Canine Leishmaniasis: Clinical and Diagnostic Aspects*

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ABSTRACT: Canine leishmaniasis is a chronic parasitic disease transmitted by small blood-sucking insects. Clinically, affected patients present with progressive wasting, slight or marked reduction in appetite, lymphadenomegaly, splenomegaly, and cutaneous lesions (e.g., dry desquamation [dry seborrhea], ulcers, nodules). A correct diagnosis is based on specific serologic and molecular tests and nonspecific hematologic tests, which show principally normocytic, normochromic, poorly regenerative anemia and an increase in total serum protein levels associated with a polyclonal gammopathy.

Leishmaniasis is a zoonotic protozoal disease caused by various species of the genus *Leishmania*. It is transmitted by small bloodsucking insects of the genera *Sergentomyia*, *Warileya*, *Brumptomyia*, *Lutzomyia*, and *Phlebotomus*. *Leishmania* is present in numerous countries in both the Old and New World. There are about 12 million cases of human leishmaniasis worldwide, and there are 400,000 to 1,200,000 new cases annually, principally in equatorial and subequatorial regions. The clinical features of the disease vary widely. In humans, it can be divided into visceral, cutaneous, and mucocutaneous forms. The clinical features in dogs are similar to those in the human visceral form associated with serious cutaneous complications. Dogs and humans are the principal definitive hosts. The principal etiologic agents responsible for visceral leishmaniasis are *Leishmania infantum* in the Mediterranean area and North America, *Leishmania donovani* and *L. infantum* in Asia (particularly in India and China) and Africa, and *L. donovani* and *Leishmania chagasi/L. infantum* in Central America (e.g., Brazil, Venezuela) (Figure 1).

Isolated foci of canine visceral leishmaniasis have been recently reported in areas of the New World previously considered to be disease-free. Endemic canine leishmaniasis has also been rarely reported in the United States. The only reported autochthonous cases originated in Ohio, Texas, Maryland, and Oklahoma, and antileishmanial antibodies have been detected in asymptomatic cases.

*A companion article on therapeutic aspects of the disease appears on p. 370 of this issue.
animals in Alabama and Michigan. These data indicate an evolution of the disease and an increase in its zoonotic potential. Recent studies carried out in numerous US states and two Canadian provinces have demonstrated a seropositivity of 1.8% in hunting dogs; the parasite has been typed as *L. infantum* MON 1.9

In nonendemic areas of the world, knowledge of the disease is fundamental in making an early clinical and/or serologic diagnosis in animals arriving from endemic areas and in establishing a suitable therapeutic protocol to reduce the number of parasites and limit the potential danger to infected animals. In endemic areas, veterinarians play a primary role in the development and execution of suitable protocols for animal control, with the aim of reducing the incidence of disease in dogs and, therefore, humans.

This article describes the clinical diagnosis of canine visceral leishmaniasis to help veterinarians recognize and understand the disease. It includes the differential diagnosis with respect to other canine pathogens, such as *Ehrlichia*, *Rickettsia*, and *Hepatozoon*, found endemically in the Old and New World.

**CAUSE AND BIOLOGIC CYCLES**

*Leishmania* are dixenic protozoal parasites belonging to the order Kinetoplastida, family Trypanosomatidae, which need a bloodsucking intermediate host as vector and a vertebrate as final host. They have a characteristic mitochondrial structure containing DNA (kinetoplast), which is attached to a basal body that gives rise to a single flagellum. There are three different morphologic forms:

- **Amastigotes** are 2- to 5-µm oval bodies containing an eccentric nucleus and no flagellum and are found within the cells of the vertebrate host.
- **Promastigotes** are 10 to 15 µm, with a long flagellum of up to 20 µm, and are typically found in the invertebrate host.
- **Paramastigotes** are found in the invertebrate host but have a shorter, stouter flagellum.

The parasite is transmitted to humans and animals by sand flies of the genus *Lutzomyia* in the New World and *Phlebotomus* in the Old World.18 Soil flies are very small insects (2 to 3 mm) that depend on a blood meal for the maturation of their eggs. The meal generally occurs during the night, with peak feeding at 3:30 AM to 5:30 AM. Only the females are bloodsuckers. In temperate climates, sand fly maturation (i.e., embryo, larva, and pupa) is very slow during the cold season, whereas there can be two complete cycles of development during the warm season.9

The duration of the biologic cycle in the invertebrate host varies from a minimum of 4 days to a maximum of 20 days, depending on the external climatic conditions.10 The amastigotes ingested by the female insect vector multiply and transform into paramastigotes and promastigotes within the intestines. *Leishmania* then migrate toward the proboscis, from which they can subsequently be transferred to the final host11 (Figure 2). The parasites are inoculated into the skin of the final host and are phagocyted by cutaneous macrophages (Langerhans cells). They are then transformed into amastigotes.

Reports of granulomatous endometrial lesions caused by *Leishmania* in bitches aborting in the late stages of gestation (i.e., days 46 to 48)12 and the death of a 4-day-old puppy born to a 4-year-old bitch with chronic leishmaniasis suggest the possibility of transplacental transmission13 (Figure 2).

**PATHOGENESIS**

Experimental studies on laboratory animals have shown that...
the outcome of *Leishmania* infection depends on the type of immune response evoked. Sixty percent to 80% of dogs from endemic areas have had contact with the parasite, as demonstrated by the presence of specific antibodies or a specific cellular immune response; but only a few dogs show clinical signs of disease. Protective immunity is cell-mediated (CD4+) and depends on the phenotype of the T helper activated.\(^{14}\) Thus production of interferon-\(\gamma\), interleukin (IL)-2, tumor necrosis factor--\(\alpha\), and IL-12 by the phenotype Th 1 is associated with resolution of the infection and, consequently, the protection of the animal. However, production of IL-4, IL-6, and IL-10 by the phenotype Th 2 is associated with progression of the infection into the disease state.\(^{15-17}\) In sick dogs, phagocytes have a diminished capacity to kill engulfed parasites. The abnormal production of nonprotective antibodies, which include autoantibodies, causes immunologic alterations that can lead to conditions such as vasculitis, polyarthritis, cutaneous ulceration, uveitis, and glomerulonephritis.\(^{17}\)

**CLINICAL FINDINGS**

Leishmaniasis in dogs is generally subacute or chronic; the acute form with fever has been noted in only 4% of dogs.\(^{18}\) Although sand flies are prevalent in temperate endemic areas during the spring and summer, the disease does not manifest a seasonal variation. This is probably due to a long incubation period that has been shown experimentally to vary from a minimum of 1 year\(^{19}\) to a maximum of 4 years.\(^{20}\) Affected animals are usually outdoor dogs of both sexes that are 3 to 7 years of age. The incidence of infection is low in toy breeds and puppies younger than 6 months of age;\(^{21}\) presumably because their indoor environment reduces the possibility of nocturnal contact with sand flies.

Clinically, the disease may appear as progressive wasting, with slight or marked reduction in appetite and cutaneous lesions. The most common clinical signs are lymphadenomegaly (i.e., one or more lymph nodes) and splenomegaly, which are present in 89% and 54% of cases, respectively (see the box on p. 362). Skin lesions are generally characterized by nonpruritic, dry exfoliative dermatitis, with hypotrichosis or alopecia of the head (e.g., pinnae, dorsum of the nose, eyelids), the dorsal region, and, occasionally, the whole body. In chronic, immunodepressed, untreated dogs, repeated cutaneous infections (e.g., mycosis, pyo-

![Figure 2—Biologic cycle of *Leishmania* spp.](https://www.VetLearn.com)

![Figure 3—Severe dermatitis with alopecia, hypotrichosis, and hyperparakeratosis in an immunodepressed, untreated dog.](https://www.VetLearn.com)
The clinical signs of leishmaniasis can result in characteristic skin thickening (elephant skin; Figure 3). The typical signs of bilateral peri-orbital alopecia together with onychogryposis and weight loss can give affected animals the characteristic appearance of an old dog (Figure 4). Skin ulcers are observed in 40% of sick animals and are usually distributed over the limbs (e.g., plantar pads, bony protuberances), the margin of the auricular pinnae, and the nostrils or other mucous membranes (e.g., mouth, genitals; Figure 5). On examination of the skin, it is also possible to observe the presence of nonulcerated nodules of varying diameters (1 to 10 cm), similar to the anergic macrophagic granuloma found in human cutaneous leishmaniasis. Needle aspiration of these nodules shows numerous *Leishmania* amastigotes (Figure 6). Mucous membranes are often pale, depending on the degree of anemia, or reddish-brown as a result of hepatorenal damage.

Twenty-four percent of sick dogs show ocular lesions, varying from simple blepharoconjunctivitis to severe panophthalmitis; in 16% of cases, these lesions are the only clinical signs. The ocular signs most commonly involve the anterior segment of the eye and manifest as conjunctivitis (often nodular), keratoconjunctivitis,

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**Clinical Signs of Leishmaniasis**

1. Lymphadenomegaly
2. Pale mucous membranes
3. Dry exfoliative dermatitis
4. Ulcerations
5. Splenomegaly
6. Weight loss
7. Onychogryposis
8. Anorexia
9. Depression
10. Periorbital alopecia
11. Ocular signs
   - Blepharoconjunctivitis
   - Keratoconjunctivitis
   - Uveitis
   - Panophthalmitis
12. Alopecia
13. Epistaxis
14. Nonulcerated nodules
15. Arthropathies
16. Ascites
17. Hepatic involvement
18. Polyuria/polydipsia
19. Diarrhea

*Listed in order of descending frequency.
and/or anterior uveitis (Figure 7). The possible formation of posterior synechiae can cause glaucoma resulting from pupillary obstruction.22,23

Another clinical sign of Leishmania infection is epistaxis, which is usually unilateral and intermittent. It can be caused by severe thrombocytopenia, immune complex vasculitis, or localized ulceration of the nasal cavity mucosa. Animals affected by leishmaniasis may also present with lameness of varying severity caused by polymyositis and/or immune-mediated arthrosynovitis. Palpation of the diaphysis of the long bones provokes pain, as does flexion and extension. The Leishmania amastigotes can often be isolated from bone biopsies and synovial aspirates.24,25

Polyuria or polydipsia can indicate renal damage and are often the only clinical signs of disease. Affected animals present with severe nephritis or nephrotic syndrome derived from type 1 or 2 proliferative mesangioendothelial glomerulonephritis. Renal damage can also manifest as peripheral edema, ascites, proteinuria, and increased serum levels of urea and creatinine.26

Chronic colitis is a rare clinical finding and results in watery diarrhea with fresh blood or mucus accompanied by tenesmus and increased frequency of defecation27 (see the box on p. 362). Endoscopic examination shows extensive hyperemia of the colonic mucosa with the possible presence of small bleeding ulcers. Biopsy reveals infiltration by lymphoplasmocytes, histocytes, and numerous Leishmania amastigotes.

Chronic granulomatous hepatitis is found in about 5% of cases18,21,28 and causes vomiting, polyuria, polydipsia, poor appetite, and weight loss. Liver biopsy is essential for diagnosis and, in positive cases, shows diffuse infiltration of macrophages rich in amastigotes and small areas of necrosis.21

An even more infrequent clinical finding is granuloma of the penis, the so-called “petechial penis,” characterized by rings of about 4 cm in diameter containing small bright-red areas (1 to 2 mm) rich in Leishmania amastigotes.29,30

**LABORATORY FINDINGS**

Laboratory tests are essential for the diagnosis, prognosis, and monitoring of clinical progress during therapy (see the box on this page).

### Specific Tests Serologic

The use of serologic tests to detect anti-Leishmania circulating antibodies (mainly IgG, subclass IgG1) is fundamental for diagnosing canine leishmaniasis. The most widely used of these tests is the indirect immunofluorescent antibody (IFA) test.11 A titer of 1:80 or greater is considered a positive response.18 The sensitivity of this serologic test is high (98.7%), and a diagnostic approach to canine leishmaniasis can be formulated on the basis of the IFA result (Figure 8). Other serologic diagnostic tests include ELISA (with its modifications; e.g., Dot ELISA, Falcon assay test [FAST] ELISA), Western blot, complement fixa-

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**Laboratory Findings for Leishmaniasis**

<table>
<thead>
<tr>
<th>Specific Tests</th>
<th>Nonspecific Tests</th>
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<tbody>
<tr>
<td>IFA</td>
<td>Hematology&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ELISA</td>
<td>BUN,&lt;sup&gt;a&lt;/sup&gt; creatinine&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Molecular diagnosis:</td>
<td>Serum protein</td>
</tr>
<tr>
<td>PCR</td>
<td>electrophoresis&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Parasitology</td>
<td>Immunology</td>
</tr>
<tr>
<td>Cytologic test&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Latex test</td>
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<tr>
<td>Culture test</td>
<td>Coombs’ test</td>
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<tr>
<td>Antinuclear antibody test</td>
<td>ALT,&lt;sup&gt;a&lt;/sup&gt; AST, ALP</td>
</tr>
<tr>
<td></td>
<td>Creatine phosphokinase</td>
</tr>
<tr>
<td></td>
<td>Urinalysis&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Minimum database.

*ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase.
tion, indirect hemagglutination, and counterimmuno-electrophoresis. The sensitivity and specificity of these serologic tests is related to the quality of the antigens employed: Techniques using whole organism antigens give more reliable and repeatable results than those employing soluble antigens. However, some serologic tests (e.g., immunodiffusion in agar gel) have recently been readapted to provide results comparable to IFA.

Serologic results must always be correlated with clinical features and laboratory findings before it is possible to make a definitive diagnosis of leishmaniasis. In canine leishmaniasis, no direct correlation has been observed between serologic titer and severity of the clinical signs. The serologic titer has little value as a marker for monitoring treatment, but titer negativity at the end of treatment is a more valuable indicator, and the reappearance of seroconversion is an early indication of relapse.

Parasitologic Examination
The diagnosis of canine leishmaniasis is confirmed by direct observation of amastigotes in a free state or within macrophage cytoplasm obtained by needle aspiration of lymph nodes, spleen, and bone marrow (using May-Grünwald Giemsa stain; Figure 9) or by culture of tissues from these sites when previous direct microscopic examination is negative. A negative cytologic examination is found in approximately 16% to 18% of cases. Evan’s modified Tobie’s medium is usually employed as a suitable substrate for Leishmania growth.

Molecular Diagnosis
Molecular diagnostic tests are the most interesting of the new methods of biologic diagnosis. This is due to their high sensitivity and specificity. Molecular probes and polymerase chain reaction (PCR) can be used to detect an extremely small number of microorganisms or fragments of their genetic material. PCR can be used to identify animals that have been exposed to the parasite. A positive result must always be correlated to the clinical signs and/or other laboratory findings before a diagnosis of canine leishmaniasis can be made. The PCR test is helpful in asymptomatic cases or those with a titer less than 1:80 (Figure 8).

Nonspecific Tests
Total and Fractionated Proteins
The plasma protein profile is considered one of the most reliable markers for monitoring leishmaniasis. The results of serum electrophoresis together with estimates of the density of Leishmania in lymph nodes or bone marrow are essential for assessing the clinical course of the disease during and after therapy and for monitoring the frequent relapses.

Total serum protein levels are markedly increased in infected dogs and can reach more than 10 g/dl. This increase is due mainly to high levels of β- and γ-globulins, which often appear fused together in a characteristic electrophoretic pattern (Figure 10). Following renal or hepatic damage, a drastic fall in albumin levels can be observed. Occasionally, the loss of plasma protein is so high that it manifests, paradoxically, as hypopro-
teinemia. Mono- and oligoclonal gammopathies are occasionally found in canine leishmaniasis; therefore, neoplastic lympho- and immunoproliferative processes (such as myeloma) should be considered in the differential diagnosis.

### Hematology

Anemia is one of the most common findings in canine leishmaniasis (60% of cases). It usually manifests as normocytic, normochromic, poorly regenerative anemia with medullary hypoplasia. Thrombocytopenia and

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**Table 1. Differential Diagnosis of Leishmaniasis**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Course</th>
<th>Clinical Findings</th>
<th>Clinical Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leishmaniasis</td>
<td>Subacute; chronic</td>
<td>Lymphadenomegaly; splenomegaly; weight loss; cutaneous lesions; ocular lesions; anorexia; epistaxis; arthropathies; renal failure</td>
<td>Increase in total protein with hypergammaglobulinemia and hypoalbuminemia; anemia; possible increased serum BUN; creatinine and ALT activity; IFA test results of &gt;1:80</td>
</tr>
<tr>
<td>Hepatozoonosis</td>
<td>Subacute; chronic</td>
<td>Exposure to ticks; diarrhea; anorexia; nose and ocular discharge; paraparesis; weight loss; bone and muscular pain; fever; possible lymphadenomegaly</td>
<td>Leukocytosis; neutrophilia; monocytosis; hypergammaglobulinemia; moderate anemia</td>
</tr>
<tr>
<td>Ehrlichiosis</td>
<td>Acute; chronic</td>
<td>Exposure to ticks; fever (39.5˚C–40˚C); anorexia; depression; lymphadenomegaly; splenomegaly; pale mucous membranes; tendency to bleed; uveitis; neurologic signs</td>
<td>Severe pancytopenia; increased serum ALT, AST, and ALP activity; hypergammaglobulinemia; IFA test results of &gt;1:40</td>
</tr>
<tr>
<td>Babesiosis</td>
<td>Hyperacute; acute; chronic</td>
<td>Exposure to ticks; depression; fever; anorexia; pale mucous membranes; icterus; splenomegaly; petechiae; hemoglobinuria; disseminated intravascular coagulation</td>
<td>Regenerative anemia; positive results from a Coombs’ test; increased bilirubin serum levels; presence of the parasite in the erythrocytes; IFA test results of &gt;1:40</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>Subacute; chronic</td>
<td>Systemic or regional lymphadenomegaly; weight loss; pale mucous membranes; splenomegaly; hepatic involvement; diarrhea</td>
<td>Atypical lymphocytes in bone marrow and lymph nodes; increased serum ALT, AST, and ALP activity; increased serum calcium and bilirubin levels</td>
</tr>
<tr>
<td>Food allergy</td>
<td>Chronic</td>
<td>No seasonal pruritus; papules; plaques; erythema; ulcers; lichenification; hyperpigmentation; alopecia of the ears, distal axillae, back, and head; pyotraumatic dermatitis</td>
<td>Possible eosinophilia; hypoallergenic diet tests</td>
</tr>
<tr>
<td>Flea allergy dermatitis</td>
<td>Subacute; chronic; seasonal</td>
<td>Presence of fleas; seasonal pruritus; diffuse erythema of inguinal, perianal, and lumbar regions; broken hairs; pyotraumatic dermatitis; lichenification; hyperpigmentation</td>
<td>Presence of fleas or their feces; intradermal skin test (positive reaction: ≥2)</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>Chronic</td>
<td>Periorbital alopecia; pruritus; leg licking; sneezing; epiphora; external otitis; perianal adenitis</td>
<td>Intradermal skin tests (positive reaction: ≥2)</td>
</tr>
<tr>
<td>Demodicosis</td>
<td>Subacute; chronic</td>
<td>Young animals; periocular erythema or diffuse pustular folliculitis; generally no itch; pododermatitis</td>
<td><em>Demodex canis</em> in cutaneous scrapings</td>
</tr>
<tr>
<td>Sarcoptic mange</td>
<td>Subacute; chronic</td>
<td>Young or immunodeficient animals; inguinal, axillary, and pinna lesions (papules and scabs) or diffuse lesions; pruritus</td>
<td><em>Sarcoptes scabiei</em> in cutaneous scrapings</td>
</tr>
</tbody>
</table>
leukocytosis are observed in 29% and 24% of cases, respectively. A high erythrocyte sedimentation rate is a common finding due to factors such as anemia, high serum levels of \( \gamma \)-globulins and fibrinogen, and the presence of immunocomplexes and hypoalbuminemia.

**Renal and Liver Function**

Determining various parameters of renal function (e.g., serum creatinine, proteinuria, and blood urea nitrogen [BUN] values) can be useful for evaluating the degree of renal damage and for monitoring clinical progress during treatment. High BUN and creatinine blood levels that persist despite therapy suggest a poor prognosis. Liver damage is not usually severe. It manifests as an increase in alanine aminotransferase and aspartate aminotransferase levels, which tend to normalize after therapy. These hepatic parameters, however, do not have the same prognostic value as the renal ones.

**Clinical Immunology**

Latex, Coombs’, and antinuclear antibody tests are the most common autoimmune tests employed in diagnosing canine leishmaniasis. The positivity of these tests in the absence of a characteristic clinical picture indicates, at most, a condition of autoreactivity and not the existence of an autoimmune disease, which may subsequently manifest.

**Differential Diagnosis**

Numerous infectious and noninfectious diseases show similar clinical signs to those of canine leishmaniasis. The differential diagnosis of canine leishmaniasis is reviewed in Table 1.

**Prophylaxis**

Preliminary studies suggest that vaccination against canine leishmaniasis may be possible even though little is known about the immunologic response in animals that are predisposed to the disease or naturally resistant. More research is needed to optimize antigens, adjuvants, and immunization protocols to prepare first- and second-generation vaccines for field trials.

Mosquito netting and nocturnal isolation of animals in endemic areas may help reduce access of mosquitoes to infected animals during warm months. Environmental repellents may also prove useful as prophylactic aids. Recent experimental studies have shown the effectiveness of collars impregnated with deltamethrin; they are recommended for all animals in endemic areas, including those from *Leishmania*-free areas.

**Conclusion**

Canine leishmaniasis is often difficult to diagnose. Knowledge of the relevant clinical signs and significant laboratory findings is essential for a correct, early diag-
nosis and is useful for monitoring the clinical development of the disease in treated animals and for improving the epidemiologic methods in endemic areas.

REFERENCES


2. At what time of day are sand flies most active?
   a. 3 PM to 4 PM
   b. 6 PM to 8 PM
   c. 7 AM to 9 AM
   d. 3:30 AM to 5:30 AM

3. Which IL is considered to be protective?
   a. IL-2
   b. IL-6
   c. IL-4
   d. IL-10

4. What is the average incubation of leishmaniasis?
   a. a few days
   b. 1 to 4 years
   c. a few weeks
   d. 1 month

5. What is the most frequent clinical sign of leishmaniasis?
   a. ocular involvement
   b. granulomatous hepatitis
   c. lymphadenomegaly
   d. cutaneous involvement

6. Which of the following clinical signs indicates an immune-mediated process?
   a. periorbital alopecia
   b. lymphadenomegaly
   c. uveitis
   d. dry, exfoliative dermatitis

7. Which of the following is most frequently affected by Leishmania infection?
   a. kidney
   b. heart
   c. liver
   d. nervous system

8. When is fever most frequently present in leishmaniasis?
   a. in the terminal phase of the disease
   b. during the acute phases
   c. at any time during the course of the disease
   d. never

9. Which serologic test is most frequently employed to diagnose leishmaniasis?
   a. Western blot
   b. IFA
   c. indirect hemagglutination
   d. complement fixation

10. What are the most frequent findings on serum protein electrophoresis?
    a. beta fraction gammopathies
    b. polyclonal gammopathies associated with normo- hypoalbuminemia
    c. hypoalbuminemia
    d. a moderate increase in α protein