Leishmaniasis

Gad Baneth, DVM, PhD[a,*], Laia Solano-Gallego, DVM, PhD[b]

KEYWORDS

• *Leishmania infantum* • Canine leishmaniasis • Feline leishmaniasis • Co-infection • Allopurinol • Meglumine antimoniate • Miltefosine • Topical insecticides

KEY POINTS

- Leishmaniasis is one of the most important zoonotic diseases in large areas of Europe, Asia, Africa, and Latin America.
- Dogs are considered the main reservoir hosts of *Leishmania infantum* for humans.
- Visceral leishmaniasis of humans and canine leishmaniasis caused by *L. infantum* are potentially fatal if not treated.
- Canine leishmaniasis causes a spectrum of disease patterns and may affect cutaneous, renal, ocular, skeletal muscle, and hemolymphatic target organs.
- Dogs and cats infected by *L. infantum* may suffer similar diseases.

INTRODUCTION

The leishmaniases are a group of diseases caused by protozoa of the genus *Leishmania* and transmitted mostly by the bite of phlebotomine sand fly vectors. There are more than 20 zoonotic *Leishmania* species that infect animals and humans. Dogs are infected by at least 13 *Leishmania* species and cats have been reported to be infected by at least 6 species.[1–4] This review focuses on *Leishmania infantum*, the main *Leishmania* species associated with severe disease in dogs and cats in Europe, Asia, Africa, and the Americas.

Leishmania (order: Kinetoplastida, family: Trypanosmatidae) are diphasic parasites whose amastigote stage is found intracellularly in host macrophages and its flagellated promastigote stage develops in the sand fly gut extracellularly. Canine and feline leishmaniasis caused by *L. infantum* are endemic in regions whereby vector sand flies are present and transmit infection.[1,5]

[a] The Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, PO Box 12, Rehovot 7610001, Israel; [b] Departament de Medicina i Cirurgia Animal, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra, Spain
* Corresponding author.
E-mail address: gad.baneth@mail.huji.ac.il

Vet Clin Small Anim 52 (2022) 1359–1375
https://doi.org/10.1016/j.cvsm.2022.06.012
vetsmall.theclinics.com

CANINE LEISHMANIASIS

Epidemiology

Dogs are considered the main reservoir for human *L. infantum* infection and canine leishmaniasis is a major zoonosis endemic in more than 70 countries in an area that spans Southern Europe, Northern Africa, the Middle East, Central Asia, China, and South America.[1] *L. infantum* has also emerged in dogs in the USA and Canada, whereby transmission is thought to be mainly transplacental, rather than by sand flies.[6] The travel and importation of infected dogs and cats make the disease an important concern in endemic and nonendemic areas.[5,7]

Leishmania infection may give rise to a chronic and severe disease that can eventually be fatal in dogs and cats. However, only a small fraction of the infected animals in endemic areas develop the clinical disease while a larger part of the exposed population is subclinically infected. Longitudinal studies of natural infection with *L. infantum* have shown that some subclinically infected animals eventually develop clinical disease; however, the majority remain subclinically infected or resolve infection.[8–11] It has been estimated based on serologic surveys that 2.5 million dogs are infected with *L. infantum* in Portugal, Spain, France, and Italy. Furthermore, several million dogs are infected in South America and other endemic regions. Genetic evidence suggests that the introduction of *L. infantum* to the Americas occurred by infected dogs during the Spanish and Portuguese colonization.[12,13]

Pathogenesis

Leishmania spp. are transmitted to the skin of animals and humans when an infected female sand fly bites the host, takes a blood meal, and transmits the promastigote stage of the parasite. Promastigotes are then phagocytized by macrophages. They transform and replicate within cytoplasmic vesicles within the macrophages as amastigotes. *L. infantum* amastigotes migrate in macrophages from the skin to the local draining lymph node, and then disseminate to the spleen, bone marrow, and internal organs whereby they invade other cells.[14] After an incubation period, parasites travel back to the host skin whereby they are available for being taken up in the blood meals of feeding female sand flies. The amastigotes in the blood meal develop in the gut of a suitable sand-fly vector. They then move from the hindgut toward the fly's pharynx and are introduced into the skin of a new host when the sand fly feeds again.[9,14]

The outcome of canine infection following the infecting sand fly bite depends on the balance between the parasite virulence and infectious dose, and the immediate and long-term immune responses mounted by the infected animal host. An effective cell-mediated immune response associated with γ-interferon and reactive oxygen species production that facilitates the activation of macrophages and killing of the intracellular *Leishmania* parasites has been shown to be protective and enables infected animals to control infection. In contrast, a response that predominantly involves the secretion of interleukin 4 (IL4) and evolution of B-cell lymphocytes into plasma cells with increased IgG production and hyperglobulinemia is associated with uncontrolled infection and progression to clinical disease. T lymphocytes from dogs with chronic disease increasingly express the programmed death-1 (PD-1) cell-surface receptor and demonstrate decreased lymphocyte proliferation when stimulated with *L. infantum* antigen. These lead to a phenomenon termed T cell exhaustion in which there is a reduction in γ-interferon secretion and minimal to absent *Leishmania*-specific lymphocyte proliferation.[15,16] Hypergammaglobulinemia and high parasite load in canine leishmaniasis are associated with the formation of circulating immune complexes which are deposited in the kidneys and other organs

and induce immune-complex glomerulonephritis with proteinuria. Loss of albumin through damaged glomeruli in the urine, and an inflammatory response that decreases the production of albumin, a negative acute-phase protein, by the liver, are the reasons for the subsequent serum hypoalbuminemia. Renal disease, which is considered the main cause of death in dogs with leishmaniasis, develops gradually over time. The time of onset and severity of renal disease varies between individual infected animals. Renal disease is frequently not evident in the early stages of infection.[17]

Overall, the progression of *L. infantum* infection to clinical disease in dogs is marked by a depression in cell-mediated immunity and an excessive humoral response.[16] Dogs that are resistant to the development of disease may develop low and sometimes intermittent and borderline antibody levels and remain subclinically infected. Dogs affected by clinical disease and subclinically infected dogs are both infectious to sand flies, and therefore constitute reservoirs.[18] A factor that may enhance transmission of *L. infantum* is that clinically affected dogs were found to be more attractive to sand flies in search of blood meals than uninfected dogs.[19] Naturally infected dogs and cats may develop initial signs of clinical disease after a variable subclinical incubation period of at least 3 months (for dogs), or remain subclinically infected for their lifetime.[8,20] In other cases, animals succumb to disease long after infection often due to immune-suppression by another condition such as malignant neoplasia, endocrine disease or concurrent infectious disease. A retrospective study from the University of Barcelona in Spain found that the age distribution of the disease in dogs is bimodal with a peak of prevalence at 2 to 4 years and a secondary peak from the age of 7 years.[21] The early peak likely represents dogs susceptible to the development of clinical disease while the second peak includes older dogs that may have been harboring infection subclinically for a long time and whose immune response may have been weakened by concurrent disease conditions. Age has also been associated with differences in clinicopathological findings and disease severity in canine leishmaniasis. Young dogs less than 3 years old were found to develop systemic signs with renal and hematologic abnormalities less frequently than older dogs, while dermatologic signs were more common in young and adult dogs, compared with old dogs older than 8 years.[22]

Susceptibility or resistance to canine leishmaniasis is influenced by the host's genetics. Examples of this include the overrepresentation in canine leishmaniasis surveys of breeds that originated from nonendemic countries for leishmaniasis such as the Boxer, Rottweiler, Doberman Pinscher, and German shepherd.[21,23] In comparison, severe disease is rare and significantly lower than among other breeds such as Ibizan hounds from the endemic Balearic Islands of Spain.[24] Studies have shown that Ibizan hounds produce a predominantly cellular immune response against *L. infantum* infection.[24,25] Genetic studies have found that disease tends to develop more frequently in dogs with certain genotypic markers. However, it is clear that many genetic loci and genes contribute to susceptibility or resistance to the disease, and their relative contributions are difficult to infer. A dog leukocyte antigen (DLA) class II DLA-DRB1 genotype, which is a dog major histocompatibility complex (MHC) class II allele, was linked to an increased risk of being infected in an endemic area in Brazil.[26] Other studies have linked the polymorphism of the canine Slc11a1(NRAMP1) gene which encodes an iron transporter protein involved in the control of intraphagosomal replication of parasites and macrophage activation, and inferred that susceptible dogs have mutations in this gene.[27]

Infection with additional vector-borne disease agents has been shown to impact the risk of developing canine leishmaniasis and the progression to clinical disease.[28,29] In a longitudinal study of 214 hunting dogs in the USA, dogs infected with 3 or more tick-

borne diseases were eleven times more likely to be associated with progression to clinical leishmaniasis than dogs with no tick-borne disease. In addition, dogs with exposure to both tick-borne diseases and *Leishmania* spp. were five times more likely to die.[29] Other studies have found an association between canine leishmaniasis and canine ehrlichiosis caused by *Ehrlichia canis*. Dogs with this coinfection can develop higher skin *Leishmania* parasite loads than dogs solely infected with *Leishmania* spp.[28,30,31]

Clinical Signs

The history of dogs with canine leishmaniasis often includes weight loss, weakness, skin lesions, ocular abnormalities, epistaxis, and signs of renal disease such as polyuria and polydipsia. On physical examination, the main clinical signs found in canine leishmaniasis are dermal lesions, lymphadenomegaly, splenomegaly, ocular lesions, muscle atrophy, and poor body condition. Dogs with leishmaniasis may also present with vomiting, diarrhea, gastrointestinal disease, tongue lesions, melena, rhinitis, neurologic abnormalities, onychogryphosis (abnormal nail growth), and lameness. Fever is found in less than 20% of canine leishmaniasis cases which usually presents as a chronic disease. Sixteen to 80% of the dogs with clinical leishmaniasis have ocular or periocular lesions including uveitis and keratoconjunctivitis.[32–34]

A variety of skin lesions are found in dogs with leishmaniasis.[35] The most common is exfoliative dermatitis (**Fig. 1**), which can be generalized or localized over the face, ears, tail, and limbs. Ulcerative dermatitis is frequently found over bony prominences. Nodular dermatitis and pustular dermatitis are occasionally reported, and a mild form of papular dermatitis has also been described in dogs that have a strong cell-mediated immunity to *L. infantum* infection (**Fig. 2**).[25,36,37]

Laboratory Findings

Canine leishmaniasis is a systemic disease that affects multiple systems and organs and causes pathology which induces alterations in hematological, serum biochemistry, and urine test parameters. Anemia, which is usually mild to moderate normocytic normochromic and nonregenerative, is a common finding in dogs with leishmaniasis. About 67% of the dogs admitted for veterinary care due to the disease are anemic, and 26% have lymphopenia while 24% have leukocytosis.[33] Thrombocytopenia is apparently not common with about 6% prevalence in a study that ruled out other conditions causing decreased platelet concentrations such as *Ehrlichia canis* infection.

Fig. 1. Exfoliative dermatitis over the face and ears of a dog with *L. infantum* infection.

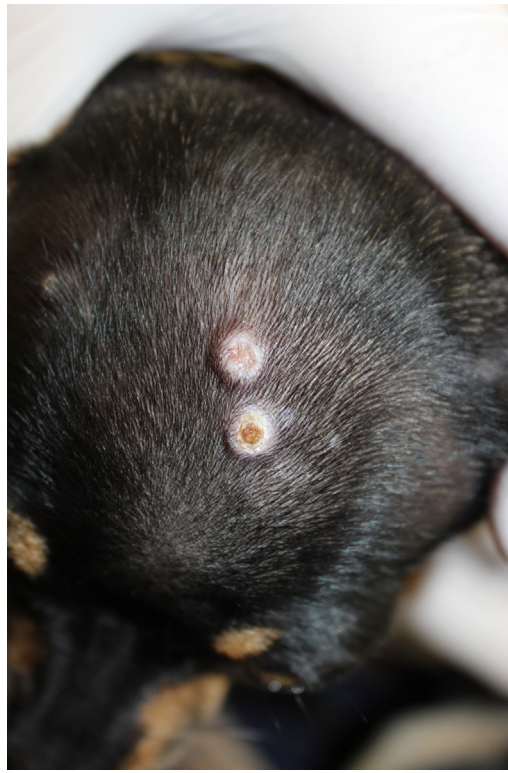

Fig. 2. Papular dermatitis due to *L. infantum* on the head of a seven-month-old Pinscher. Note the typical crater forms with central crust and indurated margins. (*Courtesy* Dr. Laura Ordeix (Dermatology Service, Fundació Hospital Clínic Veterinari-UAB).)

The most common serum biochemistry alterations in dogs with leishmaniasis are hyperproteinemia with elevated gamma-globulins in 73%, increased beta-globulins in 68%, and hypoalbuminemia in 55%, producing a decreased albumin/globulin ratio in 78% of the affected dogs.[33] Other serum biochemistry parameters including cholesterol levels and alanine aminotransferase (ALT) activity are less frequently elevated. Azotemia with increases in urea and creatinine levels is found in dogs with advanced kidney disease due to *L. infantum* infection. Proteinuria with increased urine protein to creatinine ratio (UPC) was found in 48% of dogs affected clinically by leishmaniasis whose urine was tested.[33]

The levels of some acute-phase proteins can be used as markers for the severity of inflammation in canine leishmaniasis and for following the response to treatment.[38,39] C-reactive protein (CRP), ferritin, and haptoglobin increase in canine leishmaniasis, whereas the negative acute-phase protein paraoxonase 1 (PON1) decreases in clinical disease. Albumin which is also considered a negative acute-phase protein also decreases during disease irrespective of kidney disease and urinary loss.[38]

Clinical Evaluation of Dogs Suspected of Leishmaniasis

Evaluation of dogs suspected of leishmaniasis includes a thorough physical examination, complete blood count (CBC), serum biochemistry, and urinalysis. Due to the common ocular involvement in the disease, a thorough ophthalmologic examination

is needed in infected dogs. Dogs with clinical disease will typically be hyperglobulinemic, hypoalbuminemic, anemic, and will frequently have proteinuria due to glomerular loss of albumin, even if they are not azotemic. If proteinuria is found on a dipstick test, quantification by the urine protein/creatinine (UPC) ratio is needed to evaluate the magnitude of protein loss. Specific laboratory tests for the detection of infection described in the diagnosis section are indicated to confirm the suspicion of leishmaniasis.

A clinical staging system presented by the LeishVet association divides the canine disease into 4 clinical stages based on clinical signs, clinicopathological abnormalities, and level of antileishmanial antibodies.[40] These 4 clinical stages include stage I-mild disease, stage II-moderate disease, stage III-severe disease, and stage IV-very severe disease. The severity of the disease is mainly based on the degree of renal disease. A good example of stage I-mild disease is papular dermatitis (see **Fig. 2**) as the sole clinical sign without any evidence of systemic disease including the absence of clinicopathological abnormalities. Most sick dogs which are diagnosed with leishmaniasis in Mediterranean basin countries are classified as having stage II-moderate disease or stage III-severe disease while stage IV is less common.[40] Staging is helpful for decisions regarding the most suitable treatment and for determining prognosis.

Diagnosis

Canine leishmaniasis is often illusive and challenging to diagnose because of the variety of presenting clinical signs and clinical pathologic abnormalities, and the frequent occurrence of subclinical infection. Subclinically infected dogs may have no clinical pathologic changes and remain subclinically infected for long periods of time or develop changes gradually as they progress toward clinical disease. The indications for pursuing a diagnosis of leishmaniasis are variable and differing presentations may require the use of different tests. The presentation of dogs with clinical signs or clinical-pathological abnormalities compatible with the disease is perhaps the most common reason for seeking the diagnosis of the disease. However, the detection of subclinical infection in blood donors or testing dogs from endemic areas for importation to certain countries such as Australia and South Africa, are also indications for testing. Follow-up of dogs during disease treatment and after recovery, and testing of dogs before vaccination against leishmaniasis or as part of a health check, are additional reasons for testing for this infection. Some diagnostic assays such as cytologic detection of amastigotes in tissues will only be positive in dogs with high *Leishmania* parasite loads, as often found in animals with clinical disease, while other tests such as quantitative serology are more likely to be positive than cytologic tests that directly demonstrate the presence of the organism in dogs with lower parasite loads, including some subclinically infected animals.[40,41]

Cytologic examination of aspirates from the bone marrow, spleen, lymph nodes or skin, or touch impressions from the skin and other tissues can be used to detect *Leishmania* amastigotes. Slides can be stained by a Romanoswsky-type stain such as May Grunwald-Giemsa or quick commercial stains and viewed by light microscopy. The *Leishmania* amastigote stage is detected in the cytoplasm of macrophages and more rarely in neutrophils, and may also be viewed outside cells as an artifactual result of cell damage that can occur while making cytologic preparations.[42,43] Amastigote forms are about 1 to 4 μm long by 1 to 2 μm wide and contain a prominent nucleus and a rod-like kinetoplast structure (**Fig. 3**). The detection of the parasite by cytology is often unrewarding due to the possibility of a low number of visible tissue amastigotes even in dogs with full-blown clinical disease.

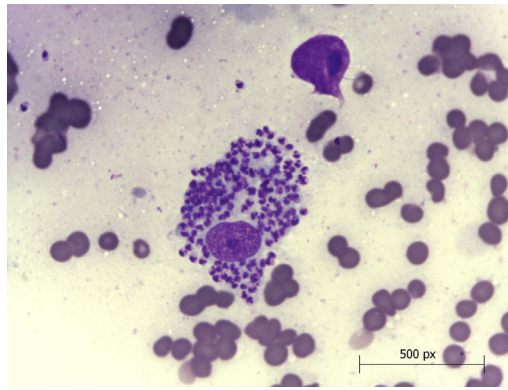

Fig. 3. Cytology of a skin lesion from the cat in Fig. 4 showing a macrophage laden with *L. infantum* amastigotes in its cytoplasm as well as free amastigotes seen in the hemodiluted background. Diff-quick staining. (*Courtesy* Dr. Laura Ordeix (Dermatology Service, Fundació Hospital Clínic Veterinari-UAB).)

Histopathology of tissues from dogs with leishmaniasis often shows granulomatous, pyogranulomatous, or lymphoplasmocellular inflammatory patterns compatible with the disease; however, the definitive detection of *Leishmania* amastigotes in biopsy sections of the skin or other infected organs is frequently difficult. In such cases, *Leishmania* immunohistochemical staining can be used to detect and verify the presence of parasite in the tissue.[43–45]

Serology is a major diagnostic technique regularly used for the diagnosis of canine leishmaniasis.[32] Several serologic methods are used for the detection of anti-*Leishmania* antibodies. These include the enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence assay (IFA), direct agglutination test (DAT), and western blotting. ELISA and IFA are the most frequently used techniques for diagnostic and research purposes. Recombinant antigens such as rK39 and k26 are also used to detect antibodies in dogs.[46] Rapid kits for the serologic evaluation of canine leishmaniasis are commercially available and provide qualitative positive or negative results. Increased sensitivity and specificity are obtained with quantitative serologic laboratory methods such as the IFA and ELISA. However, while dogs with a clinical disease are almost always seroreactive, dogs with subclinical infection are less frequently seroreactive. Therefore, serology is not the optimal assay for detecting subclinical infection.[47]

Serologic cross-reactivity with other infectious organisms and especially with trypanosomatids such as *Trypanosoma cruzi* are a problem in areas whereby canine trypanosomiasis is common, particularly in areas of Latin America and Texas.[48,49] Serologic cross-reactivity is also found between different species of *Leishmania* which infect dogs, and antibodies formed against *Leishmania tropica*, *Leishmania major, Leishmania braziliensis* and *Leishmania amazonensis* are reactive with *L. infantum* antigen.[50,51] This may present difficulties in regions whereby several species of *Leishmania* cause clinical disease in dogs such as the Middle East (*L. major, L. tropica* and *L. infantum*) and South America (*L. braziliensis, L. amazonensis*, *Leishmania mexicana,* and *L. infantum)*.

PCR to detect parasite DNA in tissues allows specific detection of *Leishmania* spp. Different PCR assays with a variety of parasite target sequences of genomic loci or kinetoplast DNA (kDNA) are used for the diagnosis of infection. kDNA PCR assays are more sensitive than genomic DNA assays which target parts of the *Leishmania*

ribosomal operon DNA such as the internal transcribed spacer (ITS) region. Nevertheless, ITS-PCR is able to identify the infecting *Leishmania* species while kDNA PCR is only indicative of *Leishmania* infection without species identification.[52] The preferred and most sensitive sampling sites for *Leishmania* PCR are the lymph nodes, bone marrow, spleen, and skin, while PCR of blood or urine are less sensitive and may be negative also in cases of overt clinical disease.[32,53] Conjunctival swab PCR is a sensitive noninvasive technique which can be used in surveys and when invasive sampling of bone marrow, lymph nodes or spleen is risky.[54,55]

The LeishVet guidelines for the practical management of canine leishmaniasis recommend using quantitative serology as the main diagnostic test in dogs with clinical signs suspected of leishmaniasis, or with hematological and serum biochemistry abnormalities compatible with the disease.[32] Moderate to high antileishmanial antibody levels with clinical findings compatible with the disease are considered sufficient to reach a diagnosis of canine leishmaniasis. PCR, cytology, and histopathology demonstrating the presence of the parasite are ancillary tests that can aid in the diagnostic process of dogs with the suspected disease in the case of uncertain serologic results.[32] Combining serology and PCR may facilitate the diagnosis of subclinical infection in apparently healthy dogs and blood donors.

Treatment

Medical treatment varies according to the clinical stage of the infected dog.[40] Treatment of canine leishmaniasis is prolonged and although it is frequently successful in achieving clinical cure, if the affected dog is not in a progressive stage of the disease, complete elimination of the parasite is often not accomplished. Treatment requires long-term monitoring to follow the dog's response and detect possible relapse, ascertain that renal disease, if present or develops during treatment, does not deteriorate, and that possible side effects of the drugs do not cause harm.

Allopurinol is the main drug used for the treatment of canine leishmaniasis. It is administered orally and acts by interfering with the purine pathway and the parasite's RNA synthesis. Allopurinol is given as long-term treatment of at least 6 months and usually a year or more (**Table 1**). The pentavalent antimony meglumine antimoniate (Glucantime) which inhibits leishmanial glycolysis and fatty acid oxidation and is injected subcutaneously is frequently used in combination with allopurinol for the first 4 weeks of treatment. Alternatively, miltefosine (Milteforan), can be used orally for the first month of treatment in combination with allopurinol instead of meglumine antimoniate. Monotherapy with meglumine antimoniate or miltefosine has not been shown to produce consistent long-term suppression of parasite loads in dogs and may result in disease relapse within 6 months to 1 year.[56,57] Other second-line drugs such as paramomycin and marbofloxacin have been shown to have antileishmanial effects.[58] The standard treatment protocol in Europe for dogs in the stable clinical condition is allopurinol at 10 mg/kg every 12 hours per-os (P.O), in combination with meglumine antimoniate at 100 mg/kg injected subcutaneously every 24 hours for 28 days, or in combination with miltefosine at 2 mg/kg P.O. every 24 hours for 28 days.[58] Dogs with severe clinical condition, particularly those with progressive renal disease due to leishmaniasis, may be treated with allopurinol alone. Long-term treatment of canine leishmaniasis is deemed a success and discontinued when all the following 3 conditions are met: (1) disappearance of clinical signs; (2) normalization of the hematology, blood biochemistry profile, and urinalysis; and (3) quantitative serology has decreased to below the cut-off value.[32]

A follow-up study of 1 year with 37 dogs that were treated with the combined allopurinol and meglumine antimoniate protocol, of which 32 dogs were in LeishVet stage

Table 1
The main antiprotozoal drugs used for the treatment of canine and feline leishmaniasis

Drug Name	Dose for Dogs	Dose for Cats
Allopurinol	10 mg/kg q 12 hr orally for 6–12 mo or more, until clinical signs disappear, clinicopathological findings return to reference values and quantitative serology reaches the cut-off value.[58]	10 mg/kg q 12 hr or 20 mg/kg q 24 h for at least 6 mo 3
Meglumine antimoniate	100 mg/kg injected subcutaneously q 24 hr for 28 d. Usually used during the first 4 weeks of the dog's treatment in combination with allopurinol administered as above.[58]	20–50 mg/kg q 24 hr subcutaneously for 30 d, alone or combined with allopurinol treatment.[3]
Miltefosine	2 mg/kg orally q 24 hr for 28 d. Usually used during the first 4 weeks of the dog's treatment in combination with allopurinol administered as above.[58]	

II and 5 in stage III, found that all dogs showed improvement in their clinical status and clinicopathological abnormalities within 30 days of treatment. There was also a substantial but incomplete drop in their blood parasite loads and antibody levels in the first 6 months of treatment. Nevertheless, despite the marked clinical improvement of most dogs, only 5 (16%) were eligible for stopping treatment at the end of 1 year of therapy.[53] In another study that included 23 dogs in LeishVet stage II treated and followed-up for 2 to 9 years, survival was generally long, although antibody levels remained positive in most dogs after 1 year of treatment. Three dogs from this study had a clinical relapse with high antibody levels and parasitemia, 8 dogs had immune-mediated lesions, such as uveitis, arthritis, and cutaneous vasculitis, and in all of these cases, the dogs had persistently high anti-*Leishmania* antibody levels at diagnosis and during follow-up. Three dogs in this study developed xanthine urolithiasis which was likely associated with their allopurinol treatment.[59]

Treated dogs can remain carriers of leishmaniasis and be infectious to sand flies and therefore dogs during and after therapy should be treated with topical insecticides to prevent transmission to other animals and to humans.[60] Owners of dogs should receive a thorough explanation about the disease, its zoonotic potential, and the prognosis of their dog.

The main adverse effects of the drugs recommended for the treatment of canine leishmaniasis include gastrointestinal signs for miltefosine, local inflammation and pain in the injection site, and potential nephrotoxicity for meglumine antimoniate, and xanthine urolithiasis and renal mineralization for allopurinol.[58]

Resistance to antileishmanial drugs including pentavalent antimonials and miltefosine has been extensively reported in humans. Disease relapse of dogs with canine leishmaniasis during allopurinol treatment has been described and was associated with allopurinol resistance of *L. infantum* isolated from these animals.[61,62] Sporadic cases of *L. infantum* isolates from dogs resistant to other drugs have been reported but clinical relapses have not been consistently described in these dogs.[63,64]

Ancillary therapy for canine leishmaniasis includes treatment with domperidone (Leishguard) which is registered in some European countries for prophylaxis of the disease.[65] Domperidone is a dopamine D2 receptor antagonist with immunostimulant properties via the stimulation of prolactin secretion which acts as a proinflammatory agent. It is claimed to reduce the probability of progression to clinical disease by

stimulating cellular immunity. A dietary supplement of nucleotides and active hexose also has been assessed as an additional adjunctive for the management of sick dogs as well as the treatment of subclinically infected dogs to prevent disease progression.[66,67]

Follow-up during the treatment of canine leishmaniasis varies according to the dogs' clinical status. Dogs in stable condition and no renal disease can be monitored 1 month after the beginning of treatment with a physical examination, CBC, serum biochemistry, and urinalysis, and then if no deterioration is noted, every 3 to 4 months during the first year of treatment. Repeated serology with a quantitative assay is recommended 3 and 6 months after the beginning of treatment, and then every 6 to 12 months. A marked increase in the antibody levels of dogs during or after the end of therapy, often precedes disease relapse, and requires additional testing and consideration of repeated treatment, or the use of additional drugs and an increase in allopurinol dose in dogs under treatment.

Prevention

Transmission of *L. infantum* from infected dogs to vector sand flies and subsequently to naïve dogs can be greatly reduced by insecticides that prevent and repel sand fly bites. Topical insecticides containing pyrethroids in collars, spot-on formulations, and sprays, have been shown to effectively reduce *Leishmania* transmission. Slow release deltamethrin and flumethrin collars and long acting spot on drops containing permethrin with imidacloprid or permethrin with fipronil have been reported to significantly reduce the number of sand fly bites to dogs under experimental transmission and also to decrease transmission of infection in field studies.[68–70] The use of commercial topical insecticides proven to prevent *L. infantum* transmission is, therefore, currently considered as the most effective mean of protecting dogs from *Leishmania* infection.[71]

Commercial vaccines against canine leishmaniasis have been approved for the protection of dogs in Europe and Brazil. Overall, vaccination decreases the likelihood of clinical disease development in dogs but is less successful in entirely preventing the establishment of infection.[72] The currently marketed vaccines, Letifend in Europe and LeishTec in Brazil are based on *Leishmania* recombinant proteins. The Letifend consists of a chimerical protein (protein Q) formed by 5 antigenic fragments from 4 *L. infantum* proteins with no adjuvant, whereas the LeishTec includes recombinant protein A2 from *Leishmania donovani* and saponin as adjuvant. Two vaccines that are no longer produced include the Leishmune based on the fucose-mannose ligand (FML) of *L. donovani* and a saponin adjuvant, and the CaniLeish composed of purified excreted–secreted proteins of *L. infantum* adjuvanted with *Quilaja saponaria* saponin. Additional studies are needed for the evaluation of current vaccines and development of future vaccines against canine leishmaniasis.[72,73]

Feline leishmaniasis

Cats have been reported to be infected with at least 6 species of the genus *Leishmania* in different parts of the word including *L. infantum*, *L. major*, *L. tropica*, *L. mexicana*, *L. braziliensis,* and *L. amazonensis*.[3,4,74] Infection with *L. infantum* in cats has been described mostly from areas whereby the disease is prevalent in dogs, although clinical disease seems to be less frequent than in canines.[3,74] A study of 249 stray cats from Madrid in Spain found 4.8% seroreactivity for *L. infantum* and a significant association with feline immunodeficiency (FIV) infection. Only 2 of the seroreactive stray cats had clinical signs compatible with feline leishmaniasis.[75] A nationwide study of *L. infantum* infection in 2659 cats from Italy found an overall prevalence of 3.9% by

Fig. 4. Ulcerative cutaneous lesion due to squamous cell carcinoma and *L. infantum* infection in an FIV-positive cat. (*Courtesy* Dr. Laura Ordeix (Dermatology Service, Fundació Hospital Clínic Veterinari-UAB).)

combined serology and PCR with a higher infection rate of 10.5% in warmer southern Italy.[76] The risk of *L. infantum* infection in cats was associated with being older than 18 months, intact and positive for FIV.[3,76,77] Naturally infected cats are infectious to sand flies and have been speculated to be a source of infection for dogs and humans[78–80]

The clinical findings in feline leishmaniasis due to *L. infantum* are usually similar to those found in dogs with the disease. The most common clinical signs include lymphadenomegaly, skin lesions with ulcerative (**Fig. 4**), crusting, exfoliative or nodular dermatitis, ocular lesions with uveitis and conjunctivitis, gingivostomatitis, poor body condition, rhinitis and signs of renal disease. The main clinicopathological alterations are hyperproteinemia with hypergammaglobulinemia, anemia, proteinuria, and azotemia. About 50% of the cats with leishmaniasis suffer from other disease conditions such as coinfections with FIV or Feline leukemia virus (FeLV), hemotrophic *Mycoplasma* infection, malignant neoplasia, endocrine diseases, and treatment with immune-suppressive drugs.[3,77]

The diagnosis of feline leishmaniasis is performed for dogs by cytology and histopathology, serology, and PCR. Using more than one diagnostic technique is often needed to confirm the diagnosis.[77] Treatment of feline leishmaniasis may include allopurinol at 10 mg/kg q 12 hr or 20 mg/kg q 24 hr for at least 6 months (see **Table 1**). Meglumine antimoniate has also been used for cats at 20 to 50 mg/kg q 24 hr subcutaneously for 30 days, alone or combined with allopurinol treatment.[3,77] The median survival time for cats with leishmaniasis treated with antileishmanial drugs described in a case series from Spain was 17 months. Cats with concomitant diseases had a mean survival of 13 months, whereas cats with no complicating diseases had a mean survival of 41 months.[77]

Testing is recommended for feline blood donors living in or originating from a *Leishmania* endemic area. Cats can be protected from infection by the use of topical insecticides licensed for use in felines. Most of the pyrethroid-based topical insecticides licensed for dogs are toxic for cats. A flumethrin and imidacloprid collar licensed for cats was effective in decreasing *L. infantum* infection in a field trial.[81]

Public Health Importance

Human visceral leishmaniasis due to *L. infantum* is a severe and potentially fatal disease. Dogs are well-known peridomestic reservoirs for this disease and cats may also serve as reservoirs for humans through transmission by sand flies. In southern Europe

whereby human visceral leishmaniasis is usually sporadic and the ratio between clinically affected people and infected dogs is high, ownership of diseased animals is often not perceived as associated with increased risk to humans. However, studies in Brazil and Iran have reported that increased prevalence of leishmaniasis in the canine population, poor socioeconomic conditions, and dog ownership are risk factors for human leishmaniasis.[82–85] Therefore, in areas whereby sand-fly vectors of leishmaniasis are present and transmission of the disease occurs, dogs and cats should be protected against sand fly-bites by topical insecticides and animal owners should be educated about the risks of this disease.

CLINICS CARE POINTS

- Leishmaniasis should be suspected in dogs and cats with compatible skin lesions, ocular lesions, renal disease, lymphadenomegaly, splenomegaly, unexplained hyperglobulinemia, epistaxis, and a variety of other clinical abnormalities.
- Dogs and cats presenting with renal disease, ocular lesions, or other pathologies caused by *L. infantum* in the absence of characteristic skin lesions should not be overlooked.
- Subclinical infection with *L. infantum* is more common than clinical disease in endemic areas.
- *Leishmania infantum* can be transmitted by blood transfusion. Blood donors from endemic areas and kennels whereby disease is present need to be tested for infection.
- Transmission of canine leishmaniasis in North American kennels and hunting dogs is mainly transplacental rather than vector borne.
- Quantitative serology is considered the most useful method for the detection of leishmaniasis in dogs and cats with suspected clinical disease.
- PCR of blood is frequently negative in dogs with clinical disease. Bone marrow, lymph node, spleen, conjunctiva, and skin samples are more likely to yield a positive PCR.
- Treatment of leishmaniasis is long-term and should not be stopped before clinical signs disappear, hematology and serum biochemistry abnormalities normalize, and quantitative serology becomes negative.
- The most effective way to prevent canine infection is protection with topical insecticides tested and approved for the prevention of leishmaniasis.

DISCLOSURE

The authors declare that they have no conflict of interest related to any of the topics presented in this publication.

REFERENCES

1. Dantas-Torres F, Solano-Gallego L, Baneth G, et al. Canine leishmaniosis in the old and new worlds: unveiled similarities and differences. Trends Parasitol 2012;28:531–8.
2. Iatta R, Mendoza-Roldan JA, Latrofa MS, et al. *Leishmania tarentolae* and *Leishmania infantum* in humans, dogs and cats in the Pelagie archipelago, southern Italy. PLoS Negl Trop Dis 2021;15:e0009817.
3. Pennisi MG, Cardoso L, Baneth G, et al. LeishVet update and recommendations on feline leishmaniosis. Parasit Vectors 2015;8:302.

4. Paşa S, Tetik Vardarlı A, Erol N, et al. Detection of *Leishmania major* and *Leishmania tropica* in domestic cats in the Ege Region of Turkey. Vet Parasitol 2015; 212:389–92.
5. Schäfer I, Volkmann M, Beelitz P, et al. Retrospective evaluation of vector-borne infections in dogs imported from the Mediterranean region and southeastern Europe (2007-2015). Parasit Vectors 2019;12:30.
6. Toepp AJ, Schaut RG, Scott BD, et al. *Leishmania* incidence and prevalence in U.S. hunting hounds maintained via vertical transmission. Vet Parasitol Reg Stud Rep 2017;10:75–81.
7. Schäfer I, Kohn B, Volkmann M, et al. Retrospective evaluation of vector-borne pathogens in cats living in Germany (2012-2020). Parasit Vectors 2021;14:123.
8. Oliva G, Scalone A, Foglia Manzillo V, et al. Incidence and time course of *Leishmania infantum* infections examined by parasitological, serologic, and nested-PCR techniques in a cohort of naive dogs exposed to three consecutive transmission seasons. J Clin Microbiol 2006;44:1318–22.
9. Baneth G, Koutinas AF, Solano-Gallego L, et al. Canine leishmaniosis - new concepts and insights on an expanding zoonosis: part one. Trends Parasitol 2008;24: 324–30.
10. Solano-Gallego L, Llull J, Ramis A, et al. Longitudinal study of dogs living in an area of Spain highly endemic for leishmaniasis by serologic analysis and the leishmanin skin test. Am J Trop Med Hyg 2005;72:815–8.
11. Grimaldi G Jr, Teva A, Santos CB, et al. The effect of removing potentially infectious dogs on the numbers of canine *Leishmania infantum* infections in an endemic area with high transmission rates. Am J Trop Med Hyg 2012;86:966–71.
12. Momen H, Pacheco RS, Cupolillo E, et al. Molecular evidence for the importation of Old World *Leishmania* into the Americas. Biol Res 1993;26:249–55.
13. Maurício IL, Stothard JR, Miles MA. The strange case of *Leishmania chagasi*. Parasitol Today 2000;16:188–9.
14. Serafim TD, Iniguez E, Oliveira F. *Leishmania infantum*. Trends Parasitol 2020; 36:80–1.
15. Solano-Gallego L, Montserrrat-Sangrà S, Ordeix L, et al. *Leishmania infantum*-specific production of IFN-γ and IL-10 in stimulated blood from dogs with clinical leishmaniosis. Parasit Vectors 2016;9:317.
16. Toepp AJ, Petersen CA. The balancing act: Immunology of leishmaniosis. Res Vet Sci 2020;130:19–25.
17. Koutinas AF, Koutinas CK. Pathologic mechanisms underlying the clinical findings in canine leishmaniasis due to *Leishmania infantum/chagasi*. Vet Pathol 2014;51:527–38.
18. Laurenti MD, Rossi CN, da Matta VL, et al. Asymptomatic dogs are highly competent to transmit *Leishmania (Leishmania) infantum chagasi* to the natural vector. Vet Parasitol 2013;196:296–300.
19. Chelbi I, Maghraoui K, Zhioua S, et al. Enhanced attraction of sand fly vectors of *Leishmania infantum* to dogs infected with zoonotic visceral leishmaniasis. PLoS Negl Trop Dis 2021;15:e0009647.
20. Otranto D, Paradies P, de Caprariis D, et al. Toward diagnosing *Leishmania infantum* infection in asymptomatic dogs in an area where leishmaniasis is endemic. Clin Vaccin Immunol 2009;16:337–43.
21. Miranda S, Roura X, Picado A, et al. Characterization of sex, age, and breed for a population of canine leishmaniosis diseased dogs. Res Vet Sci 2008;85:35–8.
22. Cabré M, Planellas M, Ordeix L, et al. Is signalment associated with clinicopathological findings in dogs with leishmaniosis? Vet Rec 2021;189:e451.

23. Gharbi M, Jaouadi K, Mezghani D, et al. Symptoms of canine leishmaniosis in Tunisian dogs. Bull Soc Pathol Exot 2018;111:51–5.
24. Solano-Gallego L, Llull J, Ramos G, et al. The Ibizian hound presents a predominantly cellular immune response against natural *Leishmania* infection. Vet Parasitol 2000;90:37–45.
25. Burnham AC, Ordeix L, Alcover MM, et al. Exploring the relationship between susceptibility to canine leishmaniosis and anti-*Phlebotomus perniciosus* saliva antibodies in Ibizan hounds and dogs of other breeds in Mallorca, Spain. Parasit Vectors 2020;13:129.
26. Quinnell RJ, Kennedy LJ, Barnes A, et al. Susceptibility to visceral leishmaniasis in the domestic dog is associated with MHC class II polymorphism. Immunogenetics 2003;55:23–8.
27. Sanchez-Robert E, Altet L, Utzet-Sadurni M, et al. Slc11a1 (formerly Nramp1) and susceptibility to canine visceral leishmaniasis. Vet Res 2008;39:36.
28. Mekuzas Y, Gradoni L, Oliva G, et al. *Ehrlichia canis* and *Leishmania infantum* co-infection: a 3-year longitudinal study in naturally exposed dogs. Clin Microbiol Infect 2009;15(Suppl 2):30–1.
29. Toepp AJ, Monteiro GRG, Coutinho JFV, et al. Comorbid infections induce progression of visceral leishmaniasis. Parasit Vectors 2019;12:54.
30. Attipa C, Solano-Gallego L, Papasouliotis K, et al. Association between canine leishmaniosis and *Ehrlichia canis* co-infection: a prospective case-control study. Parasit Vectors 2018;11:184.
31. Andrade GB, Barreto WT, Santos LL, et al. Pathology of dogs in Campo Grande, MS, Brazil naturally co-infected with *Leishmania infantum* and *Ehrlichia canis*. Rev Bras Parasitol Vet 2014;23:509–15.
32. Solano-Gallego L, Miró G, Koutinas A, et al. LeishVet guidelines for the practical management of canine leishmaniosis. Parasit Vectors 2011;4:86.
33. Meléndez-Lazo A, Ordeix L, Planellas M, et al. Clinicopathological findings in sick dogs naturally infected with *Leishmania infantum*: Comparison of five different clinical classification systems. Res Vet Sci 2018;117:18–27.
34. Oliveira CS, Ratzlaff FR, Pötter L, et al. Clinical and pathological aspects of canine cutaneous leishmaniasis: A meta-analysis. Acta Parasitol 2019;64:916–22.
35. Saridomichelakis MN, Koutinas AF. Cutaneous involvement in canine leishmaniosis due to *Leishmania infantum* (syn. *L. chagasi*). Vet Dermatol 2014;25:61–71,e22.
36. Colombo S, Abramo F, Borio S, et al. Pustular dermatitis in dogs affected by leishmaniosis: 22 cases. Vet Dermatol 2016;27:9.e4.
37. Lombardo G, Pennisi MG, Lupo T, et al. Papular dermatitis due to *Leishmania infantum* infection in seventeen dogs: diagnostic features, extent of the infection and treatment outcome. Parasit Vectors 2014;7:120.
38. Ceron JJ, Pardo-Marin L, Caldin M, et al. Use of acute phase proteins for the clinical assessment and management of canine leishmaniosis: general recommendations. BMC Vet Res 2018;14:196.
39. Daza González MA, Fragío Arnold C, Fermín Rodríguez M, et al. Effect of two treatments on changes in serum acute phase protein concentrations in dogs with clinical leishmaniosis. Vet J 2019;245:22–8.
40. Solano-Gallego L, Cardoso L, Pennisi MG, et al. Diagnostic Challenges in the era of canine *Leishmania infantum* vaccines. Trends Parasitol 2017;33:706–17.
41. Moreira MA, Luvizotto MC, Garcia JF, et al. Comparison of parasitological, immunological and molecular methods for the diagnosis of leishmaniasis in dogs with different clinical signs. Vet Parasitol 2007;145:245–52.

42. Oliveira GA, Sarmento VAS, Costa EWDS, et al. Detection of *Leishmania infantum* amastigotes in neutrophil from peripheral blood in a naturally infected dog. Rev Bras Parasitol Vet 2021;30:e004821.
43. Paltrinieri S, Gradoni L, Roura X, et al. Laboratory tests for diagnosing and monitoring canine leishmaniasis. Vet Clin Pathol 2016;45:552–78.
44. Ordeix L, Dalmau A, Osso M, et al. Histological and parasitological distinctive findings in clinically-lesioned and normal-looking skin of dogs with different clinical stages of leishmaniosis. Parasit Vectors 2017;10:121.
45. Kost WO, Pereira SA, Figueiredo FB, et al. Frequency of detection and load of amastigotes in the pancreas of *Leishmania infantum*-seroreactive dogs: clinical signs and histological changes. Parasit Vectors 2021;14:321.
46. Porrozzi R, Santos da Costa MV, Teva A, et al. Comparative evaluation of enzyme-linked immunosorbent assays based on crude and recombinant leishmanial antigens for serodiagnosis of symptomatic and asymptomatic *Leishmania infantum* visceral infections in dogs. Clin Vaccin Immunol 2007;14:544–8.
47. Mettler M, Grimm F, Capelli G, et al. Evaluation of enzyme-linked immunosorbent assays, an immunofluorescent-antibody test, and two rapid tests (immunochromatographic-dipstick and gel tests) for serological diagnosis of symptomatic and asymptomatic *Leishmania* infections in dogs. J Clin Microbiol 2005;43:5515–9.
48. Duprey ZH, Steurer FJ, Rooney JA, et al. Canine visceral leishmaniasis, United States and Canada, 2000-2003. Emerg Infect Dis 2006;12:440–6.
49. Meyers AC, Edwards EE, Sanders JP, et al. Fatal Chagas myocarditis in government working dogs in the southern United States: Cross-reactivity and differential diagnoses in five cases across six months. Vet Parasitol Reg Stud Rep 2021;24:100545.
50. Baneth G, Yasur-Landau D, Gilad M, et al. Canine leishmaniosis caused by *Leishmania major* and *Leishmania tropica*: comparative findings and serology. Parasit Vectors 2017;10:113.
51. Paz GF, Rugani JMN, Marcelino AP, et al. Implications of the use of serological and molecular methods to detect infection by *Leishmania* spp. in urban pet dogs. Acta Trop 2018;182:198–201.
52. Talmi-Frank D, Nasereddin A, Schnur LF, et al. Detection and identification of old world *Leishmania* by high resolution melt analysis. PLoS Negl Trop Dis 2010;4:e581.
53. Solano-Gallego L, Di Filippo L, Ordeix L. Early reduction of *Leishmania infantum*-specific antibodies and blood parasitemia during treatment in dogs with moderate or severe disease. Parasit Vectors 2016;9:235.
54. Strauss-Ayali D, Jaffe CL, Burshtain O, et al. Polymerase chain reaction using noninvasively obtained samples, for the detection of *Leishmania infantum* DNA in dogs. J Infect Dis 2004;189:1729–33.
55. Di Muccio T, Veronesi F, Antognoni MT, et al. Diagnostic value of conjunctival swab sampling associated with nested PCR for different categories of dogs naturally exposed to *Leishmania infantum* infection. J Clin Microbiol 2012;50:2651–9.
56. Slappendel RJ, Teske E. The effect of intravenous or subcutaneous administration of meglumine antimonate (Glucantime) in dogs with leishmaniasis. A randomized clinical trial. Vet Q 1997;19:10–3.
57. Manna L, Gravino AE, Picillo E, et al. *Leishmania* DNA quantification by real-time PCR in naturally infected dogs treated with miltefosine. Ann N Y Acad Sci 2008;1149:358–60.

58. Miró G, Petersen C, Cardoso L, et al. Novel areas for prevention and control of canine leishmaniosis. Trends Parasitol 2017;33:718–30.
59. Torres M, Bardagí M, Roura X, et al. Long term follow-up of dogs diagnosed with leishmaniosis (clinical stage II) and treated with meglumine antimoniate and allopurinol. Vet J 2011;188:346–51.
60. Miró G, Gálvez R, Fraile C, et al. Infectivity to *Phlebotomus perniciosus* of dogs naturally parasitized with *Leishmania infantum* after different treatments. Parasit Vectors 2011;4:52.
61. Yasur-Landau D, Jaffe CL, David L, et al. Allopurinol Resistance in *Leishmania infantum* from dogs with disease relapse. PLoS Negl Trop Dis 2016;10:e0004341.
62. Yasur-Landau D, Jaffe CL, David L, et al. Resistance of *Leishmania infantum* to allopurinol is associated with chromosome and gene copy number variations including decrease in the S-adenosylmethionine synthetase (METK) gene copy number. Int J Parasitol Drugs Drug Resist 2018;8:403–10.
63. Maia C, Nunes M, Marques M, et al. In vitro drug susceptibility of *Leishmania infantum* isolated from humans and dogs. Exp Parasitol 2013;135:36–41.
64. Gonçalves G, Campos MP, Gonçalves AS, et al. Increased *Leishmania infantum* resistance to miltefosine and amphotericin B after treatment of a dog with miltefosine and allopurinol. Parasit Vectors 2021;14:599.
65. Sabaté D, Llinás J, Homedes J, et al. A single-centre, open-label, controlled, randomized clinical trial to assess the preventive efficacy of a domperidone-based treatment programme against clinical canine leishmaniasis in a high prevalence area. Prev Vet Med 2014;115:56–63.
66. Segarra S, Miró G, Montoya A, et al. Prevention of disease progression in *Leishmania infantum*-infected dogs with dietary nucleotides and active hexose correlated compound. Parasit Vectors 2018;11:103.
67. Segarra S. Nutritional modulation of the immune response mediated by nucleotides in canine leishmaniosis. Microorganisms 2021;16(9):2601.
68. Brianti E, Napoli E, Gaglio G, et al. Field evaluation of two different treatment approaches and their ability to control fleas and prevent canine leishmaniosis in a highly endemic area. PLoS Negl Trop Dis 2016;10:e0004987.
69. Gálvez R, Montoya A, Fontal F, et al. Controlling phlebotomine sand flies to prevent canine *Leishmania infantum* infection: A case of knowing your enemy. Res Vet Sci 2018;121:94–103.
70. Yimam Y, Mohebali M. Effectiveness of insecticide-impregnated dog collars in reducing incidence rate of canine visceral leishmaniasis: A systematic review and meta-analysis. PLoS One 2020;15:e0238601.
71. Dantas-Torres F, Nogueira FDS, Menz I, et al. Vaccination against canine leishmaniasis in Brazil. Int J Parasitol 2020;50:171–6.
72. Velez R, Gállego M. Commercially approved vaccines for canine leishmaniosis: a review of available data on their safety and efficacy. Trop Med Int Health 2020;25:540–57.
73. Calzetta L, Pistocchini E, Ritondo BL, et al. Immunoprophylaxis pharmacotherapy against canine leishmaniosis: A systematic review and meta-analysis on the efficacy of vaccines approved in European Union. Vaccine 2020;38:6695–703.
74. Rivas AK, Alcover M, Martínez-Orellana P, et al. Clinical and diagnostic aspects of feline cutaneous leishmaniosis in Venezuela. Parasit Vectors 2018;11:141.
75. Montoya A, García M, Gálvez R, et al. Implications of zoonotic and vector-borne parasites to free-roaming cats in central Spain. Vet Parasitol 2018;251:125–30.

76. Iatta R, Furlanello T, Colella V, et al. A nationwide survey of *Leishmania infantum* infection in cats and associated risk factors in Italy. PLoS Negl Trop Dis 2019;13: e0007594.
77. Fernandez-Gallego A, Feo Bernabe L, Dalmau A, et al. Feline leishmaniosis: diagnosis, treatment and outcome in 16 cats. J Feline Med Surg 2020;22: 993–1007.
78. Asfaram S, Fakhar M, Teshnizi SH. Is the cat an important reservoir host for visceral leishmaniasis? A systematic review with meta-analysis. J Venom Anim Toxins Incl Trop Dis 2019;25:e20190012.
79. Batista JF, Magalhães Neto FDCR, Lopes KSPDP, et al. Transmission of *Leishmania infantum* from cats to dogs. Rev Bras Parasitol Vet 2020;29:e017820.
80. Vioti G, da Silva MD, Galvis-Ovallos F, et al. Xenodiagnosis in four domestic cats naturally infected by *Leishmania infantum*. Transbound Emerg Dis 2021. https://doi.org/10.1111/tbed.14216.
81. Brianti E, Falsone L, Napoli E, et al. Prevention of feline leishmaniosis with an imidacloprid 10%/flumethrin 4.5% polymer matrix collar. Parasit Vectors 2017; 10:334.
82. Gavgani AS, Mohite H, Edrissian GH, et al. Domestic dog ownership in Iran is a risk factor for human infection with *Leishmania infantum*. Am J Trop Med Hyg 2002;67:511–5.
83. Margonari C, Freitas CR, Ribeiro RC, et al. Epidemiology of visceral leishmaniasis through spatial analysis, in Belo Horizonte municipality, state of Minas Gerais, Brazil. Mem Inst Oswaldo Cruz 2006;101:31–8.
84. da Silva Santana Cruz C, Soeiro Barbosa D, Oliveira VC, et al. Factors associated with human visceral leishmaniasis cases during urban epidemics in Brazil: a systematic review. Parasitology 2021;148:639–47.
85. Matsumoto PSS, Hiramoto RM, Pereira VBR, et al. Impact of the dog population and household environment for the maintenance of natural foci of *Leishmania infantum* transmission to human and animal hosts in endemic areas for visceral leishmaniasis in Sao Paulo state, Brazil. PLoS One 2021;16:e0256534.