

Leishmaniosis

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General information

Answers to most questions regarding leishmaniosis can be found within the LeishVet guidelines <http://www.leishvet.org/fact-sheet/>. They also provide guidelines in several European languages (very useful for clients that struggle with English language).

Leishmaniosis

Leishmaniosis is caused by the protozoan parasite *Leishmania infantum*, which is transmitted by sand flies of the *Phlebotomus* species. Dogs are the major reservoir for this infection. This infection causes disease in humans, particularly immunosuppressed adults and children. It is extremely common in countries surrounding the Mediterranean and in South America. Most recently it has spread across the USA and Canada, particularly in the fox hound breed, even though there are no competent sand fly vectors in these areas. Online maps are available to help determine if a country a dog has travelled to/from has leishmania infection there. Direct dog-to-dog transmission (DOI: 10.1136/vr.105157; DOI: 10.1136/vr.11268), vertical transmission (DOI: 10.1371/journal.pntd.0007058; DOI: 10.1371/journal.pntd.0001019), and iatrogenic transmission through contaminated blood products (DOI: 10.1016/j.vetpar.2005.12.011) have been reported.



In endemic areas the infection prevalence in dogs may approach 90% but most dogs are asymptomatic as their immune response plays a large part in determining the outcome of infection; an ineffective cell mediated immune response with a pronounced humoral response is associated with clinical disease. Leishmaniosis is a chronic disease with a long incubation period – it can be 3 months to 7 years after infection that clinical signs develop.

Diagnosing leishmaniosis

What clinical signs consistent with leishmaniosis?

Clinical signs include: skin lesions (scaling is common, also alopecia, ulceration) especially head and pressure points; lymphadenopathy; ocular signs (e.g. uveitis, conjunctivitis, keratoconjunctivitis sicca); splenomegaly; polyarthropathy; weight loss & poor body condition; fever. The dermatological changes are often not that pruritic unless there is secondary bacterial infection.

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Other clinical signs include: onychogryphosis (abnormal nails); epistaxis; kidney disease-associated signs e.g. polyuria & polydipsia; evidence of mild to moderate bone marrow suppression (pallor; opportunistic infection; increased bleeding after venepuncture).

What laboratory changes can occur with leishmaniosis?

The following non-specific changes are not infrequently seen with clinical leishmaniosis:

- Routine haematology: non-regenerative anaemia (common) or regenerative anaemia due to immune-mediated haemolysis (less common). Occasionally (typically only mild to moderate) thrombocytopenia or neutropenia.
- Serum biochemistry: Hyperproteinaemia (common) due to hyperglobulinaemia and hypoalbuminaemia (compensatory to hyperglobulinaemia or increased losses). Azotaemia and / or raised liver parameters can occur.
- Urine analysis: poor urine concentrating ability (USG <1.030) and / or proteinuria (UPC ≥ 0.5).

In dogs where leishmaniosis has been confirmed, full haematology (including blood film examination), serum biochemistry, and urinalysis (including UPC) at an external laboratory are recommended stage the disease if not already performed. Some clinicians also advocate serum protein electrophoresis, but in most cases results of this test are unlikely to facilitate making the diagnosis or alter treatment recommendations so we do not routinely perform this

Checking for other imported diseases that may require treatment, or may be contributing to clinical signs, is sensible. Based on the country of import / prior residence the map on <http://www.cvbd.org/> can give you an indication of which to check for based on the country of import. Co-infections, particularly *Ehrlichia* and *Dirofilaria* are common. We also recommend performing *Brucella canis* serology, following increased detection in travelled dogs

How do I diagnosis a dog with leishmaniosis?

Serology

The detection of *Leishmania*-specific serum IgG antibodies in the serum of dogs using quantitative techniques is very useful in the diagnosis of *Leishmania* infection. However, serology indicates exposure to *Leishmania*, and should be considered alongside clinical signs and additional clinicopathological testing.

Antibodies are typically detected by either ELISA or immunofluorescence (IFAT) and both are regarded as being good tests although some subjectivity occurs in the interpretation of IFAT titres. Serial monitoring should be performed using the **same assay** at the **same laboratory** – *results obtained from different laboratories are not comparable*. It should be noted that in travelled dogs' concurrent chronic infectious agents may also be present / causing clinical disease.

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Although it can take a few months for dogs to seroconvert (up to 22 months, although median is 5 months) the long incubation period with *Leishmania* means that most sick dogs are likely to be antibody positive. High antibody levels are consistent with clinical leishmaniosis. Low antibody levels are not usually indicative of disease (but do not rule out clinical disease). Some dogs do not seroconvert (i.e. a negative result does not rule out infection). If leishmaniosis is suspected with low or negative serology results further monitoring (repeat serology every 3-6 months) and / or additional diagnostic tests such as PCR are indicated to help confirm or exclude clinical *Leishmania* disease. Serology results can also help determine when treatment courses can be completed.

Confirmation of *Leishmania* as the cause of clinical signs is preferable but is not always possible. A positive response to treatment is also an indicator that *Leishmania* is the underlying problem.

Direct visualisation (demonstration of Leishmania organisms in cytology or histopathology samples)

Finding *Leishmania* organisms on stained smears or sections prepared from fine needle aspirates, impression smears or tissue biopsies from affected tissues is useful to confirm infection, although the examiner needs to be experienced in identifying organisms. Organisms can be scarce and only present in certain organs, reducing the sensitivity of this technique. Samples should only be obtained in dogs in which clinical leishmaniosis is suspected due to the presence of consistent clinical signs and / or clinicopathological changes.

Suitable samples include bone marrow, lymph node, skin or joint fluid. Samples showing pathology consistent with *Leishmania* infection e.g. (pyo)granulomatous or lymphoplasmacytic inflammation or lymph node reactive hyperplasia, as well as organisms, are more likely to represent clinically significant *Leishmania* infection.

Polymerase chain reaction (PCR)

PCR is a sensitive and specific technique that can amplify DNA from *Leishmania infantum* in various samples. PCRs based on amplifying *Leishmania* kinetoplast DNA (kDNA), such as the PCR offered by Langford Vets Diagnostic Laboratories (Acarus), are particularly sensitive due to the many thousands of kDNA copies present in each *Leishmania* organism. Real-time (quantitative) PCR, as offered by Acarus, additionally allows quantification of *Leishmania* DNA in the sample; thus, the level of any *Leishmania* infection present can be determined (higher levels consistent with severe disease), and additionally response to treatment can be monitored (as *Leishmania* organism numbers should fall quite quickly with effective treatment; if previously positive, negative PCR results should be obtained before treatment is stopped).

Samples suitable for PCR are tissues in which lesions are believed to be present based on clinical examination or clinicopathological results. Otherwise, the sensitivity of detection by PCR: bone

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marrow or lymph node aspirates > skin aspirates > conjunctival swabs > buffy coat > whole blood. So, although blood is often positive by PCR in clinical *Leishmania* disease, a negative result cannot be used to rule out disease, and in these cases concurrent serology testing is important to determine if clinical *Leishmania* disease is likely in cases with consistent clinical signs and / or clinicopathological changes.

PCR can sometimes be positive in dogs that are *Leishmania*-infected, but which do not have clinical disease. Serology results can be used in conjunction to stage disease and predict whether disease is likely to occur. Repeat testing of asymptomatic dogs is always recommended to assess for any progression although repeat serology in this context may be more useful than PCR.

Treatment

When to treat

Treatment is indicated if a dog has **active** *Leishmania* infection with associated clinical signs. The only time dogs without clinical signs may need treatment is if *Leishmania* infection has been demonstrated (e.g. by cytology or PCR) in association with an antibody titre which is found to significantly rise on repeat testing 2-3 months later (although treatment in these cases is controversial).

Treatment options

Treatment with allopurinol plus either meglumine (antimonate) or miltefosine is recommended. Treatment with allopurinol alone is not recommended in cases with clinical disease, unless very localised with no evidence of systemic disease. In dogs with clinical disease, sole treatment with allopurinol is associated with a slower response and treatment failures are more likely – but if owner finances are very limited it could be considered (with the above caveats).

The choice of meglumine vs. miltefosine is mostly dependent on owner finances and degree of organ dysfunction (e.g. presence of azotaemia). Most Mediterranean vets preferentially use meglumine (despite the adverse effects on the kidneys and need to inject). Miltefosine is easier to administer (oral liquid), and in our experience associated with few adverse effects; however, it is associated with increased frequency of relapses as compared to meglumine in the longer term.

- Protocol 1. Meglumine antimonate and allopurinol for 28 days, followed by allopurinol alone for at least 6-12 months.
- Protocol 2. Miltefosine and allopurinol for 28 days, followed by allopurinol alone for at least 6-12 months.

	Drug	Dose	Adverse effects
Protocol 1	Meglumine antimonate (Glucantime)	100 mg/kg SC q24h for 28 days (ideally given	Pain, swelling at injection site – try and use different injection sites daily and massage well afterwards (sometimes anti-inflammatory prednisolone is required

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		divided twice daily)*	for 3-5 days if severe reactions). Fever, loss of appetite and diarrhoea are occasionally reported. Transient increase in liver enzymes sometimes seen. Transient acute kidney injury (tubular) is rare.
Protocol 2	Miltefosine (Milteforan)	2mg/kg PO q24h with food for 28 days	Vomiting, diarrhoea (usually occurs within 5-7 days of starting treatment and is self-limiting over 1-2 days, so no treatment usually required)
Protocol 1 & 2	Allopurinol	10 mg/kg PO BID for at least 6-12 months	Xanthine uroliths – can monitor using ultrasound. Although xanthine crystalluria is common, urolithiasis seems to be relatively uncommon. If occurs then need dietary management and/or reduce dose of allopurinol to 5 mg/kg BID or 2.5 mg/kg BID, ensuring control of <i>Leishmania</i> remains.

*Consider starting at 50mg/kg divided daily, and re-assess urea, creatinine and UPC after 3 days – if there is concern regarding kidney function.

Supportive care may also be required e.g. antibiotics if 2° bacterial pyoderma, treatment for *Malassezia* if present, intravenous fluid therapy if kidney disease. It is possible to use prednisone in dog with leishmaniosis when there are clinical signs associated with inflammation secondary to deposition of immunocomplexes (e.g. uveitis, glomerulonephritis or polyarthritis). Usually a short course (7-15 days), starting at 0.5-1 mg/kg/day, tapered to stop.

Monitoring is also important (see below) to assess response and decide when allopurinol treatment can be stopped.

Various alternative (lower) doses and dosing schedules for allopurinol are anecdotally used in the long-term management of leishmaniosis. They are often used to either reduce costs or reduce the risk of urolithiasis. There are little to no data to support these schedules as being equivalent or superior to the current widely recommended one - having said that evidence base regarding treatment is limited and primarily based upon expert opinion (of specialist vets that live and work in areas where *Leishmania* is endemic).

Obtaining the meglumine / miltefosine

Typically, this is not possible via routine veterinary wholesalers. Both meglumine (Glucantime; 1.5g/5mL; packets of 5 ampoules of 5mL; France / Spain) and miltefosine (Milteforan; 20mg/ml; 30mL or 60mL bottles; France / Spain) need a special import certificate (SIC) for the individual patient, but this is readily available from the VMD website (<https://www.vmd.defra.gov.uk/sis/default.aspx>). Select the 'Apply for Special Import Certificate (To import a veterinary medicinal product authorised within the EU)' option, which will then require

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you to enter your RCVS membership number to continue. The first time you do this, you will need to register yourself and your practice. This on-line application is free.

Milteforan (as a 60mL bottle) is available directly from the manufacturer Virbac. Merlin vet exports (www.merlinvet.co.uk) are also able to supply both Milteforan (as 30mL or 60mL bottles) and Glucantime. Other importers (e.g. Henry Schein www.henryschein.co.uk) are also available. Check with them directly regarding cost. These items are invariably non-refundable.

Any alternative treatments

Other therapeutic protocols for leishmaniosis less favoured include amphotericin B, metronidazole in combination with spiramycin, marbofloxacin. and domperidone. Further studies are required for these medications. We most frequently use domperidone in dogs that have failed standard therapy.

Once clinical remission is achieved and >6 months of being clinically normal. Depending on the patient circumstances and owner concerns (e.g. history of prior relapses; finances; serum titres), you can either continue on allopurinol alone or consider domperidone (Leisguard is the imported canine preparation; generic liquid and tablet preparations are available in the UK from human pharmacies) or nucleotides (Impromune; Bioiberica) as alternatives. Domperidone is 0.5 mg/kg/day for 30 days every three months (for extra info on this drug see DOI: 10.1590/0074-02760180301). The Impromune is more expensive than the domperidone and the dosage published for leishmaniosis is daily ongoing. The objectives of both domperidone and Impromune nucleotides are not to cure or control infection, but to reduce the risk of relapse. If there is relapse restarting induction treatment is recommended.

Vaccination has not been shown to be effective in the face of clinical signs, and it can interfere with serological testing.

Urolithiasis

When there is only xanthinuria, you could use allopurinol but 5 mg/kg twice daily (or 2.5mg/kg twice daily) with a low purine diet. If there is not xanthinuria in subsequent samples, you could maintain this treatment. When there is mineralization, urolithiasis, or no control of xanthinuria the allopurinol is stopped (if possible), with alternative treatments to maintain remission (e.g. domperidone or Impromune) considered.

Dietary support

Dogs should be encouraged to drink water, to reduce the risk of stone formation. Assuming there is no evidence of kidney dysfunction, there is a specific diet that has been marketed for the adjunctive management of dogs with leishmaniosis www.affinity-petcare.com/advanceold/en/dogs/leishmaniasis. Royal Canin Dalmatian, Royal Canin

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hypoallergenic and Purina HA Hypoallergenic are also formulated to have lower levels of purines cf. standard dog food, to reduce the risk of stone formation yet have normal protein levels.

In adult dogs where xanthine stones have formed more extreme low protein, low purine diets that should be considered include Hill's U/D (canned and dry), and Royal Canine Urinary U/C Low Purine. When these diets are fed close monitoring of serum protein levels is recommended (especially if there is proteinuria).

Some of these diets might only be available via the internet.

Monitoring response to treatment

Response to therapy is good to poor depending on the degree of organ dysfunction. The majority of dogs improve clinically during the 1st month of treatment, usually after a week or so. Dogs with kidney dysfunction have a poorer response but can still do well. A reduction in parasitaemia (as determined using quantitative PCR) is usually seen quite quickly in dogs that were initially positive; the UPC and globulin levels may also improve quite quickly, but this is variable. Antibody titres remain elevated for longer periods of time and usually only start to decline a few months (4-6 months) after starting treatment (there is usually little point in looking for a decline in antibody levels until >4 months of treatment).

Monitoring of dogs is recommended at the following approximate time points after starting treatment: 1 month, at 4 months, at 6 months and then every 6-12 monthly thereafter as required depending on progress.

Monitoring at 1- and 4-month checks should include evaluation of any known organ dysfunction, serum albumin and globulin levels, urine protein: creatinine ratio, and blood qPCR (if previously positive). If there has been a response to treatment, these will often normalise during this period. The 1-month testing is useful to evaluate the efficacy of the meglumine / miltefosine given and a marked improvement in test results suggests that the maintenance phase of treatment (i.e. allopurinol alone) can be entered; however, this maintenance phase should be continued for at least 6-months. Rarely the improvement is not as good as expected and, in these cases, consideration can be made to giving meglumine or miltefosine for another month (please ask for advice before doing this).

For the subsequent checks, antibody levels should be evaluated in addition to haematology, serum biochemistry, and urinalysis.

When can I finish treatment?

After approximately one year of successful treatment, consideration may be given to discontinuing allopurinol, particularly in a non-endemic region. All clinicopathological parameters should be within reference range, the antibody titres borderline or negative, and (if previously positive) blood

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qPCR negative, before allopurinol treatment is stopped. If a bone marrow sample is available, a negative PCR provides further support for discontinuation.

Discontinuation of therapy is ultimately dependent on recovery of the dog's immunological ability to control the infection. Some dogs never regain this ability and will require continual therapy (allopurinol plus intermittent meglumine or miltefosine as required) to keep the infection under control.

Persistent / increasing titres in the absence of clinical signs

Some clinically well dogs *without* a history of clinical disease will have rising titres on routine monitoring. A very careful physical examination should be performed alongside haematology, serum biochemistry, and urinalysis (including UPC) – to confirm the absence of subtle signs and underlying organ dysfunction, cytopenias, or hyperglobulinaemia. C-reactive protein measurement may also be considered. These are tricky cases and consensus as to how to manage them has not been achieved. It is not clear that these dogs will ever develop clinical signs – and as such antiparasitic treatment is not indicated (there is concern that use of antiparasitics could lead to resistance). The use of immune support medication (e.g. domperidone 0.5mg/kg once daily for 1 month, then 2-3 months off, or Impromune nucleotides) could be considered – particularly where C-reactive protein is increased and otherwise unexplained. Repeat serology should be considered after 6 months, sooner if clinical signs develop.

Some clinically well dogs *with* a prior history of clinical disease and receiving maintenance allopurinol (typically following an induction agent) will have rising titres or persistently increased titres in the absence of clinical signs. A very careful clinical examination should be considered alongside haematology, serum biochemistry, and urinalysis (including UPC) – to confirm the absence of subtle signs. These are tricky cases and consensus as to how to manage them has not been achieved. It is not clear that these dogs are in the process of relapse – although you could consider C-reactive protein measurement as an early warning (NB: there are very little data to support this and increases are usually subtle). In most cases the allopurinol is continued (there is concern that there is increased risk of relapse were it to be stopped) and the use of immune support medication (e.g. domperidone 0.5mg/kg once daily for 1 month, then 2-3 months off, or Impromune nucleotides) could be considered.

Prognosis

Although clinical response is good in most dogs, it is guarded to poor in dogs with evidence of disseminated immune-mediated disease, and those with severe kidney disease or failure. Treatment does not clear infection; but many dogs with low infection loads (low serology, qPCR usually negative) remain asymptomatic for long periods – monitoring is important as described

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above. These dogs should never be used as blood donors or bred from. Immunosuppression should be avoided, if possible.

Vaccination against leishmaniosis

In addition to sand fly repellent, vaccination against leishmaniosis may be considered to reduce the risk of acquiring clinical leishmaniosis in dogs that travel to the Mediterranean region (NB: not protective against infection). Vaccination induces antibody production that may cause seropositive results on conventional ELISA and IFAT *Leishmania* antibody testing. Vaccination will not induce PCR positivity so PCR can still be used for diagnosis of *Leishmania* infection in a vaccinated dog. A good review regarding the effect of vaccination on testing can be found online (DOI: 10.1016/j.pt.2017.06.004)

Despite the existence of vaccination, the importance of vector (sand fly) control in the prevention of leishmaniosis cannot be overemphasised. All owners must be advised to do this, even in vaccinated dogs. The use of synthetic pyrethroids that repel sand flies and keeping dogs indoors between 7pm and 7am is recommended when sand flies are active (May to October for Europe).

Vaccination is not recommended for dogs with clinical disease.

In contact animals

Even though the UK does not have the known vector for *Leishmania* spp. (i.e. the sand fly) there have been cases of dog-to-dog transmission in the UK from clinical cases to naive dogs with no travel history. These dogs have had a history of being in close contact with cases of clinical *Leishmania* infection, either by sharing the same household or by spending extended periods of time in close contact (e.g. 'doggy day care').

Regular treatment with flea / tick medication is recommended. NB: it is useful to know the heartworm status of these dogs prior to giving them anything that is microfilaricidal.

In contact humans

In the absence of the sand fly vector the risk of zoonotic transmission is currently considered to be very low. Direct transmission of *Leishmania* infection in the absence of sandfly vectors from dogs to humans has never been documented. However, in the presence of cutaneous lesions (particularly given that alternative differentials include zoonoses such as dermatophytosis) the wearing of gloves and routine hand hygiene would be considered prudent.

The Human Animal Infections and Risk Surveillance group (HAIRS) has produced guidance regarding human health implications:

<https://www.gov.uk/government/publications/hairs-risk-assessment-canine-leishmaniosis>

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