Network for wildlife health surveillance in Europe

Diagnosis Card

Leishmaniosis

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Last update

January 2025

Etiology

Leishmaniosis[1] is caused by diphasic protozoans of the genus *Leishmania* (class Kinetoplastea, family Trypanosomatidae). In Europe, *Leishmania* (*Leishmania*) *infantum* is the primary agent causing zoonotic visceral and cutaneous leishmaniosis. However, other human-pathogenic species such as *L. donovani*, *L. tropica* or *L. major* circulate in the neighboring countries and may infect animals. Some European reptiles are infected with *Leishmania* (*Sauroleishmania*) *tarentolae*, a non-pathogenic species that was recently detected in human blood donors and in sheltered dogs.

Affected species (wildlife, domestic animals, humans)

A broad range of mammals are susceptible to *L. infantum* infection. The main peridomestic reservoir is considered to be the dog, but infection has been confirmed in cats, ferrets, horses, wild (either free-ranging or captive) carnivores, rodents, chiropterans, lagomorphs, and hedgehogs, as well as in captive primates and macropods. Different studies have observed that under certain circumstances, some wild hosts can act as alternative reservoirs for the parasite, and xenodiagnoses experiments have confirmed the ability of a handful of wild species to transmit the infection to sandflies.

Epidemiological characteristics and disease course

Leishmania infantum main way of transmission is through the bites of sandflies of the genus *Phlebotomus* (Old World). Other non-vectorial routes of transmission (in utero, venereal, blood transfusion) are possible but infrequent. Dogs act as main peridomestic reservoirs for human infection and themselves are also affected. Canine leishmaniosis is a chronic disease and clinical signs may develop 3 months to years after infection. Prevalence of subclinical infections is high in dogs, and infected animals can keep a clinically healthy status during long periods although they can transmit the parasite to sandflies. It has been proposed the existence of domestic and sylvatic cycles with eventual events of spill-over between them. For example, an outbreak of human leishmaniosis in Madrid was linked to Iberian hares. The disease is endemic in the Mediterranean basin but due to the global warming, cases of leishmaniosis in dogs and/or detection of DNA of *Leishmania* in wildlife are being reported at higher latitudes. Cases tend to be more frequent during the sandfly season, i.e. around summer, although DNA or seropositivity in different hosts can be found all year round.

Clinical signs

The majority of the information about clinical signs and lesions is derived from domestic carnivorans because the information about these features in European wildlife is almost nonexistent. Most studies did not find apparent general clinical signs in infected and/or seropositive animals. Nevertheless, some individuals can develop clinical leishmaniosis with lesions resembling those in sick dogs, including weight loss, lymphadenomegaly, cutaneous lesions (e.g., exfoliative, nodular, and/or ulcerative dermatitis). Clinicopathologic abnormalities can include non-regenerative anemia, thrombocytopenia,

[1] *In this card, Leishmaniosis is used over Leishmaniasis, following the recommendations of the Standardized nomenclature of animal parasitic diseases (SNOAPAD). See Kassai et al. 1988. Standardized nomenclature of animal parasitic diseases (SNOAPAD). Vet Parasitol 29:299-326.*

hyperproteinemia, hyperglobulinemia, hypoalbuminemia, azotemia, proteinuria, among others.

Gross lesions

In dogs, severe cases include cachexia and amyotrophy. Skin lesions and lymph nodes enlargement are primary lesions well described. Other findings may include hepatomegaly, splenomegaly, glomerulonephritis, arthritis, and ocular lesions. In rare occasions it causes orchitis, pancreatitis, meningitis, and myocarditis. In a wolf with visceral leishmaniosis, chronic dermatitis, orchitis, lymphadenomegaly, and hepatosplenomegaly were observed.

Histological lesions

Histologically, the most common patterns of skin inflammation are granulomatous perifolliculitis, interstitial dermatitis, and superficial and deep perivascular dermatitis, with inflammatory infiltrate composed primarily of macrophages and fewer lymphocytes and plasma cells. Leishmania amastigotes are found intracellularly within parasitophorous vacuoles mainly in the cytoplasm of macrophages, or extracellularly. Other typical findings are acanthosis, hyperkeratosis and superficial crusting. Histological lesions in other organs include macrophage proliferation and focal granulomas in the spleen, granulomatous hepatitis and fibrosis, glomerulonephritis and interstitial nephritis. Other organs such as the eyes, nasal cavity, testis, and gastrointestinal tract may also develop inflammatory patterns.

Differential diagnosis

Dermatoses: demodicosis, sarcoptic mange, pyodermatitis, ringworm, autoimmune skin diseases (e.g., lupus erythematosus, pemphigus foliaceus), and cell squamous carcinoma. Systemic disorders: lymphoma, ehrlichiosis, anaplasmosis.

Criteria for diagnosis

Confirmation of *Leishmania* infection: microscopic observation of *Leishmania* amastigotes; amplification of *Leishmania* DNA; *Leishmania* parasites isolation; quantitative serology for the detection of *Leishmania*-specific antibodies. High antibody levels due to an exacerbated humoral response associated with clinical signs and/or clinicopathological abnormalities compatible with leishmaniosis usually confirm the diagnosis.

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

Quantitative serological tests (IFAT, ELISA) are the standard techniques for the diagnosis of sick dogs while qualitative rapid tests (dot-ELISA and/or immunochromatographic assays) are only indicated for a first screening of healthy animals. Cytology, histology, immunohistochemistry, and culture (NNN medium) from aspirates or biopsies of target organs or tissues (bone marrow, lymph nodes, spleen, skin lesions are the most likely testing for detecting the parasite). Conventional PCR, nested PCR and real-time PCR targeting genes (SSUrRNA, kDNA, ITS1, hsp70), followed by DNA sequencing are the most sensitive tests for demonstrating the infection in target organs previously mentioned. Other tissues such as conjunctival and oral swabs are considered useful as non-invasive samples. Peripheral blood is not recommended due to its reduced sensitivity. Bone marrow and lymph node aspirates should be placed in tubes containing anticoagulant (EDTA), NET-10 buffer (10 mM NaCl, 10 mM EDTA,10 mM Tris-HCl, pH 8) or a similar buffer. Tissue samples for molecular diagnosis should be preserved in tubes without additives and analyzed directly, or stored at -20ºC or in 95% ethanol until further analysis.

EWDA proposed harmonized protocol (for harmonization at large scale)

Quantitative serology (IFAT or ELISA) in serum samples with validated tests. PCR-based molecular methods performed on the preferred samples: bone marrow, lymph node, spleen, skin. Species typing by sequencing is recommended in case of DNA detection. Cytological examination (Giemsa's stain) of skin lesions, mucosal or muco-cutaneous lesions (tissue imprints or aspirates), bone marrow, or lymph node aspirates for the microscopic observation of amastigotes infecting macrophages or extracellular fluid. Bone marrow and lymph node aspirates should be placed in NET-10 buffer (200 µl) or EDTA tubes. Biopsies and swabs should be kept in tubes without additives or with ethanol (70%). All samples must be kept refrigerated (-4ºC) until transport to the laboratory (within 24-48 hours) for direct analysis or stored at -20ºC until serological and molecular processing.

Laboratories that can be contacted for diagnostic support

- Reference laboratory of the WOAH for *Leishmania* diagnosis (see: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/). Istituto Zooprofilattico Sperimentale della Sicilia (IZSSi), National Reference Centre for Leishmaniasis (C.Re.Na.L.) (https://crenal.izssicilia.it/).
- Centro Nacional de Microbiología. Madrid, Spain. WHO Collaborating Center for Leishmaniasis (https://cnm.isciii.es/en/w/leishmaniasis-y-enfermedad-de-chagas-1/-/categories/38382?&category=38382)
- PetParasiteLab (Animal Health Dept. Veterinary Faculty. Universidad Complutense de Madrid, Spain)(https://petparasitelab.com/).

Recommended literature

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