



CANINE LEISHMANIOSIS

VECTOR BORNE DISEASE

PROF. GUADALUPE MIRÓ

Professor Guadalupe Miró qualified from the Madrid Veterinary School where she also completed her PhD. She is Full Professor in Parasitology and Parasitic Diseases at the Animal Health Department, Universidad Complutense of Madrid (UCM), a Diplomate of the European Veterinary Parasitology College (EVPC) and Director of the UCM Pet Parasite Laboratory. Professor Miró is also currently in charge of the Infectious Diseases Consultancy at the Veterinary Teaching Hospital of the Madrid Veterinary School with a special focus on infectious diseases of small animals. Her areas of research specialisation are vector-borne diseases of pets and wildlife, canine & other animal leishmaniosis and shelter medicine involving the epidemiology and control of zoonotic diseases. She is a member of the World Association for the Advancement of Veterinary Parasitology (WAAVP), the International Society for Companion Animal Infectious Diseases (ISCAID), the Spanish Society of Parasitology (SOCEPA). She is also founding member of ESCCAP (Spain representative) and LeishVet group (President) and member of the main Spanish Associations of Veterinary Practitioners' (AMVAC and AVEPA). Her scientific output consists of numerous scientific articles in peer reviewed journals and is author of several books in the field.



INDEX

VECTOR BORNE DISEASE

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.

WHERE IS THE DISEASE MOST LIKELY TO BE FOUND?

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.



WHERE IS THE DISEASE MOST LIKELY TO BE FOUND?

Favorable 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.

Under 10 °C...



WHERE IS THE DISEASE MOST LIKELY TO BE FOUND?

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 lifecycle

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 (dog or other species)
- 🌿 An invertebrate (the phlebotomine sand fly vector)

The protozoa have a distinct form in each host type.



HOW DOES A DOG **BECOME INFECTED?**

The protozoa have a distinct form in each host type.



Leishmania spp.
parasitic stages

HOW DOES A DOG **BECOME INFECTED?**

Vector (lifecycle)

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.

They can also have a **seasonal life cycle with peak activity in spring-summer in cooler areas**. They can survive the winter in diapause or hypobiosis as a fourth larval stage. The known habitat can reach latitudes of 50° N and 40° S with some variation depending on the great diversity of species and subspecies.

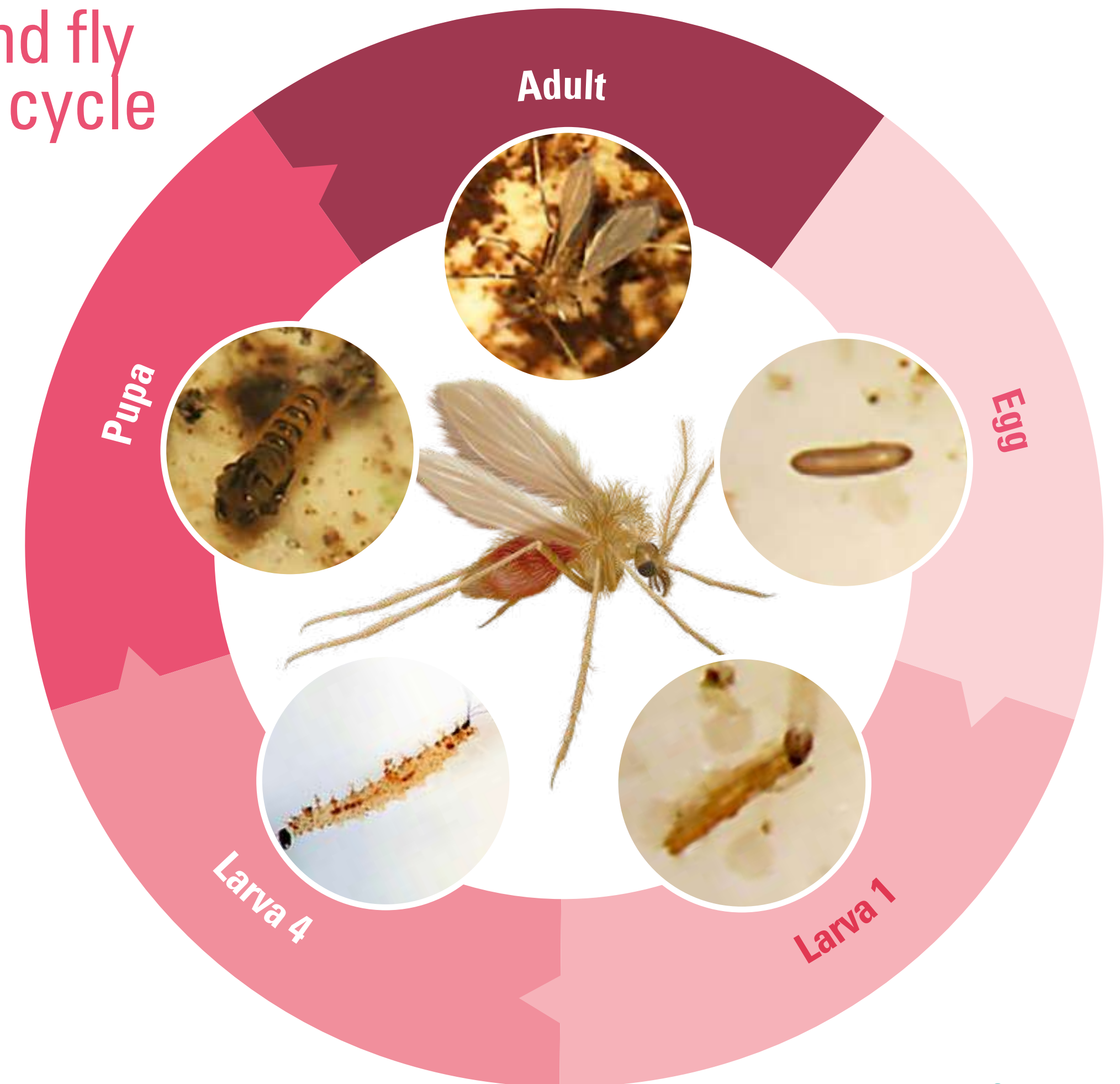


HOW DOES A DOG **BECOME INFECTED?**

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.

Click on  for more information

Sand fly life cycle



HOW DOES A DOG **BECOME INFECTED?**

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.



HOW DOES A DOG **BECOME INFECTED?**

Reservoirs

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

HOW DOES A DOG **BECOME INFECTED?**

Probability of transmission and 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.

Vertical transmission

Sexual transmission

Blood transfusion

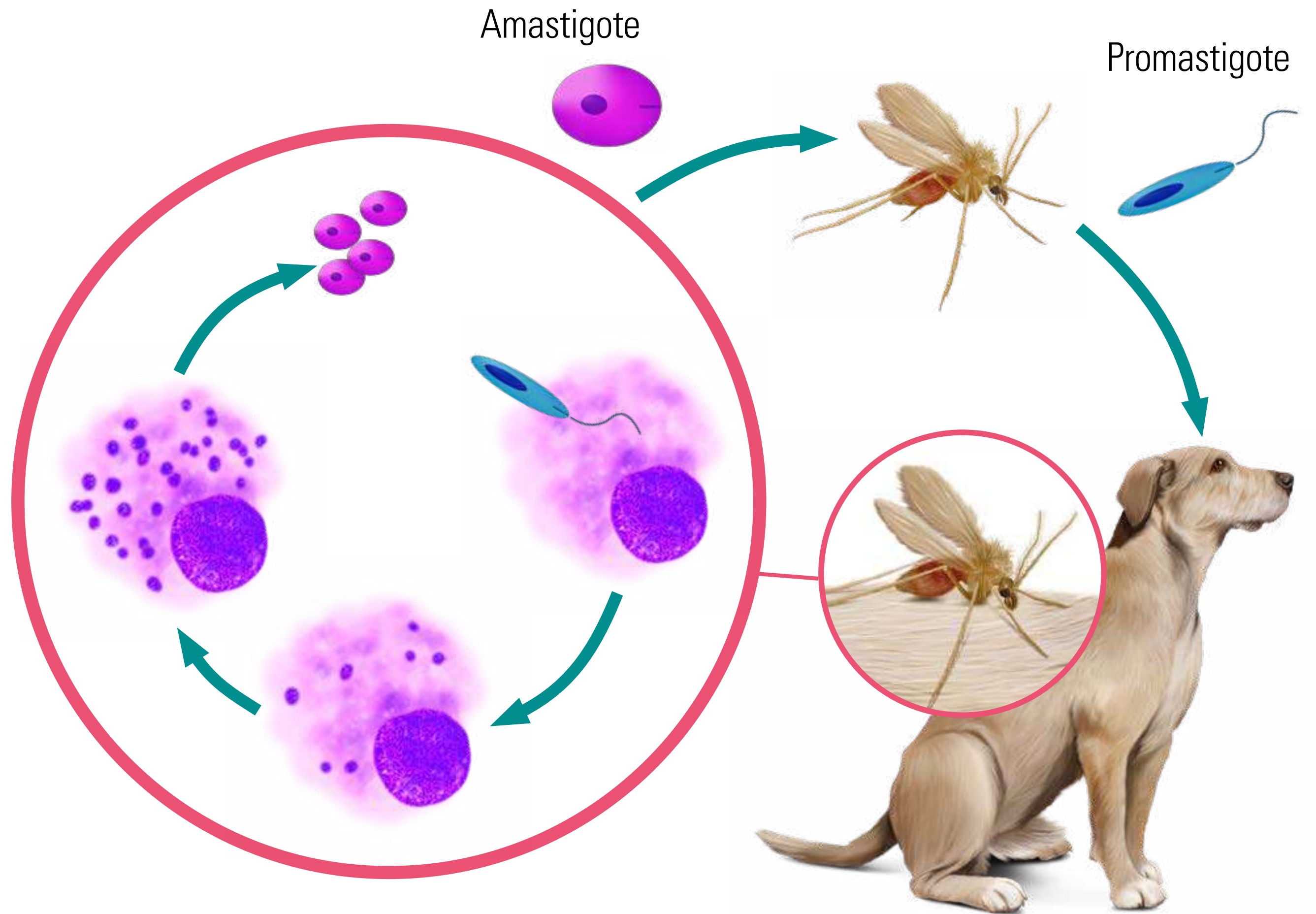
Dog to dog

Click on  for more information

HOW DOES A DOG **BECOME INFECTED?**

Transmission mechanisms

Click on  for more information



Schematic view of the *Leishmania infantum* life cycle.

WHAT BEHAVIORS PUT A DOG AT RISK FOR THE DISEASE?

VECTOR BORNE DISEASE

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

Infection vs disease

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

1st type

🌿 Anti *L. infantum* antibodies can be detected in both types of dogs at different levels, although levels are usually higher in the sick dogs.

2nd type

🌿 The parasite can be observed in both types of dogs in hemato-poietic organs by cytology and/or molecular diagnosis.

Click on  for more information

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



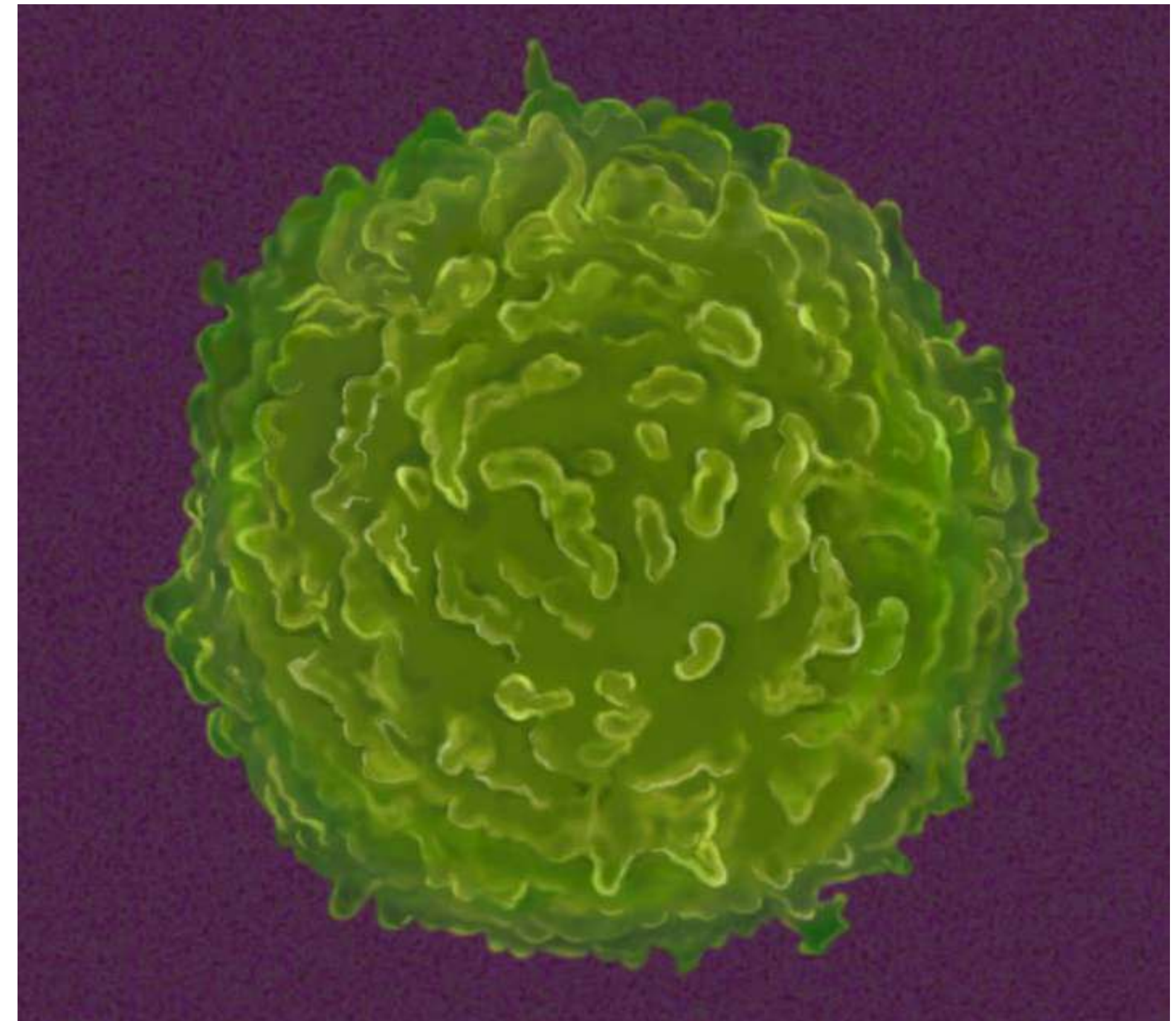
VECTOR **B**ORNE **D**ISEASE

WHAT CLINICAL SIGNS DOES A SICK DOG SHOW AND WHY?

Pathogenesis

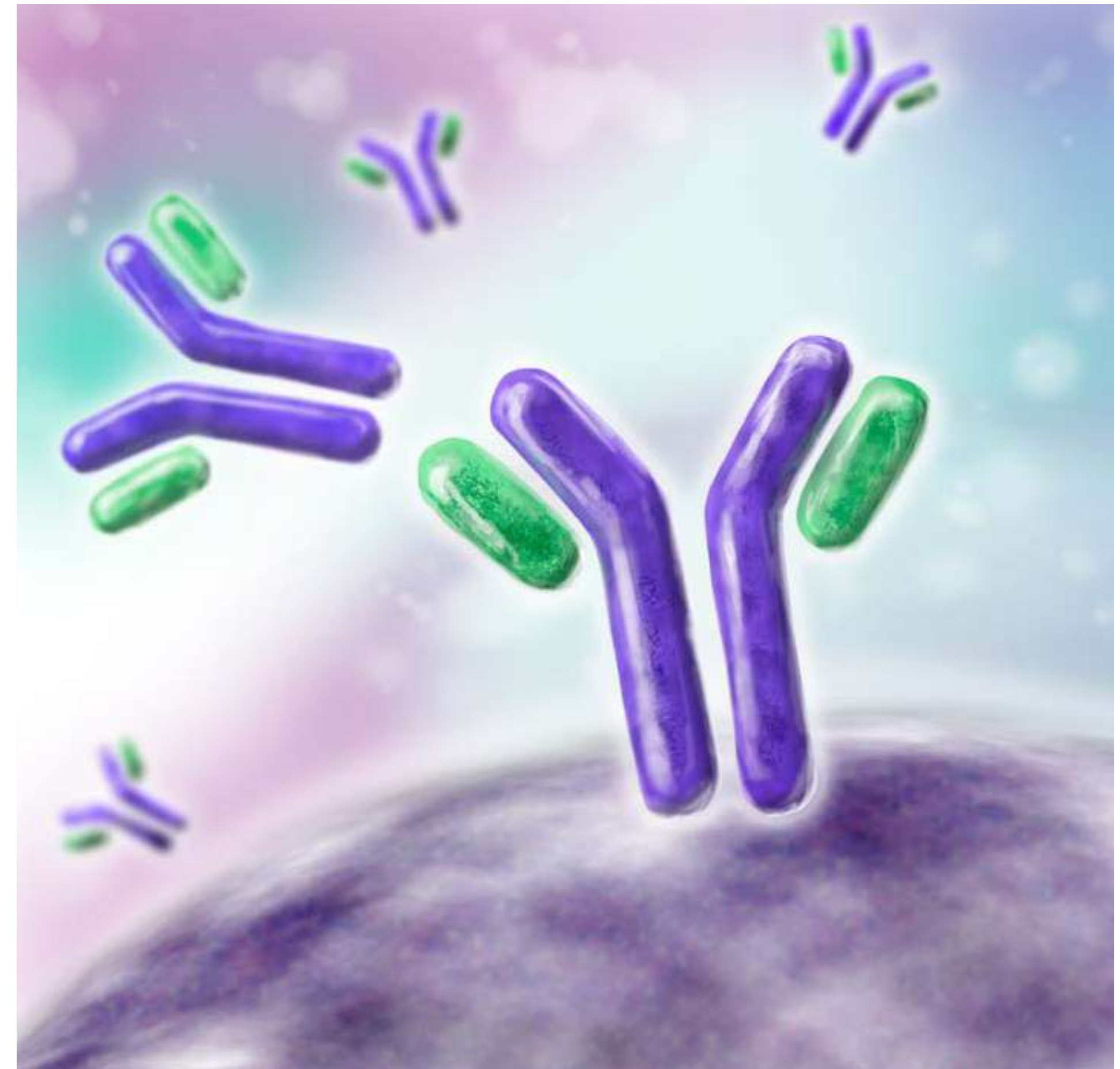
Once infection is established in the dog, there are **two potential types of T-cell mediated immune response** that may develop.

- 🌿 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.





WHAT CLINICAL SIGNS DOES A SICK DOG SHOW AND WHY?

- ❧ 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.

WHAT CLINICAL SIGNS DOES A SICK DOG SHOW AND WHY?

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:

Other, **less common clinical signs** have been described:



WHAT CLINICAL SIGNS DOES A SICK DOG SHOW AND WHY?

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.

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:**

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 **hematopoietic organ** or skin or **other tissue.**

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.

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 anemia.
- Mild neutrophilia with lymphopenia and monocytosis (stress leukogram).
- Thrombocytopenia.
- Leukopenia (less common).

Serum protein electrophoretogram alterations are:

- Hyperproteinemia with hyperglobulinemia and hypoalbuminemia, either compensatory or as a result of renal proteinuria.
- In dogs with immune-mediated glomerulonephritis there will be renal azotemia and proteinuria.

WHAT DIAGNOSTIC TESTS SHOULD BE RUN

IN A DOG THAT IS SUSPECTED TO HAVE THE INFECTION/DISEASE?

Specific techniques

Include serological and parasitological diagnosis (cytology and PCR).



Available serological methods can be:

- 🍃 **Qualitative** (based on immunochromatography, dot-ELISA).
- 🍃 **Quantitative** (immunofluorescence-IFAT, enzyme-linked immunosorbent assay-ELISA technique, western blotting-WB).

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).

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

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.

HEALTHY	CLINICALLY HEALTHY INFECTED	SICK
 <ul style="list-style-type: none">PCR <input type="checkbox"/>Serology <input type="checkbox"/>Clinical signs <input type="checkbox"/>Laboratory alterations <input type="checkbox"/>	 <ul style="list-style-type: none">PCR <input checked="" type="checkbox"/>Serology (Low titers) <input checked="" type="checkbox"/>Clinical signs <input type="checkbox"/>Laboratory alterations <input type="checkbox"/>	 <ul style="list-style-type: none">PCR <input checked="" type="checkbox"/>Serology (Medium-high titers) <input checked="" type="checkbox"/>Clinical signs <input checked="" type="checkbox"/>Laboratory alterations <input checked="" type="checkbox"/>

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

WHAT DIAGNOSTIC TESTS SHOULD BE RUN

IN A DOG THAT IS SUSPECTED TO HAVE THE INFECTION/DISEASE?

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 localized cutaneous leishmaniosis. This localized version of the disease is characterized 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 antimonials** (n-methyl glucamine) were almost exclusively used.

These work as a highly effective leishmanicide molecule by...

Short half-life



6-9 hours

Excreted by kidneys



two daily subcutaneous doses are recommended to maintain a higher inhibitory concentration.

WHAT GENERAL TREATMENT STRATEGY IS RECOMMENDED FOR SICK DOGS?

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 feces



no hepato-renal impact

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 minimizes relapses. **(Table)**

WHAT GENERAL TREATMENT STRATEGY IS RECOMMENDED FOR SICK DOGS?

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. **(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, ...

Kidney patients may benefit from...



WHAT GENERAL TREATMENT STRATEGY IS RECOMMENDED FOR SICK DOGS?

Monitoring for response to treatment

Dogs under treatment with an appropriate protocol for their clinical and parasitological situation need to be **monitored.**



30 days after treatment

During the first year

From the second year onward



Click on  for more information

WHAT GENERAL TREATMENT STRATEGY IS RECOMMENDED FOR SICK DOGS?

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.**

It is essential in the case of a suspected co-infection to follow a comprehensive diagnostic plan including a complete hematological 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 flies. In addition, people can apply fly repellent treatments to reduce their own chance of being bitten.



ARE OTHER PETS OR PEOPLE IN THE HOUSE AT RISK?

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. braziliensis*, *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.



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.

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 post infection. 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.

WHAT ARE SOME RECOMMENDATIONS AROUND PREVENTION STRATEGIES?

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 favorable 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.



WHAT ARE SOME RECOMMENDATIONS AROUND PREVENTION STRATEGIES?

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.

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



WHAT ARE SOME RECOMMENDATIONS AROUND PREVENTION STRATEGIES?

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?



FURTHER READING

Bibliography

-  Baneth G *et al.* Canine leishmaniosis - new concepts and insights on an expanding zoonosis: part one. *Trends in Parasitology* 24 pp 324 - 330 2008.
-  Boggiatto PM *et al.* Transplacental transmission of *Leishmania infantum* as a means for continued disease incidence in North America. *PLoS Neglected Tropical Diseases* 5 p e1019 2011.
-  de Freitas E *et al.* Transmission of *Leishmania infantum* via blood transfusion in dogs: potential for infection and importance of clinical factors. *Veterinary Parasitology* 137 pp 159-167 2006.
-  Gálvez R *et al.* Controlling phlebotomine sand flies to prevent canine *Leishmania infantum* infection: A case of knowing your enemy. *Research Veterinary Science* 121 pp 94-103 2018
-  Grinnage-Pulley T *et al.* A Mother's Gift: Congenital Transmission of *Trypanosoma* and *Leishmania* Species. *PLoS Pathog.* 12 p e1005302 2016.
-  Karkamo V *et al.* The first report of autochthonous non-vector-borne transmission of canine leishmaniosis in the Nordic countries. *Acta Veterinaria Scandinavica* 56 p 84 2014.
-  Koch LK *et al.* Modeling the climatic suitability of leishmaniasis vector species in Europe. *Scientific Reports* 17 7 p 13325 2017.
-  Koutinas AF *et al.* Clinical considerations on canine visceral leishmaniasis in Greece: a retrospective study of 158 cases (1989-1996). *Journal of the American Animal Hospital Association* 3 pp 376 - 383 1999.
-  Maia C and Cardoso L. Spread of *Leishmania infantum* in Europe with dog travelling. *Veterinary Parasitology* 213 pp 2 - 11 2015.
-  Millán J *et al.* Role of wildlife in the epidemiology of *Leishmania infantum* infection in Europe, *Parasitology Research* 113 pp 2005 - 2014 2014.
-  Miró G *et al.* Canine leishmaniosis, new concepts and insights on an expanding zoonosis: part two. *Trends in Parasitology* 24 pp 371 - 377 2008.
-  Miró G *et al.* Novel areas for prevention and control of canine leishmaniosis. *Trends in Parasitology* 33 pp 718 - 730 2017.
-  Naucke TJ *et al.* First report of transmission of canine leishmaniosis through bite wounds from a naturally infected dog in Germany. *Parasites and Vectors* 9 p 256 2016.
-  Petersen CA and Barr SC. Canine leishmaniasis in North America: emerging or newly recognized? *Veterinary Clinics of North America Small Animal Practice* 39 pp 1065 - 1074 2009.
-  Quilez J *et al.* Genetic control of canine leishmaniasis: genome-wide association study and genomic selection analysis. *PLoS One* 7 p e35349 2012.
-  Ready PD. Leishmaniasis emergence and climate change. *Rev Sci Tech* 27 pp 399 - 412 2008.
-  dos Santos Nogueira F *et al.* Use of miltefosine to treat canine visceral leishmaniasis caused by *Leishmania infantum* in Brazil. *Parasites & Vectors* 12 p 79 2019.

FURTHER READING

- da Silva SM *et al.* First report of vertical transmission of *Leishmania* (*Leishmania infantum*) in a naturally infected bitch from Brazil. *Veterinary Parasitology* 166 pp 159 - 162 2009.
- Solano-Gallego L *et al.* Prevalence of *Leishmania infantum* infection in dogs living in an area of canine leishmaniasis endemicity using PCR on several tissues and serology. *Journal of Clinical Microbiology* 39 pp 560-563 2001.
- Solano-Gallego L *et al.* LeishVet guidelines for the practical management of canine leishmaniosis. *Parasites & Vectors* 4 p 86 2011.
- Svobodova V *et al.* Canine leishmaniosis in three consecutive generations of dogs in Czech Republic. *Veterinary Parasitology* 237 pp 122-124 2017.



Copyright © 2020 Intervet International B.V., also known as MSD Animal Health. All rights reserved.

VECTOR BORNE DISEASE

