Review

Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniosis

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ABSTRACT

Canine leishmaniosis (CanL) due to Leishmania infantum is a life threatening zoonotic disease with a wide distribution in four continents and importance also in non-endemic regions. The purpose of this report is to present a consensus of opinions on the diagnosis, treatment, prognosis and prevention of CanL in order to standardize the management of this infection. CanL is a disease in which infection does not equal clinical illness due to the high prevalence of subclinical infection among endemic canine populations. The most useful diagnostic approaches include serology by quantitative techniques and PCR. High antibody levels are associated with severe parasitism and disease and are diagnostic of clinical leishmaniosis. However, the presence of lower antibody levels is not necessarily indicative of disease and further work-up is necessary to confirm CanL by other diagnostic methods such as cytology, histopathology and PCR. We propose a system of four clinical stages, based on clinical signs, clinicopathological abnormalities and serological status. Suitable therapy and expected prognosis are presented for each of the stages. The combination of meglumine antimoniate and allopurinol constitutes the first line pharmaceutical protocol. However, although most dogs recover clinically after therapy, complete elimination of the parasite is usually not achieved and infected dogs may eventually relapse. Follow-up of treated dogs with blood counts, serum biochemistry, urinalysis, serology and PCR is essential for prevention of relapses. Protection against sand fly bites by topical insecticides is effective in reducing infection, and recent development of vaccines has indicated that prevention by vaccination is feasible.

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1. Introduction

Leishmania infantum infection was first described in dogs in 1908 by Nicolle and Comte in Tunisia (Nicolle and Comte, 1908). Since then, knowledge on L. infantum in dogs has continually expanded improving the understanding of this infection. The development of new sensitive and specific diagnostic techniques showing high prevalence of infection versus lower prevalence of clinical leishmaniosis, the evidence of its wide spectrum of clinical manifestations, the lack of ultimately effective therapy, and the different measures proposed for disease prevention have been accompanied with increased interest in canine leishmaniosis (CanL) and disparity of opinions concerning this infection in veterinary medicine. Several consensus statements have been published on the management of other canine infectious diseases such as ehrlichiosis (Neer et al., 2002) and borreliosis (Littman et al., 2006). However, there is insufficient scientific agreement on the management of CanL and therefore an increasing need for debate leading to shared standardization of criteria for clinical studies. These include evaluation of efficacy of different
therapeutic protocols or vaccines, and assisting veterinary practitioners in providing the best clinicodiagnostic approach to their canine patients. LeishVet is a group of veterinary scientists from academic institutes in Europe and the Mediterranean basin with a main clinical and scientific interest in CanLeish. It was formed during the WorldLeish congress in 2005 and officially registered as an association in May 2008. One of the first goals of LeishVet was to develop consensus recommendations that would represent the most current understanding of *L. infantum* infection in dogs and bridge controversies in the veterinary literature. The directions presented here reflect general uniformity of opinion following an in-depth debate on controversial issues. For issues without clear consensus, the pros and cons are presented. These recommendations are based on current literature and clinical experience combined to provide evidence-based medicine guidelines. The questions presented primarily address the diagnosis, clinical staging, treatment, clinical monitoring, prognosis and prevention of CanLeish to offer a consensus opinion to scientists and veterinary practitioners on these topics.

2. Epidemiology and pathogenesis

2.1. Why is leishmaniosis important to human and veterinary medicine?

Human leishmaniosis, caused by several species of *Leishmania*, comprises a group of diseases which are mostly zoonotic. These include visceral leishmaniosis (VL), which involves internal organs and is fatal if untreated, and the cutaneous and mucocutaneous forms (CL), which affect the skin or mucocutaneous junctions and may heal spontaneously, leaving disfiguring scars (Murray et al., 2005). This group of infections is the third most important vector-borne disease after malaria and lymphatic filariasis. It is endemic in many tropical and sub-tropical regions of the Old and New World. Leishmaniosis is endemic in 88 countries, with more than 350 million people at risk. The estimated incidence is 2 million new cases per year, 0.5 million of VL and 1.5 million of CL (Desjeux, 2004). Visceral leishmaniosis causes an estimated 59,000 deaths annually (a rate surpassed among parasitic diseases only by malaria), and 2,357,000 disability-adjusted life years (DALYs) lost, placing leishmaniosis 9th in a global analysis of infectious diseases (Desjeux, 2004). Leishmaniosis is one of the major infectious diseases afflicting the world’s poorest population living mainly in rural and suburban areas (Alvar et al., 2006).

In veterinary medicine, leishmaniosis caused by *L. infantum* is mostly important in dogs. Dogs are considered the main reservoir of this parasite for humans (Gramiccia and Gradoni, 2005). Canine leishmaniosis is one of the major zoonoses globally causing severe fatal disease in dogs. Dogs are a common companion animal found in a large number of households in developed and developing countries and the health of pet dogs is of great concern to their owners. Infection in cats (Martin-Sanchez et al., 2007), wild canids (Sobrino et al., 2008) and horses (Fernandez-Bellon et al., 2006) has also been reported in areas where disease is common in dogs.

2.2. What species of Leishmania infect dogs? What is the geographical distribution?

The *Leishmania* species that infect the dog and their geographical distribution are listed in Table 1. The most important species is *L. infantum* in the Old World which is synonymous to *L. chagasi* in Central and South America. In fact, *L. infantum* is thought to have been imported into the Americas by the dogs of European settlers during the colonization of South America (Tuon et al., 2008). Dogs have been found infected by several other *Leishmania* species responsible for cutaneous, mucocutaneous and visceral leishmanioses in humans (Lemrani et al., 2002; Dantas-Torres, 2007). For these species, dogs do not appear to be a significant reservoir of infection for people (Dantas-Torres, 2007).

Table 1
Geographical distribution of *Leishmania* species infecting dogs and their vector sand fly species.

<table>
<thead>
<tr>
<th>Leishmania species</th>
<th>Geographical distribution</th>
<th>Proven sand fly vectors</th>
<th>Suspected sand fly vectors</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. infantum</em></td>
<td>Mediterranean basin, Middle East</td>
<td>Phlebotomus perniciosus, P. ariasi, P. perfiliewi, P. neglectus, P. langeroni, P. tobbi</td>
<td>P. longicuspis, P. syriacus, etc.</td>
</tr>
<tr>
<td>L. infantum = L. chagasi</td>
<td>Central and South America</td>
<td>Lutzomyia longipalpis, L. evansi, L. olmeca olmeca</td>
<td>P. brevis, P. halepensis, etc.</td>
</tr>
<tr>
<td><em>L. donovani</em></td>
<td>East Africa</td>
<td>P. orientalis, P. martini</td>
<td>P. rodhaini</td>
</tr>
<tr>
<td><em>L. tropica</em></td>
<td>North Africa</td>
<td>P. Sergenti, P. arabicus</td>
<td>P. chabaudi, P. saevus</td>
</tr>
<tr>
<td><em>L. braziliensis</em></td>
<td>Central to South America</td>
<td>Lu. whitmani, Lu. yucumensis, Lu. wellcomei, Lu. spinicrassa</td>
<td>Lu. amazonesis, Lu. migonii, Lu. panamensis, Lu. paraensis, Lu. complexus, Lu. pessoai, etc.</td>
</tr>
<tr>
<td><em>L. peruviana</em></td>
<td>Peruvian Andes</td>
<td>Lu. trapiodi, Lu. ylephiletor, Lu. umbratlitis</td>
<td>Lu. noguchi, Lu. pescei</td>
</tr>
<tr>
<td><em>L. panamensis</em></td>
<td>Central America</td>
<td>Lu. peruensis, Lu. verrucarum, Lu. ayacuchensis</td>
<td>Lu. shannoni, Lu. ovalesi, etc.</td>
</tr>
</tbody>
</table>
2.3. How is Leishmania transmitted to the dog? How does Leishmania develop in the vector?

*Leishmania* is a diphasic parasite that completes its life cycle in two hosts, a phlebotomine sand fly vector which harbours the flagellated extracellular promastigote form and a mammal where the intracellular amastigote form develops. The final promastigote form, the metacyclic promastigote is transmitted to a new vertebrate host completing the *Leishmania* life cycle. When the parasitized phlebotomine sand fly feeds on blood from a vertebrate, it inoculates promastigotes from its gut via the proboscis. Only females are hematophagous whereas males feed on plants (*Killick-Kendrick, 1999*). Sand flies feed on dogs mainly in scarcely haired skin areas such as the head, nasal bridge, ear pinnae and inguinal and perianal areas. Once the parasite gains access to the vertebrate’s host dermis, phagocytosis by macrophages ensues. The macrophage surrounds the parasite in a phagosome vacuole and tries to eliminate it through a cascade of oxygen-based metabolites, such as nitric oxide, and liberation of lysosomal hydrolases discharged into the interior of the parasitophorous vacuole. *Leishmania* evades these non-specific defenses to survive and multiply in the macrophage. The progress of infection depends on the efficiency of the host’s immune response (*Alvar et al., 2004*). Parasites can be ingested by other phlebotomine sand flies in which the amastigotes are freed from their mammalian host cells and transformed into promastigotes. The latter develop only in the anterior segment of the gut (*Bates, 2007*). There is a strong association between sand fly vector species and *Leishmania* species transmitted due to specific enzyme activities and ligands present in the gut of the insect that allow only specific *Leishmania* spp. to remain attached to its wall and replicate without being excreted from the gut with the digested blood meal (*Volf et al., 2008*). As a consequence, natural transmission occurs only in those areas where adapted (competent) species of vectors are present (*Killick-Kendrick, 1999; Volf et al., 2008*). The survival of the parasite during the winter is mainly sustained on infected dogs, as no transovarial transmission of *Leishmania* has been shown in the sand fly vector (*Bates, 2007*).

2.4. What are the geographical distribution and vectorial characteristics of sand flies?

Female sand flies from the genera *Phlebotomus* (Old World) or *Lutzomyia* (New World) are the principal vectors of *Leishmania* (*Killick-Kendrick, 1999; Sharma and Singh, 2008*). The geographical distribution of sand flies species is shown in Table 1. Sand flies are primarily present in tropical countries or are active during the relatively warm months of the year in temperate countries. The activity of adult sand flies is crepuscular and nocturnal from early spring to late autumn in the Mediterranean basin and all year round in South America (*Killick-Kendrick, 1999; Sharma and Singh, 2008*). Its range of activity is between 15 and 28 °C, and is always associated with high relative humidity and absence of wind or rain. Sand flies can fly distances from 200 m to 2.5 km and may enter houses at night due to their positive phototropism (*Killick-Kendrick, 1999; Sharma and Singh, 2008*).

2.5. Is there evidence of non-sand fly transmission in dogs?

Sand flies are the only arthropods that are adapted to biologic transmission of *Leishmania*. Some species of vector sand flies are adapted to one specific *Leishmania* spp. while other “permissive” vector sand fly species are able to transmit several *Leishmania* spp (*Volf et al., 2008*). Ticks and fleas have been evaluated as potential vectors of *Leishmania* but no evidence has been shown that they have a role in natural transmission of the protozoan (*Coutinho et al., 2005; Coutinho and Linardi, 2007*). Direct dog-to-dog transmission has been implicated as being responsible for transmission of infection among foxhounds in the USA in the absence of apparent vectors; however, this has not been confirmed yet by experimental evidence (*Duprey et al., 2006*). Transplacental transmission of infection in dogs appears to be rare but possible (*Rosypal et al., 2005*). Recently, venereal transmission has been reported in dogs (*Silva et al., 2009*). Transmission of infection by infected canine blood products has been documented and is of special concern in areas where blood donors could be carriers of infection (*de Freitas et al., 2006; Tabar et al., 2008*). Nevertheless, non-sand fly modes of transmission probably play only a marginal role in the natural history and epidemiology of leishmaniosis (*Baneth et al., 2008*).

2.6. What is the importance of canine leishmaniosis in non-endemic areas?

The disease can be diagnosed in non-endemic countries in dogs that have been living or have traveled to endemic areas (*Shaw et al., 2003*). The increased numbers of dogs travelling to Southern Europe and imported as companion animals from endemic areas have raised serious concerns about the introduction of vector-borne diseases, such as CanL, into the non-endemic areas of Europe (*Shaw et al., 2003*). A study from the Netherlands found that about 58,000 dogs travel yearly from Holland to Southern Europe with their owners for vacations and the risk for acquiring CanL is 0.027–0.23% (*Teske et al., 2002*). Infected dogs in non-endemic areas may also contribute to the maintenance of the parasite within the canine population through rare but possible non-vector transmission modes of infection. In addition, *L. infantum* infection has spread north in Europe, reaching the foothills of the Alps in Northern Italy (*Maroli et al., 2008*). Transmission could occur in a new area if infected dogs are imported and if the population of sand fly vectors is large enough. Changes in the dynamics of sand fly populations may lead to the creation of new permanent foci.

2.7. What are the public health considerations of canine leishmaniosis?

Dogs are considered the most important peridomestic reservoir of *L. infantum* infection to humans. However, only one study carried out in Iran has shown that the ownership of an infected dog is a risk factor for human infection.
(Gavgani et al., 2002b). The presence of infected dogs in the vicinity of humans is certainly associated with transmission of infection; but, the actual presence of an infected dog in the household does not appear to greatly increase the risk of infection to the family when transmission is already taking place in the region. Therefore, the danger to owners of dogs with leishmaniosis appears to be small. The efficiency of elimination of seropositive dogs in Brazil in decreasing human infection is disputable and several reports have claimed that it is not helpful in decreasing human or canine infection in the long run (Dantas-Torres and Brandao-Filho, 2006; Nunes et al., 2008).

3. Immunology and clinicopathological findings

3.1. Is there a difference between infection and disease in canine leishmaniosis?

The traditional concept that all dogs infected with *L. infantum* will eventually develop severe clinical leishmaniosis after a variable incubation period has been disproved (Ferrer et al., 1988). CanL is a disease in which infection does not equal clinical illness due to the high prevalence of subclinical infection (Solano-Gallego et al., 2001a; Baneth et al., 2008). In the past, researchers used the clinical classification of asymptomatic, oligosymptomatic and polysymptomatic dogs based only on the results of physical examination (Mancianti et al., 1988). This classification has a limited value because it does not consider clinicopathological abnormalities and disregards dogs that have widespread organ dysfunction without apparent visual manifestations (Solano-Gallego and Baneth, 2008). The authors define dogs with clinical leishmaniosis when they present clinical signs and/or clinicopathological abnormalities and have a confirmed *L. infantum* infection. Dogs with subclinical infection, or clinically healthy infected dogs, are defined as those that present neither clinical signs on physical examination nor clinicopathological abnormalities by routine laboratory tests (CBC, biochemical profile and urinalysis) but have a confirmed *L. infantum* infection.

3.2. What is the prevalence of disease, seroprevalence and prevalence of infection in endemic regions?

The majority of dogs infected with *Leishmania* do not develop clinical signs or clinicopathological abnormalities and the prevalence of disease is frequently lower than 10% in endemic regions (Table 2). Although seropositivity is found in nearly all dogs with clinical disease, it is evident only in some of the clinically healthy infected ones. Epidemiological studies using molecular techniques in areas where CanL is endemic have shown that the prevalence of canine *Leishmania* infection may be considerably higher than seroprevalence and prevalence of disease (Baneth et al., 2008). *L. infantum* infection in dogs is endemic in approximately 50 countries in Europe, Africa, Asia and the Americas (Alvar et al., 2004), with variable rates of prevalence, depending on ecological and climatic conditions that determine the abundance of vectors (Table 2). Several reports have revealed the emergence of infection in new locations as well as its increase in previously established areas of endemcity (Maroli et al., 2008).

3.3. What role does the immune response play in the different clinical manifestations of Leishmania infection?

A broad range of immune responses and clinical manifestations have been described in CanL. *Leishmania* infection in dogs may be manifested as a subclinical infection, a self-limiting disease (Bottero et al., 2006), or a non-self-limiting and severe illness. In dogs, the two

### Table 2

Examples of prevalence of disease, seroprevalence and prevalence of *L. infantum* infection, based on both serology and molecular techniques, in several countries where CanL is endemic.

<table>
<thead>
<tr>
<th>Country (region)</th>
<th>Number dogs</th>
<th>Prevalence of disease</th>
<th>Seroprevalence</th>
<th>Prevalence of infection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>France (south)</td>
<td>30</td>
<td>(apparently healthy dogs)</td>
<td>3% (IFAT); 67% (WB)</td>
<td>90%</td>
<td>Berrahal et al. (1996)</td>
</tr>
<tr>
<td>Greece (Athens)</td>
<td>253</td>
<td>26%</td>
<td>30%</td>
<td>83%</td>
<td>Lachaud et al. (2002)</td>
</tr>
<tr>
<td>(centre)</td>
<td>1638</td>
<td>(apparently healthy dogs)</td>
<td>22.4%</td>
<td>ND</td>
<td>Sideris et al. (1999)</td>
</tr>
<tr>
<td>(northwest)</td>
<td>73</td>
<td></td>
<td></td>
<td>65.8%</td>
<td>Leontrides et al. (2002)</td>
</tr>
<tr>
<td>Israel (selected areas)</td>
<td>122</td>
<td>8.2%</td>
<td>11.5%</td>
<td>ND</td>
<td>Papadopoulou et al. (2005)</td>
</tr>
<tr>
<td>(West Bank, Palestine)</td>
<td>148</td>
<td>ND</td>
<td>6.8%</td>
<td>25% (n = 60)</td>
<td>Naseredin et al. (2006)</td>
</tr>
<tr>
<td>Italy (northwest)</td>
<td>4456</td>
<td>ND</td>
<td>26.4%</td>
<td>ND</td>
<td>Zaffaroni et al. (1999)</td>
</tr>
<tr>
<td>Portugal (north)</td>
<td>294</td>
<td>3.1%</td>
<td>20.4%</td>
<td>ND</td>
<td>Cardoso et al. (2004)</td>
</tr>
<tr>
<td>Spain (Mallorca island)</td>
<td>100</td>
<td>13%</td>
<td>26%</td>
<td>67%</td>
<td>Solano-Gallego et al. (2001a)</td>
</tr>
<tr>
<td>(79 (37 + 42)</td>
<td>8–29%</td>
<td>8–20%</td>
<td>64–73%</td>
<td>Fernandez-Bello et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>Croatia (centre)</td>
<td>306</td>
<td>8.2%</td>
<td>15%</td>
<td>ND</td>
<td>Zivinjak et al. (2005)</td>
</tr>
<tr>
<td>Cyprus (selected areas)</td>
<td>301</td>
<td>3.7%</td>
<td>10%</td>
<td>ND</td>
<td>Deplazes et al. (1998)</td>
</tr>
<tr>
<td>Turkey (west)</td>
<td>490</td>
<td>1.1%</td>
<td>5.3%</td>
<td>ND</td>
<td>Ozbek et al. (2000)</td>
</tr>
<tr>
<td>Morocco (north)</td>
<td>1013</td>
<td>0.4%</td>
<td>8.6%</td>
<td>ND</td>
<td>Nejar et al. (1998)</td>
</tr>
<tr>
<td>Tunisia (south-central)</td>
<td>250</td>
<td>(apparently healthy dogs)</td>
<td>6%</td>
<td>ND</td>
<td>Chargui et al. (2007)</td>
</tr>
<tr>
<td>Brazil (northeast)</td>
<td>1381</td>
<td>11%</td>
<td>24%</td>
<td>ND</td>
<td>Rondon et al. (2008)</td>
</tr>
</tbody>
</table>

N.D.: not determined.

* The prevalence of infection includes cellular immunity tests.
opposite extremes of this clinical spectrum are characterized by: (1) protective immunity that is CD4 T cell mediated by the release of γ-interferon, IL-2 and TNFα that induce macrophage anti-Leishmania activity, and (2) disease susceptibility that is associated with the production of a marked humoral non-protective immune response and a reduced or depressed cell mediated immunity with a mixed Th1 and Th2 cytokines response (Alvar et al., 2004; Baneth et al., 2008). Within this spectrum, clinical disease can range from a mild papular dermatitis associated with specific cellular immunity and low humoral responses (Ordeix et al., 2005) to a severe disease characterized by renal damage with glomerulonephritis due to immune complex deposition associated with a massive humoral response and high parasite loads (Costa et al., 2003).

3.4. What patterns and frequencies of immune responses are found in dogs living in endemic regions?

The patterns of immune responses found in dogs exposed to Leishmania infection based on published studies on naturally and experimentally infected dogs are described in Fig. 1. When describing the situation in an endemic area at one point of time using several techniques for the detection of exposure to the parasite such as serology, evaluation of cellular immunity with leishmanin skin test or lymphocyte proliferation test and clinical evaluation, typically 5–10% of the dogs are sick and about 90–95% are clinically healthy. In a hypothetical model based on previous studies (Cabral et al., 1998; Solano-Gallego et al., 2000), the apparently healthy dogs group can be divided into a subgroup of about one third which is not infected and two thirds that are infected. Twenty-two percent of the infected dogs will be considered susceptible to the development of clinical disease due to depressed cellular response and presence of humoral response. These will probably develop clinical signs and/or clinicopathological abnormalities later on. Forty-seven percent of the infected dogs are considered “resistant” due to the presence of a demonstrable cellular response against Leishmania. Twenty-seven percent of “resistant” dogs will present cellular and humoral response and 18% will present a cellular response only (Cabral et al., 1998; Solano-Gallego et al., 2000). However, subclinical infection is not necessarily permanent and factors such as immunosuppression and concomitant diseases could break the equilibrium and lead to the progression of clinical disease in dogs as has been observed in humans coinfected with human immunodeficiency virus and Leishmania (Alvar et al., 2008).

3.5. What determines if an infected dog will remain clinically healthy or become severely sick?

It is not known for certain what mechanisms in dogs are responsible for resistance or susceptibility, nor the way factors such as age, gender, nutrition, host genetics, coinfections and/or concomitant disease, immunosuppressive conditions, cytokine environment, parasitic burden, virulence of Leishmania strain, previous infections and method of transmission can affect the polarity of clinical manifestations of Leishmania infection. Some dog breeds such as the Boxer, Cocker spaniel, Rottweiler and German shepherd seem to be more susceptible to the development of disease (Sideris et al., 1999; Franca-Silva et al., 2003) while others such as the ibizian Hound rarely develop clinical signs (Solano-Gallego et al., 2000). The Slc11c1 (Solute carrier family 11 member a1) gene, formerly named N-RAMPI, and certain alleles of the MHC II genes have been associated with susceptibility to CanL (Quinnell et al., 2003; Sanchez-Robert et al., 2008). Age seems to be an important factor. Peaks of the highest disease prevalence have been reported in dogs younger than 3 years and older than 8 years (Abranches et al., 1991; Cardoso et al., 2004). Experimentally infected male hamsters seem to be more
susceptible to developing disease than females (Travi et al., 2002). In dogs, some epidemiological studies have not reported gender predisposition (Abranches et al., 1991; Miró et al., 2007b) while other studies have reported a higher risk for the disease in male dogs (Zaffaroni et al., 1999; Živicnjak et al., 2005). In addition, canine experimental infections have demonstrated how different routes of infection and life stages of the parasite used for infection will lead to divergent clinical manifestations. Intradermal infection and inoculation with promastigotes will lead most likely to a subclinical infection while intravenous and amastigotes infections will manifest as a severe disease (Moreno and Alvar, 2002).

3.6. What are the most common clinical manifestations of leishmaniosis?

The clinical features of leishmaniosis vary widely as a consequence of the numerous pathogenic mechanisms of the disease process, the different organs affected, and the diversity of immune responses mounted by individual hosts (Baneth et al., 2008). Canine leishmaniosis is a systemic disease that may potentially involve any organ, tissue and biological fluid and is manifested by non-specific clinical signs. The main clinical findings found on physical examination in classical CanL include skin lesions, generalized lymphadenomegaly, progressive weight loss, muscular atrophy, exercise intolerance, decreased appetite, lethargy, splenomegaly, polyuria and polydipsia, ocular lesions, epistaxis, onychogryphosis, lameness, vomiting and diarrhea (Ciaramella et al., 1997; Koutinas et al., 1999; Baneth et al., 2008). The variable and non-specific clinical signs make the list of differential diagnosis to CanL widely extensive.

3.7. How common are cutaneous lesions in dogs with leishmaniosis?

Skin lesions are the most common manifestation of CanL in dogs admitted for treatment due to the disease (Ciaramella et al., 1997; Koutinas et al., 1999). Cutaneous lesions may be seen along with other clinical signs and/or clinicopathological abnormalities, be the sole reported abnormality or be absent. Several dermatological entities have been described (Ferrer et al., 1988; Koutinas et al., 1992): (1) non-pruritic exfoliative dermatitis with or without alopecia which can be generalized or localized over the face, ears and limbs, (2) ulcerative dermatitis over bony prominences, mucocutaneous junctions, paws, ear pinnae, (3) focal or multifocal nodular dermatitis, (4) mucocutaneous proliferative dermatitis and (5) papular dermatitis (Ordeix et al., 2005; Bottero et al., 2006). Atypical cutaneous manifestations such as depigmentation, panniculitis, digital and nasal hyperkeratosis, pustular eruption, an alopecia areata-like disease or pempisus follicieus-like disease and erythema multiforme are relatively uncommon (Blavier et al., 2001; Papadogiannakis et al., 2005). Staphylococcal pyoderma, either superficial or deep, is a common complication. The most common cutaneous histopathological picture is a perianexial nodular to diffuse pyogranulomatous/granulomatous dermatitis, together with orthokeratotic to parakeratotic hyperkeratosis, acanthosis, crusting and ulceration. However, other histopathological patterns such as subcorneal pustular dermatitis, lichenoid dermatitis, vasculitis and panniculitis, have also been described (Ferrer et al., 1988; Koutinas et al., 1992; Blavier et al., 2001; Solano-Gallego et al., 2004; Papadogiannakis et al., 2005).

3.8. How common are renal disease and ocular manifestations in dogs with leishmaniosis?

In sick dogs, it is essential to evaluate the renal function and to stage possible renal disease using the International Renal Interest Society (IRIS) recommendations (IRIS, 2006a) since CanL is associated with a high prevalence of chronic renal disease (Costa et al., 2003). The early diagnosis of renal disease is beneficial to the patient and may prolong its life. Renal disease may be the sole clinical manifestation of CanL in sick dogs and can progress from mild proteinuria to nephrotic syndrome or end stage renal disease. Chronic renal failure (CRF) is a severe manifestation of disease progression and is the principal cause of animal death in CanL. Several studies have described the presence of histological lesions in 100% of the dogs evaluated (Costa et al., 2003; Zatelli et al., 2003). Despite the high prevalence of renal pathology, azotemia typical of renal failure is an uncommon laboratory finding and it is evident only when the majority of nephrons become dysfunctional rather late during disease progression. Glomerulonephritis and tubulointerstitial nephritis are the most common pathological findings while amyloidosis is very rare (Costa et al., 2003; Zatelli et al., 2003). Glomerulonephritis is frequently associated with the glomerular deposition of immune complexes and is mainly membranoproliferative and/or mesangioproliferative (Plevraki et al., 2006). Other histological types of glomerular disease (membranous, focal segmental, chronic, minimal change) have also been described (Costa et al., 2003; Zatelli et al., 2003; Plevraki et al., 2006). Membranoproliferative glomerulonephritis is more frequently associated with CRF, whereas in dogs without clinicopathological evidence of renal disease, histopathological evaluation usually reveals mesangioproliferative lesions and minimal change glomerulonephritis (Plevraki et al., 2006).

The relative prevalence of ocular and periocular lesions in CanL has been reported to range from 16% to 80% (Ciaramella et al., 1997; Koutinas et al., 1999; Pena et al., 2000). Eye lesions are the sole presenting complaint and clinical manifestation of the disease in up to 15% of clinical cases (Pena et al., 2000). The most common manifestations are conjunctivitis, blepharitis (exfoliative, ulcerative, or nodular), anterior uveitis and keratoconjunctivitis, either common or sicca (Koutinas et al., 1999; Naranjo et al., 2005). Ocular consequences of systemic hypertension (e.g. retinal detachment and/or hemorrhages, retinal arterial tortuosity, hyphema) are quite uncommon as they were seen in only 5.7% of the hypertensive dogs with CanL (Cortadellas et al., 2006). Ocular histopathology of 60 CanL cases has revealed a lymphoplasmacytic to granulomatous inflammation in a perivasculary to nodular to diffuse pattern, along with the presence of the parasite (56.2%)
in ocular tissues, especially in the conjunctiva, limbus and ciliary body (Pená et al., 2008).

3.9. What are the uncommon clinical forms of leishmaniosis?

Uncommon clinical forms (Blavier et al., 2001) include mucosal lesions (oral cavity, tongue and genital organs), joint swelling with erosive or non-erosive polyarthritis, osteolytic and osteoproliferative bone lesions, chronic hepatitis (Rallis et al., 2005), chronic relapsing colitis (Adamama-Moraitou et al., 2007), neurological disease due to meningitis and masseter muscle atrophic myositis or polymyositis (Vanvakidis et al., 2000), autoimmune disorders and cardiovascular disorders such as pericarditis, systemic vasculitis, thromboembolism and serum hyperviscosity syndrome.

3.10. What additional clinicopathological findings should alert the clinician to the possibility of canine leishmaniosis?

Clinicians should suspect the possibility of CanL when dogs present persistent renal proteinuria (urinary protein creatinine ratio (UPC) \( \geq 0.5 \)) or renal azotemia (IRIS stages II, III or IV of CRF) (IRIS, 2006a), non-regenerative anemia (chronic disease and/or CRF), leukocytosis or leukopenia, serum hyperproteinemia, polyclonal beta and gamma hyperglobulinemia, hypoalbuminemia, decreased albumin/globulin ratio and elevated liver enzyme activities (Ciaramella et al., 1997; Koutinas et al., 1999). Serum hyperviscosity, thrombocytopenia (Petanides et al., 2008), thrombocytopathy, impaired secondary hemostasis and fibrinolysis (Ciaramella et al., 2005) may also be detected.

The type of inflammatory infiltrate found in tissue cytology (aspirates, impression smears) or histopathology in organs such as skin, liver, intestine, eye, spleen, lymph nodes, striated muscles, synovium, nasal mucosa, etc., is commonly either pyogranulomatous to granulomatous and/or lymphoplasmacytic (Solano-Gallego et al., 2004; Adamama-Moraitou et al., 2007; Pená et al., 2008; Petanides et al., 2008). Typically, there is lymphoid reactive hyperplasia in lymphoid organs such as lymph nodes and spleen along with bone marrow/spleen monocytic hyperplasia (Mylonakis et al., 2005; Barrouin-Melo et al., 2006; Giunchetti et al., 2008) and variable numbers of Leishmania amastigotes.

4. Diagnosis

4.1. What are the various purposes for which *L. infantum* infection diagnosis is carried out?

Diagnosis is usually performed for two main reasons: (1) to confirm ‘disease’, e.g. to find out if a dog with clinical signs and/or clinicopathological abnormalities compatible with CanL indeed has the disease and (2) to investigate the presence of ‘infection’ for epidemiological studies, for screening clinically healthy dogs living endemic regions usually requested by the owners, to prevent transmission from subclinical carriers by blood transfusion, to avoid importation of infected dogs to non-endemic countries, and to monitor response to treatment (Miró et al., 2008). For these reasons, it is important to separate *Leishmania* infection from disease and to apply different diagnostic techniques accordingly.

4.2. How is clinical canine leishmaniosis diagnosed?

The diagnosis of CanL is complex as the clinical spectrum and clinicopathological abnormalities are both wide and not specific. Accurate diagnosis of CanL often requires an integrated approach consisting of clinicopathological diagnosis and specific laboratory tests. Pertinent clinical history, a thorough physical examination and several routine diagnostic tests such as CBC, biochemical profile, urinalysis and serum electrophoresis can help to raise the suspicion index for this disease. Other diagnostic tests such as coagulation profile, radiographs, abdominal ultrasound, cytological and histological evaluation of tissues or evaluation of biological fluids would be performed on an individual basis. Several specific diagnostic techniques have been developed to facilitate the diagnosis. It is essential to understand the basis of each diagnostic test and its limitations and appropriate interpretation. Reliable and specific diagnostic tests are essential for the detection of *Leishmania* infection in sick dogs although they lack 100% sensitivity and specificity.

4.3. What tests are available for the evaluation of *Leishmania* infection in dogs with suspected clinical leishmaniosis and in clinically healthy infected dogs?

In dogs with clinical signs and/or clinicopathological abnormalities consistent with leishmaniosis, the diagnostic methods include the detection of amastigotes in stained cytological smears of aspirates from cutaneous lesions, lymph nodes, bone marrow and spleen (Alvar et al., 2004; Saridomichelakis et al., 2005). Less commonly cytology is carried out on other tissues or body fluids (Agut et al., 2003; Dantas-Torres, 2006). Search for amastigotes by cytology could be unrewarding due to the low to moderate numbers of detectable parasites present even in dogs with full blown clinical disease (Moreira et al., 2007). *Leishmania* parasites may also be viewed in histopathologic biopsy sections from the skin or other infected organs. *Leishmania* infection should be suspected when either pyogranulomatous, granulomatous or lymphoplasmacytic inflammations in different tissues (Solano-Gallego et al., 2004; Adamama-Moraitou et al., 2007; Pená et al., 2008; Petanides et al., 2008) and/or lymph node reactive hyperplasia are observed (Mylonakis et al., 2005; Giunchetti et al., 2008). Definite identification of parasites within tissue macrophages may be difficult and an immunohistochemical staining method can be employed to detect or confirm the presence of *Leishmania* in the tissue. The isolation in culture of parasites from infected tissues is not suitable for rapid diagnosis and has a lower sensitivity than PCR and serology. Parasite culture is currently used more often for research purposes (Miró et al., 2008).

The most useful diagnostic approaches for investigation of infection in sick and clinically healthy infected dogs include: (1) detection of specific serum anti-leishmanial antibodies by several serological techniques and (2)
demonstration of the parasite DNA in tissues by applying molecular techniques.

4.4. How should serology be used for the diagnosis of canine leishmaniosis?

The diagnosis of CanL can be made by the detection of specific serum antibodies (IgG) using quantitative serological techniques, such as the immunofluorescence antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA). High antibody levels are associated with high parasitism and disease (Reis et al., 2006). Some dogs remain seronegative for variable periods after being infected with *Leishmania* (Strauss-Ayali et al., 2004). However, due to the relatively long incubation period, sick dogs are likely to be seropositive (Oliva et al., 2006). It is important to submit samples to a laboratory that runs quantitative serological techniques and can provide an endpoint titer (IFAT) or optical density reading (ELISA) and a classification of the levels of antibodies (negative, doubtful, low, medium and high positive levels). A high level of antibodies is conclusive of a diagnosis of CanL. However, the presence of low antibody levels is not necessarily indicative of the disease and further work-up is necessary to confirm or exclude clinical leishmaniosis by other diagnostic methods such as cytology, histopathology and PCR (Miró et al., 2008) (Fig. 2).

4.5. What reference and commercial antibody tests for *Leishmania* are currently available?

The IFAT, ELISA and immunochromatographic devices are the most commonly used techniques for detection of antileishmanial antibodies (Alvar et al., 2004; Maia and Campino, 2008; Miró et al., 2008). False positive results due to serological cross-reactivity with other pathogens have been described in all of the serological techniques mentioned above, especially in areas of *Trypanosoma cruzi* infection in North, Central and South America or with other species of *Leishmania* (Ferreira Ede et al., 2007; Porrozzi et al., 2007) and with tests using whole-parasite crude antigens. Cross-reactions are less likely to occur when using recombinant peptides such as rA2, rK9, rK26 and rK39 (Boarino et al., 2005; Porrozzi et al., 2007).

IFAT, which generally uses whole promastigotes antigen is highly specific and sensitive for the detection of clinical CanL, but may lack sensitivity to detect clinically healthy but infected dogs (Mettler et al., 2005). The cut-off titre to

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**Fig. 2.** Algorithm of diagnostic approach in dogs with clinical signs and/or clinicopathological abnormalities compatible with leishmaniosis.
distinguish positive and negative results varies from 1:40 to 1:160 between different laboratories (Ferroglio and Vitale, 2006).

ELISA sensitivity and specificity greatly depend on the antigens employed, which mainly include soluble promastigote extracts and recombinant or purified proteins (Miró et al., 2008). Whole-parasite extracts are sensitive for the detection of subclinical or clinical canine infections but provide a somewhat lower specificity (Mettler et al., 2005; Ferreira Ede et al., 2007). On the other hand, ELISA with recombinant peptides is very specific but may lack sensitivity for the detection of clinically healthy infected dogs, depending on the antigen employed (Mettler et al., 2005; Porrozzetti et al., 2007). An ELISA based on recombinant antigens (K9, K26 and K39 sub epitopes), has shown high specificity and sensitivity in infected dogs (Boarino et al., 2005). Studies evaluating IgG1 and IgG2 using polyclonal antibodies have frequently reached conflicting outcomes and are therefore not used routinely in the diagnosis of CanL (Day, 2007).

Immunochromatography-based assays are easy to use and provide qualitative results on the spot. These kits usually have good specificity but their sensitivity is variable and their performance is still not optimal (Mettler et al., 2005). Several rapid tests kits and antigenic preparations for IFAT and ELISA are also commercially available.

4.6. How should PCR be used for the diagnosis of canine leishmaniosis?

PCR assays have greatly improved the sensitivity of parasitological diagnosis in CanL. Detection of parasite-specific DNA in tissues by PCR allows sensitive and specific diagnosis. Several different assays with various target sequences using genomic or kinetoplast DNA (kDNA) have been developed for CanL. Assays based on kDNA appear to be the most sensitive for direct detection in infected tissues (Gomes et al., 2008; Maia and Campino, 2008; Miró et al., 2008). PCR can be performed on DNA extracted from tissues, blood, biological fluids or even from histopathologic specimens. PCR on bone marrow, lymph node, spleen or skin is most sensitive and specific for the diagnosis of CanL (Maia et al., 2009; Manna et al., 2008a; Reis et al., 2009). PCR on whole blood, buffy coat, and urine is less sensitive than the aforementioned tissues (Solano-Gallego et al., 2001a; Manna et al., 2008a). Sampling using non-invasive conjunctival swabs has proven to be very sensitive and specific for the detection of *L. infantum* in groups of seropositive dogs with clinical leishmaniosis (Strauss-Ayali et al., 2004; Ferreira Sde et al., 2008). PCR on aspirates of lymph node and bone marrow has been shown to be more sensitive than microscopic detection of amastigotes in stained smears or parasite culture (Moreira et al., 2007). Currently, three different PCR techniques are available: conventional PCR, nested-PCR and real-time PCR (Gomes et al., 2008; Maia and Campino, 2008; Miró et al., 2008). Quantitative real-time PCR is an advanced technique that can detect extremely low parasitic loads when compared with conventional PCR (Francino et al., 2006). Real-time PCR allows the quantification of *Leishmania* loads in tissues of infected dogs which is important for diagnosis as well as for follow-up during the treatment of CanL (Pennisi et al., 2005b; Manna et al., 2008a). It is important to highlight that information provided by PCR should not be separated from the data obtained from clinicopathological and serological evaluations. These should all be combined together for a comprehensive assessment.

4.7. How should PCR be used in clinically healthy dogs?

The presence of *Leishmania* DNA in the blood or other tissues of clinically healthy dogs living in endemic areas indicates that these dogs harbour infection (Solano-Gallego et al., 2001a) but, they may never develop clinical disease (Oliva et al., 2006). The interpretation of PCR results should be done cautiously in clinically healthy dogs and with consideration of the diagnostic procedure’s purpose. For instance, for the purpose of identifying infected dogs and preventing their importation to non-endemic areas where infection might spread via local sand flies, or for the purpose of preventing transmission of infection via blood products from infected donors, PCR would be an appropriate technique in combination with quantitative serological tests. However, the decision to treat clinically healthy dogs with anti-leishmanial medication based on positive PCR alone is not recommended.

4.8. Are there other useful immunodiagnostic tests for the prognosis in sick dogs?

Dogs that have a progressive infection manifested as disease have a depressed cellular response to *Leishmania* antigens expressed by no apparent cellular response or little response in cell-stimulation assays while clinically healthy infected dogs are considered “resistant” due to the presence of a demonstrable cellular response (Baneth et al., 2008). The *in vivo* leishmanin skin test, the *in vitro* lymphocyte stimulation with *Leishmania* antigen and the detection of IFN-γ are tests that evaluate the cellular response to *Leishmania* and they might be used to predict susceptibility or resistance to infection in the near future (Dos-Santos et al., 2008). However, these tests are available only in research laboratories and need further standardization (Maia and Campino, 2008). The clinical usefulness of lymphocyte sub-population size, such as the CD4 and CD8 in assessing the severity of *Leishmania* infection and response of dogs to therapy is questionable. A recent study has not found differences between lymphocyte sub-populations in sick dogs, before and after the antileishmanial treatment when compared to healthy dogs (Miranda et al., 2007).

5. Treatment

5.1. What is the most effective specific treatment in canine leishmaniosis?

The drugs most commonly used in the treatment of CanL and current therapeutic protocols are listed in Table 3. Meglumine antimoniate, aminosidine and miltefosine are, up to date, the only drugs licenced in Europe specifically for treatment of CanL. The combination of meglumine antimoniate with allopurinol is considered as
5.2. What is the expected clinical response to treatment in canine leishmaniosis? What are the most common side effects of different therapeutic protocols?

The expected clinical response to treatment of sick dogs can vary from poor to good depending on their overall initial clinicopathological status (Table 4). Dogs with renal insufficiency are expected to have a lower recovery rate in comparison to those without kidney compromise. The majority of dogs experience clinical improvement within the first month of therapy (Pennisi et al., 2005b; Manna et al., 2008a), although in others, a longer period of therapy is required before any apparent improvement. Serum antibody titres and serum protein alterations are expected to require a longer period of time before normalization. Some dogs show side effects to therapy that must be distinguished from deterioration of the disease and are listed in Table 3 (Noli and Auxilia, 2005).

5.3. What clinicopathological parameters should be monitored during the treatment of canine leishmaniosis?

The clinicopathological parameters to be monitored during treatment would depend on the individual abnormalities. However, in general, it is recommended to perform complete CBC, biochemical profile and urinalysis including urine protein/creatinine ratio in proteinuric dogs. The frequency of monitoring clinicopathological parameters would vary in each patient but, clinicopathological parameters should, in most cases, be monitored more frequently initially, i.e. after the first month of treatment and then every 3–4 months. Later on, if the dog is fully recovered clinically with treatment then a recheck would be recommended every 6 months or once a year.

5.4. How should serology be used for monitoring the effectiveness of treatment?

The use of antibody levels to assess clinical improvement after treatment is controversial. Older studies reported that antibody titer did not decrease within the...
first months after the beginning of the therapy (Ferrer et al., 1995). However, recent studies have demonstrated a slow and progressive decrease in IgG and IgA antibody levels which is associated with clinical improvement (Solano-Gallego et al., 2001b; Rodriguez et al., 2006). Therefore, we recommend repeating a quantitative serological test in the same laboratory 6 months after the initial treatment due to the relatively long half life of IgG (~3 weeks in humans) (Anderson et al., 2006) and the common presence of initial high levels of antibodies in sick dogs. Ideally, it would be better to evaluate the antibody kinetics by running sera from the initial and follow-up dates simultaneously in the same assay. For example, decrease of IFAT titer would be considered significant if there is more than a twofold dilution difference between the first and the following sample. Some dogs would present a significant decrease in antibody levels associated with clinical improvement within 6 months to 1 year of treatment while others might not have a decrease in antibody titers despite the clinical improvement. In contrast, a marked increase of antibody levels should be interpreted as a marker of relapse, especially in dogs following the discontinuation of treatment.

5.5. When should allopurinol be discontinued?

The clinical decision on timing of allopurinol discontinuation is not well defined and should be based on clinicopathological, serological and parasitological assessments. However, the authors consider the combination of following criteria sufficient to discontinue allopurinol treatment: (1) presence of complete physical and clinicopathological recovery evaluated by a thorough physical examination, CBC, full biochemistry panel and urinalysis at least 1 year after the initial allopurinol administration, and (2) marked decrease of antibody levels (negative or borderline positive by a quantitative serological assay).

It is important to highlight that some extremely susceptible dogs never arrive at a point that would allow the discontinuation of allopurinol while some less susceptible dogs would be capable of controlling the infection without extremely lengthy treatment.

5.6. Is there any evidence for anti-Leishmania drug resistance in dogs?

While the occurrence of *Leishmania* resistance to pentavalent antimonials, amphotericin B, aminosidine

### Table 4
Clinical staging of canine leishmaniosis based on serological status, clinical signs, laboratory findings, and type of therapy and prognosis for each clinical stage.

<table>
<thead>
<tr>
<th>Clinical stages</th>
<th>Serologya</th>
<th>Clinical signs</th>
<th>Laboratory findings</th>
<th>Therapy</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I: mild disease</td>
<td>Negative to low positive antibody levels</td>
<td>Dogs with mild clinical signs such as peripheral lymphadenopathy, or poplar dermatitis (Ordeix et al., 2005; Bottero et al., 2006)</td>
<td>Usually no clinicopathological abnormalities observed; normal renal profile: creatinine &lt; 1.4 mg/dl; non-proteinuric: UPC &lt; 0.5</td>
<td>Scientific neglect/ allopurinol alone/ allopurinol + meglumine antimoniate or miltefosine</td>
<td>Good</td>
</tr>
<tr>
<td>Stage II: moderate disease</td>
<td>Low to highb positive antibody levels</td>
<td>Dogs, which apart from the signs listed in stage I, may present: diffuse or symmetrical cutaneous lesions such as exfoliative dermatitis/onychogryphosis, ulcerations (planum nasale, footpads, bony prominences, mucocutaneous junctions), anorexia, weight loss, fever, and epistaxis (Petanides et al., 2008)</td>
<td>Clinicopathological abnormalities such as mild non-regenerative anemia, hypergammaglobulinemia, hypoalbuminemia, serum hyperviscosity syndrome (Petanides et al., 2008). Substage—(a) normal renal profile: creatinine &lt; 1.4 mg/dl; non-proteinuric: UPC &lt; 0.5. (b) Creatinine &lt; 1.4 mg/dl; UPC &lt; 0.5–1</td>
<td>Allopurinol + meglumine antimoniate or miltefosine</td>
<td>Good to guarded</td>
</tr>
<tr>
<td>Stage III: severe disease</td>
<td>Medium to high positive antibody levels</td>
<td>Dogs, which apart from the signs listed in stages I and II, may present signs originating from immune-complex lesions: vasculitis, arthritis, uveitis and glomerulonephritis</td>
<td>Clinicopathological abnormalities listed in stage II Chronic kidney disease (CKD) IRIS stage I with UPC &gt; 1 or stage II (creatinine 1.4–2 mg/dl) (IRIS, 2006a)</td>
<td>Allopurinol + meglumine antimoniate or miltefosine Follow IRIS guidelines for CKD (IRIS, 2006b)</td>
<td>Guarded to poor</td>
</tr>
<tr>
<td>Stage IV: very severe disease</td>
<td>Medium to high positive antibody levels</td>
<td>Dogs with clinical signs listed in stage III. Pulmonary thromboembolism, or nephrotic syndrome and end stage renal disease</td>
<td>Clinicopathological abnormalities listed in stage II CKD IRIS stage III (creatinine 2–5 mg/dl) and stage IV (creatinine &gt; 5 mg/dl) (IRIS, 2006a) Nephrotic syndrome: marked proteinuria UPC &gt; 5</td>
<td>Allopurinol (alone) Follow IRIS guidelines for CKD (IRIS, 2006b)</td>
<td>Poor</td>
</tr>
</tbody>
</table>

a Dogs with negative to medium positive antibody levels should be confirmed as infected with other diagnostic techniques such as cytology, histology/immunohistochemistry and PCR.

b High levels of antibodies are conclusive of a diagnosis of CanL and are defined as three- to four fold increase of a well established laboratory reference cut-off.
and miltefosine is well known in human medicine (Croft et al., 2006), only limited information is available for the dog. A decreased sensitivity to meglumine antimoniate or antimonials of *L. infantum* isolated from dogs after several treatment courses has been reported (Gramiccia et al., 1992; Carrio and Portus, 2002). In contrast, no differences in susceptibility to antimonials of intracellular amastigotes of *L. infantum* strains isolated from untreated dogs were found, despite repeated in vitro passages and hamster infection (Carrio and Portus, 2002). Unfortunately, there is no data available about the occurrence of *Leishmania* resistance to other drugs in dogs.

6. Prognosis

6.1. Can treated dogs with leishmaniosis truly be cured or cleared of the infection?

Treatment with antileishmanial drugs and especially with the combination of meglumine antimoniate/allopurinol or allopurinol alone often leads to clinical cure (Noli and Auxilia, 2005). However, some dogs that initially respond well to therapy can experience clinical relapse after the cessation of treatment or during it suggesting that infection had not been eliminated. Furthermore, parasitological cure is rarely achieved and treated dogs, even those on a prolonged allopurinol regime, continue to harbour the parasite and be infectious to sand flies, although to a lesser extent (Ikeda-Garcia et al., 2007; Manna et al., 2008a; Ribeiro et al., 2008).

6.2. Can clinically healthy but infected dogs clear the infection spontaneously?

It remains unclear if some healthy infected dogs are able to clear *Leishmania* infection spontaneously. Based on current knowledge in rodent and human studies (Dereure et al., 2003; Soliman, 2006; Okwor and Uzonna, 2008), it is likely that persistent infection occurs in some dogs without ever producing apparent lesions or clinical disease, and that complete clearance of infection in dogs would be an exceptional event. Long-term experimental studies have demonstrated that healthy infected dogs harbour the parasite in lower numbers in the blood, liver, spleen, lymph node and skin when compared to sick dogs which have high parasitic loads and wide tissue dissemination of the parasite (Rodriguez-Cortes et al., 2007). A longitudinal study in a cohort of dogs introduced into an endemic area has demonstrated that some of them were PCR positive in bone marrow and converted to negative over time (Oliva et al., 2006). However, this study was limited due to the sensitivity of conventional PCR and the sampling of only one tissue. Further studies are required to elucidate this question.

6.3. Can infected dogs be reinfected?

There is no conclusive information in the literature about re-infection in the dog. Experimental re-challenge infections in dogs indicated that a previous exposure could confer some degree of resistance in some dogs (Santos et al., 2003). No data is available on reinfection under natural conditions. However, the authors hypothesize that reinfection in endemic areas where dogs live outdoors is likely. Dogs living in endemic areas are often continuously exposed to infective sand fly bites. Studies have shown that dogs may be bitten by sand flies hundreds of times during one night. In addition, a relatively low to moderate proportion of sand flies harbours *L. infantum* (0.5–3%) in endemic areas (Gomez-Saladin et al., 2005; Martin-Sanchez et al., 2006). It is most probable that dogs that are initially able to resist the establishment of infection may eventually succumb when the parasite is continuously introduced and reintroduced. It is also possible that dogs can be infected with one strain of the parasite and may then be infected with a different and possibly more virulent strain.

6.4. How could prognosis be established on the basis of the different clinicopathological features?

Prognosis in CanL is difficult to establish and there is limited information available. In addition, no controlled studies have been attempted to evaluate the prognostic factors. Nevertheless, the prognosis for each patient will vary according to its clinicopathological status. Clinical staging systems are aimed to group patients in which the severity of clinical picture and prognosis are the same. These systems are useful to evaluate the efficacy of different therapies, to decide on the therapy most suitable for each patient and also to consider a prognosis. The criteria of classification have to be simple and clinical, with the use of uncomplicated diagnostic methods. According to the established criteria, each patient is staged at a certain moment in time. Later on, the stage can change as the disease deteriorates or improves. We propose a system of four clinical stages, based on clinical signs, clinicopathological abnormalities and serological status, along with the type of therapy and prognosis suitable for each stage (Table 4).

7. Management of clinically healthy dogs in endemic areas

7.1. Should clinically healthy dogs be screened for *Leishmania* antibodies?

Healthy dogs should be screened for *Leishmania* antibodies as an initial indication for the presence of infection if they are imported, scheduled to travel to non-endemic areas, serve as blood donors, employed in epidemiological/research studies or if their owners wish to have them monitored for early infection and the potential to develop disease. It is well known that high antibodies titers are correlated with high parasitism and disease (Reis et al., 2006) and for this reason a high positive antibody titer may indicate that an infected dog is heading toward the development of a widespread infection and future development of clinical disease. Consequently, early detection of the disease is beneficial for the patient. Therefore, it is appropriate to screen dogs living in endemic areas for *Leishmania* antibodies, at least every 6–12
months, for proper institution of both therapeutic and preventative measures.

7.2. Should clinically healthy dogs be assessed for carrying Leishmania DNA?

In general, healthy dogs in endemic regions should only be screened for Leishmania DNA if they are to be exported to non-endemic areas, and if they are considered to be used as blood donors, as pets for immunosuppressed people, or in epidemiological/research studies. Otherwise, we recommend using serology alone or the combination of serology with PCR for screening healthy dogs. It is better to avoid screening clinically healthy dogs only with PCR.

7.3. How should a clinically healthy but seropositive dog be managed?

Clinically healthy but seropositive dogs should be managed according to their antibody level. Dogs with high antibody levels should be treated as sick dogs with the same medication and dosage given to clinical cases and by applying the same type of monitoring during the treatment. Seropositive dogs with low antibody titers should be confirmed by retesting. Confirmed seropositive dogs with low antibody titers should be monitored with physical examinations, routine laboratory tests and serological tests on a regular basis every 3–6 months to assess the progression of the infection. If antibody titers increase significantly during the monitoring, irrespective of clinical signs and clinicopathological abnormalities, these dogs should be treated as sick dogs. Healthy seropositive dogs have been found to infect sand flies (Molina et al., 1994; Michalsky et al., 2007). Therefore, they should be protected with topical insecticide repellents to minimize the transmission of infection during the vector season.

7.4. How should a clinically healthy but PCR-positive and seronegative dog be managed?

Clinically healthy PCR-positive and seronegative dogs can be monitored clinically and serologically overtime (every 6 months or at least once a year) to evaluate seroconversion and possible development of illness. Treatment is not recommended. However, they should be put on preventative measures against parasite transmission (see Section 8.1).

8. Prevention

8.1. What preventive measures should be applied to decrease the risk of canine leishmaniosis?

Effective prevention of sand fly bites can be achieved when the following steps are taken together (Alexander and Maroli, 2003): (i) keeping the dog indoors during the sand fly season from dusk to dawn; (ii) reducing the microhabitats favorable to sand flies in the vicinity of the house or in locations where the dog spends time; (iii) usage of environmental insecticide treatment, and (iv) usage of topical insecticides with proven activity against the sand flies which bite dogs.

In recent years, various insecticide formulations have been evaluated under laboratory and field conditions with encouraging results. Deltamethrin impregnated collars release the insecticide gradually and it is distributed in the subcutaneous adipose tissue of the animals within 1–2 weeks (Halbig et al., 2000). Under optimal conditions, the repellent effect of these collars can last up to 6 months (Killick-Kendrick et al., 1997). Spray or spot-on formulations also offer high levels of protection, though of a shorter duration (2 and 3 weeks, respectively). Topical application of permethrin provides good repellent and insecticidal effects against P. perniciosus that last several weeks (Molina et al., 2001). These topical formulations require some days for the insecticide to spread throughout the stratum corneum. In contrast, powder formulations achieve an immediate effect, but have a shorter lasting effect. The synergizing action of imidacloprid, a nicotinoid insecticide, enhances the properties of pyrethroids like permethrin. In this way, a strong repellent effect against P. papatasii and P. perniciosus, which lasts for 3–4 weeks, has been achieved (Mencke et al., 2003; Miró et al., 2007a). Field studies have shown that some topical insecticides used in canine populations have been effective in reducing the transmission of infection to both dogs and humans (Maroli et al., 2001; Gavgani et al., 2002a; Otranto et al., 2007).

Veterinarians and dog owners are advised to check the label recommendations of products and follow the manufacturer’s instructions, especially for the correct application of the product and frequency of reaplication. Client education on the maintenance of appropriate insecticide throughout the period of sand fly activity is also crucial for the protection of dogs.

8.2. What preventive measures should be used in dogs from non-endemic areas travelling to endemic areas?

The preventive measures should be the same as described for healthy or sick dogs that live in endemic areas (see Section 8.1). It is recommended that collars should be applied 2 weeks before travelling and be changed every 5 months. Alternatively, pyrethroids in spot-on or spray formulations should be applied 2 days before travelling and repeatedly administered every 2–3 weeks, depending on the type of product. As recommended for dogs living in the endemic areas, advice should be given for follow-up visits after returning for clinical and laboratory check up (see Sections 7.3 and 7.4).

8.3. Are there vaccines available for canine leishmaniosis?

After decades of attempts to produce safe and effective Leishmania vaccines with very limited success, several canine vaccines are under experimental or field evaluation, some with promising results (Miró et al., 2008). Purified Leishmania fraction vaccines appear to be the most successful. The main representative of this group of vaccine antigens is the glycoprotein GP63 enriched fraction of L. donovani also known as the “fucose mannose
ligand” (FML). An FML-based vaccine has been evaluated in field studies in Brazil with a good protection rate and it was licensed in that country as the first commercial vaccine for CanL (Leishmune®) (Borja-Cabrera et al., 2002; Nogueira et al., 2005). The FML vaccine has also been proposed for immune therapy of sick dogs (Borja-Cabrera et al., 2004) and as a transmission-blocking vaccine (Saraiva et al., 2006). However, separately naturally infected from FML-vaccinated dogs is difficult and has lead to reluctance to use this vaccine by some veterinarians in Brazil where seropositive dogs are officially culled (Dantas-Torres and Brandao-Filho, 2006). A second vaccine based on an excrated/secreted antigen purified from specific-medium culture supernatant of \textit{L. infantum} promastigotes with muramyl dipeptide as adjuvant has been studied in France with good protection against \textit{L. infantum} experimental infection (Lemesre et al., 2005) and a high efficacy rate in a double blinded field trial (Lemesre et al., 2007).

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