A case report of typical leishmaniasis in dog

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Abstract *Leishmania* spp. are vector-borne flagellates transferred by sand flies. They cause cutaneous, mucocutaneous and visceral infections in mammals, especially in humans and dogs. A mature male boxer with ulcerative nodules around his eyes and snout was referred to Small Animal Hospital, Faculty of Veterinary Medicine, University of Tehran. Multiple cutaneous lesions were seen in physical examination. Mild leukocytosis, neutrophilia, left shift, lymphopenia, and thrombocytopenia were reported by the laboratory. Diagnosis was confirmed by the observation of amastigotes in blood samples and inside tissue macrophages. The infection was treated using pentavalent antimonial drug for four weeks.

Keywords Leishmaniasis · Zoonosis · Dog · Sand fly · Iran

Introduction

Leishmaniasis is a zoonosis caused by species of the genus *Leishmania* (Pasa et al. 2005). *Leishmania* spp. are parasitic protozoa belonging to the Order Kinetoplastida and Family Trypanosomatidae (Ikeda et al. 2008). The parasite infects many mammals such as humans, cats, canines, equines and rodents (Handman and Bullen 2002). Dogs and rodents are the major reservoirs of cutaneous leishmaniasis (CL) while wild carnivores such as jackals, foxes and wolves are reservoirs of human visceral leishmaniasis (VL) (Mohebbi et al. 2005). Rodents are known as the major reservoirs of mucocutaneous leishmaniasis (MCL). Species that cause disease in dogs include *Leishmania infantum*, *L. chagasi*, *L. tropica*, *L. major* and *L. braziliensis* (de Andrade et al. 2006; Reithinger and Davies 1999). *Leishmania* spp. are transferred by sand flies, mainly by the genera *Phlebotomus* and *Lutzomyia* (Rassi et al. 2004).

Leishmaniasis is endemic in 88 countries, mostly located in Central and East Asia, North and East Africa, North and South America, and Europe (Salotra and Singh 2006). Canine leishmaniasis (CanL) is found in similar geographic regions. In Iran, the first case of CanL was reported from Tehran by Neligan (1913). Later in 1949, Pouya reported a CanL case in Caspian area, where he also reported the first human case of VL in Iran. Moreover, infected dogs were found in other area of Iran (Bokai et al. 1998; Hamidi et al. 1982).

Leishmania exists in two forms: amastigotes that are obligate intracellular parasites of mammalian macrophages, and promastigotes that are found in guts of invertebrate hosts (Barbieri 2006). Skin is the major site of CL infection, in which a granulomatous reaction with numbers of amastigotes develops, depending on the activation of macrophages by Th1 lymphokines (dos-Santos et al. 2004).

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Typical CL symptoms in dogs include fatigue, anorexia, weight loss, lymphadenopathy, alopecia and non-pruritic exfoliative dermatitis especially on the face, ears and feet (Handman and Bullen 2002). Other symptoms that may be seen include fever, pruritic ulcers, nasal and digital hyperkeratosis, elongated and deformed nails (onychogryphosis) and ocular involvements (Ozbel et al. 2000).

The early diagnosis and treatment of CanL cases are important for the control of human leishmaniasis (Maia and Campino 2008). Laboratory diagnosis includes specific methods such as serology, parasitology and molecular diagnosis, and nonspecific methods such as hematology, biochemistry and clinical immunology (Ciaramella and Corona 2003). Treatment mainly aims intracellular amastigotes in tissue macrophages (Pasa et al. 2005). Antimonials (e.g. meglumine antimoniate), amphotericin B and allopurinol are currently used for the treatment of CanL. However, treatment does not cure the parasite in all cases and relapse of leishmaniasis, especially in symptomatic animals, is common (Roze 2005). Prevention of leishmaniasis is based on the limitation of reservoirs (screening programs, vaccination and chemotherapy), control of vector populations (insecticides) and prevention of sand fly bites (Roze 2005).

Case presentation

A mature male boxer showing ulcerative nodules on his snout and around eyes was referred to Small Animal Hospital, Faculty of Veterinary Medicine, University of Tehran (Fig. 1). According to the owner, these lesions had gradually occurred 10 days before the visit. Multiple cutaneous lesions were seen on physical examination. No symptoms of fever, diarrhea, arthritis or eye lesions were found. Direct microscopy of cutaneous lesions was performed, using impression smear technique. Furthermore, a blood sample was taken for hematology.

Results

In light microscopy of Giemsa-stained blood samples and lesion impression smears, amastigotes (Leishman–Donovan bodies, $1.5\text{--}2.0 \times 2.5\text{--}5.0 \mu\text{m}$) were seen inside and outside macrophages (Fig. 2). Mild leukocytosis (WBC: $21,000/\mu\text{l}$), neutrophilia (segmented: $18,900/\mu\text{l}$), left shift (bands: $630/\mu\text{l}$) and lymphopenia (lymphocytes: $1,050/\mu\text{l}$) were reported by the laboratory. Other findings included hypochromia and thrombocytopenia. The animal was treated using meglumine antimoniate (100 mg/kg day, IV) and allopurinol (20 mg/kg day, PO) for four weeks.

Fig. 1 Lesions on the animal's face

Fig. 2 Intracellular amastigotes in tissue macrophages

Discussion

In this case, leishmaniasis was approved based on the clinical symptoms and laboratory findings. Cutaneous lesions were seen on the animal's face, which often appear in 60 % of all symptomatic dogs (Jafari Shourijeh et al. 2006). Leishmaniasis was finally diagnosed by direct observation of amastigotes in light microscopy of the stained smears prepared from lesions and blood samples, which is the gold standard (Xavier et al. 2006). Different staining methods such as Giemsa, Romanowsky, Wright or Diff-Quik can be used for the staining of amastigotes obtained by needle aspiration of lymph nodes, spleen and

bone marrow or by the culture of infected tissues (Castillo and Rojas 1997). The method features 100 % specificity but the sensitivity is poor (Kassi et al. 2003). Other methods such as DNA probes, electron microscopy (EM), fluorescent antibody technique (FAT) and immunoperoxidase staining are more sensitive but need specific materials and methods (Koehler et al. 2002).

Blood abnormalities such as leukocytosis, neutrophilia, left shift and lymphopenia are usually associated with infected dogs (Jafari Shourijeh et al. 2006; Bogdan et al. 2001), which were clinically found in the present case. Anemia is another common finding in canine leishmaniasis. The anemia usually presents in normocytic normochromic form in association with medullary hypoplasia (Costa-Val et al. 2007). It seems that anemia is related to the clinical stage of the disease (Dias et al. 2008). Neutrophilia, which usually results in leukocytosis, has been related to cutaneous lesions caused by the secondary infection (Ikeda et al. 2008). Lymphopenia may occur due to the immunosuppressive nature of the disease, medullary dysfunction or leukocyte recruitment in involved organs (Reis et al. 2006). However, these parameters may vary depends on the stage of the disease (Amusatogui et al. 2003). In summary, direct microscopy is a rapid and reliable method to detect *Leishmania* spp. in dogs.

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