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Review

Visceral leishmaniasis in zoo and wildlife

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ABSTRACT

Visceral leishmaniasis (VL) is an emerging zoonosis caused by *Leishmania* (*Leishmania*) *infantum*. Although the domestic dog is the main vertebrate host, many zoo and wild mammal species have been diagnosed with *L. infantum* infection, especially in endemic areas. There are many available diagnostic approaches, including serological, parasitological and molecular tests. Among wild animals, carnivores and primates are more often clinically affected, with some species, such as the bush dog (*Speothos venaticus*) being especially susceptible to development of clinical signs. There are also reports and research articles of VL in felids, rodents, and marsupials. This work aims to review the occurrence of VL in zoo and wildlife and raise awareness of its importance in the field of conservational veterinary medicine.

Contents

1. Introduction

Visceral leishmaniasis (VL) is an important systemic, often fatal, emerging zoonosis with a broad geographic distribution, particularly in tropical and Mediterranean regions. The causative agent is *Leishmania* (*Leishmania*) *infantum* (synonym *L. chagasi*), a protozoan belonging to the *L. donovani* complex of the Order Kinetoplastida and Family Trypanosomatidae (Baneth et al., 2008). The disease is primarily transmitted by phlebotomine sand flies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the Americas. Importantly, sexual, vertical and iatrogenic transmission has been reported (Morillas-Marquez et al., 2002; Pangrazio et al., 2009; Silva et al., 2009).

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The disease has been diagnosed in several parts of the world with an increasing frequency in recent decades. Originally distributed within the Mediterranean coast, Europe and Asia, the protozoan was introduced in the Americas during the colonization process (Kuhls et al., 2011). Historically, once established in a new area, the disease becomes endemic and its control has proven to be very challenging (Diniz et al., 2008; Petersen, 2009). Microsatellite analysis of 98 strains of *L. infantum* from seven South and Central American countries were compared to profiles of 308 *L. infantum* strains from different countries in Southern Europe, North Africa, Middle East, and Asia. These strains have been isolated from humans, dogs or wild animals, namely *Cerdocyon thous* and *Didelphis marsupialis*. The results indicate that *L. infantum* was introduced in the New World by multiple waves of immigration from European countries and the strains from wild animals did not differ from those isolated from humans or domestic animals (Kuhls et al., 2011).

VL has been documented in several mammalian species, including carnivores (Beck et al., 2008; Luppi et al., 2008), primates (Malta et al., 2010), marsupials (Santiago et al., 2007), edentates (Araújo et al., 2013), lagomorphs (Molina et al., 2012), bats (Lima et al., 2008), rodents (Papadogiannakis et al., 2010), and horses (Rolão et al., 2005). Although the domestic dog is the most important vertebrate host and the main reservoir for human VL, several wild mammal species are susceptible to *L. infantum* infection. Wild animals are subjected to challenges imposed directly or indirectly by anthropic influence that impacts ecosystems and may exposure wildlife to pathogens, which under some circumstances may lead to or increase the risk of extinction of a given species. Importantly, wild animal health reflects environmental health and may serve as an indicator of human risk of exposure to zoonosis in that environment (Aguirre, 2009). Therefore, the importance of wild animals as "sentinels" must be considered, although analytical methods for linking animal and human data, thus predicting human risk are still underdeveloped (Scotch et al., 2009). Zoo animals maintained in captivity in endemic urban environments are under high risk of infection, and therefore diagnostic tests are advised for prevention and control of the disease in zoo populations, especially when exchanging or introducing susceptible animals (Malta et al., 2010; Jusi et al., 2011; Libert et al., 2012).

2. Visceral leishmaniasis in zoo and wild animals — diagnostic methods and pathology

As occurs in man and domestic dogs, infected wild mammals may be symptomatic or asymptomatic. The disease develops similarly in domestic and wild mammals. Common necropsy findings in wild animals with VL include gross changes that are commonly seen in domestic dogs such as emaciation, pale mucous membranes, liver enlargement and discoloration, splenomegaly, lymphadenopathy. Additionally, less specific changes may also be observed including pulmonary congestion and edema, hemorrhages in skin, lung, heart, and intestines (Beck et al., 2008; Luppi et al., 2008; Malta et al., 2010). Cutaneous crusts, ulcers and alopecia may be observed particularly in canids (Beck et al., 2008; Luppi et al., 2008; Souza et al., 2010).

Microscopically, a macrophage infiltrate containing intracytoplasmic amastigotes is usually present in many organs (lymph nodes, spleen, liver, kidney, lung, and small intestine) along with plasma cells and lymphocytes (Beck et al., 2008; Luppi et al., 2008; Malta et al., 2010) (Fig. 1). As frequently observed in domestic canids, membranoproliferative glomerulonephritis has been described in a bush dog (*S. venaticus*) with VL (Luppi et al., 2008) (Fig. 1).

Diagnostic methods currently applied to wild animals include serological assays such as indirect immunofluorescence (IFA), enzyme-linked immunosorbent assay (ELISA) (Luppi et al., 2008), fucose–mannose ligant-ELISA (FML-ELISA) (Santiago et al., 2007), immunochromatographic strip assays (ICT) (Molina et al., 2012; Rosypal et al., 2010), direct agglutination test (DAT) (Mohebali et al., 2005), and Western blot (WB) (Sobrino et al., 2008). In addition, molecular assays have been employed for the diagnosis of *Leishmania* infection, including *in situ* hybridization (Oliveira et al., 2005), polymerase chain reaction—PCR (Luppi et al., 2008), real-time quantitative PCR—qPCR (Sastre et al., 2008), PCR-restriction fragment length polymorphism—PCR-RFLP (Souza et al., 2010), among other methods including culture (Figueiredo et al., 2008), direct parasitological analysis (Lima et al., 2009), histopathology and immunohistochemistry (Malta et al., 2010).

3. *Leishmania* infection in zoo and wild carnivores

The Carnivora order includes many wild species that are susceptible to VL. Serological and molecular surveys of *L. infantum* in wild animals from different parts of the world have been published, and natural disease has been described in free-ranging and captive wild canids (Sastre et al., 2008; Beck et al., 2008; Luppi et al., 2008; Souza et al., 2010; Libert et al., 2012). Table 1 summarizes reports of Leishmania infection in wild animal from several regions in the world.

There are several reports of *Leishmania* infections in wild canids in Europe. Gray wolves (*Canis lupus*), which are considered a wild reservoir host for *L. infantum*, red foxes (*Vulpes vulpes*), Egyptian mongooses (*Herpestes ichneumon*), genets (*Genetta genetta*), and Iberian lynxes (*Lynx pardinus*), pine martens (*Martes martes*), and beech martens (*Martes foina*) have been diagnosed as asymptomatic carriers in Spain and Portugal (Sastre et al., 2008; Sobrino et al., 2008; Millán et al., 2011; Muñoz-Madrid et al., 2013; Criado-Fornelio et al., 2000). Diagnosis of *Leishmania* sp. infection in red foxes (*V. vulpes*) has also been reported in Italy and France (Mancianti et al., 1994; Dipineto et al., 2007; Davoust et al., 2014), as well as a report of a *Leishmania*-infected gray wolf (*C. lupus*) from Croatia (Beck et al., 2008).

There has also been a few reports of Leishmania infection in wild canids in the Middle East and North Africa, including jackals (*Canis aureus*) from Iran, Israel, and Algeria (Shamir et al., 2001; Mohebali et al., 2005; Bessad et al., 2012), and foxes (*V. vulpes*) and wolves (*C. lupus*) from Iran (Mohebali et al., 2005).