

Review articles

Canine leishmaniosis – an emerging disease

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ABSTRACT. Canine leishmaniosis (CanL) is an invasive disease of dogs, caused by *Leishmania* spp. parasites transmitted by the bite of an infected phlebotomine sand fly. CanL is declared an important disease by World Organisation for Animal Health (OIE). Due to its zoonotic potential is of a great importance the prevention of this disease in non endemic areas. Canine leishmaniosis is endemic disease in more than 70 countries and is a common disease in Mediterranean region. Recently, many cases have been reported in non endemic areas, like United Kingdom, Germany and Poland as well, where this disease is considered exotic. The aim of this article is to summarize shortly canine leishmaniosis, it's transmission, clinical manifestations, diagnostics procedure, treatment, prognosis and prevention. Increasing knowledge about this disease can be of a great use for veterinary surgeons from countries where CanL is an emerging disease. Multiple clinical presentations of CanL should aware clinicians to include leishmaniosis in the differential diagnosis of most clinical cases. Unfortunately, even if dogs recover clinically after treatment, complete elimination of *Leishmania* spp. is rarely achieved, and they remain infected and may relapse.

Key words: canine leishmaniosis, dogs, *Leishmania* spp.

Introduction

Canine leishmaniosis (CanL) is declared by World Organization for Animal Health (OIE) to be an important disease due to its zoonotic potential. Canine leishmaniosis is consider to be an endemic disease in more than 70 countries all over the world and about 2,5 million dogs suffer from this disease. In Europe, canine leishmaniosis was considered to be restricted only to Mediterranean region. Recently, numerous cases of canine leishmaniosis have also been reported in northern countries such as: Germany, the Netherlands, United Kingdom and Poland as an emerging disease [1–5]. Taking into consideration that Mediterranean territory is a region of main tourist interests for the European citizen, spreading of CanL cases is not surprising [6]. International trade and transport of dogs support this possibility [7].

The etiological agent of disease is a parasite *Leishmania infantum* and domestic dogs are its main reservoir hosts. *Leishmania* spp. is flagellated protozoan from the family Trypanosomatidae, which belongs to the Kingdom of Protista [8]. There are various species of *Leishmania* spp., but the parasite responsible for disease in dogs in Mediterranean region is *L. infantum.* The transmission of this parasite in Europe is *via* vectors, such as sand flies from genus *Phlebotomus* spp. There are two species: *Phlebotomus perniciosus* and *Phlebotomus ariasi*, being the first a more efficient transmitter of that protozoan. Even though, *Phlebotomus ariasi* has a wider distribution in Europe. This fly has a nocturnal activity from early spring to late autumn. Until the present, the presence of phlebotomine sand fly, being the sole vector of this parasite, in non endemic regions was not confirmed. However, dogs that traveled or have

lived in endemic regions are at risk [5–8].

The aim of this article is to perform a review of canine leishmaniosis, including transmission, clinical manifestations, diagnostic procedures, treatment, prognosis and prevention, because this knowledge can be of a great use for veterinary surgeons from countries where CanL is an emerging disease.

Transmission of invasion

In order to complete the full life cycle *Leishmania* parasite needs two hosts: an insect – sand fly and a mammal host (e.g. dog). Only female sand flies are hematophagous. *Leishmania* spp. exists in two forms: amastigote and promastigote. The promastigotes are flagellate form. When phagocyted by dog's macrophages may stay alive inside them, multiplicate and transform into amastigote, which is an unflagellate form [9]. When a fly bites an infected animal it ingests also macrophages with amastigotes. Inside fly's intestine macrophages bursts and the promastigotes are released. Then dogs get infected when bitten by an infected sand fly and the cycle is repeated (Fig. 1).

It is considered that dog is the principal host of this parasite, but other infected mammals may play role as a reservoir too [9]. Other forms of transmission such as infection during blood transfusion [10] or derivatives coming from infected donators [11,12] as well as transplacental [13,14], vertical [15] and venereal transmission [16] were reported. However, only one study in Iran has shown that the ownership of an infected dog may be a risk for a human to suffer from visceral leishmaniosis [17,18]. Taking into consideration the ways of transmission of CanL, the risk for owners of the dogs seems relatively to be small.

In endemic regions where the prevalence of infection is about 60%, only 10–30% dogs become symptomatic [4]. Whenever a dog presents a medium-high level of antibodies together with clinical signs it suggests that suffers from CanL.

Immune response

The immune response plays a crucial role in clinical manifestations of canine leishmaniosis. Some dogs present a subclinical form of disease, while others present a severe clinical manifestation

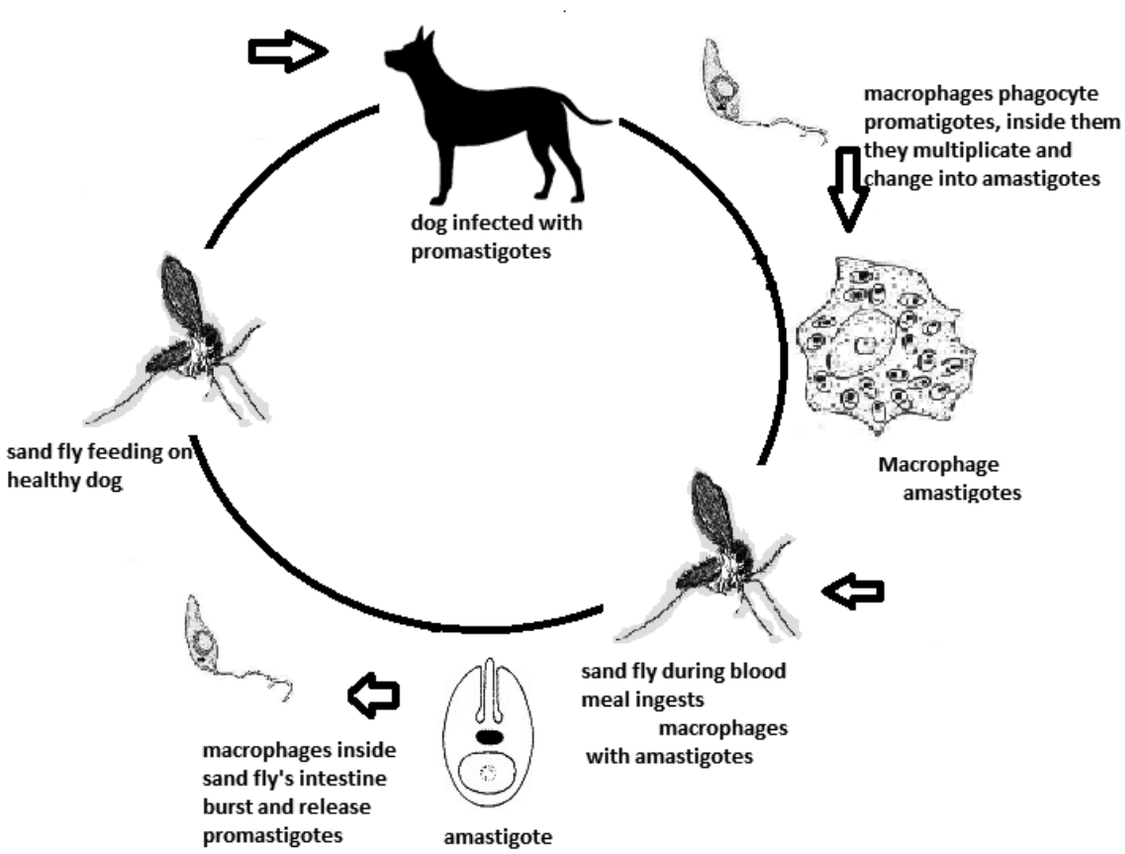

Fig. 1. The life cycle of *L. infantum*

of canine leishmaniosis. The statistics shows that in endemic regions only about 10–30% of infected dogs develop clinical disease or clinopathological abnormalities [3,4]. Those differences are due to the individual immune response. An appropriate cellular immune response is essential to control the infection. The protective immunity is produced by lymphocytes T $CD4^+$, Th1 like type which release cytokines: γ-interferon, IL-2, TNF-α that induce macrophage anti-*Leishmania* activity. Dogs with appropriate immune response usually present a very poor humoral immune response, while the ill ones usually present an enhanced humoral response together with reduced cellular immunity with mixed Th1 and Th2 dependent cytokines response [3,19].

A case-based studies [20] shown that certain breeds: Boxer, Cocker Spaniel, Rottweiler, German Shepherd have a predisposition to develop clinical symptoms of CanL, while others like Ibizian Hound rarely develop signs of CanL [21]. Age, sex, body condition may be other contributing factors.

Clinical manifestation of CanL

Canine leishmaniosis is chronic systemic disease and clinical signs may remain non-specific [22,23]. There is wide variety of clinical manifestations due to different types of immune response, but pathogenic mechanism always remains the same. The lesions are a consequence of an inflammatory reaction against parasite, which can take place in skin, visceral organs like: liver, kidneys or intestine, eyes, bones and mucous membranes. The deposits of immune-complexes (IgG and IgM) in different anatomical zones is very characteristic. Skin lesions are the most frequent owner's complain. Among skin disorders the most frequent manifestations are: non pruritic exfoliative dermatitis with or without alopecia, erosive-ulcerative dermatitis, nodular, papular or pustular dermatitis [2]. Other common clinical presentations are renal, ocular and articular lesions. Ocular problems may include: blepharitis, anterior uveitis and keratoconjuntivitis. Sometimes lameness, epistaxis, vascular or neurological problems are observed. However, clinical signs may be variable and nonspecific. In the majority of cases lymphadenomegaly, apathy, emaciation and muscular atrophy is also observed. Nevertheless, renal failure is the main cause of mortality due to CanL [2]. Subclinically infected dogs may develop an overt CanL when they receive immunosuppressive drugs or when they suffer from concomitant diseases.

Fig. 2. The most frequent observed clinical manifestations of canine leishmaniosis (source: Ilona Kaszak, Marta Planellas)

The broad spectrum of clinical presentations should aware clinicians to include leishmaniosis in the differential diagnosis of most other clinical cases (Fig. 2).

Diagnosis

The initial approach of a patient suspected of leishmaniosis is based on blood analysis. Complete blood count (CBC) can reveal some abnormalities like: non regenerative anemia, thrombocitopenia, leucocytosis or leukopenia. Biochemical analysis

Table 1. Policlonal gammopathy in a dog with CanL (source: Marta Planellas)

Total proteins	10.43 g/dL	Normal values
Albumin	3.13 g /dL	2.6 - 3.3 g/dL
Alfa 1	0.29 g/dL	0.2 - 0.5 g/dL
Alfa 2	1.26 g/dL	0.3 - 1.1 g/dL
Beta	2.48 g/dL	0.9 - 1.6 g/dL
Gamma	3.25 g/dL	0.3 - 0.8 g/dL

can result in: hypercholesterolemia, renal azotemia, elevated liver enzymes activities and hyperproteinemia. Serum proteins electrophoresis is indicated in the case of hyperproteinemia. Monoclonal or polyclonal gamma-globulinemia with or without hypoalbuminemia may be observed in dogs with CanL (Table 1). Hypoalbuminemia is common in dogs with CanL and can be due to protein loss in consequence of renal disease or due to inhibition of protein synthesis in an acute phase of disease. The evaluation of urine analysis is crucial in these cases: urine specific gravity, urinary protein creatinine ratio (UPC) should be determined. Values of UPC above of 0.5 suggest proteinuria, which is a common alteration in dogs with leishmaniosis [2] (Table 1).

In order to perform a definitive diagnosis of leishmaniosis parasitological, serological and molecular diagnostic methods for the detection of

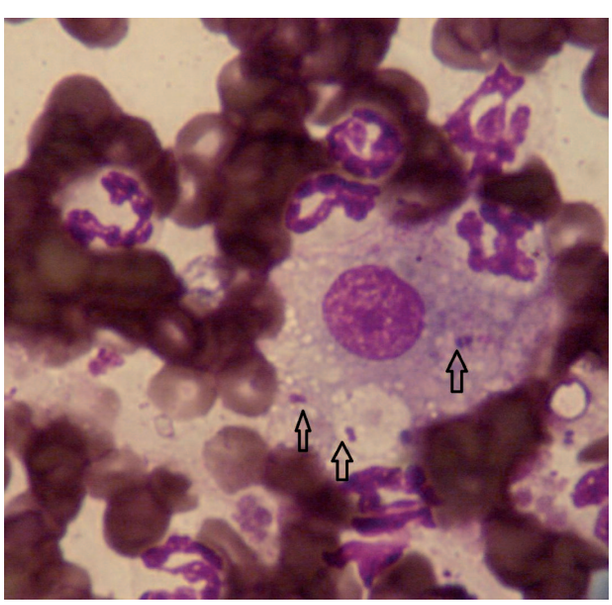

Fig. 3. Cytology from fine needle aspirate of a reactive lymph node from dog with clinical leishmaniosis, showing *Leishmania infantum* intracellular amastigotes (arrows) (source: Marta Planellas)

Leishmania infantum infection can be considered [2,24,25]. To obtain a final diagnosis it is important to use more than one method. Cytology, histopathology, immunohistochemistry and parasite culture are direct methods (Fig. 3). More sensitive and specific is the detection *Leishmania* DNA by PCR directly in dogs tissues. Real time PCR, which allows quantification of the *Leishmania* parasite load, is useful especially for follow-up during treatment. PCR can be performed on DNA extracted from tissues, blood, biological fluids and even histopathological samples [26] (Fig. 4).

Serologically CanL can be diagnosed through the detection of specific antibodies (IgG) using quantitative techniques like immunoflorescence antibody test (IFAT), enzyme linked immunosorbent assay (ELISA). High level of anti-*Leishmania* antibodies together with clinical signs suggests leishmaniosis. However, low level of antibodies cannot exclude CanL.

Disease monitoring should consist of both serological and molecular methods (Fig. 4).

Treatment

There are various protocols of treatment CanL [27], but combination of antileishmanial drugs like meglumine antimoniate together with allopurinol, which is a specific leishmaniostatical drug, is the most frequently chosen protocol. The duration of the treatment depends on the severity of the disease, individual tolerance of drugs and clinical response to treatment. Nevertheless, parasitological cure is rarely achieved. There are also several side effects, like xantine urolithiasis in case of long treatment with allopurinol. Meglumine antimoniate can be potencially nephrotoxic and miltefosine can produce gastrointestinal upset. It is important to determine the clinical stage of each patient with CanL in order to apply the adequate treatment and prognosis (Table 2) [27].

The most common treatment for leishmaniosis in dogs is antimoniate meglumine administered subcutaneous at the dose of 100 mg/kg once a day for 1 month together with allopurinol administered orally 10 mg/kg every 12 hours during six months minimum. This combination may be administered to all dogs in stage B, C or D. Once leishmaniosis is diagnosed, dogs in stage B or C should have a follow up as continues: first control after one month of treatment, including of complete physical examination, CBC, serum biochemical analysis and

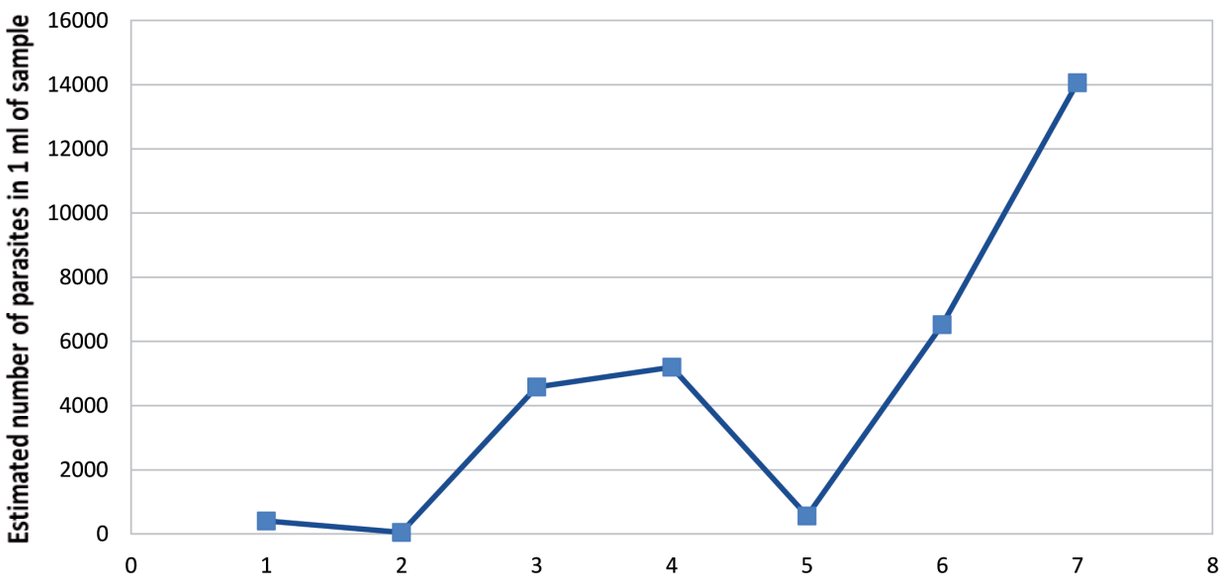

Fig. 4. Evolution of *L. infantum* parasitemia by Real time PCR in blood sample of a 3-year-old dog (source: Marta Planellas)

Table 2. Stages of CanL, according to [27]

Stage of Canine Leishmaniosis	**Features**
A – Exposed	Includes dogs having negative cytologic, histologic, parasitological and molecular findings and low titer of antibodies against *Leishmania* spp. Those dogs are clinically healthy or may suffer from other diseases. They live or have lived in region where *Leishmania* spp. vector (sand fly) is present.
B – Infected	Includes dogs in which parasites have been detected through direct methods (eg, microscopic evaluation, organism cultivation or PCR assay) and with low titer of antibodies against *Leishmania* spp. Those dogs are clinically healthy or may suffer from other diseases. In endemicareas detection of *Leishmania* DNA via PCR assay in skin or peripherally obtained blood samples collected during the infection transmission period, in the absence of evident lesions, may not be sufficient to consider a dog infected.
C – Sick (clinically evident disease)	Includes dogs with positive cytologic results regardless serologic results, dogs with high titer of antibodies against *Leishmania* spp., and rarely, infected dogs. One or more clinical signs characteristic for leishmaniosis may be present. Due to wide variety of clinical manifestations of leishmaniosis, clinical signs may be different and uncommon but as long as they can be associated with this disease they are significant. Even the dogs don't present clinical signs during physical examination acan be considered sick, when hematologic, biochemical or urinary alternations common to leishmaniosis are present.
D – Severly sick	Includes dogs with severe clinical manifestetion. Presenting one of the following abnormalities: evidence of proteinuria nephropathy or chronic renal failure; presence of concurrent problems (eg, ocular or joint) related or unrelated to leishmaniosis that require immunosuppressive treatment; concomitant infections, cancer diseases endocrine or metabolic diseases and clinical unresponsiveness to repeated courses of anti-*Leishmania drugs.*
Ea – Sick unresponsive	Includes sick dogs unresponsive to recommended anti-*Leishmania* treatment
Eb – Sick – early relapse	Includes sick dogs treated in accordance with anti-*Leishmania* protocol but that relapse soon after treatment ceases.

urinalysis. Afterwards, the patient should be reevaluated every 6 months, adding serology investigation against *Leishmania* spp. antibodies and/or real-time PCR evaluation of parasitemia additionally to other blood tests. The dogs in D stage should be evaluated every 1–2 months during treatment with particular emphasis on evaluating affected organs (e.g. kidney, liver) [27].

Prognosis

Unfortunately, even if dogs recover clinically after treatment, complete elimination of *Leishmania* spp. is rarely achieved, and dogs remain infected and may relapse. However, dogs in stages B or C have better prognosis. Revision every 6 months, once treatment is finished, is enough to control the evolution of the disease. The prognosis for patients in stage D depends on evaluation of affected organs (e.g. kidney, liver) is usually reserved (Table 2).

Prevention

One of the most effective method of prevention is to use repellent for fighting with the vectors, sand flies [2,28]. Veterinary products containing synthetic pyrethroids, permethrin, deltamethrin have a repellent effect against these flies [29]. Spot on or collars are the two available forms of recommended products. Other measures of prevention could be keeping the dog indoors from dusk to dawn during the sand fly activity season and trying to reduce microhabitats favorable to sand flies, such as piles of wood or stagnant water. There are also some recommendation for application of immunomodulators like domperidone as a preventitive mesure or treatment of mild CanL [4].

Vaccines are also available. The most effective vaccines are made of purified *Leishmania* spp. fractions [2]. The vaccine of fucose-mannose-ligand (FML) based, is a fraction of *L. donovani* and is available in Brasil. The excreted/secreted antigen purified from specific-medium culture supernatant of *L. infantum* is the vaccine approved for dogs in Europe. Nevertheless, neither of those preventive measures can protect the dog in 100%.

Conclusions

Canine leishmaniosis has a worldwide distribution, being present on four continents, in over 70 countries and causes severe fatal disease in dogs. In Europe CanL was considered to be a disease restricted to Mediterranean region due to clima conditions specific for a life cycle of a sand fly which transmits *Leishmania infantum*. However, international trade, international dog's exhibitions, transport and travel of dogs contribute to the disease appearance in new non endemic areas. Apparently these cases are sporadic, but the risk of possible transmission to other dogs exist. Apart from that, the dynamic changes in phlebotomine sand fly population can lead to establishment of new habitats of this insect. Therefore, European countries in which CanL is endemic should take available preventive measures in order to prevent the expansion of this disease, whereas in non endemic areas early diagnosis of CanL is crucial for animal's recovery. The course of CanL may be unpredictable to due its complex pathogenesis and clinical manifestations may be various and non-specific. That is the reason why, multiple diagnostic methods should be performed in order to detect CanL. The course of the disease may range from mild to severe, afftecting multiple organs. Most of the patients respond well to the treatment and clinical cure can be achieved.

That is why, CanL should be included in differential diagnosis of most of the clinical cases, also in non endemic regions.

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