

# Canine leishmaniasis

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## KEY POINTS

- Canine visceral leishmaniasis is a severe systemic disease caused by the diphasic protozoan parasite *Leishmania infantum* in the Old World and *L. chagasi* in the New World.
- The geographical distribution of the disease is dependent on its insect vector, the sandfly *Phlebotomus* spp. in the Old World and *Lutzomyia* spp. in the New World.
- Common clinical signs are skin lesions, weight loss, anorexia, lymphadenopathy, ocular lesions, nasal bleeding, locomotory problems, and muscle atrophy.
- Chronic enteritis and renal failure are the most common cause of death.
- Frequent clinical laboratory findings are polyclonal hyperglobulinemia, hypoalbuminemia, hyperproteinemia, a slight increase in liver enzymes, non-regenerative anemia and thrombocytopenia, azotemia, and creatininemia.
- The definitive diagnosis is obtained by direct observation of the parasites or serological means.
- The best available therapy today is the combined use of pentavalent antimonial agents with allopurinol.
- The prognosis for a complete cure is guarded.
- Dogs are the main reservoirs for human visceral leishmaniasis.

## INTRODUCTION

Leishmaniasis is a disease of human beings and animals caused by the protozoan parasite of the genus *Leishmania*. Dogs usually develop the systemic (visceral) form of infection, with a highly variable clinical appearance. Canine leishmaniasis may be difficult to diagnose and frustrating to treat. Dogs are considered the main reservoir for visceral leishmaniasis in humans.

## ETIOLOGICAL AGENT

*Leishmania* organisms belong to the genus *Protozoa*, the order *Kinetoplastida* and the family *Trypanosomidae*. The parasite requires two different hosts, a vertebrate and an insect, to complete its cycle. The flagellate (promastigote) form is about 10–15  $\mu\text{m}$  long and is found in the insect vector and in laboratory cultures (Figure 1). In the vertebrate host the parasite is observed in the amastigote form (i.e., without flagellum), smaller (2–5  $\mu\text{m}$ ), and with a visible rod-shaped kinetoplast (Figure 2). The latter is a mitochondrial structure rich in DNA, associated with the flagellum. The amastigotes are often seen in the intracellular position, particularly in macrophages, where they are able to survive and reproduce by binary fission.

Several *Leishmania* species and subspecies, responsible for different clinical diseases in man and in the dog, have been identified (Table 1). Their classification is very complicated: initially they were classified following parasite morphology, insect vector, type of lesions, serological tests, and geographical distribution (1). Today more advanced techniques, involving isoenzymatic patterns, DNA peptide mapping, monoclonal antibodies, cell membrane structure

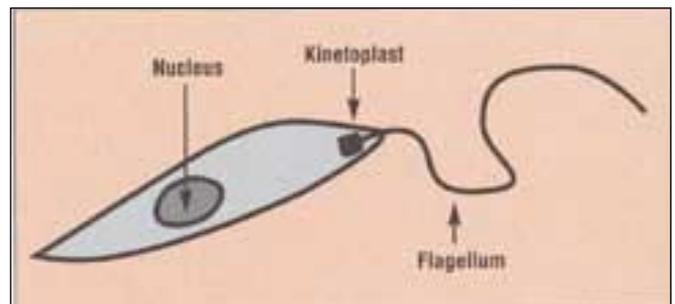


Figure 1 Schematic drawing of *Leishmania* spp. promastigotes.



Figure 2 Amastigotes of *Leishmania infantum* in a macrophage from a cytological preparation of a fine needle aspiration biopsy of an affected lymph node-Hemacolor, 1000 $\times$ .  
Courtesy of Dr. Fabia Scarampella.

analysis, and fatty acid analysis have identified at least 17 species and subspecies (2). The most accepted classification worldwide is based on isoenzyme electrophoretic analysis. Populations of parasites with similar isoenzyme patterns are called **zymodemes**. Another classification is based on analysis of kinetoplast DNA with restriction enzymes and recognizes different **schizodemes**.

## EPIDEMIOLOGY

*Leishmania infantum* is responsible for the Old World canine visceral leishmaniasis. This species, with different zymodemes, is present in all countries around the Mediterranean Sea, in Portugal, West, East and North Africa, the Middle East, India, and China (2, 3). Its geographical distribution reflects the living area of the insect vector *Phlebotomus* spp. This is a small (2–3 mm), silent, nocturnal, blood-sucking sandfly belonging to the family *Psychodidae*. *Phlebotomi* are mostly found in rural areas between 100 and 800 m above sea level and spend their life in a limited area, not exceeding 1.5 km around their birthplace.

In some endemic areas of the Mediterranean the incidence of seropositivity is about 5–15% of the canine population (4–8). Of seropositive dogs, 20–40% are asymptomatic carriers and may represent an unrecognized reservoir of the infection for other dogs and human beings (6–8). In some areas the incidence of clinical disease is about 3.5 new cases per 100 dogs per year.

The endemic area, and the total number of affected animals, has been increasing in recent years (4, 6). This could reflect greater mobility of dog owners with their pets and/or a change in the climatic conditions that favor the sandflies' survival in new areas.

*Leishmania chagasi* is the microorganism that causes the visceral disease in dogs and humans in the New World. This parasite is inoculated by the bloodsucking sandfly *Lutzomyia* spp. and its distribution includes Central and South America and small endemic areas in North America (Ohio, Alabama, Michigan) (9). In Oklahoma a case involving *L. infantum* has been described (2). The visceral disease caused by *L. chagasi* is very similar to the Old World visceral leishmaniasis, which will be described later.

Other species of *Leishmania* which may infect dogs and humans are *L. braziliensis* in Brazil, *L. tropica* in the Mediterranean area, and *L. mexicana* in Central America and Texas, USA (2). While the latter has never been associated with clinical disease in dogs (although antibodies against it have been found in the serum of animals living with affected owners), *L. braziliensis* and *L. tropica* can cause cutaneous nodules and mucosal ulceration in dogs (10, 11). Recently *L. mexicana* has been isolated from nodular cutaneous lesions in a cat in Texas (12). Rodents, and not dogs, are considered the main reservoir of these species of *Leishmania*.

The cases of canine leishmaniasis diagnosed in non-endemic areas are usually in animals which have been imported from endemic areas or which have been living there for some time. Occasionally, infections following even a short vacation have been reported. However, autochthonous cases have occasionally been described in countries where *Phlebotomus* spp. are absent (13, 14). In these cases the affected animals were born in non-endemic countries and had never traveled abroad, although they had been in contact with infected animals. This suggests the existence of an alternative insect vector – possibly a tick – or other ectoparasite. In one case a dog born in a non-endemic area from an affected bitch developed the disease, suggesting the possibility of transuterine transmission (14). The disease may occasionally be transmitted as a result of blood transfusions from infected animals.

There is no age, breed, or sex predilection for the infection, although it is thought that toy breeds are less affected, as they often have an indoor lifestyle. Furthermore, in endemic areas, the disease

**Table 1**  
***Leishmania* species, their distribution and the disease they cause in dogs and humans**

	<i>Dog</i>	<i>Human</i>
<b>Old World</b>		
<i>Leishmania infantum</i>	Visceral	Visceral, cutaneous
<i>Leishmania tropica</i>	Visceral, cutaneous	Cutaneous
<b>New World</b>		
<i>Leishmania chagasi</i>	Visceral	Visceral
<i>Leishmania mexicana</i>	Asymptomatic	Cutaneous
<i>Leishmania braziliensis</i>	Mucocutaneous	Mucocutaneous

is rarely seen in very young and very old dogs because of the long incubation period (usually more than four months) and the very low cure rate.

Visceral leishmaniasis in human beings is caused by the same parasites but is much less common than the canine variety. Most human patients have an immunosuppressive disease, such as HIV, or are receiving anti-tumor chemotherapy. Direct transmission from dogs to humans without an insect vector is probably impossible, although whether contact with open mucosal lesions is entirely without risk is not known.

## PATHOMECHANISM OF INFECTION

The insect vector sucks blood from an infected vertebrate and ingests the amastigote parasites. These multiply in the intestinal tissues and transform into promastigotes. The flagellum enables them to migrate into the insect's sucking apparatus. With each blood meal parasites are deposited into the skin of a new host. They are internalized by macrophages and other dendritic cells, where they can survive and multiply. Leishmanial organisms are able to live in the endothelial reticulum of host cells because they neutralize the host cell's pH and detoxify oxygen metabolites. In macrophages the parasites multiply by binary fission until they rupture the cell and spread to other macrophages.

It has recently been recognized (15) that Langerhans cells and other dendritic cells may also be infected by the parasite. These cells process and present parasitic antigens on their surface and are able to prime naive T helper (Th) cells and direct their response to the infection (16). Resistance to infection depends on a strong Type 1 T-helper response (Th1), which involves production of cytokines such as interferon gamma (IFN $\gamma$ ), tumor necrosis factor (TNF), interleukin (IL) 2 and IL 12. These cytokines stimulate the cell-mediated immunity which eliminates the infection. Animals that mount a strong Th1 response may have transitory positive serum antibody titers or may temporarily harbor the parasites before they eliminate them.

In other individuals disease occurs as a consequence of a Type 2 T-helper response (Th2), with production of IL4, IL5, IL6 and IL10, which in turn promote B-cell proliferation and antibody production. Unfortunately these antibodies are not protective and may even be detrimental, due to the formation of immune complexes and their subsequent deposition in basement membranes. Antibody-mediated opsonization of parasites may even increase phagocytosis by macrophages and their subsequent parasitosis, according to some authors (2). The cell-mediated immune system of susceptible dogs is impaired, and lymphocytes have decreased proliferative capacities *in vitro* if stimulated with *Leishmania* antigens (17, 18).

Infected animals may present with either reaction pattern – that is, either they develop a progressive disease with a fatal outcome, or



**Table 2**  
**Relative prevalence (%) of different signs in cutaneous leishmaniasis (signs with a prevalence of >4% only) (2, 3, 20, 25)**

Generalized lymphadenomegaly, symmetrical	71.2–96.1
Skin lesions (see <b>Table 3</b> )	75.0–89
Pale mucous membranes	58–94.2
Weight loss	30.7–70
Pyrexia	23.0–70
Lethargy	18–70
Anorexia	18–70
Splenomegaly	15–53.3
Renal failure	16–32
Ocular lesions	16–50
Epistaxis	10–37
Arthropathies	4–6.4
Acute form of leishmaniasis: fever and generalized lymphadenopathy and absence of skin lesions	4
Severe renal failure without other signs of leishmaniasis	4

they stay asymptomatic. However, in the natural disease both Th1 and Th2 cellular subtypes are activated and the variability of the clinical signs and severity of the disease depends on the balance between these two systems (17). Asymptomatic animals represent 20–40% of the seropositive population (6–8). Of these about 80% will eventually develop the disease.

The incubation period is very long – between one month and seven years. In this period the microorganisms disseminate widely, with a predilection for the bone marrow, lymph nodes, spleen, and liver.

Damage caused by the parasite depends on two factors (19):

- Direct action on the tissues, resulting in the formation of non-suppurative inflammatory lesions in skin, liver, intestines, kidneys, eyes, and bones.
- Indirect damage caused by immune complex deposition in the joints and in the basement membranes of kidneys, blood vessels, and eyes, resulting in vasculitis, glomerulonephritis, polyarthritis, and uveitis.

## CLINICAL DISEASE

Several organs may be affected, as parasites have been found in every part of the body, except, probably, the central nervous system. For this reason leishmaniasis may have several different clinical features. The relative prevalence of the different signs reported in the literature is summarized in **Table 2**.

The main presenting signs are weakness, decreased physical activity, skin disease and weight loss (**Figure 3**). The dogs usually appear much older than they are because of the prominent muscular atrophy, particularly on the head. Anorexia, if present, is probably related to renal failure. Weakness and decreased activity may be the consequences of anemia, muscle atrophy, polyarthropathy, or chronic renal failure. Locomotory problems are not very frequent and include shifting leg lameness, due to immune-mediated polyarthritis, polymiositis, and bone lesions, in which parasites are found in granulomatous inflammatory groups (20, 21).

Leishmanial organisms multiply in the macrophages of the liver, causing a chronic active hepatitis and, occasionally, palpable liver enlargement, vomiting, polyuria and polydipsia, anorexia, and weight loss. Chronic ulcerative colitis with large bowel diarrhea and melena



**Figure 3** Dog with leishmaniasis: weight loss and generalized scaling skin disease are apparent.

Courtesy of Dr Fabrizio Fabbrini.

**Table 3**  
**Relative prevalence (%) of different skin lesions (on total of affected animals) (3, 20, 25)**

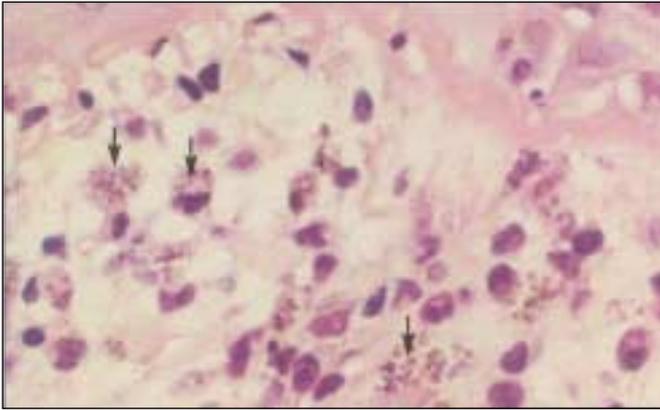
Dry exfoliative dermatitis	56–90.9
Ulceration	32.8–40
Periorbital alopecia	18
Diffuse alopecia	14
Onychogryphosis	24–54.5
Paronychia	13.6
Sterile pustular dermatitis	1.6–13.6
Nasal depigmentation	4.5
Nasal/digital hyperkeratosis	4.5
Non-ulcerated nodules	4.5–16.8



**Figure 4** Dog with alopecia and scaling dermatitis on the ear.

(22) as well as acute fatal hemorrhagic enteritis (20) have both been described associated with leishmaniasis. Enteritis may be the result of direct parasitic damage (granulomatous enteritis) or consequence of the renal failure. A case of acute hemorrhagic pancreatitis has also been reported (23).

A moderate to severe renal insufficiency is often seen in affected dogs. Histopathologically, two types of renal lesions have been described (14): membranous glomerulonephritis and an extra-membranous glomerulonephritis, both a consequence of immune-complex depositions. Proliferative lesions are rarely seen. Associated proteinuria may lead to nephrotic syndrome and uremia, which is the main cause of death in affected dogs. An acute, rapidly fatal renal insufficiency, without other signs of



**Figure 5** Histological appearance of leishmaniasis in the skin (alopecic scaling form): numerous parasitic bodies are seen in macrophages in the subepidermal dermis-Hematoxylin-Eosin, 500×. Arrows: macrophages full of parasitic bodies.



**Figure 6** Nasal depigmentation and ulceration and epistaxis. Courtesy of Dr Fabrizio Fabbrini.

leishmaniasis, may occur (20, 25).

Heart disease and thrombosis have been described (26, 27), but seem rather infrequent. Epistaxis, often unilateral, is frequently seen, and is considered the result of both ulcerative lesions of the nasal mucosa and/or impaired coagulation, due to hyperglobulinemia and thrombocytopenia (2). **Clinicians should note that concurrent ehrlichiosis may complicate leishmaniasis in some areas of the world, and, particularly, may contribute to the hematological abnormalities cited above.**

Lymphadenopathy, local or generalized, is one of the most consistent signs, although occasionally it may be absent. There is a proliferation of B-cell regions, and a depletion of T-cells. Cytologically the lymph nodes appear strongly reactive, with the presence of plasma cells, eosinophils, and macrophages, some of which may contain parasitic bodies.

Antibodies in the aqueous humor and cerebrospinal fluid, spongiform neuronal degeneration in the brain and cerebellum, mobilization of glial cells, and accumulation of amyloid have recently been described, associated with visceral leishmaniasis in dogs (28).

Skin lesions are frequent in canine leishmaniasis. The relative prevalence of the different cutaneous signs is shown in **Table 3**. A study by Ferrer and co-workers established an association between the type of skin lesions and the level or immunocompetence of the affected dogs (29). They recognized four main patterns of macroscopic and microscopic skin lesions, with different prognostic values:

**Symmetrical alopecia and a silvery scale (Figure 4)** is the most common presentation, and is reported in 60% of dogs with skin disease. The lesions often start on the head and then spread to the



**Figure 7** Characteristic appearance of ulceration due to vasculitis on the pinnae.



**Figure 8** Nodular leishmaniasis: several small cutaneous nodules are visible on the elbow.

rest of the body. Histologically, a diffuse infiltrate of macrophages, lymphocytes and plasma cells is observed in the dermis and occasionally subcutis. Parasites, which may be seen in high numbers inside macrophages (**Figure 5**), reach the skin by vascular dissemination and their presence in the dermis represents the direct cause of the dermatitis. Dogs with this form are the most immune competent of all affected animals (16).

**Ulceration (23%) (Figures 6 and 7)**, particularly affecting bony prominences, mucocutaneous junctions and extremities. Histological features include ulceration with a mixed infiltrate and very few parasites. Ulceration may occur due to the direct action of the parasites or to the necrotizing vasculitis caused by immune complex deposition (25). Dogs with this pattern probably have an intermediate immunocompetence (16).

**Multiple nodules** of variable sizes, from a few millimeters to 10 cm (12%) (**Figure 8**). Histologically there are accumulations of macrophages and high numbers of parasites are observed. Dogs with this reaction pattern may have an extremely ineffective immune response against the infection (16) as Langerhans cells are completely absent in the skin.

A generalized **sterile pustular dermatosis (4%)** on the trunk. Histological features include subcorneal pustules with mild dermal, non-suppurative infiltrate and very low numbers of parasites. The pathogenesis of this form is unknown.

In some dogs more than one pattern may be present, perhaps reflecting the balance between a cell-mediated and a predominantly antibody-mediated response. Other less frequent skin lesions associated with leishmaniasis are nasal and digital hyperkeratosis, onychogriphosis (**Figure 9**), paronychia, nasal and oral depigmentation, localized hyperpigmented hyperkeratotic plaques similar to nevi, ulcerative stomatitis, and nodular dermatofibrosis without renal lesions, which disappear with treatment (20).

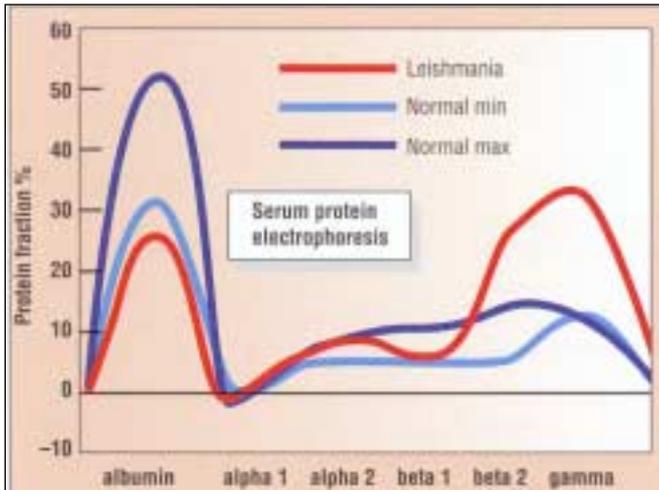


**Figure 9**  
*Moderate onychogryphosis.*



**Figure 10** *Ocular lesions: blepharitis, keratoconjunctivitis sicca and uveitis.*

*Courtesy Dr Fabrizio Fabbrini.*



**Figure 11** *Characteristic appearance of serum protein electrophoresis (red line) as it appears during leishmaniasis.*

Recently a mucosal form, characterized by tissue proliferation on the penis, tongue, in the nose, and in the mouth, has been described (30). In addition, histological features of interface band-like lymphoplasmocytic cellular dermatitis mimicking lupus erythematosus have been described with leishmaniasis (3).

Ocular lesions involve mainly the anterior segment of the eye (Figure 10). Periorbital dermatitis and associated blepharitis is one of the most common clinical signs. Keratoconjunctivitis sicca may occur, probably due to the direct destructive action of the parasites on the lachrymal apparatus (31). Other lesions include granulomatous conjunctivitis refractory to usual treatments, keratitis, granulomatous uveitis, immune complex-mediated anterior uveitis associated with corneal edema and closed-angle glaucoma,

**Table 4**  
**Clinical laboratory findings in dogs with visceral leishmaniasis (2, 3, 19, 20, 25)**

Hyperglobulinemia	70–100
Low albumin/globulin ratio	76
Hypoalbuminemia	68–94
High total serum protein (up to 13 g/l)	63.3–91
Hyporegenerative, normocytic normochronic anemia	21–94.2
Leukopenia (normal or low lymphocyte count)	22
Leukocytosis with left shift	8–24
Thrombocytopenia	29.3–50
Azotemia and creatininemia	38–45
Increase liver enzymes	16–61
Proteinuria	29–91
Positive ANA test	31–47
Positive Coombs test (mostly weak)	10–84

scleritis, and retinal hemorrhage. Iridocyclitis has been observed in dogs undergoing treatment and is considered by one author (13) as an allergic manifestation, similar to post-kala-azar leishmaniasis in humans.

## CLINICOPATHOLOGICAL FINDINGS

Clinical laboratory abnormalities and their relative prevalence (as reported in the literature) are summarized in Table 4.

The most frequent abnormality is hyperglobulinemia, due to polyclonal B-cell activation and antibody production. Hypoalbuminemia may be present and is the result of protein-losing nephropathy, liver disease, and malnutrition. Serum protein electrophoresis has a typical form, strongly suggesting leishmaniasis (Figure 11): a decrease of albumin is associated with an increase in beta- and gamma-globulins. Beta-1 and beta-2 globulins increase in the initial phase of the disease, followed by beta-3 and gamma-globulins. Monitoring serum protein electrophoresis is considered the best way of assessing response to treatment.

Thrombocytopenia and non-regenerative anemia may be the result of chronic leishmaniasis but the potential for concurrent diseases to complicate the hematological picture should not be overlooked.

## DIFFERENTIAL DIAGNOSES

The differential diagnoses of leishmaniasis are legion, since its clinical appearance is extremely variable. The alopecic desquamative dermatitis, if not associated with systemic signs, may look similar to demodicosis, keratinization disorders, sebaceous adenitis, and pyoderma. The ulcerative lesions have to be differentiated from lupus erythematosus, other causes of vasculitis, deep mycoses, and cutaneous neoplasia. Differential diagnoses of the cutaneous nodules are several skin tumors, sterile or infective granulomas, and nodular dermatofibrosis. Any pustular diseases, including pyoderma, pemphigus foliaceus, and demodicosis, are differential diagnoses.

If systemic signs are present, leishmaniasis may be confused with other infections, such as ehrlichiosis; clinically they may be very similar, although in ehrlichiosis platelet counts are often extremely low and petechiae and hemorrhages are often seen. The generalized lymphadenopathy has to be differentiated from neoplastic lymphoproliferative diseases (malignant lymphoma). Polyarthrititis, glomerulonephritis, vasculitis, and ulcerative skin

lesions are features observed also in systemic lupus erythematosus (SLE). Furthermore the histological appearance of lupus erythematosus and of leishmaniasis may be very similar. The differentiation of leishmaniasis from SLE may be very difficult, as up to 30% of the dogs with visceral leishmaniasis may have a (weakly) positive antinuclear antibody (ANA), 10% may have a (weakly) positive Coombs test (19) and 13% may have a positive lupus erythematosus-cell test (13).

Affected animals may have other concomitant diseases, possibly due to their weak cell-mediated immune responses, such as demodicosis (25), dermatophytosis (3), and hemoparasitosis such as ehrlichiosis (3, 9, 25).

## DIAGNOSTIC PROCEDURES

The diagnosis of canine visceral leishmaniasis is difficult for three main reasons (33):

- The clinical signs are very variable and may look similar to other diseases.
- The histopathological appearance is extremely nonspecific, and may be similar to that of other infectious or immune-mediated diseases.
- Currently, no available diagnostic test can offer a specificity and a sensitivity of 100%.

The clinical appearance of the affected dog may suggest the diagnosis but confirmation of the diagnosis is necessary. Three main types of tests are available:

- Parasitological techniques, whose aim is to visualize the microorganisms.
- Serologic tests, which identify circulating anti-leishmanial antibodies.
- Molecular methods (polymerase chain reaction), where parasite DNA is amplified and detected in host tissues.

### Parasitological techniques

These have a specificity of 100%, but may have a very low sensitivity. The identification of leishmanial organisms in cytological preparations made from fine needle aspiration biopsies of lymph nodes and bone marrow is rapid and easy. Bone marrow samples may be collected with a normal needle from the costochondral junctions or with a spinal needle from the iliac crest or sternum. The cytological smears are best stained with May-Gruenewald-Giemsa, and the parasites appear as small oval bodies (2–5  $\mu\text{m}$ ) containing a dark nucleus and a small kinetoplast in a perpendicular position (**Figure 2**). In bone marrow smears leishmanial organisms are found almost exclusively in macrophages, whereas in cytological preparations from lymph nodes they are frequently observed in an extracellular position, probably due to cell rupture during the sample collection and preparation. No relationship has been observed between the severity of the clinical disease and the number of parasites found in cytological smears (20).

Leishmanial organisms may also be observed occasionally in impression cytological preparations obtained from beneath crusts and scales, or by fine needle aspiration biopsies from cutaneous nodules. Unfortunately, the sensitivity of cytology as a diagnostic tool for canine leishmaniasis is low: bone marrow smears may be positive in only about 50–70% of the infected animals (25, 33), and lymph node smears in only about 30% of the cases. This technique also relies strongly upon the skill of the person performing it and on the time devoted to searching for the parasites in the smear, which should not be less than 10 minutes per sample.

Leishmanial organisms may also be observed in histological sections stained with Hematoxylin-Eosin or with Giemsa. They are

more numerous in the alopecic hyperkeratotic or nodular dermatitides, but can be very scarce or absent in other cutaneous forms, such as the ulcerative or the pustular form. In order to increase the sensitivity of this test, immunohistochemical and immunocytochemical techniques have been developed (34). These methods achieve the selective demonstration of the parasites by immunoperoxidase staining.

*Leishmania* spp. can be cultured and isolated from infected tissues in Novy-MacNeal-Nicolle (NMN) medium, where they can be observed in the promastigote form. Sensitivity tests to different drugs may be performed on these cultures. Parasite cultures are not routinely used in veterinary medicine, because they are difficult and expensive to perform and because they do not always yield positive results.

### Serological tests

Several serological tests, which measure circulating antibodies, have been developed. Among these, indirect immunofluorescence (IFAT), Dot-ELISA and direct agglutination test (DAT) are currently commercially available (18, 35–38). These tests usually have a high sensitivity and specificity (80–100%) but cannot be used as the only means of diagnosis as they may give false positive results in some healthy, resistant dogs which have previously come into contact with the parasite. False negative results may also occur in some affected dogs which have not produced antibodies (prepatent phase) (20, 36). In the latter case it is advisable to repeat the test after six to eight weeks. Furthermore, the serologic titers are not proportionate to the severity of the clinical disease (36, 38) and are not suitable as a means of monitoring treatment efficacy because antibody titers may remain measurable, even after clinical cure (33).

### Molecular methods

A polymerase chain reaction (PCR) technique has been developed recently and is a highly sensitive, and specific, diagnostic test for leishmaniasis (39). Parasitic kinetoplast DNA in liver, spleen, skin, lymph node, and bone marrow biopsies or blood is selectively identified and amplified (33), and both fresh and formalin-fixed paraffin embedded tissue can be used for this technique. This test can identify the presence of parasites, even in animals which have been clinically cured for years (40). Unfortunately, PCR for *Leishmania* spp. is currently only available in specialized laboratories.

## TREATMENT

Although treatment in the dog achieves clinical cure, it rarely results in complete elimination of the parasites and recurrences are frequent. No drug has yet been developed that safely and quickly eliminates the infection. Current research aims at new therapeutic protocols with drugs already in use and the development of new drugs (41). Current treatment protocols are summarized in **Table 5**.

Before starting treatment, a complete blood count, biochemical profile and urine analysis (including a quantitative test such as urine protein:creatinine ratio) should be performed in order to assess the renal and hepatic status. Furthermore, it is necessary to obtain a serum protein electrophoresis profile as a basal value for subsequent comparison and evaluation of treatment efficacy.

### Pentavalent antimonials

N-methylglucamine (meglumine) antimonate is currently the drug of choice for canine visceral leishmaniasis. In some countries this drug is not available and sodium stibogluconate is used in its place. Their mechanism of action is not clear: they may inhibit some parasite glycolytic enzymes. Meglumine antimonate is not absorbed



**Table 5**  
**Treatment regimens for canine visceral leishmaniasis**

Drug	Dose	Duration of treatment
Meglumine antimonate	50 mg/kg every 12 hours SC	20–40 days
Sodium stibogluconate	10–50 mg/kg daily SC	10–30 days
Allopurinol	10–30 mg/kg every 12 hours PO	9–12 months
Amphotericin B	0.5–0.8 mg/kg twice weekly	Up to a total dose of 6–16 mg/kg control plasma creatinine weekly interrupt for 2 weeks if creatinine > 25 mg/l
Aminosidine	5–10 mg/kg twice daily	2–3 weeks. Nephrotoxic and ototoxic
Meglumine antimonate + Allopurinol	As above for each drug	As above for each drug. Repeat meglumine antimonate at each administration
Amphotericin B + Allopurinol	As above for each drug	As above for each drug. In cases of meglumine antimonate resistance

if given by mouth and must be injected, preferably every 12 hours, as it has a very short half-life and is quickly eliminated in urine (42). Subcutaneous injection is preferred to intramuscular or intravenous administration because of higher bioavailability (43) and a longer half-life (42, 44). Meglumine antimonate, since it is readily excreted through the kidneys, does not significantly accumulate and has a very low toxicity. Side effects in the dog are painful local swellings at the site of injection, gastrointestinal problems (44), anorexia, locomotory problems, and fatigue (20).

The initial course of treatment is 50 mg/kg every 12 hours for 20 days. This is repeated if no obvious improvement is observed. If, after 40 days of treatment, no response is obtained, the *Leishmania* strain is considered resistant and other therapeutic options must be chosen. Resistant strains have been repeatedly described, probably due to inappropriate use of the drug by veterinarians or medical practitioners (45).

The use of meglumine antimonate does not prevent recurrences, which take place in about 75% of cases after 6–8 months (41, 44). Combination with allopurinol increases efficacy and decreases recurrence rate. Recent protocols (20, 36) advocate the use of meglumine antimonate (100 mg/kg every day) until the remission of clinical signs in combination with allopurinol (10 mg/kg every 8 hours or 20 mg/kg every 12 hours) for 9–12 months. The dogs are monitored with serum protein electrophoresis and if a recurrence is suspected then meglumine antimonate is administered again. If the disease does not recur within the first year then allopurinol is withdrawn and the dog is monitored every 3–6 months for the rest of its life.

Liposome-encapsulated meglumine antimonate may be commercially available in the future. This formulation has the advantage of obtaining higher serum concentrations, with decreased renal excretion and lower toxicity. Furthermore, liposomes are phagocytosed by macrophages and can exert their action in the cytoplasm and lysosome system where the parasites are located (46).

## Allopurinol

Allopurinol is a hypoxanthine analog. Leishmanial organisms are not able to synthesize purines and have to use the host molecules. Allopurinol is incorporated into the parasite's RNA and alters protein synthesis, inducing synthesis of abnormal proteins. As mammals are able to synthesize purines, this drug has a very low toxicity for these species. Allopurinol has a parasitostatic effect, and is best combined with other antileishmaniacal drugs, such as meglumine antimonate (see above) or amphotericin B (see below) in order to potentiate their effect and obtain longer remission periods. There are few reports of the successful use of allopurinol alone (38), although this can represent the sole therapeutic choice in countries where antimonial compounds are not readily available (47).

## Amphotericin B

Amphotericin B is a polyenic antibiotic produced by *Streptomyces nodosus*, currently indicated by intravenous administration for systemic and deep mycoses. It acts by irreversibly binding to ergosterole, a main component of the leishmanid cell membrane, causing its disruption and parasite death. Amphotericin B has some affinity to mammalian cholesterol and is toxic, causing a decrease in renal blood perfusion, renal filtration, creatinine clearance, and focal tubular necrosis. Other side effects include phlebitis, hyperthermia, and vomiting (48). In order to minimize the side effects it is diluted in 5% dextrose or in saline and administered by slow intravenous infusion (up to 5 hours). The drug is administered on alternate days at a dose rate of 0.15–0.5 mg/kg until a cumulative dose of 8–12 mg/kg has been given. Plasma creatinine should be measured before each administration in order to assess renal function. Although amphotericin B is 400 times more effective than meglumine antimonate against *Leishmania* spp. its use is limited to cases resistant to meglumine antimonate because of its toxic potential and difficult administration. Recently a new protocol has been successfully used by Lamothe to treat 39 cases (48). Intravenous injections (over 5–30 seconds) at a dosage of 0.5–0.8 mg/kg are given twice weekly until a total dosage of 6–16 mg/kg is reached. Creatinine is measured weekly during this period. Drug administration is interrupted for two weeks if creatinine values become significantly (> 20%) elevated. With this protocol the investigator obtained up to a 90% clinical cure rate, with only 10% recurrence after one year.

Recently, the efficacy and toxicity of a liposome-encapsulated amphotericin B have been evaluated in dogs (49). This formulation is much less nephrotoxic, as less than 1% is excreted through the kidneys, and seems to be effective, although frequent recurrences have been observed. The major problem with this formulation of the drug is that it is extremely expensive.

## Aminosidine

Aminosidine is an aminoglycosidic antibiotic, derived from *Streptomyces chrestomyceticus*, with antimicrobial action against a wide range of microorganisms. It acts by inhibiting normal ribosomal function and causes abnormal protein production. It is administered parenterally (IM or SC), as it is not absorbed by mouth, at a dose of 5–10 mg/kg twice daily. As with every other aminoglycoside, aminosidine can be nephro- and ototoxic and should be given only to dogs with adequate renal function. Some studies have observed that it gives better results in combination with meglumine antimonate than when the two products are used alone (50).

## Immunomodulation

Prednisolone may be used in dogs with renal insufficiency, if the use of meglumine antimonate is contraindicated. A dose of 1 mg/kg of prednisolone is given daily, with allopurinol and appropriate

supportive therapy until renal values (plasma creatinine and urea) return to normal. The prednisolone is then suspended and meglumine antimonate administered at half-dose (50 mg/kg daily) and then, if tolerated, at full dose (20).

Therapy with IFN  $\gamma$  and IL12 has been investigated in human medicine. These cytokines induce the shift from a Th2 to a Th1 type of reaction, thus potentiating the cell-mediated immune system which is able to eliminate the infection. It is possible that this treatment option may be available for dogs in the future.

### Monitoring therapy

The best way to monitor the therapeutic effect is serial evaluation of total plasma proteins and serum protein electrophoresis. These sequential values are compared with baseline values obtained before treatment is initiated. Serological titers alone are not suitable for the evaluation of the response to treatment as they are not related to the severity of the disease (36, 38). A complete cure should be confirmed by a negative cytological examination, disappearance of all clinical signs, normalization of all blood values, and two negative PCR results performed six months apart (33).

### PROGNOSIS

The aim of therapy is to eliminate the parasite and definitively cure the dog. Unfortunately, this is the exception rather than the rule, as recurrence rates are very high. It has been stated that affected dogs have a 75% probability of surviving at least four years if they are given a course of 21–42 days of meglumine antimonate and subsequent treatments at every recurrence (2). Dogs having a compromised renal function have the worst prognosis. A recent study reported that dogs can be maintained in clinical remission for years with long-term intermittent administration of allopurinol (51). In the USA, where sporadic outbreaks of the disease have been described, pentavalent antimonial compounds are not readily available and are not registered for use in dogs. Euthanasia of infected animals is often performed in order to prevent the transmission of the disease (which is potentially hazardous to human beings). However, it has been determined that a dog's infective potential correlates with the presence of clinical signs (especially skin lesions), and treated animals in clinical remission are not a source of infection (52). Thus euthanasia should be discouraged in endemic areas.

### PREVENTION AND CONTROL

Prevention and control of canine and human leishmaniasis can be achieved by control of sandflies and by taking steps to avoid exposure to them. In endemic areas dogs should not spend the night outdoors and fine mesh nets should be applied to the windows. A recent study demonstrated that the application of a repellent deltamethrin collar to dogs can protect them from sandflies' bites and prevent *Leishmania* infection (53).

The elimination of all clinically affected dogs has not improved the human infection rate where it has been imposed (54). However, treatment of all seropositive (symptomatic and asymptomatic dogs) has significantly decreased the prevalence of new infection cases in endemic areas (4). In the future, new prophylactic tools, such as vaccination, may be available.

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### REFERENCES

- Hommel, M. The genus *Leishmania*: biology of the parasites and clinical aspects. *Bulletin de l'Institut Pasteur* 1978; **75**: 5–102.
- Slappendel, R. J., Ferrer, L. Leishmaniasis. In: Green, C. E. (ed). *Infectious Diseases of the Dog and Cat*, 2nd edn. Philadelphia: Saunders, 1998: 450–458.
- Kontos, V. J., Koutinas, A. F. Old World Canine Leishmaniasis. *Compendium* 1993; **7**: 949–959.
- Mancianti, F., Gramiccia, M., Gradoni, L., Pieri, S. Studies on canine leishmaniasis control. Evolution of infection of different clinical forms of canine leishmaniasis following antimonial treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1988; **82**: 566–567.
- Mansueto, S., Miceli, M. D., Di Rosa, S. et al. Unusual increase of visceral leishmaniasis of adult in Sicily. *Giornale di Malattie Infettive e Parassitologia* 1987; **39**: 83–88.
- Moretti, A., Piergili Fioretti, D., Farinelli, M. Leishmaniosi canina. *Obiettivi e Documenti Veterinari* 1995; **10**: 19–25.
- Ozon, C., Marty, P., Veyssière, C., Haas, P., Le Fichoux, Y. Résultats d'une enquête sur la leishmaniose canine effectuée pendant une courte période chez les vétérinaires praticiens des Alpes-Maritimes. *Pratique Médicale Chirurgienne des Animaux de Compagnie* 1995; **30**: 199–201.
- Fisas, R. *Estudio sobre la estructura y dinamica del foco de leishmaniasis del Priorat*. Doctoral Thesis, University of Barcelona, 1992.
- Bravo, L., Frank, L. A., Brenneman, K. A. Canine leishmaniasis in the United States. *The Compendium on Continuing Education* 1993; **15**: 699–705.
- Pirmez, C., Coutinho, S. G., Marzochi, M. C. A., Nunes, M. P., Grimaldi, G. Canine American cutaneous leishmaniasis: A clinical and immunological study in dogs naturally infected with *Leishmania braziliensis* in an endemic area of Rio de Janeiro, Brazil. *American Journal of Tropical Medicine and Hygiene* 1988; **38**: 52–58.
- Morsy, T. A., Schnur, L. F., Feinsod, F. M. et al. Natural infections of *Leishmania tropica major* in domestic dogs from Alexandria, Egypt. *American Journal of Tropical Medicine and Hygiene* 1987; **37**: 49–52.
- Barnes, J. C., Stanley, O., Craig, T. M. Diffuse cutaneous leishmaniasis in a cat. *Journal of the American Veterinary Medical Association* 1993; **202**: 416–418.
- Slappendel, R. J. Canine Leishmaniasis. A review based on 95 cases in the Netherlands. *Veterinary Quarterly* 1988; **10**: 1–16.
- Diaz-Espineira, M. M., Slappendel, R. J. A case of autochthonous canine leishmaniasis in the Netherlands. *Veterinary Quarterly* 1997; **19**: 69–71.
- Saint-André Marchal, I., Marchal, T., Moore, P. F., Magnol, J. P., Bourdoiseau, G. Infection of canine Langerhans cells and interdigitating dendritic cells by *Leishmania infantum* in spontaneous canine leishmaniasis. *Revue de Médecine Vétérinaire* 1997; **148**: 29–36.
- Fondevila, D., Vilafranca, M., Ferrer, L. Epidermal immunocompetence in canine leishmaniasis. *Veterinary Immunology and Immunopathology* 1997; **56**: 319–327.
- Pinelli, E., Killink-Kendrick, R., Wagenaar, J. et al. Cellular and humoral immune response in dogs experimentally and naturally infected with *Leishmania infantum*. *Infection and Immunity* 1994; **62**: 229–235.
- Bourdoiseau, G., Bonnefont, C., Hoareau, E. et al. Specific IgG1 and IgG2 antibody and lymphocyte subset levels in naturally *Leishmania infantum*-infected treated and untreated dogs. *Journal of Veterinary Immunology and Immunopathology* 1997; **59**: 21–30.
- Ferrer, L. Leishmaniasis. In: Kirk, R. W., Bonagura, J. D. (eds). *Current Veterinary Therapy XI*, Philadelphia: Saunders, 1992: 266–270.
- Denerolle, P. Leishmaniose canine: difficulté du diagnostic et du traitement. *Pratique Médicale et Chirurgienne des Animaux de*



*Compagnie* 1996; **31**: 137–145.

21. Spreng, D. Leishmanial polyarthritis in two dogs. *Journal of Small Animal Practice* 1993; **34**: 559–563.

22. Ferrer, L., Juanola, B., Ramos, J. A., Ramis, A. Chronic colitis due to *Leishmania* infection in two dogs. *Veterinary Pathology* 1991; **28**: 342–343.

23. Carrasco, L., de Lara, F. C., Martin, E. et al. Acute haemorrhagic pancreatitis associated with canine visceral leishmaniasis. *Veterinary Record* 1997; **141**: 519–521.

24. Benderriter, T., Casanova, P., Nashkidachvili, L., Quilici, M. Glomerulonephritis in dogs with canine leishmaniasis. *Annals of Tropical Medicine and Parasitology* 1988; **82**: 1–7.

25. Ciaramella, P., Oliva, G., De Luna, R. et al. A retrospective clinical study of canine leishmaniasis in 150 dogs naturally infected by *Leishmania infantum*. *Veterinary Record* 1997; **141**: 539–543.

26. Font, A., Durall, N., Domingo, M. Cardiac tamponade in a dog with visceral leishmaniasis. *Journal of the American Animal Hospital Association* 1991; **21**: 481–487.

27. Font, A., Closa, J. M., Molina, A., Mascort, J. Thrombosis and nephrotic syndrome in a dog with visceral leishmaniasis. *Journal of Small Animal Practice* 1993; **34**: 466–470.

28. Garcia-Alonso, M., Nieto, C. G., Blanco, A. et al. Presence of antibodies in the aqueous humour and cerebrospinal fluid during *Leishmania infantum* infections in dogs. Pathological features at the central nervous system. *Parasite immunology* 1996; **18**: 539–546.

29. Ferrer, L., Rabanal, R., Fondevila, D., Ramos, J. A., Domingo, M. Skin lesions in canine leishmaniasis. *Journal of Small Animal Practice* 1988; **29**: 381–388.

30. Font, A., Roura, X., Fondevila, D. et al. Canine mucosal leishmaniasis. *Journal of the American Animal Hospital Association* 1996; **32**: 131–137.

31. Roze, M. Manifestations oculaires de la leishmaniose canine. *Recueil de Médecine Vétérinaire* 1986; **162**: 19.

32. Moreno, P., Lucena, R., Ginel, P. J. Evaluation of primary haemostasis in canine leishmaniasis. *Veterinary Record* 1998; **142**: 81–83.

33. Ferrer, L. Leishmaniasis: update in diagnosis and therapy. In: *Proceedings of the Fourteenth Annual Congress of the European Society of Veterinary Dermatology*, Pisa, 1997: 33–36.

34. Ferrer, L., Rabanal, R., Domingo, M., Ramos, J., Fondevila, D. Identification of *L. donovani* amastigotes in canine tissues by immunoperoxidase staining. *Research in Veterinary Science* 1988; **44**: 194–196.

35. Mancianti, F., Pedonese, F., Poli, A. Evaluation of dot enzyme-linked immunosorbent assay (dot-ELISA) for the serodiagnosis of canine leishmaniasis as compared with indirect immunofluorescent assay. *Veterinary Parasitology* 1996; **65**: 1–9.

36. Ferrer, L., Aisa, M. J., Roura, X., Portus, M. Serological diagnosis and treatment of canine leishmaniasis. *Veterinary Record* 1995; **136**: 514–516.

37. Harith, A. E., Slappendel, R. J., Reiter, I. et al. Application of a direct agglutination test for detection of specific anti-leishmania antibodies in the canine reservoir. *Journal of Clinical Microbiology* 1989; **27**: 2252–2257.

38. Vercammen, F., De Deken, R. Antibody kinetics during allopurinol treatment in canine leishmaniasis. *The Veterinary Record* 1996; **139**: 264.

39. Rodgers, M. R., Popper, S. J., Wirth, D. F. Amplification of kinetoplast DNA as a tool in the detection and diagnosis of *Leishmania*. *Experimental Parasitology* 1990; **71**: 267–275.

40. Berrahal, F., Mary, C., Roze, M. et al. Canine leishmaniasis:

identification of asymptomatic carriers by polymerase chain reaction and immunoblotting. *American Journal Tropical Medicine and Hygiene* 1996; **55**: 273–277.

41. Oliva, G., Cortese, L., Ciaramella, P., De Luna, R. Trattamento terapeutico della leishmaniosi del cane. *Veterinaria* 1996; **10**: 115–127.

42. Tassi, P., Ormas, P., Madonna, M. et al. Pharmacokinetic of N-methylglucamine antimoniate after intravenous, intramuscular and subcutaneous administration in the dog. *Research in Veterinary Science* 1994; **56**: 144–150.

43. Valladares, J. E., Alberola, J., Esteban, M., Arboix, M. Disposition of antimony after the administration of N-methylglucamine antimoniate to dogs. *Veterinary Record* 1996; **138**: 181–183.

44. Slappendel, R. J., Teske, E. The effect of intravenous or subcutaneous administration of meglumine antimoniate (Glucantime) in dogs with leishmaniasis. A randomised clinical trial. *The Veterinary Quarterly* 1997; **19**: 10–13.

45. Gramiccia, M., Gradoni, L., Orsini, S. Decreased sensitivity to meglumine antimoniate (Glucantime) of *Leishmania infantum* isolated from dogs after several courses of drug treatment. *Annals of Tropical and Medical Parasitology* 1992; **86**: 613–620.

46. Valladares, J. E., Freixas, J., Alberola, J. et al. Pharmacokinetics of liposome-encapsulated meglumine antimoniate after intramuscular and subcutaneous administration in dogs. *American Journal of Tropical Medicine and Hygiene* 1997; **57**: 403–406.

47. Lester, S. J., Kenyon, J. E. Use of allopurinol to treat visceral leishmaniasis in a dog. *Journal of the American Veterinary Medical Association* 1996; **209**: 615–617.

48. Lamothe, J. Essai de traitement de la leishmaniose canine par l'amphotéricine B (39 cas). *Pratique Médicale et Chirurgienne des Animaux de Compagnie* 1997; **32**: 133–141.

49. Oliva, G., Gradoni, L., Ciaramella, P. et al. Activity of liposomal amphotericine B (AmBisome ND) in dogs naturally infected with *Leishmania infantum*. *Journal of Antimicrobial Chemotherapy* 1995; **36**: 1013–1019.

50. Oliva, G., Gradoni, L., Cortese, L. et al. Comparative efficacy of meglumine antimoniate and aminosidine sulphate, alone or in combination, in canine leishmaniasis. *Annals of Tropical Medicine and Parasitology* 1998; **92**: 165–171.

51. Ginel, P. J., Lucena, R., Lopez, R., Molleda, M. J. Use of allopurinol for maintenance of remission in dogs with leishmaniasis. *Journal of Small Animal Practice* 1998; **39**: 271–274.

52. Alvar, J., Molina, R., San Andreas, M. et al. Canine leishmaniasis: clinical, parasitological and entomological follow-up after chemotherapy. *Annals of Tropical Medicine and Parasitology* 1994; **88**: 371–378.

53. Killick-Kendrick, R., Killick-Kendrick, M., Focheux C. et al. Protection of dogs from bites of phlebotomine sandflies by deltamethrin collars for control of canine leishmaniasis. *Medical and Veterinary Entomology* 1997; **11**: 105–111.

54. Dietze, R., Barros, G.B., Teixeira, L. et al. Effect of elimination of seropositive canines on the transmission of visceral leishmaniasis in Brazil. *Clinics Infectious Diseases* 1997; **25**: 1240–1242.