C.L.W.G.

CANINE LEISHMANIASIS
WORKING GROUP

Diagnosis, treatment and prevention of
Canine Leishmaniasis

Information about CLWG can be found on the website **www.gruppoleishmania.org**.
The extended Italian version of the guidelines on diagnosis, treatment and prevention of Leishmaniasis, and the management of the proteinuric patient is available on the website and it has been published in the journal "**Veterinaria**". It has also been published in English in the **Journal of the American Veterinary Medical Association**.
The activity of CLWG is supported by Hill's Pet Nutrition.

Diagnosis of Canine Leishmaniasis

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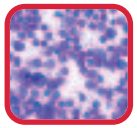
Clinical and laboratory findings

Leishmaniasis should be suspected in a dog coming from (or which has lived in) an endemic area, and which has one or more of the following clinical signs:

Alopecia and desquamative dermatitis (particularly in the nasal and periocular regions)

Ulcerative dermatitis associated with vasculitis (in this case observed on the foot pad)

Onychopathy



Generalized lymphadenopathy (cytologically consistent with lymph node hyperplasia)

Weight loss

Cachexia

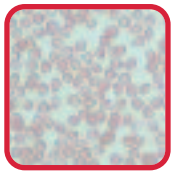
Epistaxis

PU/PD and renal failure due to glomerulonephritis

Ocular lesions: conjunctivitis, keratoconjunctivitis, episcleritis, anterior and posterior uveitis. Complications: glaucoma and endophthalmitis

Hyperproteinemia with low albumin/globulin ratio, hypoalbuminemia, polyclonal gammopathy (left picture, capillary electrophoresis) often associated with increased α and β-globulins (right pictures, agarose gel electrophoresis)

Proteinuria (increased UPC ratio)



Anemia (usually, non regenerative)

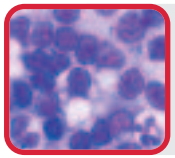
Other findings observed in Leishmaniasis: lameness, spleen or liver enlargement, fever nodular or papular dermatitis, hyperfibrinogenemia, serum biochemical values consistent with renal or liver injuries or failure increased acute phase proteins. In addition, mainly in endemic areas, atypical clinical presentation can be observed (i.e. neurological or intestinal disorders).

Diagnostic assays

Leishmaniasis should always be diagnosed by demonstrating evidence of the parasite or the host's immune response. The most useful tests for this are:

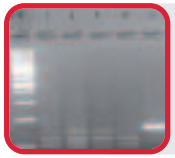
Serology

- In-clinic assays can be used but it is suggested to then verify the results with ELISA and IFAT at reference laboratory.
- ELISA and IFAT also provide the serum titer which is important to assess the strength of the immune response.
- In order to avoid misleading interpretations due to variability of operator or method used:
  - Always use the same method and the same laboratory to evaluate the seroconversion;
  - Consider a serum titer as 'high' when it is at least 4 fold higher than the cut-off positive value from the reference laboratory.



Cytology

- Cytology can be performed in the following tissues with lesions:
  - Skin (in case of papular, nodular and ulcerative injuries);
  - Enlarged lymph nodes or bone marrow in case of anemia;
  - Other fluids such as synovial fluid or CSF* (in case of arthritis or neurological signs respectively).
- In the absence of lesions, tissues with the highest sensitivity are bone marrow, lymph nodes, spleen, and blood.
- Samples can be also stored to perform PCR studies, if necessary.
- In case of negative cytology, if the clinical suspicion persists, lesions should be analyzed by means of histology, immunohistochemistry or PCR.



PCR

- The ideal gene target is the kinetoplast DNA.
- Tests more commonly used are the nested-PCR or the quantitative-PCR .
- PCR assays can be performed in tissues with lesions (fresh, frozen, or paraffin embedded).
- If lesions are absent, tissues with the highest sensitivity are: bone marrow, lymph nodes, skin, conjunctiva, buffy coat from blood.
- In endemic areas, a positive PCR in skin or at bone marrow level does not necessarily indicate an "active" infection.

Interpretation of results

Dogs can be classified as: Affected by Leishmaniasis, Infected by *L. infantum* or Exposed to the infection based on the following diagnostic flow-chart:

Serology: **positive or negative**
Cytology of lesions: **positive**
→ **LEISHMANIASIS**

Serology: **positive (high titer)**
Cytology of lesions: **negative**
→ **LEISHMANIASIS**

Serology: **positive (high titer)**
Citologia delle lesioni cutanee: **negative**
→ Histology, immunohistochemistry, PCR in skin biopsies

Serology: **positive (low titer)**
Absence of lesions to be sampled
→ PCR in bone marrow/lymph nodes

Only if lesions or clinical signs are clearly associated with positive serology, histology or PCR

LEISHMANIASIS ← **INFECTED** ← **positive** / **negative** → **"EXPOSED"**

- Look for other diseases
- Monitor with ELISA/IFAT

*Cerebrospinal fluid

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Staging of dogs affected by Leishmaniasis

STAGE	DEFINITION	DESCRIPTION
A	Exposed	Dogs without overt clinico-pathological alterations, where parasitological diagnostic assays are negative and where specific serum antibody titers can be demonstrated not higher than 4-fold the cut off value of the reference laboratory. Usually exposed dogs which live or had lived in an area where the occurrence of sand-flies have been ascertained.
B	Infected	Dogs without any clinico-pathological evidence, but where it is possible to observe the parasite either with direct methods (microscopy, culture, or PCR) or indirect methods (occurrence of specific antibodies).
C	Clinically ill	Infected dogs, where it is possible to observe clinico-pathological evidence of Leishmaniasis and where it is possible to show the parasite directly or that the dog serum has antibody titers higher than 4-fold the cut-off value of the reference laboratory.
D	Clinically ill with a severe clinical feature	Clinically ill dog affected by: (i) nephropathy with proteinuria; (ii) chronic renal failure; (iii) severe ocular disease which leads to vision loss and/or where immunosuppressive treatment is required; (iv) severe joint diseases which leads to deficit of movements and/or where immunosuppressive treatment is required;(v) severe concurrent diseases from infectious, parasitological, neoplastic, endocrine or metabolic origin.
E	Refractory relapsing	(Ea) Clinically ill dog refractory to the treatment. (Eb) Clinically ill dog that underwent the treatment, but had an early relapse.

Therapeutic protocols (Aetiological treatment)

STAGE	PRIMARY PROTOCOL	ALTERNATIVE PROTOCOLS
A	For patients in this stage no treatment should be provided.	
B	The therapeutic protocol that has large consensus is the combination of N-methyl-glucamine antimoniate (dosage of 100 mg/kg SID SC for four weeks) and Allopurinol (10 mg/kg BID PO for at least 4-6 months). The dosage of N-methyl-glucamine antimoniate can be subdivided in two doses of 50 mg/kg BID, if clinical judgement indicates, for a minimum of 4 to a maximum of 8 weeks. For subjects in stage D, this protocol may not ensure the clinical cure of these dogs. The prognosis of these patients is strictly related both to the initial clinical condition at presentation and the response to essential supportive treatment.	In cases of low efficacy of the primary protocol, occurrence of side effects, low compliance or relapses, the following alternative protocols can be considered: • **Allopurinol, as exclusive therapy for several months** (10 mg/kg, BID, PO); • **Miltefosine, for 28 days** (2 mg/kg SID, PO) **in combination with Allopurinol**. Other drugs such as Aminosidine, Amphotericin B, Pentamidine, Spiramicin/Metrodinazole combination, Enrofloxacin, Marbofloxacin and Domperidone are not recommended, as they have severe adverse effects or there is incomplete demonstration of therapeutic efficacy.
C		
D		
E	For dogs in this stage, once other possible disease and concurrent factors which influence drug efficacy have been ruled out, **alternative therapy** can be considered.	

DIETARY MANAGEMENT

A diet with selected/restricted protein content along with the administration of an ACE inhibitor is suggested in patients in Stage 1 IRIS with UPC ratio >2.0 and in patients in Stage 2, 3, and 4 IRIS with UPC ratio of >0.5.

Monitoring (stage B and C)

WHEN	HOW
After 4-8 weeks	• Complete physical exam and hemato-biochemical investigations at the end of the treatment th the combination of N-methyl-glucamine antimoniate and Allopurinol; • If the above investigations are within the normal ranges, Allopurinol should be continued as indicated before.
After 6 months	• Periodic monitoring (as before described), with the determination of antibody titers and, possibly, parasite load amount with bone marrow or lymph node qPCR; • If necessary, restart the treatment with the primary protocol or with alternative protocols in case of early relapses.

If the clinical evaluation and/or hemato-biochemical parameters do not come back to within the normal ranges, or there is no trend to normalization at the end of the treatment, the dog should be assigned to group Ea or Eb and proceed as recommended above.

Recommendation

1) Be confident of the diagnosis and rule in/out other concurrent diseases;
2) Correctly classify the disease into one of the proposed stages, giving special emphasis to the difference between the infected and diseased dog;
3) Choose the appropriate therapeutic protocol, giving priority to drugs for which there is a large international literary support;
4) Avoid using drugs which may have anti-Leishmaniosis indications, but which may induce adverse side effects or which may have unpredictable or poorly supported efficacy;
5) When necessary apply appropriate supportive therapy;
6) Apply the chosen therapeutic protocol correctly, taking care to observe the correct dosage, administration and necessary length of treatment according to the pharmacokinetics of the chosen pharmaceutical; in other words do not adapt the therapy according to the circumstances;
7) Monitor the patient appropriately during and after the treatment.

Warning In Europe, Amphotericin B is the first choice drug to treat human Leishmaniasis. For this reason, WHO strongly discourages its use in the dog affected by Leishmaniasis, in order to avoid the possible development of Amphotericin-resistant parasite strains.

Prevention of Canine Leishmaniasis

Which dogs should be protected

- It is recommended that any Leishmania-infected dogs living in areas endemic for Leishmaniasis should be protected from sand fly bites as a measure to reduce infection risk in the human and canine community (mass protection).
- Because dogs on therapy for canine Leishmaniasis can be still infectious to sand fly vectors despite clinical cure and reduction in parasite load, these patients should also be protected;
- It is also recommended that any healthy dogs living in or visiting areas endemic for Leishmaniasis for purposes such as tourism, or working activities like military, disaster or sentry work, should be protected from sand fly bites to prevent Leishmania infections (individual protection)

Ectoparasitacides Chemical approach to the prevention

Among synthetic pyrethroids, permethrin and deltamethrin have received the marketing approval for indications of efficacy against bites and toxic effects of Leishmania vectors. Based on the literature, permethrin, alone or in combination with imidacloprid as a topical application (spot-on), and deltamethrin (deltamethrin-triphenylphosphate complex) administered by slow release collar, are the ectoparasiticides of choice because of their high efficacy in preventing sand fly bites. The different starting periods of protective activity exhibited by different permethrin and deltamethrin formulations should be carefully considered when prescribing a drug. In particular, owners, when taking their pets from non-endemic to endemic areas of Leishmaniasis during the sand fly activity period, should be advised to take into account that the length of time required for the chosen ectoparasitacide to achieve full protection may vary from 0 to 1 week, according to products used.

Active ingredients currently labeled for cutaneous treatment of dogs for the prevention from Leishmania vector bites

DERMAL APPLICATION BY	ACTIVE INGREDIENT (%)	START OF PROTECTION AFTER APPLICATION	ESTIMATED DURATION OF PROTECTION
Spot-on	Permethrin (50)+imidacloprid (10)	24-48 hours	3 weeks
Spot-on	Permethrin (65)	24-48 hours	4 weeks
Spray	Permethrin (2)+piriproxifene (0.2)	immediate	3 weeks
Collar	Deltamethrin (4)+carrier [triphenyl phospate[a]	1 week	5 months

[a] - included in a protector band slow release.

When to protect

The relatively shorter duration of activity of the spot-on and spray formulations (3-4 weeks) against sand flies requires owner's to comply with frequent applications, whereas slow-release collar formulations do not need to be replaced more than twice a year in environments where Leishmania vectors are active throughout the year, or once a year in temperate areas, where no adult flies are found during cold months.

Important Although the preventative treatments ensure high efficacy against sand fly bites, they may not provide 100% protection. Thus, it is always recommended to perform control tests for Leishmaniasis following potential exposure to Leishmania vectors.

Mechanical protection

In endemic areas for Leishmaniasis, when dermal application of synthetic pyrethroids is contraindicated (e.g. in very young puppies, in case of side effects or when owner's compliance is low), an alternative preventive measure may exist in housing pets at dusk in suitable compounds provided with entrances protected by nets with very small meshes (2-3 mm). It should be noted that, although theoretically being an effective approach to protecting dogs from sand fly bites, no studies are yet available on this mechanical approach in the canine species.