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# »Wichtige autoimmune/ immunvermittelte Erkrankungen«

**Leishmaniose - eine zunehmend wichtige Erkrankung auch in Deutschland: Neueste Erkenntnisse insbesondere zu Therapie und Langzeitmanagement**

## WorkShop

**Freitag 11. Juni 2010**

# Workshop on Canine Leishmaniosis Treatment and Prevention

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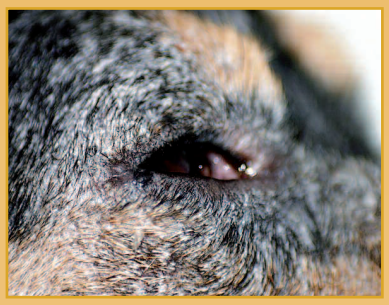
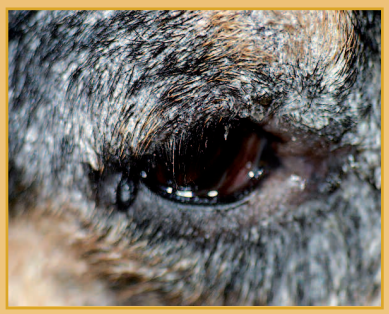




## Introduction

Of the 10 species of the genus *Leishmania* that have been isolated from dogs, *L. infantum* (Syn L. chagasi) is the most important. It has a wide geographical distribution, infecting dogs and humans mainly in Mediterranean countries, Portugal, West Africa, Southern Asia, Latin America and the USA. In recent years, this parasite tends to expand towards northern European countries, probably due to the global warming that favors the life cycle of the sandfly vectors. The remaining of this presentation will be devoted exclusively to the infection of dogs by *L. infantum* (Syn *L. chagasi*) and the term canine leishmaniosis (CanL) will refer to the relevant clinical disease.
For the needs of this workshop, the authors have elected to answer some important questions regarding the treatment and prevention of CanL as it appears in Europe and try to highlight any differences that may exist between endemic and non-endemic areas.

## Should dogs with leishmaniosis be treated or euthanized? Does decision making differ depending on whether the dog lives in an endemic or a non-endemic area? Are there cases where euthanasia should be considered?

(M. Saridomichelakis)

Euthanasia has been recommended for serolopositive dogs living **in endemic areas** in order to eliminate the reservoir of the parasite and block the transmission cycle. There are three conditions for euthanasia to be effective: a) dogs should be the only reservoir of *L. infantum*. This is not the case, since many other animal species can become infected; although their epidemiological role is of low significance, it is hard to predict what will happen in the hypothetical scenario that the primary reservoir will disappear, b) the percentage of dogs capable to transmit the parasite is low, so that their euthanasia is feasible and acceptable from an ethical and social point. Although dogs with CanL (2-5% of the canine population) are the most infectious, it is known that seropositive dogs (10-30% of the canine population), even when asymptomatic, can also transmit the parasite. There is still an open question regarding the infectivity of seronegative asymptomatically infected dogs that comprise 50-80% of the canine population living in endemic areas; if they are also infectious, blocking the transmission cycle using euthanasia would be practically equivalent to genocide, c) an effective program of massive screening and elimination of reservoirs should be implemented. This is not currently the case, at least in Europe. Also, the effectiveness of such a program would be compromised by the large numbers of stray dogs present in some endemic areas and, if serolonegative asymptomatically infected dogs are sources of infection, by the necessity of expensive diagnostic testing (e.g. PCR). These explain the limited effectiveness of programs of massive screening and elimination of serologically positive dogs in Latin America. An additional argument in favor of euthanasia is that drug-resistant parasites that may be transmitted to humans are avoided.
However, this can also be achieved by avoiding using the most effective drugs for human visceral leishmaniasis (e.g. amphotericin) in the treatment of CanL and by applying insect repellents on all infected dogs. Euthanasia has also been proposed for infected dogs living **in non-endemic areas** in an effort to prevent establishment of CanL. Non-endemic areas may be divided into those that are free of sandfly vectors and those where such vectors do exist, usually at a low density. In the former, introduction of infected dogs does not pose a real danger and in the latter the parasite will probably become established in the future and autochthonous cases will eventually appear. For all these reasons, this author does not favor euthanasia of infected dogs.
There are some CanL cases where **euthanasia may be considered**, including those with advanced renal (common) or liver (uncommon) failure. Most of these dogs will eventually succumb to the infection, despite antileishmanial and supportive treatment. Our usual approach is to emphasize the poor prognosis and, if the owner is still willing, to proceed with intensive treatment. Most of these patients die or are euthanized within a few weeks because of progressive clinical deterioration. The few dogs that may survive usually need lifelong supportive treatment and their organ failure will probably deteriorate.

## What are the aims of the treatment in canine leishmaniosis? Do they differ depending on the clinical picture or between dogs living in endemic or in non-endemic areas?

(M. Saridomichelakis)

In theory, the goals of **CanL treatment in endemic areas** are: a) to control the clinical manifestations of the disease, the clinicopathological abnormalities (e.g. anemia) and the CanL-associated organ pathology (e.g. renal lesions): although clinical cure and amelioration of most laboratory abnormalities is usually achievable, the evolution of some pathologic changes, such as glomerulonephritis, is unpredictable and they may improve, remain stable or even deteriorate, b) to prevent the recurrence of CanL due to either relapse or re-infection, c) to minimize the infectivity of treated dogs to sandfly vectors, d) to avoid the induction of drug-resistant strains of the parasite, and e) to treat any concurrent diseases. In order to achieve the first four goals, the aim of CanL treatment would be to completely eliminate the parasite and/or to change the immune response of the host. In theory, complete elimination of the parasite (parasitological cure) would control CanL-associated clinical signs and laboratory abnormalities, and would prevent recurrences, transmission to sandflies and induction of drug-resistant strains. However, it is now well known that this is rarely feasible, perhaps because parasitized cells are present in organs and tissues where therapeutic drug concentrations are not achieved and because the immune system of susceptible dogs is unable to completely eliminate the organism. Also, in the endemic areas it would be meaningless to focus on a target like this disregarding the immune status of the dog: even a parasitologically cured dog would probably become re-infected during the next transmission season and would develop CanL if its immune system was still unable to control unrestricted parasite multiplication. For these reasons, treatment of CanL should focus on the reduction of parasitic burden along with the induction of protective immune responses against *L. infantum*.
Regarding the **clinically normal, serologically positive or negative, infected dogs that live in endemic areas**, the goals of any medical intervention would be slightly different: instead of controlling and preventing the recurrence of CanL-associated clinical signs, laboratory abnormalities and organ pathology, avoiding their appearance should be the goal of any medical intervention. Also, the reduction of infectivity to sandfly vectors, although important for serologically positive asymptomatic dogs, it may or may not be an issue for their serologically negative counterparts. Most serologically negative and many of the serologically positive asymptomatically infected dogs (especially those with low antibody titers, negative lymph node and bone marrow cytology and positive leishmanin skin test) have already effective *Leishmania*-specific immune responses; for these dogs the avoidance of possible insults to their immune system (e.g. continuous exposure to sandfly bites, concurrent diseases, immunosuppressive treatment) is of paramount importance. On the contrary, for those dogs that are asymptomatic simply because they are in the incubation peri-

od of CanL, treatment should aim to the induction of protective immune responses, similar to the symptomatic dogs.
Finally, for symptomatic and asymptomatically infected dogs that live **in non-endemic areas** the only difference regarding the objectives of treatment is that re-infections and infectivity to sandflies are not a consideration.

### Should we treat a (healthy, serologically negative) *Leishmania*-PCR positive dog?
(C. Favrot)

PCR is a very sensitive test and some assays may be able to amplify the DNA of only one organism within one hundred cells. Regarding CanL, a positive PCR means that the dog has been infected with *leishmania* and still harbors some organisms. Some studies have shown that almost all dogs leaving in some endemic areas are PCR positive although most of them were healthy and negative in serology. In other words, infected does not mean ill. The fate of the disease will depend on the immune status of the dog. A healthy, serologically negative but PCR positive dog does not need any treatment. It is however wise to monitor the evolution of the disease carefully. A serology and protein profile every year may be regarded as a sufficient monitoring for such dogs. In case of lymphadenopathy however, lymph node cytology should be performed

### Is a (healthy, serologically negative) *Leishmania*-PCR positive dog dangerous for other dogs/humans?
(C. Favrot)

The high sensitivity of PCR assay allows the detection of individuals infected by very few organisms. The skin of PCR positive dogs is generally infected but in most cases with very few organisms. Additionally, direct contamination infected dog-other dog or human being is probably very rare or, at least, not epidemiologically significant, (Except vertical contamination and may be, via bite). Sandflies are necessary for all almost contaminations. It is however not proven yet that *leishmania*-permissive sandflies do exist in Northern Europe (the contrary is however not proven). It does mean that *leishmania*-permissive sandflies must be contaminated by the PCR positive dog (which is very unlikely because of the very low amount of organism in the skin/ blood of the PCR positive dogs) and that these sandflies have a blood meal on other dogs or humans.

This contamination is consequently very unlikely.

One must however keep in mind that a healthy, serologically negative, PCR positive dog is healthy and serologically negative because its immune system prevent any *leishmania* growth. The immune status of this dog may change and leishmania may subsequently begin to grow.
As well, sandflies seems to develop in Northern Europe and one cannot exclude the presence of *leishmania*-permissive sandflies in this part of Europe.
It is worth noticing that the best test for infectivity of infected animal is the so-called xenodiagnosis: laboratory sandflies are fed on the infected dog and subsequently tested for *leishmania* promastigotes. This test is however not available for routine analysis and is time consuming.

### Is it positive to predict if a healthy *Leishmania*-PCR positive dog will develop the disease?
(C. Favrot)

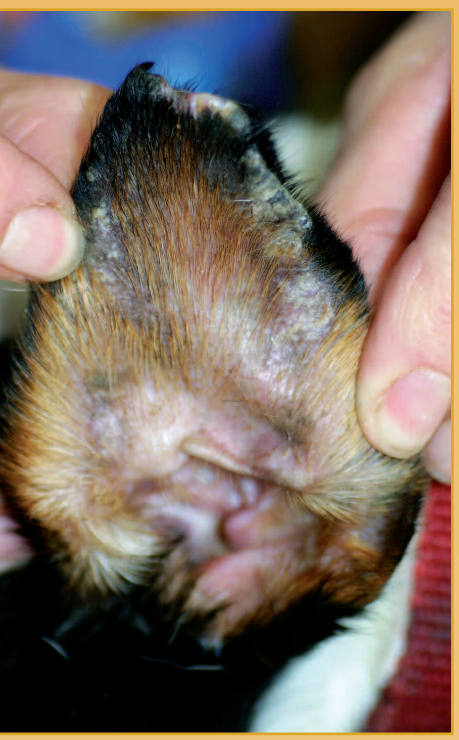
Unfortunately, it is not possible to predict the development of the disease in *leishmania*-infected healthy individuals. This development depends on the immune status of the dogs and this immune status may vary. Healthy infected individuals get a protective cellular immune response. Repetitive exposure to *leishmania*, concurrent infectious diseases, hormone imbalances, tumors may impair this response and lead to a less protective answer. It is consequently mandatory to monitor the evolution of the infection. Serology, protein profile and lymph node cytology are adequate for such monitoring.
It should be emphasized that repetitive exposures to leishmania dramatically increase the risk to develop the disease. Infected dogs should consequently be protected adequately against further sandflies bites in endemic areas.

### What are the characteristics of an ideal drug for the treatment of canine leishmaniosis? Does this drug exist?
(M. Saridomichelakis)

The **ideal drug** for the treatment of CanL should: a) be effective in accomplishing the goals (amelioration of clinical signs, clinicopathological abnormalities and organ pathology or prevention of their appearance; prevention of relapses; elimination of infectivity to sandflies; no induction of drug-resistant strains of the parasite) and aims (induction of protective immune responses; reduction or elimination of parasitic burden) of the treatment in most, or even all, treated dogs, b) be administered orally (this is of particular importance because it dictates whether dogs with CanL can be treated by their owners at home or if they need hospitalization that will greatly increase the cost of the treatment; for this reason this author prefers to classify the available drugs into those that are administered orally and those that necessitate parenteral administration), c) be safe, d) be reasonably priced, e) be registered for the treatment of CanL, and f) not be used as a first-line or res-cue drug for the treatment of human visceral leishmaniasis in the same area.
Besides many advances in the medical management of CanL, in-cluding refinement of dosing regimens (e.g. for pentavalent antimonials) and availability of new molecules (i.e. miltefosine), it is this author's opinion that **the ideal drug for the treatment of CanL does not currently exist**. For this reason combination treat-ment (e.g. allopurinol and miltefosine, allopurinol and pentavalent antimonials) is usually employed; the addition of allopurinol (that is administered orally, is generally safe and very cheap) in the chemotherapeutic protocol will increase its effectiveness, especially in terms of prevention of future relapses.

### What drugs can be used in the treatment of canine leishmaniosis and what's their mode of action?
(M. Saridomichelakis)

The **orally administered** drugs include miltefosine, allopurinol, azoles, fluoroquinolones, and metronidazole. **Miltefosine** leads to apoptosis-like cell death. It accumulates into macrophages, interacts with amastigote signal transduction pathways and inhibits phospholipid, sterol and plasma membrane synthesis.
Allopurinol is converted to allopurinol riboside and then to 4-aminopyrazolopyrimidine, a toxic analogue of ATP, ADP and AMP that blocks RNA and protein synthesis. Azoles (e.g. ketoconazole) inhibit cytochrome P450-mediated ergosterol synthesis thus leading to alteration of cell membrane fluidity and permeability.
Fluoroquinolones (enrofloxacin, marbofloxacin) may bind to topoisomerase (DNA gyrase) and inhibit DNA synthesis (similar mode of action against *Leishmania* and bacteria). **Metronidazole** interacts with protozoal DNA damaging its helical structure and causing strand breakage (similar mode of action against *Leishmania* and bacteria).
The **injectable drugs** include pentavalent antimonials, aminosidine, amphotericin B, and pentamidine. The active molecule of **pentavalent antimonials** (meglumine antimonate, sodium stibogluconate) is actually the trivalent antimony that is produced



by the reduction of pentavalent antimony in the macrophage (this explains why there drugs are far more effective for intracellular than for extracellular parasites) and subsequently taken by the amastigotes. The exact biochemical mechanisms that lead to amastigote apoptosis are still a matter of debate: they may include inhibition of ATP and GTP synthesis, disruption of glucose and fatty acid metabolism through structural and functional alternations of glucosomes, and inhibition of key enzymes (e.g. phosphofructokinase, pyruvic dehydrogenase). **Aminosidine (paromomycin)** achieves high intracellular concentrations in amastigotes, where it binds to 30S ribosomal subunit and interrupts normal protein synthesis (similar mode of action against *Leishmania* and bacteria). **Amphotericin B** induces apoptosis after irreversible binding to amastigote cell membrane ergosterol; it creates pores that alter membrane permeability and disrupt the ion gradient and osmotic balance of the parasite (similar mode of action against *Leishmania* and fungal organisms; similar mode of anti-*Leishmania* action with azoles). Also, at least in humans, amphotericin B activates macrophages and augments their oxidative burst through the production of TNF-a and IL-1. **Pentamidine** mainly damages parasitic DNA although some additional modes of action (inhibition of specific metabolic pathways, direct mitochondrial damage) have been proposed.

## Does it make sense to use terms like leishmanicide/ leishmaniostatic?

(C. Favrot)

The term leishmanicide is probably misleading as it suggests that such treatment may be associated with a parasitical cure, which is often not the case. It is very important to explain the owners that *leishmania*-infected dogs will remain infected and that the goal of the treatment is to control *leishamnia* growth and the consequence of the disease. The ability of one specific drug to kill the organism *leishmania* (leishmanicide) in comparison with other drugs that only prevent further growth (leishmaniostatic) does not mean that all organisms in the infected dog will be killed (same situation as for staphylococci for example). It may however mean that the number of living organisms within the host will be reduced more quickly and that the clinical improvement will be marked. The main goal of the therapy is to reduce the parasite burden in order to positively contribute to the restoration of the macrophage ability to kill the parasites.

## Is miltefosine the best available treatment for canine leishmaniosis?

(C. Favrot)

It is probably too early to answer this question thoroughly. The first clinical studies are encouraging but the clinical experience of the some practioners may be slightly different. In comparison with glucatime, miltefsosine presents some advantages like the lack of kidney side-effects (even if the nephrotoxicity of glaucantime appears limited) and the way of administration. When compared to allopurinol, one should mention that miltefosine is presented as leishmanicide (allopurinol is leishmaniostatic): see above for comments on this aspect. Studies directly comparing the effeicacy of allopurinol and miltefosine in monotherapy have not been made but are needed.
One should however confirm the long-term efficacy and tolerance of the drug

## Is it reasonable to treat with allopurinol only?

(C. Favrot)

Most clinician who had used allopurinol in monotherapy reported dramatic clinical improvement and maintenance of this improvement during therapy, at least in non-endemic areas. One controlled-study also confirms this efficacy in endemic areas. On the other hand, it is also obvious that some allourinol-treated leishmanosis dogs do not respond anymore after several years of treatment, suggesting that *leishmania* may become allopurinol-resistant. However allopurinol-resistance has only been proven in vitro but was never firmly demonstrated in vivo. Other possible explanations for this loss of efficacy could be the lack of compliance or concurrent diseases. The potential development of allopurinol-resistant strains in endemic areas can be regarded as a major concern. On the contrary, in non endemic areas, such resistant strains have virtually no chance to develop.
As resistances seem to develop after several months to years of continuous therapy, an option would be to discontinue the treatment after initial improvement and to treat recurrence. This option should only be considered after several negative re-checks assessing that serology titers are low, globulinemia in normal ranges and proteinuria and lymph nodes cytology negative. This option requires however a subsequent careful monitoring. It is also worth noticing that no study has demonstrated the benefit of such an approach.
Another option would be to use an allopurinol pulse-therapy (one week per month). This approach has been documented in a field study.
It is worth noticing that LeishVet- a group of experts- supports life-long daily treatment to prevent relapses.

## Why resistance to antileishmanial medication may develop? How we can prove it? What can we do to avoid or delay it?

(M. Saridomichelakis)

The same cellular and subcellular **mechanisms** that underline bacterial resistance may result in *Leishmania*-resistance to every medication we may use. Apart from innate resistance, mechanisms of acquired resistance include: a) reduced penetration of the medication into the infection site. This may be one of the reasons why parasitological cure is rarely if ever achieved, since most of the drugs do not achieve therapeutic concentrations in organs and tissues such as intraocular structures and central nervous system, b) amastigote efflux pumps that expel the agents. For example, one mechanism of resistance to pentavalent antimonials is the induction of P glucoprotein that results in 2-5 times lower intracellular concentrations of the drug, c) inactivation of the drug (e.g. mutation or dowregulation of the enzyme ACR2-Pentostam reducatase that normally reduces pentavalent antimony to its trivalent counterpart), and d) modifications of the target of the drug. For a dog with CanL resistance to antileishmanial medication may be present from the beginning (i.e. the dog has been infected by an already resistant strain) or it may develop during the course of the treatment.
Some **laboratory methods** for the *in vitro* evaluation of *Leishmania* susceptibility to various chemotherapeutic agents have been developed. However, they are not usually available to the practitioner and the correlation between their results and treatment outcome has not been extensively evaluated. Resistance is **clinically suspected**: a) when the goals of the treatment are not achieved after an adequate time period. For example, it is known that the combination of meglumine antimonate (for a month) and allopurinol (on a long-term basis and sometimes for life) should result in dramatic clinical and clinocopathological improvement within 15-30 days, whereas the relevant figures for pentavalent antimonial monotherapy, for allopurinol and for metronidazole-spiramycin are 30-60 days, 30-180 days, and 15-45 days, respectively, b) when some parameters deteriorate (e.g. re-appearance of clinical signs and/or laboratory abnormalities, increased antibody titers, increased lymph node parasitic density etc) despite ongoing treatment. This is of particular importance in the case of allopurinol that is the only medication given on a long-term basis, due to its oral administration, safety and low cost. However, it is emphasized that true resistance can only be confirmed *in vitro* and before attributing a treatment failure to resistant strains the numerous alternative explanations for a poor therapeutic outcome

should be excluded (e.g. low doses, infrequent administration, short duration of the treatment, permanent pathologic changes, concurrent diseases etc).
The two most important factors that contribute to resistance are the exposure of the parasite to low drug concentrations for a short period and the repeated administration of the same medication. Therefore, to delay development of resistant strains, it is recommended to avoid underdosing at any cost and, in case of relapse, not to use many times the same "leishmanicidal" medication to the same patient (e.g. this author does not use miltefosine or pentavalent antimonials for more than two "cycles" of treatment of one-month each in relapsing patients).

## How should I monitor the treatment?
(C. Favrot)

Treatments with effective anti-leishmania drugs are usually associated with rapid improvement of the clinical signs. It is however currently not know if and when treatment should be discontinued. It is well know that serology titers are not strictly connected to the severity of the illness but rather linked to the strength of the humoral response, which is known to be non-protective. Titers however usually decline during successful therapy.
A reasonable option for monitoring disease development will be to follow serology titer, globulinemia and proteinuria as globulinemia is a very sensitive marker of the disease and the latter an important prognostic factor. Lymph nodes cytology should also be performed, especially in case of lymphadenopathy.

## Is the treatment of canine leishmaniosis lifelong or we could discontinue it at some time?
(M. Saridomichelakis)

In principle, treatment of dogs with CanL that live in endemic areas can be discontinued after parasitological cure provided that highly effective preventative measures will be instituted to avoid re-infections, whereas parasitological cure is only needed for dogs residing in non-endemic areas. Unfortunately, complete eradication of parasites is hardly, if ever, achieved and available preventative measures are not 100% effective. Therefore, we have to rely on the restoration of *Leishmania*-specific immune responses of the host to avoid relapses after treatment discontinuation.
Because of lack of strong scientific evidence, the following recommendations are mainly based on anecdotal information, experts' opinions and personal clinical experience. Treatment of CanL cannot be stopped before at least one year of continuous allopurinol administration, along with a "leishmanicidal" medication (e.g. miltefosine, meglumine antimonate) for the first month; the latter may be administered for a second one-month period (e.g. at the sixth month) if clinical and laboratory abnormalities are still present. Afterwards the dog should be re-examined, usually every six months, and every time the minimum data base should include a thorough physical examination, hematology, serum biochemistry (with or without protein electrophoresis), serology and lymph node cytology. When, at some time point, the dog is clinically normal, laboratory results are within normal limits, antibody titers markedly decreased (although they do not always become negative) and lymph node cytology is negative (at least 100 oil immersion fields should be carefully examined) a decision can be made to either discontinue treatment or to proceed with secondary prophylaxis (e.g. periodic administration of allopurinol for one week per month)-this author usually prefers the latter. In every case, the dog should be carefully monitored and the aforementioned examinations should be repeated, ideally every 6 months, for the remaining of its life. Every single indication of imminent relapse (i.e. reappearance of mild clinical signs, anemia, hyperglobulinemia or proteinuria, increased antibody titer, increased lymph node parasitic density) should prompt treatment re-institution.

Ancillary tests that may or may not be practical, depending on the particular setting, and may be helpful in the decision making process include: a) bone marrow cytology. Besides invasive sampling, the smear quality is usually superior than in lymph node cytology, b) quantitative PCR (real-time PCR) preferably in bone marrow samples. It gives more accurate information on the parasitic density (and thus indirectly for the parasiticidal activity of the medication and the effectiveness of *Leishmania*-specific cellular immunity of the dog) compared to cytology. In the past, negative bone marrow PCR on two occasions separated by 6 months had been proposed as a criterion of parasitological cure and treatment discontinuation. Currently, with the advances in PCR methodology, a negative result is a rarity; furthermore, a negative result does not guarantee the absence of viable parasites in other body tissues, c) leishmanin skin test. It examines the delayed-type hypersensitivity after intdradermal injection of *Leishmania* antigen and is usually positive in resistant dogs (where treatment may be discontinued) and negative in the susceptible ones.

## How we define the recurrences of treated dogs? What should we do in case of a relapse?
(M. Saridomichelakis)

Strictly speaking, recurrence of CanL is the re-appearance of the clinical signs at some time point after seemingly effective treatment. However, in a broader sense, recurrence may also include the re-appearance of clinically important laboratory abnormalities that indicate organ pathology (e.g. anemia, proteinuria) even without associated clinical manifestations. These recurrences are usually preceded by an increase of *Leishmania*-specific antibody titers, increase of serum globulins, alternations of proteinogram and increased parasitic density (e.g. upon lymph node cytology).

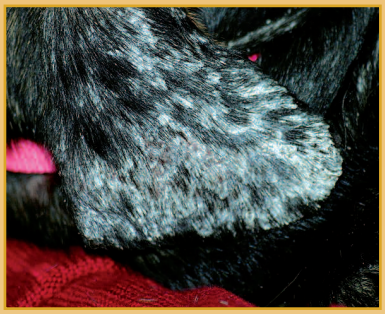
Clinician's action in the case of recurrence depends on whether the dog was or was not on maintenance treatment (i.e. continuous administration of allopurinol) or secondary prophylaxis (i.e. period administration of allopurinol). When the recurrence occurs a few months or even years after discontinuation of the medication, which is a very common scenario, allopurinol, either alone (mild or no clinical signs, mild laboratory abnormalities) or in combination with a "leishmanicidal" drug, should be re-instituted. When it occurs in a dog on secondary prophylaxis, continuous allopurinol administration with or without a "leishmanicidal" drug should be considered. Finally, recurrences of dogs already on continuous allopurinol treatment should be treated with the addition of a "leishmanicidal" drug. In every case it is emphasized to avoid the repeated use of the same "leishmanicidal" medication in order to avoid the induction of resistant strains. Also, it is highly important to thorough investigate all these dogs for concurrent diseases that may alter their immune responses and render them susceptible to CanL.

## Are glucocorticoids contraindicated in dogs with leishmaniosis?
(M. Saridomichelakis)

Systemic glucocorticoids at anti-inflammatory or even immunosuppressive doses are frequently needed to control some clinical manifestations of CanL, such as uveitis, polyarthritis and epistaxis. Also, their short-term (e.g. for three weeks) addition to the treatment regiment has been associated with faster resolution of clinical signs, improvement of thrombocytopathy and normaliza-

tion of albumin/globulin ratio. Finally, systemic glucocorticoids are occasionally needed to treat concurrent diseases or conditions such as pemphigus folliaceus, immune-mediated hemolytic anemia and immune-mediated thrombocytopenia. Topical glucocorticoids may be needed for the treatment of CanL-associated keratitis and anterior uveitis.
The disadvantages of systemic glucocorticoid administration, especially at high doses, include: a) their effects on the immune system that may downregulate *Leishmania*-specific immune responses through various mechanisms (e.g. reduced lymphocyte counts, increased expression of mannose receptors on macrophages, upregulation of Th2-like cytokines), b) enhanced parasite survival through increased serum iron concentration, increased transferin saturation and decreased serum cooper concentration, and c) deterioration of pre-existing renal failure.
For these reasons, this author uses systemic glucocorticoids only when needed (usually in cases with epistaxis, uveitis and concurrent immune-mediated diseases) and at the minimum effective dose and duration of administration.

### How can we define a case of autochthonous canine leishmaniosis? Do these cases exist in Germany/northern Europe?
(M. Saridomichelakis)



An autochthonous case means that the dog has become infected by *L. infantum* in the area of its residency. It is important to realize than a small number of autochthonous cases does not necessarily mean that the area has become endemic. Endemic foci of CanL may be divided into the stable and the unstable ones: stable endemic foci are characterized by continuous dog-to-dog transmission through sandfly bites and hence by the appearance of new cases on a yearly basis, whereas in unstable foci indisputable autochthonous cases are seen only sporadically and in low numbers. However, a few sporadic cases may also occur after infected dogs are introduced in a non-endemic area because of non-sandfly mediated transmission of the parasite (e.g. using alternative vectors such as ticks, mosquitoes and fleas; after direct or vertical transmission, through blood transfusion and after implantation of infected transmissible venereal tumor cells). Therefore, areas with sporadic autochthonous cases may represent either unstable foci of CanL or non-endemic foci where the parasite is transmitted through alternative modes.

### Do cases of autochthonous canine leishmaniosis exist in Germany/northern Europe?
(C. Favrot)

Several well (and less well-) documented cases suggest that autochtonous cases do exist in Switzerland and Germany. Affected regions are mainly Tessin and Geneva (Rhone valley) in Switzerland and Bade-Würtemberg (the author has observed two well-documented cases in dogs born and always living near Freiburg in Brisgau) and Bavaria in Germany. The explanation for that could be a) non sandfly-mediated transmission b) transmission by sandflies living in northern Europe and not identified yet as leishmania-permissive c) the extension to the north of the living area of traditional vectors such as phlebotomus perniciosus. Explanation c) is the more logical for Tessin and Geneva cases but is very unlikely for cases observed in Germany. For those cases, the more logical explanation would be the presence of autochtonous yet unidentified permissive vectors infected by untreated dogs living permanently in this area. This point emphasizes the importance not only of treating clinical cases of CanL but also to monitor carefully leishmania-infected healthy individuals.

### Are protective treatments protective enough?
(C. Favrot)

Most studies addressing this question led to similar conclusions: Protective treatments are associated with a protection rate ranging for 80 to 90%. Most of these studies were however carried out in drastic conditions (heavily infected areas, dogs living outdoor in groups etc...). It can consequently be anticipated that these treatments are very effective for pet dogs. Repetitive exposure to infected sandflies bites is known to increase the risk of developing the disease. In this regard, even a non perfect protection should be considered useful. It must however be kept in mind that these treatment alone should not be regarded as fully protective and that some other precautions should be taken.

### What should I explain to an owner who wants to go to southern Europe with his/her dog?
(C. Favrot)

a. Use protective treatment
b. Apply them at least several days before the trip
c. Renew this treatment if necessary
d. Do not walk the dog during the activity period of the sand flies ( end of the afternoon, beginning of the night)
e. Avoid places where sandflies usually develop (lakes, ponds, bushes etc. . .)
f. Preventive examination 6 months after returning in non-endemic area. For such a preventive examination serology and lymph node cytology should be performed and globulinemia and proteinuria measured.

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