

Leishmania are obligate intracellular parasites that infect both humans and animals. These parasites are transmitted by a sand fly vector and cause a wide range of diseases, such as CL, mucocutaneous leishmaniasis (ML), and VL. Over 12 million people currently suffer from leishmaniasis, and ~2 million are infected annually, making it a major global health problem and a World Health Organization (WHO) classified neglected tropical disease. In many *Leishmania*-endemic regions of the world, infected dogs serve as a significant reservoir for zoonotic transmission of VL and CL to humans (Brachelente et al. 2005). Many recent studies have also reported that dogs in the United States have tested positive for leishmaniasis, (Diniz et al. 2005) although no dog-to-human transmission has been documented.

Cutaneous leishmaniasis in dogs is a significant veterinary problem, but it is also well documented that infected dogs serve as parasite reservoirs and contribute significantly

Fig. 1 Cutaneous leishmaniasis in dog manifesting as multiple nodules or ulcerating lesion on the snout and periorbital

Fig. 2 Skin; leishmania-infected dog; note the perivascular to interstitial infiltration in the superficial and deep dermis with the predominant inflammatory cells were macrophages and then other inflammatory cells, which were present in the biopsies of the *Leishmania*-infected dog, were lymphocytes and plasma cells

to human transmission of VL and CL in many parts of the world. Cutaneous leishmaniasis in dogs manifests as localized skin lesions that may either heal or become chronic, the latter leading to significant tissue destruction and disfigurement (Mehregan et al. 1999).

In endemic areas where different *Leishmania* species or variants are transmitted, the disease can be the result of a heterogeneous infective inoculum, probably due to the accumulation of multiple independent infections (Gradoni 1999). In fact, the presence of distinct parasite populations circulating in endemic areas of CL is known, and the multiclonal origin of *Leishmania* strains has already been shown (Santaella et al. 2011). The species known to cause CL in dogs are *L. (V.) braziliensis*, *L. (V.) panamensis*, *L. (V.) guyanensis*, and *L. (V.) peruviana*. *L. (V.) braziliensis* and *L. (V.) guyanensis* are known to cause CL (Santaella et al. 2011; Vélez et al. 2012).

The clinical differential diagnosis for nodular lesions on the face of a dog includes lymphoma, mast cell tumor, squamous cell carcinoma, canine sarcoid, pemphigus foliaceus, and dermatophytosis. The histologic diagnosis of cutaneous leishmaniasis is challenging without cytology to identify kinetoplasts in amastigotes or PCR for confirmation.

The histopathologic evaluation of the skin biopsies revealed that in all *Leishmania*-infected dogs the predominant inflammatory cells were macrophages. Lymphocytes and plasma cells were the second most frequent cell type. The prevailing number between lymphocytes and plasma cells varied between individuals. Microscopically, the lesions described in humans consist of dense granulomatous inflammation with numerous giant cells, fewer lymphocytes and plasma cells, and scant amastigotes (McAdam and Sharpe 2009; Mehregan et al. 1999).

The presence of inflammatory cells in dogs without evident exfoliative dermatitis on the flank and muzzle can be explained by the current hypothesis that macroscopic lesions follow, after a certain period of time, the infiltration

of inflammatory cells (Ferrer et al. 1988). Similarly, histological lesions, such as perivascular to diffuse dermatitis, perifolliculitis and sebaceous adenitis have been reported in clinically healthy dorsal muzzle skin of 17/20 (85 %) dogs with symptomatic CL (Fondevila et al. 1977). The presence of predominantly mononuclear cells, mainly macrophages and to a lesser degree lymphocytes and plasma cells, and also neutrophils, eosinophils and mast cells in the inflammatory cutaneous infiltrate is consistent with other studies of dogs with CL (Elso et al. 2004).

Further studies with a larger number of clinical samples from naturally infected dogs are needed in order to draw correlations between histopathological profiles and pathogenicity.

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