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A Case of Visceral Leishmaniosis in a Gray Wolf (*Canis lupus*) from Croatia

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ABSTRACT: The southern habitats of Croatia's gray wolf (*Canis lupus*) population are found in central and southern parts of Dalmatia. This region is recognized as an endemic region for canine visceral leishmaniosis, caused by *Leishmania infantum*. In November 2003, a 4-yr-old male gray wolf was found dead in the northwestern border of this endemic region. Pathologic and parasitologic analysis, confirmed by polymerase chain reaction, indicated that lesions associated with infection by *Leishmania infantum* are, in this case, typical for visceral leshmaniosis commonly described in dogs. Review of the literature suggests that this is the first reported case of gray wolf death due to lesions caused by *L. infantum*.

Key words: *Canis lupus*, Croatia, gray wolf, *Leishmania infantum*, pathology, PCR, visceral leishmaniosis.

Canine leishmaniosis is a vector-borne disease that causes a variety of lesions in dogs (*Canis familiaris*) ranging from local skin changes to systemic diseases that can be fatal. *Leishmania infantum* infection is zoonotic and endemic in regions of the New World, Middle East, and Mediterranean basin. Domestic dogs represent the main reservoir host of this pathogen in the Mediterranean basin (Fisa et al., 1999; Jaffe et al., 2004). Recent reports have also indicated that wild canids such as golden jackal (*Canis aureus*), red fox (*Vulpes vulpes*), and gray wolf (*Canis lupus*) can serve as secondary reservoirs in endemic regions of Iran, Israel, and the Palestinian region and in Spain (Fisa et al., 1999; Jaffe et al., 2004; Mohebali et al., 2005). In this case study, we describe the pathologic changes of visceral leishmaniosis leading to the death of a gray wolf in Croatia. The southern habitats of gray wolf population in Croatia are located in central and southern parts of Dalmatia. Population density is two wolves/100 km^2 (Kusak, 2002). The climate in this region is characterized with hot, dry summers and mild winters with the average temperature above 12.5 C (Seletković and Katušin, 1992). In winter 2003, a 4-yr-old male gray wolf was found dead near the village of Trolokve, (altitude 220 m) at 43°38′33.4″N, 16.13°10′10.5″E. The site is located on the northwestern border of a region described previously to be endemic for canine leishmaniosis in Croatia (Živičnjak et al., 2005) where *Phlebotomus tobbi*, a vector of *L. infantum*, was identified by Bosnić et al. (2006). The wolf was frozen and transported to the Department of General Pathology and Pathological Morphology of the Veterinary Faculty, University of Zagreb.

Organ samples collected at necropsy were fixed in 10% formalin. Thick paraffin sections (3 μm) were cut, and they were stained with hematoxylin and eosin (H&E) and Grocott's methenamine silver. Blood films from both femoral veins were stained with Diff-Quick® (ThermoFisher Scientific, Kalamazoo, Michigan, USA) for light microscopic evaluation. Amastigotes of *Leishmania* sp. were found in blood films. Oval bodies, 5 μm in diameter, with prominent nucleolus and specific kinetoplast were observed inside of macrophages or free amongst blood cells (Fig. 1). DNA was extracted from frozen prescapular lymph node with a QIAGEN blood and tissue kit (QIAGEN, Valencia, California, USA) according to manufacturer's instructions. Poly-

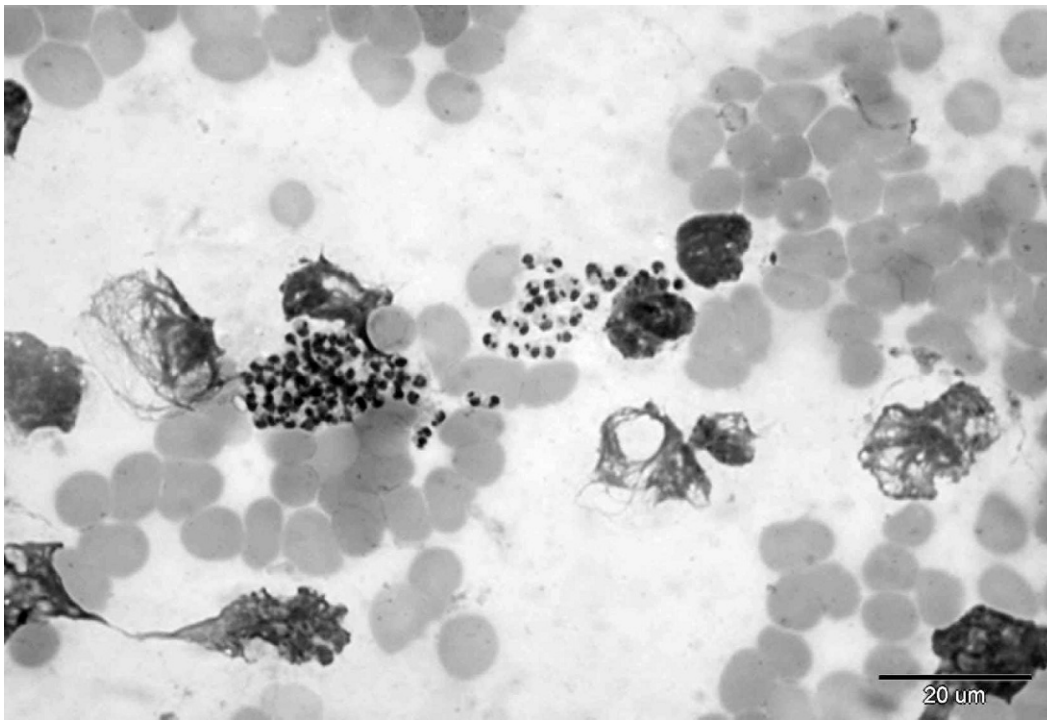

FIGURE 1. Numerous, mostly extracellular, amastigote forms of *Leishmania infantum* in blood film. Diff-Quick stain.

merase chain reaction was performed with forward primer 5′-CGTGACGCCGGT-GAAGAAT-3′ and reverse primer 5′-CGTGCACTCGGCCGTCTT-3′, according to protocol from Hide and Bañuls (2006) based on *cysteine protease b*, which discriminates between *L. infantum* and *L. donovani* by the fragment length. The amplified fragment of 702 base pairs (Fig. 2) characteristic for *L. infantum* was sequenced using the ABI PRISM® 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, California, USA) and deposited in GenBank database (accession no. EU145976). Necropsy revealed severe cachexia and dehydration. The most significant finding was generalized hair loss with reduced skin elasticity. White scaling and disseminated erosions, covered with crusts, were evident in the alopecic areas. Skin ulcerations were also present on the left hip and left fore and hind footpad. Generalized lymph node enlargement and hepatosplenomegaly were also found. Both sides of the heart were dilated, accompanied by moderate lung edema, hydropericardium, and hydrothorax. Myocardium seemed dull gray and friable. Multifocal whitish epicardial plaques were also evident. The right cranial lung lobe was consolidated. Disseminated

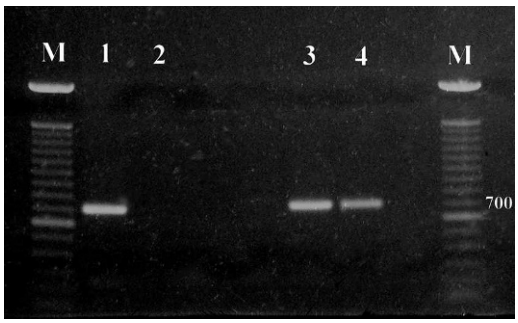

FIGURE 2. Electrophoretic separation of polymerase chain reaction products. Lane M, 100-bp ladder; lane 1, positive control of *cysteine protease b E* amplification from reference strain of *Leishmania infantum* MON-1 (702 bp); lane 2, negative control (no DNA); lanes 3 and 4, amplified products from *L. infantum*-infected wolf.

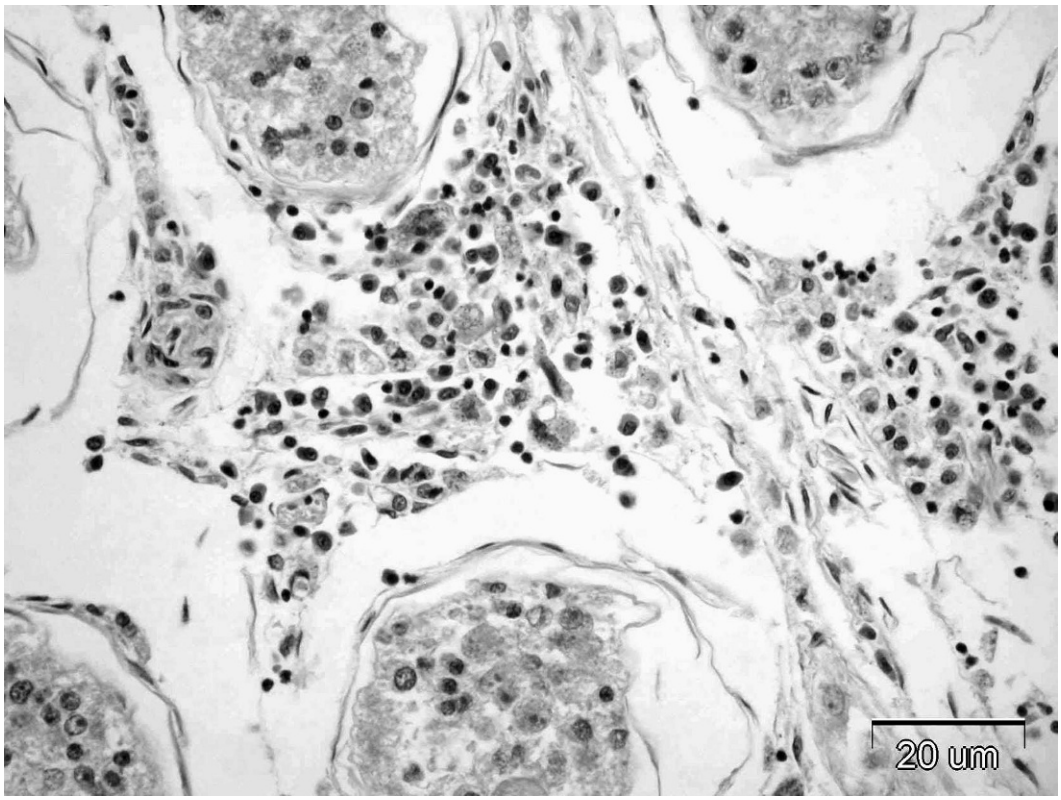

FIGURE 3. Chronic mononuclear interstitial orchitis. Plasma cells, lymphocytes, and macrophages infiltrate the interstitium. Note degeneration and azoospermia of seminiferous tubules. H&E stain.

focal hemorrhages also were present in the lungs. The stomach was filled with a mixture of mucus and hair. Both adrenal glands were enlarged. The urogenital tract showed no macroscopic changes.

The main microscopic skin lesion was severe, chronic dermatitis. Macrophages, plasma cells, and lymphocytes were found in the dermis around blood vessels and adnexa. Amastigotes were present in macrophages and fibroblasts. Hyperplastic epidermis and hair follicles had orthohyperkeratosis. Mixed cellular folliculitis and epidermal exocytosis also were evident due to secondary bacterial and also fungal infection, which was confirmed by Grocott's methenamine silver staining. Ulcerations were surrounded by hyperplastic epidermis and diffuse, mixed dermatitis. The spleen was congested and severely depleted. Red and white pulp were filled with plasma cells and macrophages containing amastigote forms of *L. infantum*, phagocytosed erythrocytes, and hemosiderin. The main finding in lymph nodes was follicular hyperplasia. Macrophages in the capsular granulomas and reticular cells of lymph nodes cords contained amastigotes. Cortical and medullary sinuses were closely packed with plasma cells, lymphocytes and macrophages that also contained amastigotes. Moderate disseminated chronic granulomatous hepatitis was accompanied by mild portal fibrosis of the liver. Amastigotes were only evident in groups of macrophages accumulated in sinusoids and particularly in the periportal areas. The liver was severely congested with dilated sinusoids. Atrophic hepatocytes containing hemosiderin in the cytoplasm were found. Multifocal, mononuclear, interstitial orchitis was found in both

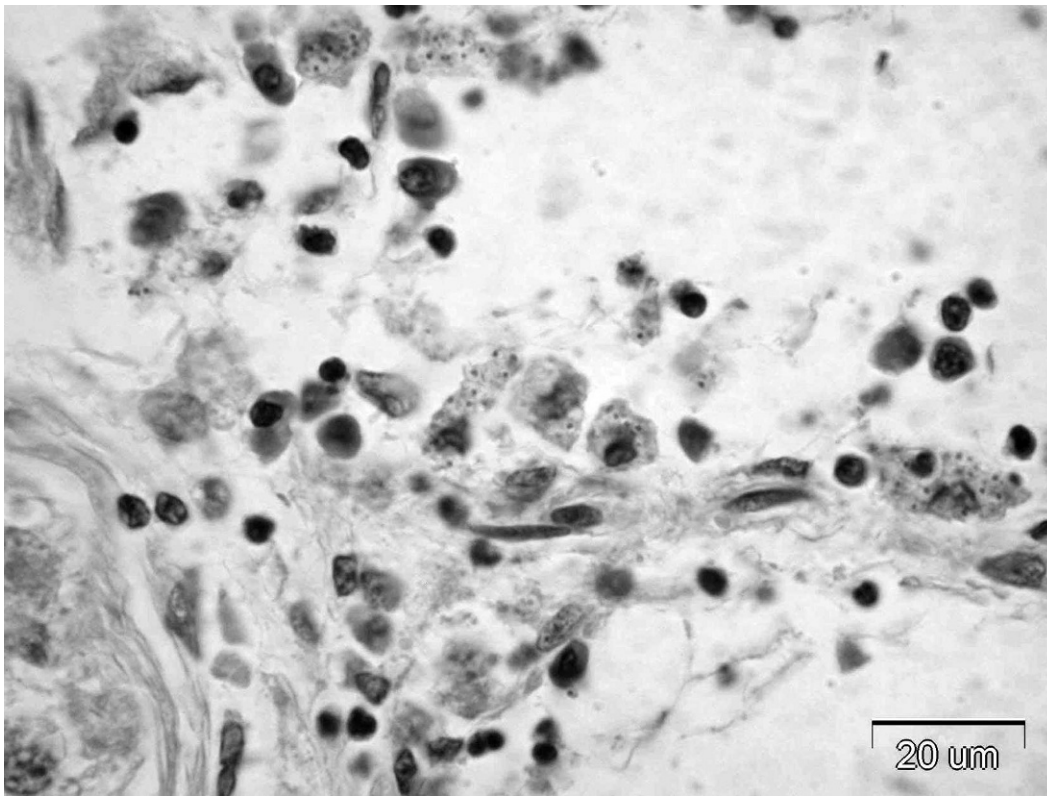

FIGURE 4. Higher magnification of chronic mononuclear orchitis showing many macrophages with intracytoplasmic amastigotes of *Leishmania infantum*. H&E stain.

testes. Among plasma cells and lymphocytes, moderate numbers of macrophages containing amastigotes were evident (Figs. 3, 4). Orchitis was associated with severe degeneration of seminiferous tubules and azoospermia. A few lymphoplasmacytic granulomas with macrophages containing amastigotes were noted throughout thickened capsules and interstitial cords of congested adrenal glands. Severe myocardial atrophy was found, muscle fibers were thin, and granules of brown pigment lipofuscin were evident around the nucleus in most fibers. Plasma cell clusters were noted around capillaries in thickened fibrotic epicardium. Lungs had alveolar edema and scattered hemorrhages. In the left consolidated cranial lobe, chronic interstitial pneumonia was identified and characterized by a mononuclear cell infiltrate that was predominantly plasma cells. Rare thromboses of alveolar capillaries were also observed. In the interstitium of the kidneys, rare plasma cell clusters were noted around blood vessels.

The results of this study showed for the first time that gray wolves could develop pathologic changes typical for visceral leishmaniosis without immune-complex deposition injuries characteristic for chronic leishmaniosis. Lesions seemed to be caused by disseminated amastigotes of *L. infantum*. Amastigotes were detected in macrophages of peripheral blood, lymph nodes, spleen, liver, skin, adrenal glands, and testes. Reticular cells of lymph nodes and spleen and skin fibroblasts were also highly infected. Consequences of this infection included chronic dermatitis, orchitis, lymphadenopathy, and hepatosplenomegaly, which are well described

for the main host of *L. infantum*, domestic dogs (Tryphonas et al., 1979; Nieto et al., 1992; Koutinas et al., 1993; Costa et al., 2003; Diniz et al., 2005; Rallis et al., 2005), and in one wild canid, a golden jackal (Hervas et al., 1996). Chronic lesions in other organs that would normally result in severe chronic immune-mediated glomerular nephritis, as described by Costa et al. (2003), were not present in this case. Kidney insufficiency is the most common cause of death resulting from leishmaniosis in dogs (Nieto et al., 1992). The gray wolf presented in this study died from heart insufficiency, which is the probable result of chronic wasting and malnutrition, characteristic symptoms of leishmaniosis (Robinson and Maxie, 1993). Only one description of a wolf positive for *L. infantum* has been described previously in the literature; however, in that case, detection was limited to PCR and no lesions were described (Mohebali et al., 2005). The low reported prevalence in wolves could be due to low infectiousness of *L. infantum* as described in crab-eating foxes (*Cerdocyon thous*) from Brazil (Courtenay et al., 2002). Our case of gray wolf visceral leishmaniosis may represent a rare spillover event from domestic dogs to a wild canid. Detailed epidemiologic investigation of *L. infantum* in gray wolf populations in Croatia will be required to determine the potential risks associated with *L. infantum* transmission between wolves and dogs and its zoonotic risk to humans.

LITERATURE CITED

Bosnić, S., L. Gradoni, C. Khoury, and M. Maroli. 2006. A review of leishmaniasis in Dalmatia (Croatia) and results from recent surveys on phlebotomine sandflies in three southern counties. Acta Tropica 99: 42–49.

Costa, F. A. L., H. Goto, L. C. B. Saldanha, S. M. M. S. Silva, I. L. Sinhorini, T. C. Silva, and J. L. Guerra. 2003. Histopathologic patterns of nephropathy in naturally acquired canine visceral leishmaniasis. Veterinary Pathology 40: 677–684.

Courtenay, O., R. J. Quinnell, L. M. Garcez, and C. Dye. 2002. Low infectiousness of a wildlife host of *Leishmania infantum*: the crab-eating fox is not important for transmission. Parasitology 125: 407–414.

Diniz, S. A., M. S. Melo, A. M. Borges, R. Bueno, B. P. Reis, W. L. Tafuri, E. F. Nascimento, and R. L. Santos. 2005. Genital lesions associated with visceral leishmaniasis and shedding of *Leishmania* sp. in the semen of naturally infected dogs. Veterinary Pathology 42: 650–658.

Fisa, R., M. Gállego, S. Castillejo, M. J. Aisa, T. Serra, C. Riera, J. Carrió, J. Gállego, and M. Portús. 1999. Epidemiology of canine leishmaniosis in Catalonia (Spain): The example of the Priorat focus. Veterinary Parasitology 83: 87–97.

Hervas, J., A. Mendez, L. Carrasco, and J. C. Gomez-Villamandos. 1996. Pathological study of visceral leishmaniasis in a jackal (*Canis aureus*). Veterinary Record 139: 293–295.

Hide, M., and A.-L. Bañuls. 2006. Species specific PCR assay for *L. infantum/L. donovani* discrimination. Acta Tropica 100: 241–245.

Jaffe, C. L., G. Baneth, Z. A. Abdeen, Y. Schlein, and A. Warburg. 2004. Leishmaniasis in Israel and Palestinian Authority. Trends in Parasitology 20: 328–331.

Koutinas, A. F., D. W. Scott, V. Kantos, and S. Lekkas. 1993. Skin lesions in canine leishmaniasis (Kala-Azar): A clinical and histopathological study on 22 spontaneous cases in Greece. Veterinary Dermatology 3: 121–130.

Kusak, J. 2002. Conditions for life of wolves (*Canis lupus* L.) in Croatia. PhD Dissertation, Biology, Faculty of Veterinary Medicine, University of Zagreb, Croatia, pp. 192–199.

Mohebali, M., H. Hajjaran, Y. Hamzavi, I. Mobedi, S. Arshi, Z. Zarei, B. Akhoundi, K. N. Naeni, R. Avizeh, and M. Fakhar. 2005. Epidemiological aspects of canine visceral leishmaniosis in the Islamic Republic of Iran. Veterinary Parasitology 29: 243–251.

Nieto, C. G., I. Navarrete, M. A. Habela, F. Serrano, and E. Redondo. 1992. Pathological changes in kidneys of dogs with natural *Leishmania* infection. Veterinary Parasitology 45: 33–47.

Rallis, T., M. J. Day, M. N. Saridomichelakis, K. K. Adamama-Moraitou, L. Papazoglu, A. Fytianou, and A. F. Koutinas. 2005. Chronic hepatitis associated with canine leishmaniosis (*Leishmania infantum*): A clinicopathological study of 26 cases. Journal of Comparative Pathology 132: 145–152.

Robinson, F. R., and M. G. Maxie. 1993. The cardiovascular system. *In* Pathology of domestic animals, K. V. F. Jubb, P. C. Kennedy and N. Palmer (eds.). Academic Press, San Diego, California, 27 pp.

Seletković, Z., and S. Katušin. 1992. Climate of Croatia. *In* Forests in Croatia, Đ. Rauš (ed.). Šumarski fakultet Sveučilišta u Zagrebu and (Hrvatske šume), Zagreb, 13–18 pp.

Tryphonas, L., Z. Zawidzka, M. A. Bernard, and E.

A. JANZEN. 1979. Visceral leishmaniasis in a dog: Clinical, hematological and pathological observations. Canadian Journal of Comparative Medicine 41: 1–12.

ŽIVICNJAK, T., F. MARTINKOVIĆ, A. MARINCULIĆ, V. MRLJAK, N. KUČER, V. MATIJATKO, Ž. MIHALJEVIĆ, AND R. BARIĆ-RAFAJ. 2005. A seroepidemiological survey of canine visceral leishmaniosis among apparently healthy dogs in Croatia. Veterinary Parasitology 131: 35–43.

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