CANINE LEISHMANIOSIS

VECTOR BORNE DISEASE

MSD Animal Health

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WHERE IS THE DISEASE MOST LIKELY TO BE FOUND?

Leishmaniosis refers to a group of diseases with a worldwide distribution caused by protozoa of the genus *Leishmania* (family Trypanosomatidae). *Leishmania* is transmitted by dipteran flies belonging to the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World (Family Psychodidae).

Geography

Canine leishmaniosis was first described in Tunisia in 1908 and currently is known to be prevalent in 50 of the 92 countries where human leishmaniosis is present. Geographic regions considered endemic are **Southern Europe, Africa, the Middle East, the Far East and Central and South America**. Currently there are estimated to be 15 million infected dogs in the world and more than 2.5 million of them suffer from clinical signs of this disease.

A dog showing classic signs of canine leishmaniosis.

Local environment

Leishmania infantum (syn. *L. chagasi*) is the most prevalent protozoa species causing disease in dogs and is endemic at a high level in the **Mediterranean Basin in Europe and Brazil in South America**. Although dogs are the main reservoir of the domestic cycle of infection, *Leishmania* spp. affect various animal species (including people). There is also a wilderness cycle of infection maintained mainly by wild canids (fox, wolf, jackal) and a long list of species (felines, ruminants, equines, rodents, lagomorphs, marsupials, primates, etc.) in which the infection has been described.

Wild canids as reservoirs of *Leishmania* spp.

WHERE IS THE DISEASE MOST LIKELY TO BE FOUND?

Favourable climate conditions

Canine leishmaniosis is endemic in areas where climatic conditions are optimal for development of the phlebotomine flies ("sand flies") that are *Leishmania* vectors. Therefore, these bioclimatic factors are critical for development of the sand fly vector and vary according to different geographic areas.

The sand fly's maximum daily activity begins after sunset and continues during the early hours of the night, provided the temperature does not drop below 17-18ºC or exceed 40ºC; there is no rain or wind, and altitudes are 1000 m and below.

Under 10 ºC the sand fly's development is greatly delayed, and freezing temperatures are lethal.

Evidence of infection / disease spread

Leishmaniosis in dogs is spreading into new areas because of the **impacts of climate change** providing new microclimates where the sand fly can survive and also from movement of animals and people.

Within Europe, many imported cases of canine leishmaniosis have been diagnosed in countries considered to be non-endemic and these dogs are suspected to have been transported from endemic areas.

Similarly, in South America, many countries have reported a significant number of cases to date, although the highest prevalence is still in Brazil.

HOW DOES A DOG **BECOME INFECTED?**

An introduction to the causative agent and life cycle

The genus *Leishmania* is divided into two subgenera: *Leishmania* (replication in the sand fly mid-gut) and *Viannia* (replication in the sand fly distal intestine).

The **protozoa species that cause disease in dogs,** *L. infantum,* is a digenic protozoa with a biological cycle that takes place in two types of hosts:

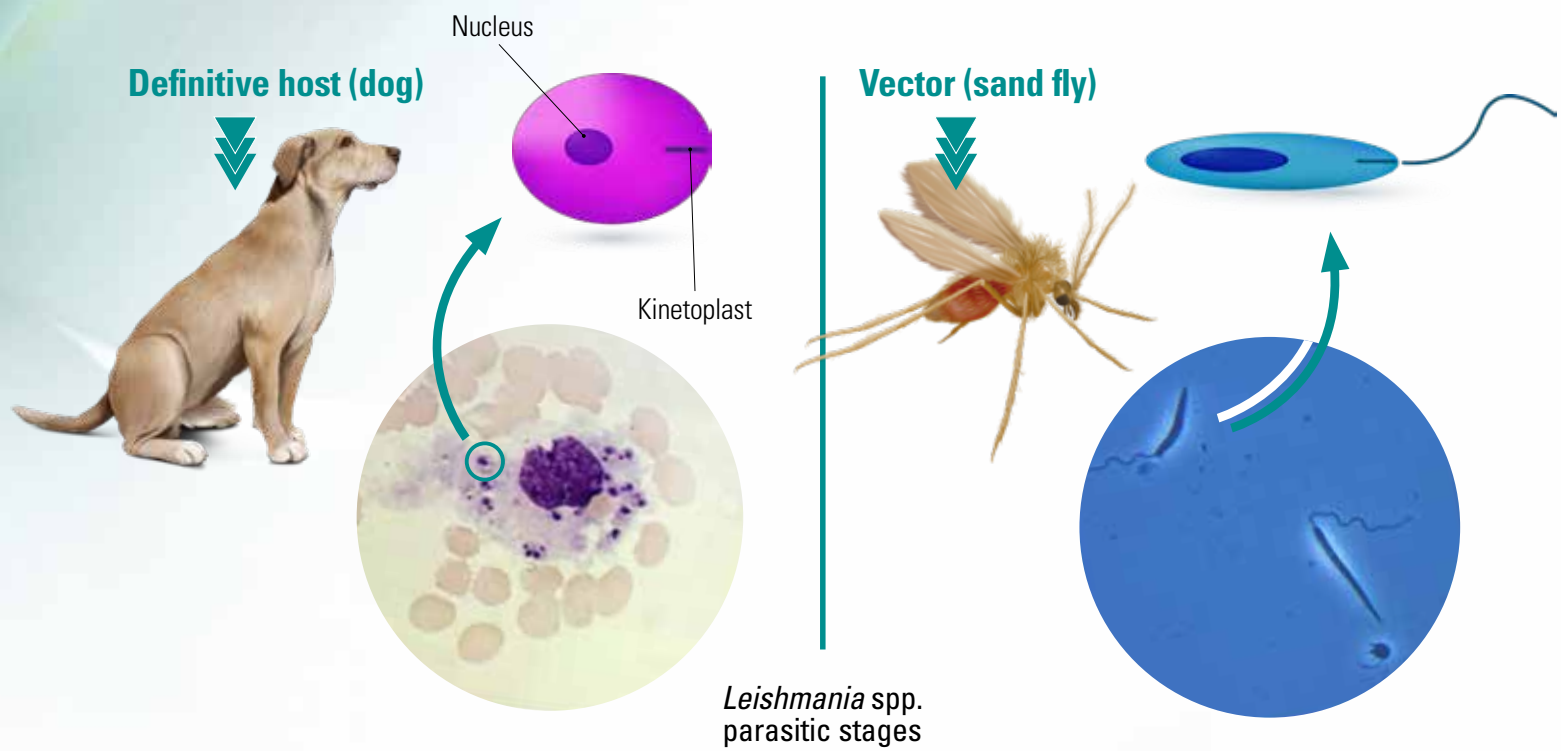
- A vertebrate host (dog or other species)
- An invertebrate host (the phlebotomine sand fly vector)

Information on *Leishmania* species and their vectors that may cause infection and disease in dogs

***Leishmania* spp.**	**Main geographic distribution**	**Hosts other than dogs**	**Sand fly species**
L. infantum syn. *L. chagasi*	Mediterranean basin, Middle east, Asia, North Africa, South and Central America	Humans, other primates, canids, cats, felids, rodents, lagomorphs, herbivorous, marsupials, bats	*Phlebotomus perniciosus, P. ariasi, P. langeroni, P. neglectus, P. perfiliewi, P. tobi* and others
L. tropica	North Africa, Middle East, Southeast Asia	Humans	*P. sergenti*
L. major	North Africa, Middle East	Humans, rodents	*P. papatasi, P. duboscqi, P. alexandri, P. ansarii*
L. donovani	Asia, Africa Cyprus (dog)	Humans	*P. argentipes, P. martini*
L. braziliensis	Brazil	Humans	*Lutzomyia intermedia*
L. mexicana	Central America, Texas	Humans, rodents	*Lu. olmeca*
L. amazonensis	Brazil	Humans, rodents	*Lutzomyia* spp.
L. peruviana	Peru	Humans	*Lu. peruensis*
L. pifanoi	Venezuela	Humans	*Lu.* spp.
L. colombiensis	Colombia, Panama, Venezuela	Humans, insectivores	*Lu.* spp.
L. panamensis	Panama	Humans	*Lu. panamensis*

HOW DOES A DOG **BECOME INFECTED?**

The protozoa have a distinct form in each host type.



Amastigote

The form found in the vertebrate is called the "amastigote". This form is immobile, intracellular, round or oval, 2-6 µm, with a very short, almost imperceptible, flagellum.

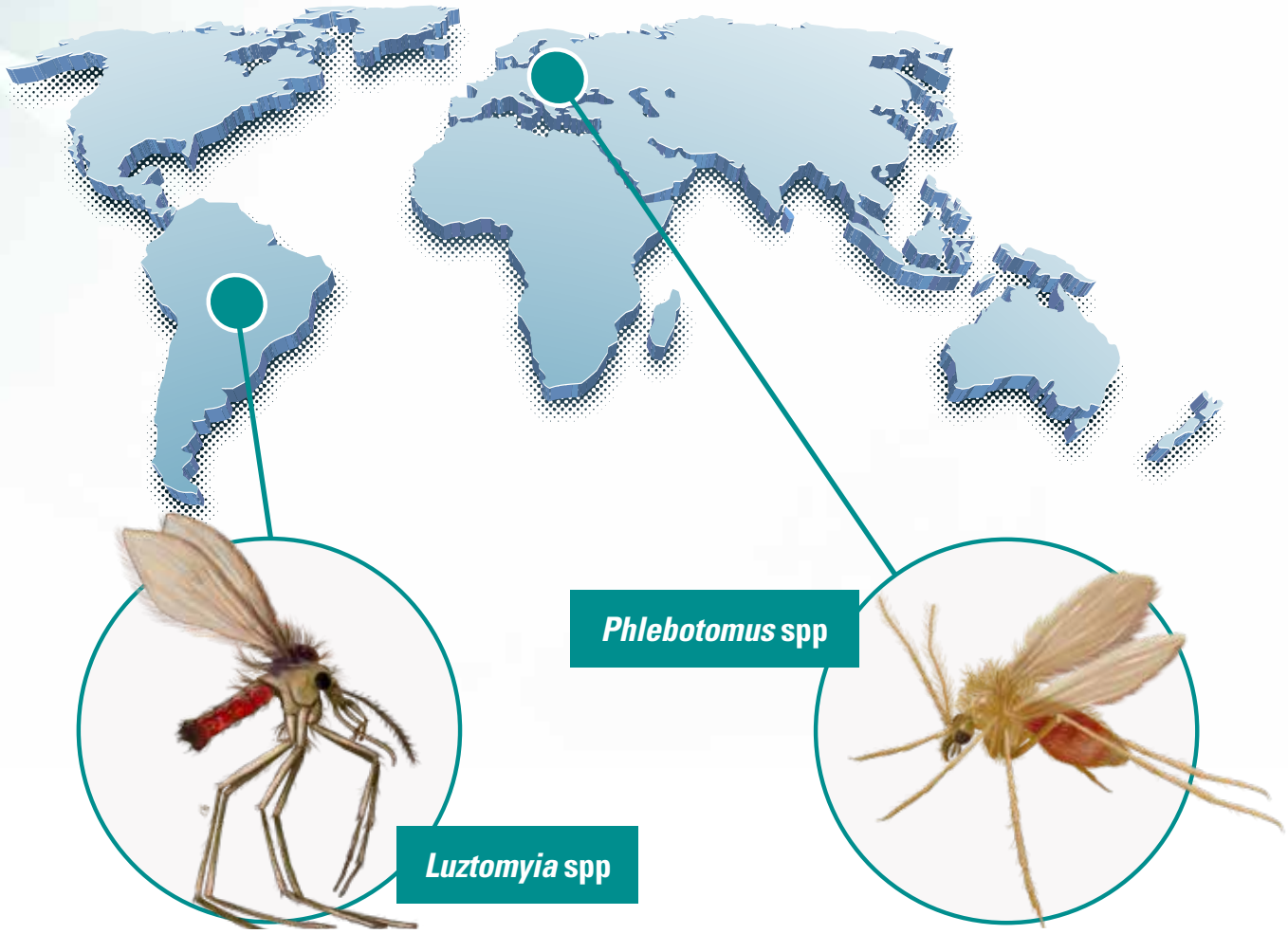
These protozoa have a basophilic nucleus and a modified mitochondrion called a kinetoplast and they parasitise cells of the mononuclear phagocytic system (MPS). Genomic DNA associated with parasite multiplication is found in the nucleus while extra-chromosomic DNA is in the kinetoplast.

Promastigote

The protozoa form found in the invertebrate is called the "promastigote" (from the Greek "mastigos"- whip) - an elongated extracellular form with an anterior flagellum that is found in the digestive tract of the vector.

Vector (life cycle)

Phlebotominae are the only arthropods capable of transmitting *Leishmania* spp infection. There are 600 known species of phlebotomine sand flies, and at least 70 transmit ***Leishmania* spp.** These species belong to the **genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World**. They are mainly distributed in tropical areas and palearctic regions. Phlebotomine sand flies have not been observed in Australia, New Zealand, the Pacific Islands or Antarctica.



They can also have a **seasonal life cycle with peak activity in spring-summer in cooler areas.** Sand flies complete their life cycle year-round in tropical regions, e.g. Brazil. They can survive the winter in diapause or hypobiosis as a fourth larval stage. The known habitat can reach latitudes of 50º N and 40º S with some variation depending on the great diversity of species and subspecies.

HOW DOES A DOG **BECOME INFECTED?**

Adult female sand flies feed on the blood of mammals and birds and are opportunistic because they will bite the most accessible host. However, they prefer dogs compared to other potential hosts, including people.
Sand flies have a characteristic short, silent movement, characterised by small zig-zag jumps (100-200 meters). They can travel up to 2-3 km and this increases their ability to spread *Leishmania* infection. The species are endophilic with positive phototropism, meaning that they will enter homes to feed and they have been found as high as the 4th floor.

Adult fed female sand fly (*Phlebotomus* sp.).

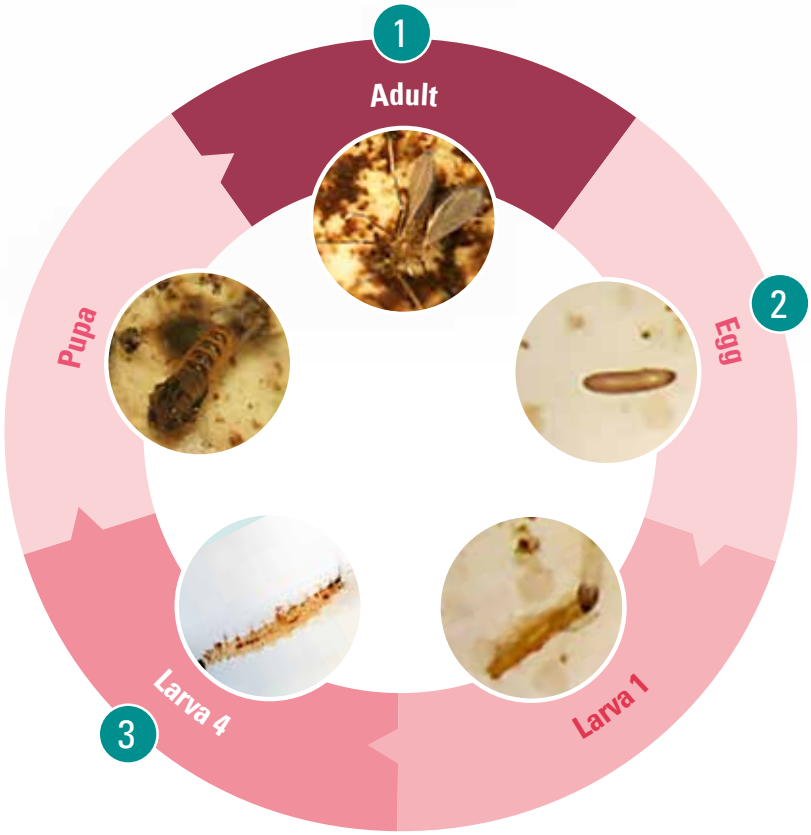
Sand fly life cycle

Sand flies belong to the Order Diptera. They are small (2-3 mm), yellowish in colour, have erect lanceolate wings that form an angle of 45º with the axis of the body, and have abundant hair covering the whole body. They are holometabolic and undergo complex metamorphosis with four life stages. All life stages of the fly are terrestrial, unlike mosquitoes which have aquatic larval stages.

1. Adult sand flies live an average of 30 days and adult females feed 3-4 times, depending on weather conditions.

2. Females lay their eggs approximately 4-5 days after feeding, preferring sandy places (hence the common name "sand flies"), burrows, stables, basements, woods, sewers, wall cracks, garbage dumps, etc.

3. Ideal insect "breeding" places are characterised by moderate temperatures, high relative humidity, dim light, and abundant animal or plant organic matter, which will form the fundamental food supply for larvae.



Proportion of infected vectors

The proportion of infected phlebotomine vectors in endemic areas is relatively low (0.5 - 3%). However, significant increases in vector infection density have been reported in specific situations (e.g. a human leishmaniosis outbreak that occurred in Madrid in 2009) and there is an extended period of vector activity, mainly due to higher temperatures associated with climate change.

Xenodiagnostic studies show that sick dogs with a more severe clinical picture are potentially more infective to sand flies than dogs with a subclinical infection. The degree of infectivity is inversely proportional to CD4+ levels in dogs.

Introduction of infected dogs into disease-free areas where phlebotomine sand fly vectors are present creates the potential risk of developing a new focus of leishmaniosis.

Xenodiagnosis is a sensitive diagnostic technique that involves feeding uninfected sand flies on dogs and then testing these sand flies for the parasite.

Reservoirs

The dog is a natural reservoir for *L. infantum* infection (syn. *L. chagasi*) and **other mammals** can act as accidental or secondary reservoirs.

This peri-domestic infection cycle includes primary reservoirs such as the dog, and secondary reservoirs, such as the horse, cat and black rat.

In addition, cases of clinical leishmaniosis have been reported in Equidae (first in Brazil and subsequently in the Mediterranean Basin, Germany and Switzerland). A similar phenomenon occurred in the cat, although the first feline case was reported in Algeria in 1912, and to date there are more than a hundred well-described clinical cases. Therefore, cats are also a reservoir that can play an epidemiological role in the *Leishmania* spp. cycle.

HOW DOES A DOG **BECOME INFECTED?**

A rural infection cycle also occurs and is represented by secondary reservoirs such as lagomorphs and red fox.

Additionally, there is a wildlife cycle that includes accidental reservoirs such as the wolf, Iberian lynx, wildcat, marten, and badger. *L. infantum* infection has been diagnosed in captive wildlife, with disease confirmed in Bennett's Wallaby (*Macropus rufogriseus*) and orangutan (*Pongo pygmaeus pygmaeus*) in Madrid.

Other routes of transmission

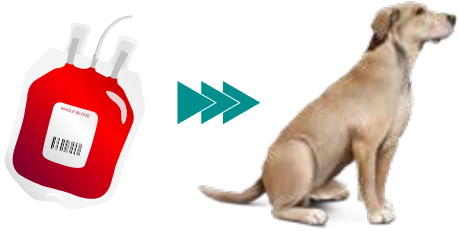
The most prevalent spread of *Leishmania* infection in endemic areas is through sand fly vector transmission. Other arthropods have not been shown to be competent vectors of the infection.

However, diagnosis of other cases in these non-endemic areas demonstrates additional potential non-vector transmission pathways of increasing concern.

In non-endemic areas without a competent arthropod vector including France, Germany, and the United States, vertical transmission is the most widespread route.

Vertical transmission

Sexual transmission

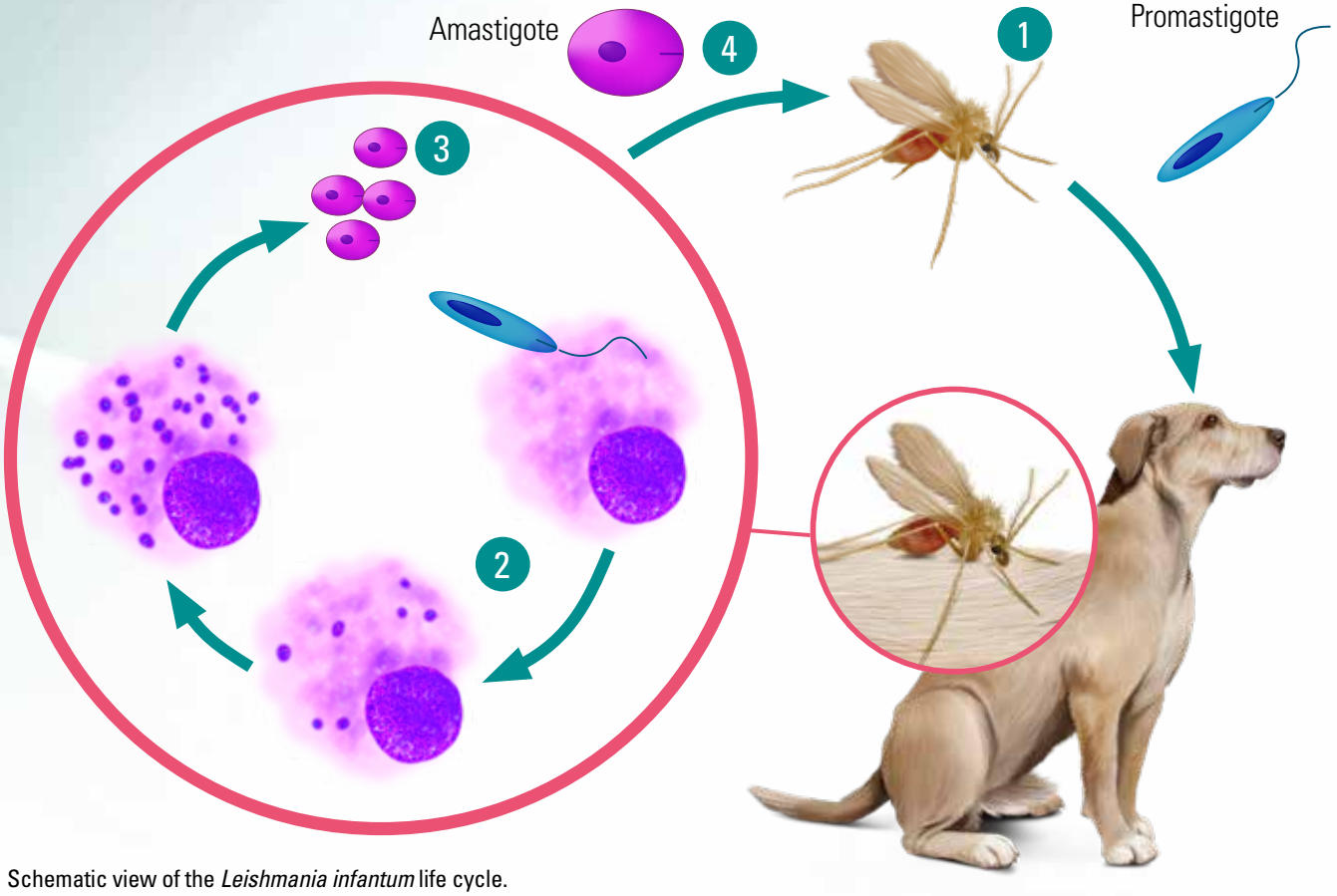


Blood transfusion

Dog to dog

HOW DOES A DOG **BECOME INFECTED?**

Transmission mechanisms



Schematic view of the *Leishmania infantum* life cycle.

1 The infected phlebotomine sand fly has flagellated *Leishmania* promastigotes present in its proboscis, and when it bites a dog it inoculates these with its saliva.

2 These promastigotes are then phagocytosed by the dog's skin macrophages and/or monocytes.

Non-vector transmission pathways are known but do not have an important role in the epidemiology of canine leishmaniosis.

3 Inside phagocytes, the promastigotes lose their flagellum and multiply by binary fission until they rupture the cell and release amastigotes. These amastigotes are in turn phagocytosed by other cells leads to massive intra-organ dissemination.

4 Amastigote-infected macrophages are taken up by another phlebotomine sand fly during a blood meal. Inside the sand fly these convert into promastigotes that actively multiply and undergo a series of modifications. They again become infectious within the proboscis and are ready to complete the life cycle by inoculation into a new vertebrate host. The cycle within the vector is completed within 7-14 days in conditions of optimal temperature and humidity.

WHAT BEHAVIOURS
PUT A DOG AT RISK FOR THE DISEASE?

Lifestyle

There are several predisposing factors known to affect disease development in infected dogs, including age, sex, breed, activity, genetic predisposition, immunocompromised status and habitat. The most important factor is the duration of exposure to the vector, that is, the hours that a dog spends outside.

Therefore, the dog's lifestyle has an impact on the risk level for contracting the infection. Guard dogs, shepherd breeds, and/or working dogs (e.g. police dogs) have the highest risk because of their increased exposure to the vector.

Time of day for increased exposure

The risk for dogs to become *Leishmania* infected is directly tied to infected sand flies present in their environment. The natural sand fly biology determines that the risk starts when minimum temperatures rise to 17° C and are sustained above this level, for example in the early summer in the Mediterranean area. The risk of transmission begins with the fly feeding time at dusk and continues through the night until dawn.

Breed-related risk

All breeds are potentially susceptible to *L. infantum* infection. However, the Ibizan Hound and crosses of this breed are more resistant to infection, associated with an active cellular immune response. Conversely, Boxers have a genetic predisposition to canine leishmaniosis that is linked to the Slc11c1 (Solute carrier family 11 member a1) gene, formerly called N-RAMPI, and certain other Major Histocompatibility Complex Type II gene alleles.

Age

Young dogs can show an increase in their serum anti-*Leishmania* antibody levels and there could be a genetic predisposition or their immune system immaturity leading to greater infection vulnerability in the early part of life. However, there can also be a higher seroprevalence observed in older dogs that may result from accumulated vector exposure time or from an increased susceptibly associated with concomitant infectious or neoplastic diseases. Additionally, some cases in older dogs could be caused by immunosuppressive therapy leading to reactivation of a latent infection acquired earlier in life.

Contact with other animals

Canine leishmaniosis is a vector-borne disease and therefore healthy dogs can cohabit with infected dogs without, in principle, any increase in risk. Direct transmission of *Leishmania* infection between dogs, in the absence of sand flies, has been demonstrated, but it is considered an unlikely scenario. The presence of a dog that acquired the infection locally is an indication of the presence of the vector and underlines the importance of vector protection for all dogs in the household and others living in the same area.

CAN A DOG BE INFECTED AND **NOT SHOW SIGNS?**

Infection *vs* disease

There are **two types of canine patients** found in endemic areas:

- Anti *L. infantum* antibodies can be detected in both types of dogs at different levels, although levels are usually higher in the sick dogs.
- The parasite can be observed in both types of dogs in haematopoietic organs by cytology and/or molecular diagnosis.

It can be difficult to differentiate these two types of dogs, except through absence of clinical signs in subclinically infected dogs and potential laboratory abnormalities in sick dogs.

1st type

The clinically healthy infected dog that, thanks to developing an active cell-based immune response type Th1, does not show clinical signs or laboratory abnormalities.

2nd type

The sick dog that develops a powerful, but non-protective, humoral response that allows the parasite to evade the immune response and become distributed throughout the body. The result in a sick dog is production of parasitic granulomas and immune complex deposition in different organs. These are the changes that lead to the wide variety of clinical signs and laboratory abnormalities described in canine leishmaniosis.

Risk of subclinical disease (frequency in the population)

In endemic areas, the proportion of **clinically healthy infected dogs** can exceed **60%** of the total population, while the percentage of **sick dogs** is usually **between 5 and 10%.** This high proportion of infected but apparently healthy dogs forms a large reservoir for the parasite in the population.

Risk to the population from subclinically diseased dogs

Subclinical dogs do not develop clinical signs or laboratory abnormalities, but they infect phlebotomine sand flies and thus perpetuate the infection. Therefore, these dogs transmit the infection despite a lack of clinical signs and an apparently low parasite load. The risk of parasite transmission from sick dogs is greater than from subclinical dogs - demonstrated by xenodiagnostic techniques (exposure of infected dogs to parasite-free phlebotomine sand fly bites under laboratory conditions). This unique technique has shown that the proportion of infected sand flies is proportional to the severity of the clinical picture in exposed dogs. There is also a positive correlation between the infecting power of sick dogs and the detection of high serum antibody levels.

Tests that reveal a subclinically infected dog

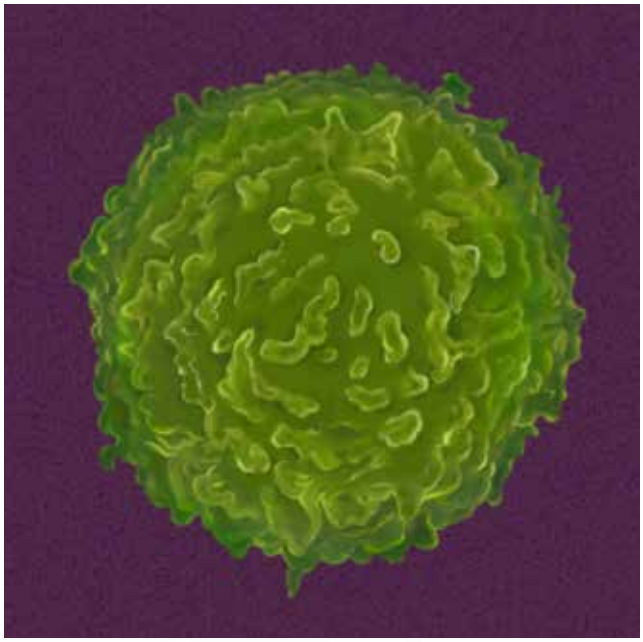
Serology for anti-*Leishmania* antibodies is the first test to run to detect *Leishmania* infection in a dog without clinical signs. A positive (even a low level) antibody test means that the dog has been exposed to the parasite. Serology, however, is an indirect diagnosis and the only way to demonstrate the infection is by evidence of the parasite. The most sensitive test is PCR to detect parasite DNA. Obtain a sample from a lymph node and /or bone marrow fine needle aspirate to provide the best material for increasing sensitivity of this molecular diagnostic technique.

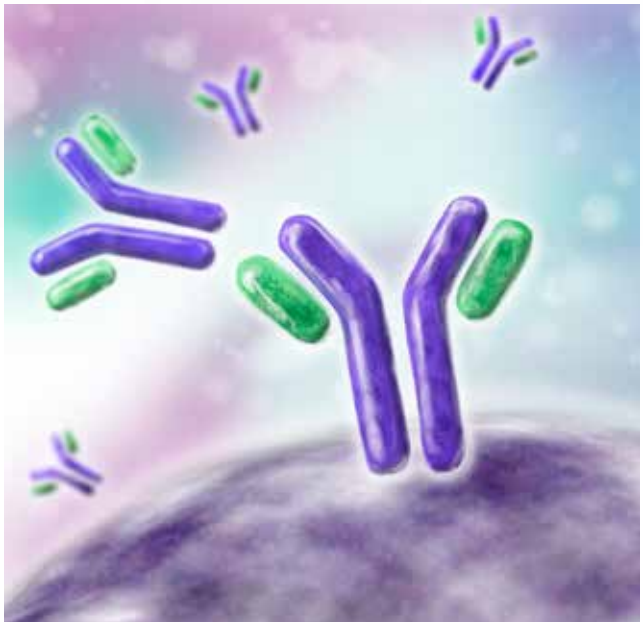
WHAT CLINICAL SIGNS
DOES A SICK DOG SHOW AND WHY?

Pathogenesis

Once infection is established in the dog, there are **two potential types of T-cell mediated immune response** that may develop.

- The **cellular response (Th1)** or protective immunity (essential to control the infection), associated with production of low levels of antibodies and activation of T cells (CD4+). T cells mediate production of cytokines, such as IFN-A, IL-2 and TNF-A, that stimulate macrophages to increase activity and produce nitric oxide, the main effector capable of inducing parasite death.
- The **humoral (Th2) response** is associated with a reduction in cell-mediated immunity and T-cell hypofunction (CD4+). This induces production of interleukins (IL-4, IL-10) that promote B lymphocyte stimulation with production of high concentrations of non-protective nonspecific gamma-globulins (IgG, IgM, IgA and IgE). This response is associated with disease progression and there is a positive correlation between anti-*Leishmania* antibody levels, parasitic load and disease level.





WHAT CLINICAL SIGNS DOES A SICK DOG SHOW AND WHY?

The **clinical signs** of canine leishmaniosis result from **two pathogenic mechanisms:**

- Inflammatory process with development of granulomas in the organs and tissues where the parasite has multiplied.
- Deposit of immune complexes (mainly immunoglobulins G and M) in different organs.

Early signs

In the early stages of disease, clinical signs are mild: lethargy, progressive weight loss, exercise intolerance, lymphadenomegaly and mild skin lesions such as alopecia and exfoliative dermatitis.

Facial cutaneous exfoliative dermatitis lesions.

Progression

If adequate treatment is not instituted or the diseased dog has a non-protective immune response, then cutaneous manifestations can develop including skin ulceration over bony prominences and mucocutaneous junctions.

Other signs indicating increasing severity are associated with immune complex deposits including:

- Vasculitis (e.g. nasal bleeding)
- Glomerulonephritis (with polyuria, polydipsia)
- Polyarthritis (erratic and sometimes intermittent limping))
- Eye lesions (conjunctivitis, kerato-uveitis, retinopathies, etc.)

Other, less common clinical signs have been described:

- Fever
- Digestive disorders (ulcerative colitis)
- Neurological (encephalitis)
- Cardiorespiratory (pneumonitis, chronic rhinitis)

Plantar ulcers

Nasal bleeding

Keratouveitis

Prognostic factors

Clinical staging of canine leishmaniosis (as developed by the LeishVet group and used most frequently) is divided into four stages (I – IV) based on clinical signs, quantitative serology, blood tests and urinalysis results. **(Table)** This classification helps to determine an appropriate treatment protocol and establish a prognosis.

Canine leishmaniosis clinical staging (LeishVet) based on the International Renal Interest Society (IRIS) staging for Chronic Kidney Disease (CKD)

Initial staging	Serology	Clinical signs	Laboratory findings	Prognosis
STAGE I Mild disease	Negative or low positive	Mild clinical signs	None Renal profile NORMAL	Good
STAGE II Moderate disease	Low / high Antibody titres	Clinical Leishmaniosis	Mild anaemia, disproteinaemia **Two sub-stages:** 1. Normal renal profile 2. Slight proteinuria, creatinine <1.4 / UPC = 0.5-1	Good / guarded
STAGE III Severe disease	Medium / high Antibody titres	Stage II + immune-mediated (vasculitis, uveitis, arthritis...)	CKD IRIS I (UPC 1-5) or IRIS II (creatinine 1.4-2.8 mg/dl)	Guarded / poor
STAGE IV Very severe disease	Medium / high Antibody titres	Stage III + nephrotic syndrome, end stage CKD	IRIS III (creatinine 2.9-5 mg/dl) IRIS IV (creatinine > 5 mg/dl) or Nephrotic syndrome (UPC > 5)	Poor / very poor

Recovery indications

In most cases, dogs classified in **stages I and II** have a good clinical response (laboratory abnormalities return to normal reference ranges and clinical signs resolve). These dogs can remain in good health for a long time, possibly years.

In contrast, dogs in the most advanced disease **stages (III and IV)** have a reduced life expectancy due to complications from chronic kidney disease, the leading cause of death in canine leishmaniosis.

WHAT DIAGNOSTIC TESTS SHOULD BE RUN IN A DOG THAT IS SUSPECTED TO HAVE THE INFECTION/DISEASE?

Rapid, table-side

Collect a detailed medical and epidemiological history when presented with a suspected case of canine leishmaniosis.

Conduct a complete physical examination with a thorough **assessment of**:

- Body condition
- Mucous membranes
- Palpation of lymph nodes
- Examination of skin and mucocutaneous junctions

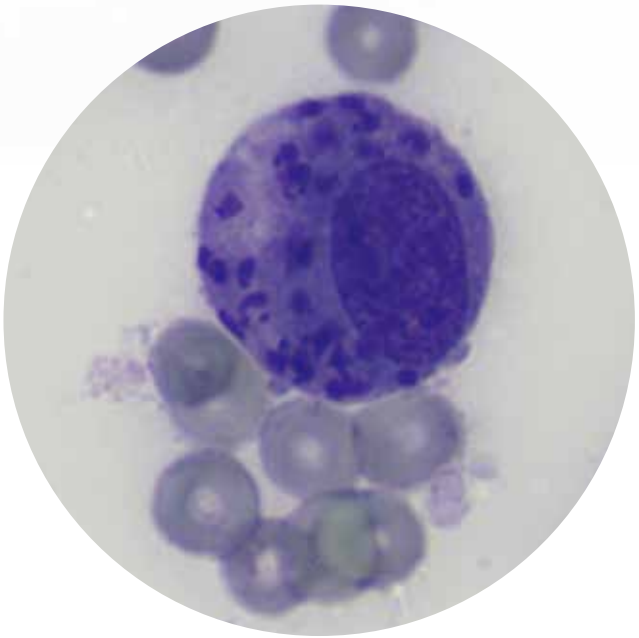
If the clinical signs are compatible with canine leishmaniosis, then carry out nonspecific tests (to assess the general condition of the patient) and specific tests (serology and fine needle aspiration for cytology/ molecular diagnosis) to uncover evidence of the parasite.

In hospital using microscope or similar equipment

Depending on the clinical picture, the parasite can be identified by evaluating a cytological sample from a **haematopoietic organ** or skin or **other tissue**.

Lymph node, bone marrow, spleen…	Skin, joint cavity, mucous membranes…

The most commonly performed procedure is fine needle aspiration of external lymph nodes, with preparation of smear, rapid Diff-quick stain, and subsequent microscopic evaluation. Finding **amastigotes inside macrophages** confirms *Leishmania* infection.



A round macrophage with many *Leishmania infantum* amastigotes in the cytoplasm.

WHAT DIAGNOSTIC TESTS SHOULD BE RUN IN A DOG THAT IS SUSPECTED TO HAVE THE INFECTION/DISEASE?

Laboratory testing

Basic laboratory tests

- Complete blood count
- Biochemical profile (including serum protein electrophoresis)
- Urinalysis

The most common **blood alterations** in canine leishmaniosis include:

- Mild non-regenerative normocytic-normochromic anaemia
- Mild neutrophilia with lymphopaenia and monocytosis (stress leucogram)
- Thrombocytopaenia
- Leucopaenia (less common)

Serum protein electrophoretogram alterations are:

- Hyperproteinaemia with hyperglobulinaemia and hypoalbuminaemia, either compensatory or as a result of renal proteinuria
- In dogs with immune-mediated glomerulonephritis there will be renal azotemia and proteinuria

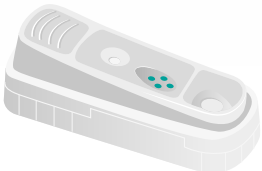
Specific technique

Include serological and parasitological diagnosis (cytology and PCR).

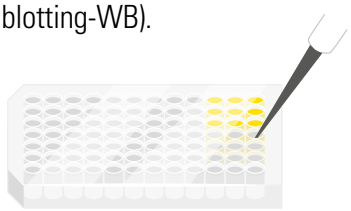
Available serological methods can be:

- **Qualitative** (based on immunochromatography, dot-ELISA).

  Easy to use in the clinic and yield a rapid **qualitative** and specific result but have a variable sensitivity that is always lower than quantitative serology.



- **Quantitative** (immunofluorescence-IFAT, enzyme-linked immunosorbent assay-ELISA technique, western blotting-WB).



There is a significant correlation between elevated antibody titres and disease.

PCR provides improved sensitivity in the parasitological diagnosis of *Leishmania* infection in dogs.
Different methods have been developed using the nuclear genome or **kinetoplast DNA** (kDNA).

Methods that use kDNA appear to be more sensitive for direct detection in infected tissues.

There are currently three **PCR methods** available: conventional PCR, nested PCR, and quantitative PCR.

PCR can be performed on DNA extracted from different tissues, blood, biological fluids and even histological material. Bone marrow, lymph node, spleen or skin are the most sensitive tissues for PCR diagnosis while blood, buffy coat and urine significantly reduce the sensitivity of this molecular diagnosis.

WHAT DIAGNOSTIC TESTS SHOULD BE RUN IN A DOG THAT IS SUSPECTED TO HAVE THE INFECTION/DISEASE?

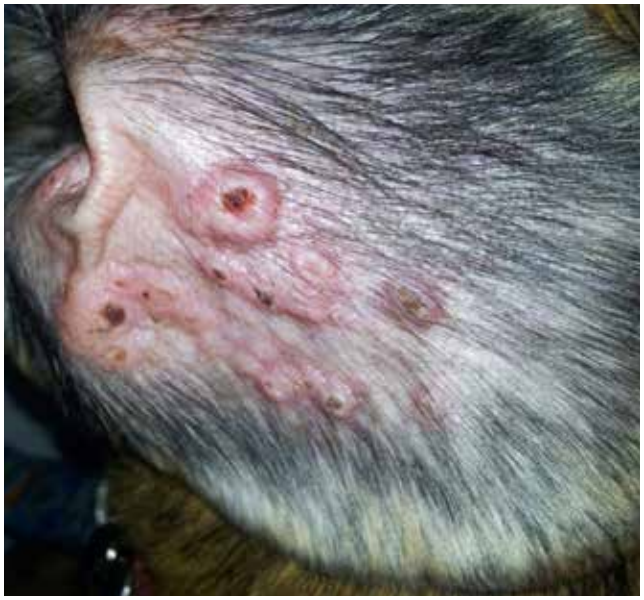
Test interpretation

Interpretation of the results in the diagnosis of canine leishmaniosis is essential because there are two types of patients: **clinically healthy infected dogs** and **sick dogs**. The key to diagnosis is to properly differentiate these two patients with the help of the results obtained.

Interpretation of canine leishmaniosis diagnostic results: infected clinically healthy compared with sick dogs.

Acute *vs* convalescent

Canine leishmaniosis is a chronic disease and therefore clinical signs do not become apparent until several months or even years after the initial infection. One exception to this occurs in dogs that develop localised cutaneous leishmaniosis. This localised version of the disease is characterised by papular lesions that usually appear in hairless areas (pinna, lips, eyelids, around the nasal planum, skin of the abdomen, etc.) These lesions are known as **"inoculation chancres"**.



"Inoculation chancres", lesions seen after the sand fly bites the ear of a dog with canine leishmaniosis.

WHAT GENERAL TREATMENT STRATEGY IS RECOMMENDED FOR SICK DOGS?

Types of drugs to use

There are a limited number of drugs effective in the treatment of canine leishmaniosis. For decades, **pentavalent antimoniates** (n-methyl glucamine) were almost exclusively used.

These work as a highly effective leishmanicide molecule by:

- blocking the formation of ATP (adenosine triphosphate) and GTP (guanosine triphosphate).
- increasing the phagocytic capacity of monocytes and neutrophils.

Short half-life	6-9 hours
Excreted by kidneys	Two daily subcutaneous doses are recommended to maintain a higher inhibitory concentration

The second leishmanicide molecule is **miltefosine**, an alkyl phosphocholine.

Induces parasite apoptosis and stimulates T cell and macrophage activity

Long half-life	6-7 days leading to accumulation in plasma after repeated oral dosing
Excreted in faeces	no hepato-renal impact

WHAT GENERAL TREATMENT STRATEGY
IS RECOMMENDED FOR SICK DOGS?

The third drug used is **allopurinol**, a structural analog of hypoxanthine.

- Inhibits ATP synthesis by altering pyrimidine metabolism and it is not considered a leishmanicide drug but a leishmaniostatic.
- Synergistic with previous leishmanicide molecules and current treatment protocols include it in combination with antimonials or miltefosine, an approach that minimises relapses.

Drug types to use for treating sick dogs based on the clinical staging	
Stage 0	Seropositive dogs without clinical signs nor laboratory abnormalities: no treatment needed
Stage I	Monitor without treatment or treat with anti-*Leishmania* short term therapy (allopurinol, domperidone, nucleotides)
Stage II	Antimoniate n-methyl glucamine 35-50 mg/kg s.c. / 12h for 4 - 6 weeks plus allopurinol (6-12 months) OR Miltefosine 2 mg/kg p.o. / 24h 4 weeks. + allopurinol
Stage III-IV	Allopurinol IRIS (www.iris-kidney.com) recommendations Individual medical approach based on clinical signs

Monotherapy or combination therapy

In addition to the leishmanicides or leishmaniostatic drugs, there are treatments available to enhance cellular immunity including: domperidone, selected nucleotides and some naturally occurring products currently under study. These may be applied in combination with parasiticide drugs to amplify the response to treatment of sick dogs and show promising results. **(Previous Table)**

WHAT GENERAL TREATMENT STRATEGY IS RECOMMENDED FOR SICK DOGS?

Supportive treatment strategies

In addition to specific treatments targeting the *Leishmania* parasite, symptomatic treatment is also important.

- Ensure the dog receives a balanced palatable diet.
- Further complementary treatments depend on the clinical situation of each dog:

In dogs with skin lesions, application of anti-seborrheal baths, antiseptics, and fatty acids are beneficial and improve their wellbeing.

Kidney patients may benefit from polyvitamins, anti-anaemic, anti-hypertensives (ACEis) and a low-protein diet.

Monitoring for response to treatment

Dogs under treatment with an appropriate protocol for their clinical and parasitological situation need to be **monitored.** It is possible to monitor the parasitic load using a quantitative PCR on bone marrow or lymph node aspirates to assess the leishmanicidal efficacy of the treatment. These results may offer some relative value in individual clinical cases and need to be validated.

30 days after treatment

Start with an initial check to evaluate the impact of administered drugs.

During the first year

Recheck every 4 months including: physical examination, blood count, hepatorenal biochemical profile, serum protein electrophoresis and quantitative serology.

From the second year onward

If the dog is stable, reduce to two check-ups per year and then every 6-12 months after that as needed, depending on the clinical course.

Management of co-infections

Outdoor dogs with high exposure to sand flies are also likely to suffer infestations from other arthropod vectors, particularly ticks and fleas. Therefore, these dogs commonly have *Leishmania* co-infection with **other pathogens.**

> *Ehrlichia canis, Hepatozoon canis, Anaplasma* spp., *Dirofilaria* spp., etc.

It is essential in the case of a suspected co-infection to follow a comprehensive diagnostic plan including a complete haematological and urinalysis to assess the presence and define the impact of each pathogen involved. Careful evaluation of all potential pathogens will lead to implementation of the optimal concurrent treatment protocol.

The infection to expect depends on the geographic distribution of the vectors. In areas where the vectors and diseases coincide, diagnosis can be complicated because some clinical signs are common to several vector-borne diseases.

ARE OTHER PETS OR PEOPLE IN THE HOUSE AT RISK?

The risks to people from an infected/sick dog

Both clinically healthy and sick infected dogs can live with people without increasing the risk of transmission. People acquire the infection separately through the bite of sand flies and therefore people who live in an endemic area where there are infected dogs and sand flies are subjected to the same risks as their dogs.

Other public health considerations

In endemic areas, use of preventive measures against sand flies can reduce the overall human disease risk. This can be achieved by applying effective repellents to all dogs in the community. This approach reduces the reservoir dog population and the chance of infection in sand flies. In addition, people can apply fly repellent treatments to reduce their own chance of being bitten.

Can cats get this infection/disease?

The cat is a proven host in the *Leishmania* epidemiological cycle. Cats can be an infection reservoir and can suffer from the disease. Most cases described in cats come from the Mediterranean Basin and refer to *L. infantum* infection, while other *Leishmania* species in the cat have been described as: *L. brazilinesis, L. mexicana, L. venezuelensis, L. amazonensis, L. tropica* and *L. major.*

Feline leishmaniosis is a chronic disease and the clinical signs and laboratory abnormalities can be like those described in the dog, although cutaneous forms predominate.

The diagnostic process is the same as for the dog and the most studied and best tolerated treatment for the cat, so far, is allopurinol.

Xenodiagnostic studies confirm that sand flies feed on cats and transmit the infection. Preventive measures for at-risk cats are therefore aimed at avoiding sand fly bites by using an effective and safe sand fly repellent.

WHAT ARE SOME RECOMMENDATIONS AROUND PREVENTION STRATEGIES?

How to avoid the vector

The main route of *Leishmania* transmission is through a bite from the sand fly vector, and the best way to avoid infection is through use of topical insecticides with proven anti-feeding efficacy against phlebotomine sand flies. These insecticides are based on molecules with repellent and insecticide action and are available as **collars, spot-on pipettes,** and **sprayers.** The synthetic pyrethroids are highly repellent molecules against these insects.

Collars

- Deltamethrin collars achieve maximum efficacy one week after application and one collar can provide protection for as long as 12 months.
- Flumethrin-based collars can be effective for 8 months.

Spot-on pipettes

For topical application of permethrin in combination with active ingredients effective against other arthropods. Spot-on products can achieve good repellent efficacy against sand flies by 48 hours after application and continue for 3-4 weeks.

Sprays can act immediately on application, but they do not have much residual effect and require weekly applications. They may be recommended for use where there is a risk of immediate exposure. **(Table).**

Principle medication active ingredients with anti-feeding efficacy that could prevent sand fly biting

Active Ingredient	Formulation	Time to efficacy onset	Sand fly species
Deltamethrin	Collar	7 days	12 months
Flumethrin + Imidacloprid	Collar	7 days	8 months
Permethrin + Imidacloprid	Spot-on	**24-48** hours	3-4 weeks
Permethrin + Fipronil	Spot-on	**24-48** hours	4 weeks
Permethrin + Dinotefuran + Piriproxyfen	Spot-on	**24-48** hours	4 weeks
Permethrin	Spray	Immediately	2-3 days

WHAT ARE SOME RECOMMENDATIONS AROUND PREVENTION STRATEGIES?

Is routine testing recommended?

Dogs living in canine leishmaniosis endemic areas should be given a serological test after the end of the sand fly season. Generally, 3 months after the end of this risk season is advised to allow time to detect antibodies that develop postinfection. Early diagnosis allows therapeutic measures to be applied as soon as possible with a greater chance of full recovery.

Dogs living in non-endemic areas that travel to endemic areas should have serology run within a few months (3-4) of returning home.

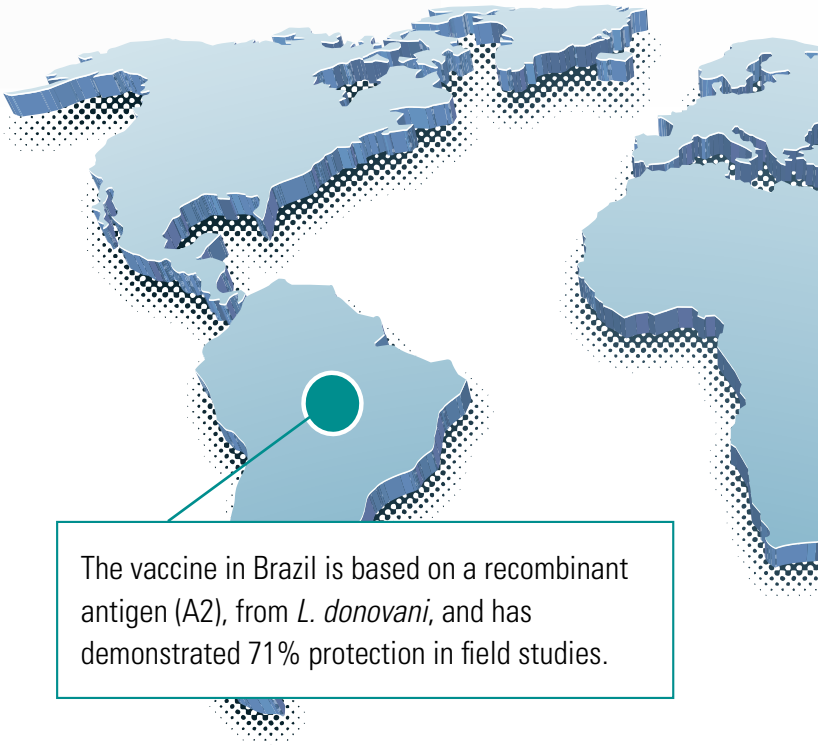
General thoughts on preventive treatments

Vector-borne disease control is always challenging. Vector control in the environment using chemical methods (e.g. periodic fumigation) is an almost impossible task given the complex vector biology and the potential for negative impacts on other animal species and the local ecosystem. Physical barriers in homes are recommended including the installation of mosquito nets in windows and the use of insecticide-impregnated mosquito net fabrics around beds during the high-risk season. In addition, it is advisable to avoid and remove favourable sand fly habitat by cleaning up decaying organic matter. Finally, keep dogs inside the house during peak sand fly activity times (dusk to dawn) to reduce the transmission risk.

Is there a vaccine?

There are currently three different vaccines available (one in Brazil and two in Europe).

- One of the European vaccines is produced from excretion/secretion antigens from *L. infantum* (LiESP/QA-21) promastigote cultures and is adjuvanted with a purified fraction of saponin (*Quillaja saponaria*, QA-21).
- The second European vaccine contains a recombinant protein of *L. infantum* MON-1 (Q protein), derived from genetic fusion of five antigenic fragments obtained from four intracellular *Leishmania* proteins.



The vaccine in Brazil is based on a recombinant antigen (A2), from *L. donovani*, and has demonstrated 71% protection in field studies.

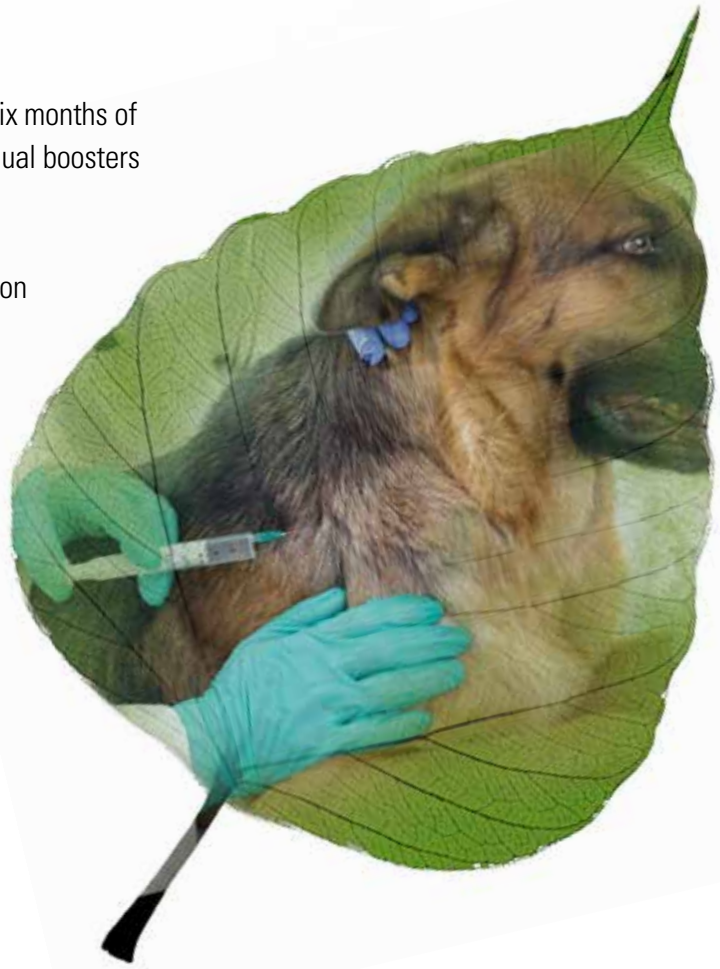
WHAT ARE SOME RECOMMENDATIONS AROUND PREVENTION STRATEGIES?

Field studies used for registration of these vaccines report an efficacy of 68.4% and 72%, respectively. **(Table)**

Vaccines against canine leishmaniosis			
Composition	**Recombinant antigen (A2) *L. donovani***	**E/S antigens *L. infantum* (LiESP/QA-21)**	**Recombinant Protein Q**
Reported Efficacy	71.4%	68.4%	72%
Indication	Seronegative dogs ≥ 6 months	Seronegative dogs ≥ 6 months	Seronegative dogs ≥ 6 months
Serology test before vaccination	Rapid test	Rapid test	IFAT / ELISA
Administration	3 doses (every 3 weeks)	3 doses (every 3 weeks)	1 dose
Onset of Immunity	30 days post 3rd dose	30 days post 3rd dose	30 days post 1st doce
Duration of Immunity	12 months	12 months	12 months
DIVA Vaccine	No	No	Yes

Vaccines can be administered to seronegative dogs over six months of age and have a duration of immunity of 12 months, so annual boosters are recommended.

None of the three vaccines can prevent *L. infantum* infection in vaccinated dogs which means that the use of repellents is essential in endemic areas, or in dogs traveling in the area. Vaccination enhances active cellular immunity in dogs that provides protection if they acquire the infection.



WHAT DOES THE FUTURE LOOK LIKE?

What are the changes being seen with the disease?

There has been an exponential advancement in canine leishmaniosis knowledge over the past three decades with many related publications in scientific journals. This research is helping to better understand canine leishmaniosis to improve the life expectancy of affected dogs.

Studies in immunology and an improved knowledge of the dog's immune response to infection, have led to development of new molecules and techniques for treatment (immunomodulatory), diagnosis (PCR) and prevention (repellents, vaccines) and helped to design better strategies for control.

Despite these advances, the disease endemic range continues to increase and canine leishmaniosis is no longer confined to the Mediterranean basin and to Brazil, historically the areas of greatest risk. The disease has been imported, and/or autochthonous cases have been documented, in many countries in Central and Northern Europe and Central and South America. Widespread and frequent movement of people and animals together with climate change favouring the sand fly vector are the main triggers for these important epidemiological changes.

Is the risk of disease increasing?

The risk of infection with *Leishmania* continues to expand because there is an increase in density of both the hosts (dogs, wild canids, other reservoirs) and the vector. In addition, increasing temperatures associated with climate change extend the period of activity of sand flies alarmingly.

In the Mediterranean basin, the **period of sand fly activity** was previously limited to 3-4 months of summer through early autumn but has now expanded to **exceed 8 months** and in some areas sand flies are now active almost year-round. This extended activity considerably increases the risk of transmission.

Has resistance to prevention or reduced treatment effect been seen?

The therapeutic arsenal against *Leishmania* is scarce and resistance has been reported to most of the actives used to treat human leishmaniosis (antimonials, amphotericin B, miltefosine, paromomycin). In veterinary medicine there is evidence of resistance to antimonials and allopurinol, but mechanisms producing resistance are not well known.

Resistance is not necessarily synonymous with therapeutic failure and it is necessary to analyze each report of apparent "resistance" because there are factors linked to **the parasite** (species, pathogenicity), **the host** (immune response, genetics) and the **drug used** (dose, tolerance, efficacy) that can be at play.

No resistance has been reported to synthetic pyrethroids used as anti-feeding treatments that prevent sand fly bites and therefore *Leishmania* infection transmission. **Apparent efficacy failure may be more likely a result of non-compliance or inadequate use.** All cases of apparent efficiency reduction should be appropriately monitored and reported to the product manufacturer and health authorities.

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