

Review

Treatment of canine Old World visceral leishmaniasis: a systematic review

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Abstract Canine visceral leishmaniasis is a systemic disease caused by *Leishmania infantum*. The aim of this systematic review was to identify and evaluate the evidence of efficacy of interventions for treatment or prevention of canine visceral leishmaniasis, and to propose recommendations for or against their use. Forty-seven articles describing clinical trials published between 1980 and 2004 fulfilled selection criteria. The evaluation of clinical trials provided good evidence for recommending the use of meglumine antimoniate at a minimum dosage of 100 mg kg⁻¹ daily for at least 3–4 weeks, combined with allopurinol in order to obtain a good clinical efficacy and a reduced relapse rate. The evaluation of the articles also provided fair evidence for recommending the use of pentamidine (4 mg kg⁻¹ twice weekly) and aminosidine (5 mg kg⁻¹ twice daily) for 3–4 weeks. There was insufficient evidence for recommending the use of allopurinol alone, amphotericin B, buparvaquone, ketoconazole, enrofloxacin, and the combinations of metronidazole with spiramicyn or metronidazole with enrofloxacin. Fair evidence against the use of aminosidine at high dosages (20–80 mg kg⁻¹ per day) was proposed due to its side effects. Evaluation of articles on repellent measures against sand fly vectors of leishmaniasis provided good evidence for recommending deltamethrin collars and fair evidence for recommending spot-on permethrin.

INTRODUCTION

Old World canine visceral leishmaniasis is a severe systemic disease caused by the diphasic protozoan parasite *Leishmania infantum*. The geographical distribution of the disease is dependent on its insect vector, the sand fly *Phlebotomus* spp., and encompasses the Mediterranean basin and the whole Iberic peninsula.^{1,2}

Common clinical signs include skin lesions such as exfoliative dermatitis, papules and small nodules, ulcerations and crusts, and partial alopecia. Systemic signs include weight loss, generalized lymphadenopathy, ocular lesions, chronic diarrhoea, epistaxis, locomotory problems and muscle atrophy. Renal failure is the most common cause of death. The most frequent clinical laboratory findings are polyclonal hyperglobulinaemia, hypoalbuminaemia, decreased albumin/globulin ratio, hyperprotidaemia, thrombocytopenia, nonregenerative anaemia and raised kidney values.^{1,2}

The most accurate diagnosis is obtained by the direct observation of parasites in cytological preparations or histological sections of lymph nodes, bone marrow or skin, and by identification of parasitic DNA in host tissues with polymerase chain reaction (PCR). Measurement of serological titres of antileishmania antibodies

by means of indirect immunofluorescence (IFA) or enzyme-linked immunosorbant assay (ELISA) may be suggestive of the disease but is considered insufficient as a sole laboratory diagnosis: positive titres must be accompanied by compatible clinical signs in order to be relevant to diagnosis.^{1,2}

The host's immune system plays a pivotal role in the establishment of infection and in outcome of therapy.^{2,3} In susceptible animals, there is an impairment of cell-mediated immunity and a high production of non-protective antibodies, which are detrimental and contribute to the development of some of the clinical signs. In resistant dogs, an efficient cell-mediated immune response is able to circumscribe and eliminate the infection, preventing the development of clinical disease.³ The type of immune response is also dependent on the number of parasites present in the body, and it is thought that treatments leading to a reduction in parasite load are able to shift the immune system towards a more efficient cell-mediated response.⁴ However, so far, hardly any therapy has been shown to be able to completely eliminate parasites from infected organisms, so that in susceptible animals, even if a temporary clinical remission is achieved, a relapse is to be expected weeks to years after drug withdrawal.^{5,6} Because recurrences are frequent, affected animals should be monitored for months to years before being declared permanently cured. Unfortunately, in spite of extensive research, no effective preventive vaccination

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has been developed so far. To date, the only possible preventive therapy against canine visceral leishmaniasis is the use of repellents against sand fly bites by means of collars or topical solutions.⁵

In past decades, several drugs have been tried for treatment of canine leishmaniasis. There are a few recent articles^{5,6} that report an overview of some of the therapies used against canine visceral leishmaniasis, but to the authors' knowledge, no attempts to systematically review and compare the efficacy of medications have ever been made. The aim of this systematic review is to identify and evaluate the evidence of efficacy of molecules and/or therapeutic protocols used to treat or prevent canine visceral leishmaniasis and, when possible, to propose recommendation for or against their use.

METHODS

Search methods

Electronic searches were performed using the MEDLINE database and CAB abstracts using 'therapy and leishman* and dog' as keywords. Furthermore, the four volumes of *Advances in Veterinary Dermatology* were searched for articles related to this review.⁷⁻¹⁰ These volumes contain peer-reviewed original articles presented at previous World Congresses of Veterinary Dermatology.

Within relevant articles, all references were examined for additional pertinent citations. Ultimately, a message was sent to the Vetderm Listserv (12 March 2004) for further detection of clinical trials in the process of being, or recently accepted for publication.

Identification of studies

This systematic review considered exclusively clinical trials published between 1980 and 2004 in scientific journals. Leishmaniasis was defined as an infection caused by *Leishmania infantum* sp. Studies evaluating therapy were included only if the diagnosis was based on the presence of clinical signs and the direct identification of leishmania through cytology, histopathology or parasite DNA by polymerase chain reaction and/or the determination of the serum antibody titre via IFA or ELISA. Typical changes of the protein electrophoresis pattern were considered as an additional criterion, whilst being inadequate and insufficient if the sole supportive diagnostic test.

Languages of publication did not represent a limitation for the evaluation of any study. Clinical trials describing less than five patients were excluded. Only clinical trials with the objective of treatment of dogs affected by leishmaniasis were evaluated, and studies had to report clinical outcome with or without laboratory evaluations.

Data extraction

Studies that met the above criteria were independently reviewed by both authors. The following aspects were analysed: study design and methodological quality, subject

enrolment quality, type of interventions and treatment outcome. Data were abstracted in tables, and results were compared. When discrepancies between assessments of authors were found, they were discussed and amended.

Quality assessment

Assessment of methodological quality. The same approach used by Olivry and Mueller¹¹ for assessment of methodological and subject enrolment qualities was applied to this study. Randomization, masking of clinicians and/or owners and intention to treat analysis were evaluated as the three main parameters to determine the risk of biased estimates of treatment success. These parameters were judged as *adequate*, *inadequate*, *unclear* or *not applicable*.

Afterwards, an overall *grade of evidence quality* derived from the scheme proposed by the authors cited above was assigned for each study, as follows:

- A Blinded randomized controlled trial (control with either active drug or placebo).
 - B Controlled trial lacking either blinding or randomization.
 - C Open, uncontrolled study.
 - D Cohort study, case-control analytic study, descriptive study, case report.
- 1 > 50 subjects per group.
 - 2 20–50 subjects per group.
 - 3 10–19 subjects per group.
 - 4 < 10 subjects per group.

Assessment of subject enrolment quality. The quality of the diagnosis of leishmaniasis of the subjects included was determined for each study as follows:

Well defined. Animals showing clinical signs compatible with leishmaniasis, in which the disease could be confirmed by parasitological methods: direct microscopic identification of the parasites through cytology or histopathology or demonstration of parasite DNA using the polymerase chain reaction.

Fairly defined. Animals showing clinical signs compatible with leishmaniasis, in which the disease was diagnosed by the sole determination of high antibody titres via IFA or ELISA serology.

Poorly defined. Animals showing clinical signs compatible with leishmaniasis, but in which the disease was not confirmed by parasitological or serological methods. Studies with such inclusion criteria were not considered further.

Assessment of outcome measures. The following main clinical outcome measures were taken into consideration:

Clinical efficacy. Expressed as percentage of treated dogs that showed either complete or nearly complete clinical remission or partial clinical remission.

Relapse rate. Expressed as percentage of animals treated that showed a relapse after treatment was stopped and within a certain time frame, which can be either the follow-up period of the entire study or another shorter period.

Survival rate. Expressed as percentage of animals treated that were still alive after a determined number of years (usually 1–5).

The following main laboratory outcome measures were taken into consideration:

Parasitological clearance. Expressed as percentage of animals treated in which parasites became undetectable after therapy, and the method used for such assessment (PCR, cytology or tissue culture).

For each treatment type, side and adverse effects were recorded and collated for evaluation.

Reporting of qualitative results

Study design, patient enrolment quality, treatment molecule and its dose and duration, clinical and laboratory outcome and side effects were reported for each type of therapy in a tabular form. The recommendation for use of these drugs for the treatment of Old World canine visceral leishmaniasis was adapted from those proposed by Olivry and Mueller¹¹ and Mueller.¹²

- 1 When more than one study, including at least one well-designed controlled trial (CT), providing sufficient outcome details and at least a 12-month follow-up period, supports high efficacy of the drug or protocol tested, there is *good evidence for* recommending the use of this medication.
- 2 When at least one well-designed CT with at least a 12-month follow-up period, or several CT studies (CTs) without a similar follow-up period support efficacy of the drug or protocol tested, there is *fair evidence for* recommending the use of this medication.
- 3 When CTs are not available, or when only one CT without a follow-up period was conducted, or when multiple studies yield controversial evidence of treatment effect, it was concluded that there is *insufficient evidence for* recommending the use of this medication.
- 4 When at least one well-designed CT or several less detailed studies support lack of efficacy of the drug or protocol tested, or efficacy is associated with common harmful events, there is *fair evidence against* recommending the use of this medication.
- 5 When more than one study, including at least one well-designed CT, supports lack of efficacy of the drug or protocol tested, or supports any efficacy with unacceptable side effects, there is *good evidence against* recommending the use of this medication.

RESULTS

About 120 articles were retrieved, 47 of which fulfilled our selection criteria. Four were written in Italian, four in German, five were in French, and the remainder were in English. The 47 trials dealt with 14 different interventions with single or multiple molecules. There was only one blinded, randomized, controlled trial (quality of evidence grade A), 20 controlled studies lacking blinding and/or randomization (grade B) and 26 open, uncontrolled studies (grade C). Only one study contained dogs treated with placebo.

Antimonial compounds (Table 1a, b, c)

Twenty studies assessing the efficacy of antimonial compounds were identified,^{13–32} of which 17 evaluated the efficacy of traditional meglumine antimoniate,^{15–30,32} one described the efficacy of liposome-encapsulated N-methylglucamine antimoniate³¹ and two reported the efficacy of sodium stibogluconate.^{13,14} Within the 20 studies mentioned, 12 were uncontrolled, three compared the efficacy of meglumine antimoniate to that of aminosidine or aminosidine and meglumine antimoniate, two compared it with that of allopurinol alone or in combination with meglumine antimoniate, one compared it to that of enrofloxacin or enrofloxacin associated with metronidazole, and finally one compared it to that of LiF2 (*Leishmania infantum*-derived Fraction 2) or LiF2 combined to meglumine antimoniate. None of these studies was blinded or randomized.

The overall number of examined dogs was 873 (including a retrospective study encompassing 227 cases), and the number of dogs treated with meglumine antimoniate was 649 (74%). Meglumine antimoniate was administered subcutaneously (every 12, 24 or 48 h), intramuscularly (every 24 or 48 h) or intravenously for an initial period varying between 1 and a half and 7 weeks. In one study, the treatment was carried out for 25 weeks. Depending upon the response and the occurrence of relapses, the initial course was often followed by further treatments of the same duration.

A complete or nearly complete clinical remission was observed in 25–100% of the cases. Treatments of less than 3–4 weeks and intermittent treatments (10 days on, 10 days off) were usually associated with a lower clinical efficacy.^{14,16,19,29}

Total daily dosages usually varied between 50 and 150 mg kg⁻¹ per day. When treatments were carried out at this dose for a minimum of 3–4 weeks, a fairly high rate of complete or nearly complete clinical remission, ranging between 66.7% and 100% was obtained.^{17,21,25–27,32} Better results were achieved if therapy was given for 4 rather than 3 weeks.

Table 1a. Trials evaluating the efficacy of antimonials for canine visceral leishmaniasis (1985–88)

Citation (reference)	13	14	15	16	17	18
Quality of evidence	B4	C3	C2	C1	C2	C1
Randomization (allocation generation)	unclear	n.a.	n.a.	n.a.	n.a.	n.a.
Randomization (allocation concealment)	unclear	n.a.	n.a.	n.a.	n.a.	n.a.
Masking of outcome assessor	unclear	n.a.	n.a.	n.a.	n.a.	n.a.
Intention-to-treat analysis	Not done	n.a.	n.a.	n.a.	n.a.	n.a.
Quality of inclusion of study subjects	well defined	fairly defined	fairly defined	fairly defined	well defined	fairly defined
# Dogs entered in trial (total)	16	13	43	81	39	n.a. (retrospective study)
# Dogs dropped out	0	3	0	0	0	n.a.
# Dogs treated with antimonial	11	10	43	81	39	227
Type of antimonial	stibogluconate	stibogluconate	meglumine	meglumine	meglumine	meglumine
Treatment of controls	none	n.a.	n.a.	n.a.	n.a.	n.a.
Dose of antimonial used	A: 50 mg kg ⁻¹ q24 h IV for 10 days, then course repeated after 10 days –3 dogs B: 10 mg kg ⁻¹ q24 h IV for 10 days, then course repeated after 10 days –3 dogs C: 10 mg kg ⁻¹ q12 h IV for 10 days one course –5 dogs	10–20 mg kg ⁻¹ q24 h IV	100–200 mg kg ⁻¹ q48 h SC for 20 injections	100 mg kg ⁻¹ q24 h IM	100 mg kg ⁻¹ q24 h	50–100 mg kg ⁻¹ IM, SC or IV q48 h for 30 days, then course repeated after 30 days until serology < 1 : 100
Length antimonial treatment (weeks)	1–2 × 1.5	1.5–2	6, repeated for each relapse or every 6 months	10 days, then 10 days off, then other 10 days	3–4 repeated at each relapse up to 10 years	minimum 2 × 4
Length of follow up (months) after treatment withdrawal	5	1–32	8–24	4–24		12–24
Other pharmacological intervention	none	none	prednisolone 1–2 mg kg ⁻¹ q24 h for 6 weeks then tapered and stopped over 2 weeks	none	fluid therapy	chophytol
Complete or nearly complete clinical remission	n.r.	40%	93%	35%	66.7%	n.r.
Partial clinical remission	n.r.	60%	n.r.	20%	2.5%	near 100%
Treatment failure	n.r.	0%	6.9%	45%	30.8%	n.r.
Relapse rate	n.r.	40%	81.4% within few months	n.r.	96% within one year	n.r.
Survival rate	n.r.	n.r.	n.r.	n.r.	61.5% after 1 year 38.5% after 2 years 15.3% after 4 years	n.r.
Parasitologic clearance	yes after 150 days in A and B	n.r.	n.r.	n.r.	n.r.	n.r.
Side-effects	0%	Diarrhea, lethargy (percentage not reported)	18.6%	n.r.	yes*	n.r.

n.r. not reported, n.d. not done, n.a. not applicable *weakness, inappetence, anorexia, vomit, diarrhoea, inflammation at injection sites.

Table 1b. Trials evaluating the efficacy of antimonials for canine visceral leishmaniasis (1992–97)

	19	20	21	22	23	24
Citation (reference)	19	20	21	22	23	24
Quality of evidence	C2	B4	B3	B2	C3	C4
Randomization (allocation generation)	n.a.	unclear	unclear	unclear	n.a.	n.a.
Randomization (allocation concealment)	n.a.	unclear	unclear	unclear	n.a.	n.a.
Masking of outcome assessor	n.a.	unclear	unclear	unclear	n.a.	n.a.
Intention-to-treat analysis	n.a.	n.d.	unclear	n.d.	n.a.	n.a.
Quality of inclusion of study subjects	well defined	well defined	fairly defined	fairly defined	well defined	fairly defined
# Dogs entered in trial (total)	36	24	22	61	10	42
# Dogs dropped out	0	0	10	0	0	0
# Dogs treated with antimonial	36	8	12	34	10	8
Type of antimonial	meglumine	meglumine	meglumine	meglumine	meglumine	meglumine
Treatment of controls	n.a.	8 only LiF2* antigen 8 meglumine + LiF2 antigen	aminosidine	meglumine + allopurinol	none	n.a.
Dose of antimonial used	200–300 mg kg ⁻¹ q24 h SC or IV for 10 days, course repeated after 10 days	300 mg kg ⁻¹ IM q48 h 20 injectons	150 mg kg ⁻¹ q24 h IV	200 mg kg ⁻¹ q48 h SC	300 mg kg ⁻¹ q48 h for 20 times IM	100 mg kg ⁻¹ SC q48 h for 20 times
Length antimonial treatment (weeks)	2 × 1.5	6	4	7	6	6
Length of follow up (months) after treatment withdrawal	18	4.5	1	12–60	2	none
Other pharmacological intervention	none	leishmania antigen LiF2 50 µg IM once weekly for three weeks	none	none	none	none
Complete or nearly complete clinical remission	50%	25%	37.5	100%	53%	100%
Partial clinical remission	25%	75	62.5	n.r.	15%	n.r.
Treatment failure	25%	n.r.	n.r.	0%	32%	0%
Relapse rate	n.r.	n.r.	n.r.	n.a.	32%	n.r.
Survival rate	n.r.	n.r.	n.r.	n.r.	53% after 2 years 15% after 5 years	n.r.
Parasitologic clearance	10% after 18 months, culture	25% cytology bone marrow	37.5% cytology bone marrow	n.r.	n.r.	100% cytology
Side-effects	local swelling 5%	n.r.	n.r.	0%	53% induration at site of injection. 15% systemic signs	n.r.

n.r. not reported, n.d. not done, n.a. not applicable *LiF2: Leishmania infantum-derived Fraction 2.

Table 1c. Trials evaluating the efficacy of antimonials for canine visceral leishmaniasis (1997–2004)

Citation (reference)	25	26	27	28	29	30	31	32
Quality of evidence	B2	B3	B3	B2, B3	B4	C4	C4	B3
Randomization (allocation generation)	unclear	unclear	unclear	inadequate	unclear	n.a.	n.a.	unclear
Randomization (allocation concealment)	unclear	unclear	unclear	inadequate	unclear	n.a.	n.a.	unclear
Masking of outcome assessor	unclear	unclear	unclear	Inadequaten.	unclear	n.a.	n.a.	unclear
Intention-to-treat analysis	adequate	unclear	unclear	inadequate	unclear	n.a.	n.a.	n.d.
Quality of inclusion of study subjects	fairly defined (well in 32/41)	fairly defined	well defined	well defined	well defined	well defined	well defined	well defined
# Dogs entered in trial (total)	41	24	32	96	16	8	6	36
# Dogs dropped out	3	0	0		1	0	0	0
# Dogs treated with antimonial	38	12	10	40	6	6	6	12
Type of antimonial	meglumine	meglumine	meglumine	meglumine	meglumine	meglumine	liposomal antimony	meglumine
Treatment of controls	n.a.	aminosidine	aminisidine 11, aminosidine and meglumine 11	allopurinol alone 11, meglumine + allopurinol 45 dogs	meglumine + allopurinol 7 dogs, allopurinol alone 3 dogs	n.a.	n.a.	enrofloxacin 20 mg kg ⁻¹ q24 h PO 12 dogs, enrofloxacin 20 mg kg ⁻¹ q24 h PO + metronidazole 10 mg kg ⁻¹ q24h PO 12 dogs 50 mg kg ⁻¹ q12 h SC
Dose of antimonial used	A: 50 mg kg ⁻¹ q12 h SC B: 100 mg kg ⁻¹ q24 h IV for 3 weeks, repeated if remission not achieved, changed from IV to SC and vice versa	150 mg kg ⁻¹ q24 h IV	100 mg kg ⁻¹ q24 h SC	200 mg kg ⁻¹ sc q48 h until clinical resolution	meglumine 100 mg kg ⁻¹ q24 h IV + allopurinol 10 mg kg ⁻¹ q12 h	75 mg kg ⁻¹ q12 h SC for 10 days, course repeated after 10 days	9.8 µg kg ⁻¹ q24 h Sb SC	
Length antimonial treatment (weeks)	3 (+ 3)	6	3	12–25	1.5, repeted after 2 weeks	1.5 + 1.5	1.5	4
Length of follow up (months) after treatment withdrawal	12–24 (for animals undergoing cross-over)	1	6	60	9–48	12	12	3
Other pharmacological intervention	none	none	none	none	none	none	none	none
Complete or nearly complete clinical remission	78%	100%	70%	55%	33%	83.3	100%	about 70%
Partial clinical remission	7.3%	n.r.	30%	n.r.	50%	n.r.	n.r.	n.r.
Treatment failure	14.6%	0%	0%	45%	0%	16.7	0%	30%
Relapse rate	74.3% within 1 year	n.a.	70%	15%	no	100% after 6–10 months	0% after 1 year	16% in 3 months
Survival rate	average 2 years, 75% 4 years	n.a.	n.r.	30% 2 years, 12.5% 3 years, 12.5% 5 years	n.r.	83.3% 1 year	100% 1 year	n.r.
Parasitologic clearance	94.2% cytology	no (culture)	40% cytology	n.r.	1/7 = 14.3% PCR	no	n.r.	0%
Side-effects	10% local swelling 25% general	0%	0%	n.r.	75% general	n.r.	n.r.	n.r.

n.r. not reported, n.d. not done, n.a. not applicable.

Every-other-day dosages varying between 50 and 300 mg kg⁻¹ per day were typically given for 6 weeks, except for one study where treatment was given for up to 25 weeks.²⁸ The responses to these protocols do not seem to be related to drug dosages, and they are quite variable and contradictory, with complete or nearly complete clinical remission ranging between 37.5 and 100%. In any case, of seven studies using meglumine antimoniate every 48 h for a minimum of 6 weeks, only three reached excellent results,^{15,23,24} while the others reported results varying between a 37.5 and 55% rate of remission, which are worse when compared to those obtained with daily administration.^{20,22,28}

If a follow-up longer than 12 months was taken into consideration, relapses ranged between 32 and 100% of treated animals. If meglumine antimoniate alone was evaluated, most of the studies, regardless of dose and length of clinical trial, showed a considerably high relapse rate, varying between 70 and 100% after 6 to 12 months.^{15,17,25,27,30} The only exceptions are represented by studies using meglumine antimoniate for an extremely long period of time (up to 25 weeks, with a relapse rate of 15%),²⁸ stibogluconate (relapse rate of 40%),¹⁴ or liposomal meglumine antimoniate (relapse rate 0%).³¹ The only report differing from those described above is a study by Denerolle,²² with an every-other-day treatment protocol of only 6 weeks and a low relapse rate of 32%.

A negative PCR, the only accurate means of measuring parasitological clearance and definitive cure, was not reported in any of the assessed studies.

Mild systemic adverse effects (lethargy, gastrointestinal signs) were observed in up to 75% (usually in about 20%) of treated animals, while injection site reactions (inflammation) were described in up to 53% of treated dogs.

Allopurinol and meglumine antimoniate (Table 2)

Nine clinical trials evaluating the efficacy of combined allopurinol and meglumine antimoniate were identified and evaluated.^{22,28,29,33–38} Four of these trials were uncontrolled.^{33,34,36} The controlled studies compared the efficacy of combined allopurinol and meglumine antimoniate to meglumine antimoniate alone (three trials), to allopurinol alone (three trials), or to metronidazole and spiramycin (one trial).^{22,28,29,35,37,38} None of the controlled studies was blinded. The total number of enrolled dogs was 288, of which 175 (61%) were treated concomitantly with allopurinol (orally) and meglumine antimoniate (SC, IM or IV). The treatment with allopurinol was carried out for 4 months to 5 years; the treatment with meglumine antimoniate was administered for 3 to 8 weeks. In one study, meglumine antimoniate was given until normalization of clinical signs and electrophoresis, without further specifying how many days of treatment were required for the 25 dogs enrolled in this trial.³⁴

By using the combination of meglumine and allopurinol, the immediate clinical results seen were probably due to the meglumine antimoniate, rather than to the allopurinol. This is suggested by the fact that the

reported efficacy of these combination trials are similar to those already described for meglumine antimoniate alone. In fact, a complete or nearly complete clinical remission was observed in 96–100% of the dogs treated with meglumine antimoniate at the dosage of 100 mg kg⁻¹ every 24 h for at least 3 weeks, in five of the assessed trials.^{29,33–35,38} Conversely, a complete or nearly complete clinical remission response of just above 65% was described in one clinical trial using the lower meglumine antimoniate dose of 40 mg kg⁻¹ every 24 h for 4 weeks.³³

However, interest in the use of allopurinol lies in the prevention of relapses, as it is a parasitostatic drug. Thus, only studies with a follow-up of longer than 10 months were considered. When allopurinol was given for periods of 5 months or more, low recurrence rates were reported (4–11%).^{22,28,34} These percentages are markedly lower than those obtained with the use of meglumine antimoniate alone, which ranged from 32 to 100%.^{15,17,22,25,27,30} However, when allopurinol was administered for a shorter length of time, relapse rates were comparable to those obtained when it was not used at all.^{33,35} One study³⁵ reported good results with long-term intermittent (1 week per month) allopurinol administration that followed an initial 12 weeks of daily treatment (0% relapses).

The dosages of allopurinol used in the assessed studies varied between 20 and 40 mg kg⁻¹ daily, normally divided into two administrations. However, the relapse rate did not seem to be influenced by the dose used.

Parasitological clearance evaluated with PCR and complete cure were only described in three of the assessed studies. Results ranged between 17 and 50% within a follow-up of 6 to 16 months and better results were obviously reported in studies with a shorter follow-up.^{29,33,36}

Side effects were only described in 30–50% of the cases in two of the studies, and they were mainly associated with the use of meglumine antimoniate. These side effects were weakness and gastro-intestinal abnormalities.^{29,37}

Allopurinol alone (Table 3)

Eight clinical trials assessing the efficacy of allopurinol alone were found.^{28,29,38–43} Four of these were controlled studies in which allopurinol monotherapy was compared to placebo,⁴³ meglumine antimoniate alone^{28,29} or to combined allopurinol and meglumine antimoniate treatments.^{28,29,38} The other four studies were uncontrolled. Only one⁴³ of the eight studies was blinded. The total number of study dogs was 218, of which 109 (50%) were treated orally with allopurinol alone.

The dosage of allopurinol used ranged between 15 and 30 mg kg⁻¹ per day orally, often divided into two or three daily doses. Generally, the treatment was carried out until resolution of clinical signs so that the length of treatment varied greatly even within each study, thereby complicating interpretation of results. When the dosages used in these trials were closely examined, higher doses of 30 mg kg⁻¹ per day did not always result in higher efficacy, as shown in the study by Denerolle and Bourdoiseau.²⁸ Nevertheless, dosages of 20 mg kg⁻¹ daily also showed contradictory

Table 2. Trials evaluating the efficacy of antimonials associated with allopurinol for canine visceral leishmaniasis

Citation (reference)	33	34	22	35	28	29	36	37	39
Quality of evidence	C4	C2	B2	B3	B2, B3	B4	C3	B3	B4
Randomization (allocation generation)	n.a.	n.a.	unclear	inadequate	inadequate	unclear	n.a.	adequate	unclear
Randomization (allocation concealment)	n.a.	n.a.	unclear	inadequate	inadequate	unclear	n.a.	unclear	n.a.
Masking of outcome assessor	n.a.	n.a.	unclear	inadequate	inadequate	unclear	n.a.	unclear	none
Intention-to-treat analysis	n.a.	n.a.	n.d.	n.d.	n.d.	unclear	n.a.	n.d.	n.d.
Quality of inclusion of study subjects	well defined	well defined	fairly defined	fairly defined	well defined	well defined	well defined	well defined	well defined
# Dogs entered in trial (Total)	6	25	61	30	96	16	15	27	12
# Dogs dropped out	0	0	0	0	0	0	0	4	0
# Dogs treated with studied drug	6	25	27	30 (15 + 15)	45	7	15	14	6
Treatment of control group	n.a.	n.a.	meglumine alone	n.a.	meglumine alone 40 dogs, allopurinol alone 11 dogs	meglumine alone 6 dogs, allopurinol alone 3 dogs	n.a.	metronidazole 25 mg kg ⁻¹ + spiramycin 150,000 UI kg ⁻¹ q24 h PO	allopurinol alone
Dose	meglumine 40 mg kg ⁻¹ q24 h IM, allopurinol 10 mg kg ⁻¹ q12 h PO	meglumine 100 mg kg ⁻¹ q24 h IM, allopurinol 20 mg kg ⁻¹ q12 h PO	meglumine 100 mg kg ⁻¹ q24 h SC, allopurinol 10 mg kg ⁻¹ q8 h PO	100 mg kg ⁻¹ q24 h SC, allopurinol 30 mg kg ⁻¹ day PO	meglumine 100 mg kg ⁻¹ q24 h SC and allopurinol 15 mg kg ⁻¹ q12 h PO	meglumine 100 mg kg ⁻¹ q24 h IV allopurinol 10 mg kg ⁻¹ q12 h PO	meglumine 80 mg kg ⁻¹ q24 h SC allopurinol 10 mg kg ⁻¹ q12 h PO	meglumine 50–100 mg kg ⁻¹ q12 h SC and allopurinol 120 mg kg ⁻¹ q12 h PO	meglumine 100 mg kg ⁻¹ q24 h SC allopurinol 15 mg kg ⁻¹ q12 h PO
Length of treatment (weeks)	meglumine 3 allopurinol 4	meglumine until normalization of clinical signs and electro-phoresis, allopurinol 9 months	meglumine 4–8, allopurinol all study long (up to 5 years)	meglumine 3 weeks then repeated after 10–15 days for other 10 days, allopurinol 12 weeks	meglumine 3 weeks then repeated after 10–15 days for other 10 days, allopurinol 12 weeks then 1 week/month at 20 mg kg ⁻¹	meglumine 4 weeks, allopurinol 8 months	meglumine 1.5 repeated after 2 weeks, allopurinol 5 weeks –20 mos	meglumine 4 allopurinol 6 months	13
Length of follow up (months) after treatment withdrawal	10	n.a.	12–36	10–44	10–44	5 years	9–16	n.a.	4
Complete or nearly complete clinical remission	66.7%	96%	81.5%	100%	100%	82%	100%	n.r.	71%
									0
									100%

Table 2. continued

Partial clinical remission	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Clinical failure	0%	18.5%	0%	0%	0%	0%	0%	29%	0%
Relapse rate	33.3% within 10 months	4% (1/25)	4% (1/25)	100% after 4.5–21 months, 86% within 14 months	0% after 10–44 months	0%	0%	0% in 4 months	0%
Survival rate	66.7% after 10 months	96% after 9 months	96% after 9 months	14% no relapse after 1 year	n.r.	n.r.	60% 2 years 15.5% 3 years 7% 5 years	n.r.	n.r.
Parasitologic clearance	16.7%	n.r.	n.r.	n.r.	n.r.	28.6%	50% PCR	0% PCR	n.r.
Method of assessment	(1/6)	n.r.	n.r.	n.r.	n.r.	PCR	after 6 months	after 6 months	n.r.
Side-effects	n.r.	n.r.	n.r.	none with allopurinol	none with allopurinol	yes 75% with meglumine*	n.r.	yes 29% with meglumine†	none

n.r. not reported, n.d. not done, n.a. not applicable.
 *weakness, inappetence, anorexia, vomit, diarrhoea; †inflammation at injection site 2 dogs, skin rash in 1 dog, high liver enzymes in 1 dog, high azotaemia 1 dog.

results of 30 to 100% complete or nearly complete clinical remission.^{29,40,42,43} When the length of treatment was considered, in three studies prolonged administration of at least 6 months was associated with good clinical efficacy.^{38,39,41,42} However, there are two exceptions – those reported by Denerolle and Bourdoiseau²⁸ and those by Martinez-Subiela and coworkers.³⁸

In a study with follow-up between 0.5 and 5 months, a relapse rate of 88.9% within 2–4 weeks after drug withdrawal was described.⁴¹ Another study with a longer follow-up (up to 72 months) also showed relapses in the majority of treated dogs.⁴² Conversely, in two clinical trials, the dogs that responded well to treatment (28.6% and 18%, respectively) also showed no relapses and complete cure.^{28,29}

Allopurinol side effects were uncommon, and a few adverse signs were described in only two studies.^{41,42}

Aminosidine (Table 4)

We identified six clinical trials documenting the efficacy of aminosidine.^{21,26,27,44–46} Three of these compared the efficacy of aminosidine to that of meglumine,^{21,26,27} while the other three were uncontrolled. None of these studies was blinded. Together these studies enrolled 180 dogs, of which 168 (93%) were treated subcutaneously with aminosidine for 2–4 weeks.

A complete or nearly complete clinical remission was observed in 33 and 54% of the animals treated for 3 weeks with the low dosages of 5 mg kg⁻¹ once daily or 3.5 mg kg⁻¹ twice daily, respectively.^{27,45} A higher rate of success (86.6–100%) was observed with a dosage of 5 mg kg⁻¹ twice daily for 3–4 weeks.^{21,26,44,45} Dosages higher than 5 mg kg⁻¹ twice daily did not increase efficacy, but were associated with more side effects.^{44,46} In the studies with follow-up longer than 3 months^{27,45,46} relapses were frequently reported (73–83%) within a few months, while a complete cure (follow-up 4 years) was reported in 19% of the animals treated with very high doses of aminosidine.⁴⁶

Side effects, such as an increase in creatinine levels and reversible bilateral deafness, were seen in 5–8% of the animals treated with the dosage of 5 mg kg⁻¹ twice daily,^{21,26,45} while they were observed in 25–50% of the animals treated with higher dosages.^{44,46} At very high dosages (40–80 mg kg⁻¹ per day), a death rate of up to 50% was reported.

Amphotericin B (Table 5)

There are four studies investigating the efficacy of intravenous amphotericin against leishmaniasis, each with a different formulation,^{47–50} with a total of 87 animals treated. Three studies evaluated the efficacy of standard amphotericin B (Fungizone®, Bristol Myers Squibb, Paris La Defence, France), diluted, respectively, in water (39 dogs),⁴⁸ Intralipid® (Pharmacia Upjohn, Guyancourt, France) (19 dogs)⁴⁹ or soybean oil (16 dogs).⁵² The fourth study by Oliva and coworkers⁴⁷ was performed with the liposomal product AmBizome® (Nextar, Paris, France), and it involved 13 dogs. None of these studies was controlled.

Table 3. Trials evaluating the efficacy of allopurinol alone for canine visceral leishmaniasis

Citation (reference)	39	40	29	41	42	28	43	38
Quality of evidence	C3	C4	B4	C3	C2	B2, B3	A2-A3	B4
Randomization (allocation generation)	n.a.	n.a.	unclear	n.a.	n.a.	inadequate	adequate	unclear
Randomization (allocation concealment)	n.a.	n.a.	unclear	n.a.	n.a.	inadequate	adequate	n.a.
Masking of outcome assessor	n.a.	n.a.	unclear	n.a.	n.a.	inadequate	unclear	none
Intention-to-treat analysis	n.a.	n.a.	unclear	n.a.	n.a.	n.d.	n.d.	n.d.
Quality of inclusion of study subjects	well defined	fairly defined	well defined	well defined	well defined	well defined	well defined	well defined
	in 7/11 dogs,	defined	defined	defined	defined	defined	defined	defined
	fairly defined							
	in the others							
# Dogs entered in trial (total)	11	7	16	10	21	96	45	12
# Dogs dropped out	0	0	0	1 renal failure	0	0	4	0
# Dogs treated with studied drug	11	7	7	9	21	11	37	6
Treatment of control group	n.a.	n.a.	meglumine alone 6 dogs, meglumine and allopurinol 3 + 4 dogs	n.a.	n.a.	meglumine 40 dogs, meglumine + allopurinol 45 dogs	none	meglumine and allopurinol
Dose	5 mg kg ⁻¹ q8 h PO	10 mg kg ⁻¹ q12 h PO	10 mg kg ⁻¹ q12 h PO	10 mg kg ⁻¹ q24 h PO	7 mg kg ⁻¹ q8 h PO	15 mg kg ⁻¹ q12 h PO	10 mg kg ⁻¹ q12 h PO	15 mg kg ⁻¹ q12 h PO
Length of treatment (weeks)	4–50	1–12	not clear	8–100	26	4–90	17	9
Length of follow up (months) after treatment withdrawal	up to 4 months	0	9–16	0	up to 72	60	0	0
Other pharmacological intervention	none	none	none	none	none	none	antibiotics, fipronil	none
Complete or nearly complete clinical remission	100% in 1–3 months	30%	43%	90% in 2–6 months	100%	18% in 9–20 months	50% in 4 months	100%
Partial clinical remission	n.r.	70%	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Clinical failure	0%	0%	57%	10%	0%	82%	50%	0%
Relapse rate	not reported	not reported	none	88.9% after 2–4 weeks	in the majority of dogs	n.r.	n.r.	n.r.
Survival rate	not reported	n.r.	n.r.	n.r.	67% 6 years 78% > 4 years	18% 2 years	n.r.	n.r.
Parasitologic clearance	n.r.	n.r.	28.6% PCR	40% culture and/or PCR	80% cytology	n.r.	0% PCR	n.r.
Method of assessment								
Side-effects	none	none	none	10% gastroenteric	minimal	none	none	none

n.r. not reported, n.d. not done, n.a. not applicable.

Table 4. Trials evaluating the efficacy of aminosidine for canine visceral leishmaniasis

Citation (reference)	21	26	44	45	46	27	
Quality of evidence	B3	B3	C2-C3	C2-C3	C3-C4	B3	
Randomization (allocation generation)	unclear	unclear	unclear	unclear	unclear	n.r.	
Randomization (allocation concealment)	unclear	unclear	unclear	unclear	unclear	n.r.	
Masking of outcome assessor	unclear	unclear	unclear	unclear	unclear	n.r.	
Intention-to-treat analysis	unclear	unclear	n.d.	n.d.	n.d.	n.r.	
Quality of inclusion of study subjects	fairly defined	fairly defined	well defined	well defined	well defined	well defined	
# Dogs entered in trial (total)	22	24	39	42	21	32	
# Dogs treated with aminosidine	10	12	39	42	21	11	
Treatment of controls	meglumine	meglumine	n.a.	n.a.	n.a.	10 meglumine, 11 aminosidine + meglumine	
# Dogs treated with aminosidine and other antileishmanial drug	n.a.	n.a.	n.a.	n.a.	n.a.	3.5 mg kg ⁻¹ q12 h SC	
Dosage	5 mg kg ⁻¹ SC q12 h	5 mg kg ⁻¹ q12 h SC	21 mg kg ⁻¹ q24 h im or sc 10.5 mg kg ⁻¹ q24 h im or sc	5.25 mg kg ⁻¹ SC q24 h (12 dogs) 5.25 mg kg ⁻¹ SC q12 h (30 dogs)	20 mg kg ⁻¹ q24 h–3 dogs 40 mg kg ⁻¹ q24 h–12 dogs 80 mg kg ⁻¹ q24 h–6 dogs		
Length of aminosidine treatment (weeks)	4	4	2 o 3	3	2–3 dogs 4–12 dogs3–6 dogs	3	
Length of follow up (months) after treatment withdrawal	1	1	3	3	48	6	
Complete or nearly complete clinical remission	100%	92%	89.7% (no differences between groups)	total 71%	low 33%	high 86.6%	low 100% medium 70% high 50%
Partial clinical remission	n.r.	8%	7.7%	24%	50%	13.3%	n.r. n.r. n.r.
Failure	0%	0%	2.6%	5%	17%	0%	30% 50%
Relapse rate	n.a.	n.a.	0%	83% low dose group 45% high dose group after 3–4 months			0% 0%
Parasitologic clearance	n.r.	no (culture)	reduction of number of parasites in 20% or animals (cytology), no clearance	0%			yes all animals w/excp of cure in 1 high dose and 3 medium dose group 0% of low dose, 25% of medium dose, 16% of high dose (cytology)
Side-effects	5%	8%	15% of dogs 1 death (2.5%)	6% renal problems			50% death high dose group 17% death in medium dose group

n.r. not reported, n.d. not done, n.a. not applicable; side-effects: increase creatinine level, reversible bilateral deafness.

Table 5. Trials evaluating the efficacy of amphotericin for canine visceral leishmaniasis

Citation (Reference)	47	48	49	50
Quality of evidence	C4	C2	C3	C3
Randomization (allocation generation)	unclear	n.a.	n.a.	n.a.
Randomization (allocation concealment)	unclear	n.a.	n.a.	n.a.
Masking of outcome assessor	unclear	n.a.	n.a.	n.a.
Intention-to-treat analysis	n.d.	n.d.	n.d.	n.d.
Quality of inclusion of study subjects	well defined	well defined	fairly defined	well defined
# Dogs entered in trial (total)	13	39	19	16
# Dogs dropped out	0	5	2 (died during treatment)	
Type of amphotericin used	liposomal (AmBizome®)	normal in water (Fungizone®)	normal (Fungizone®) in water and Intralipid®	normal (Fungizone®) in water and soybean oil
# Dogs treated with amphotericin	13	39 (of which 12 nonresponders to meglumine treatment)	19	16
# Dogs treated with amphotericin and other antileishmanial drug	none	none	17 with allopurinol after treatment stop	none
Dose of amphotericin used	total dose IV: 5 mg kg ⁻¹ –3 dogs 10 mg kg ⁻¹ –2 dogs 12 mg kg ⁻¹ –5 dogs 15 mg kg ⁻¹ –3 dogs	0.5–1 mg kg ⁻¹ 2–3 times per week IV in 5–45 s, total dose 2–16 mg kg ⁻¹	1–2.5 mg kg ⁻¹ twice weekly IV, total dose 10–17.7 mg kg ⁻¹	1–2.5 mg kg ⁻¹ twice weekly IV, total dose 9.1–17.5 mg kg ⁻¹
Length amphotericin treatment (weeks)	0.5–1.5	2–10	4–5	4–5
Length of follow up (months) after treatment withdrawal	3–5 injections 8	6–36	mean 13 injections 3	3–30
Other pharmacological intervention	none	prednisone 1 mg kg ⁻¹ in 2 dogs	none	benazepril, aspirin in 4 dogs
Complete or nearly complete clinical remission	all dogs at 12 and 15 mg kg ⁻¹	85.3% total 93% dogs > 6 mg kg ⁻¹	100% of surviving dogs (2 died)	100%
Partial clinical remission	all dogs at 10 mg kg ⁻¹	n.r.	n.r.	n.r.
Clinical failure	all dogs at 5 mg kg ⁻¹	14.7%	0%	0%
Relapse rate	92.3%	10% after 1 year at dose > 6 mg kg ⁻¹	n.r.	18.7% (3 dogs) after < 8 months
Survival rate	n.r.	in dogs treated for the first time: 82% one year, 76% two years	n.r.	62.5% after minimum 3 months – up to 2 years
Parasitologic clearance	7.7% cytology	4 of 5 dogs (80%)	82% PCR after 3 months	87.5% within 5 months
Method of assessment		culture after 1–12 month		62% after 5 months – PCR
Side-effects increase kidney values	30.7%	23%	23.6%	25%
Side-effects vomit, anorexia within 36 h of begin of therapy	n.r.	reported	79%	81.3%

n.r. not reported, n.d. not done, n.a. not applicable; AmBizome (Nextar Paris France); Fungizone (Bristol Myers Squibb, Paris La Defence, France), Intralipid (Pharmacia Upjohn, Guyancourt, France).

When diluted in water, amphotericin B was administered intravenously at a dosage of 0.5–1 mg kg⁻¹ 2–3 times per week for 2 to 10 weeks in 5–45 s until a total dose of 2–16 mg kg⁻¹ was reached.⁴⁸ A complete or nearly complete clinical remission was obtained in 85.3% of the animals; higher response rates (93%) were seen in animals treated with a total dosage greater than 6 mg kg⁻¹. The other two studies^{49,50} that used Fungizone diluted in lipids were performed with the same protocol: 1–2.5 mg kg⁻¹ IV twice weekly for 4–5 weeks until a total dose of 9.1–17.7 mg kg⁻¹ was reached. Two of the 35 (5.7%) treated dogs died during or at the end of the course due to unknown causes. Of the surviving dogs, 100% achieved a complete or nearly complete clinical remission.

The liposomal amphotericin B preparation was administered at a dosage of 1.67–3.3 mg kg⁻¹ in 3–5 injections IV over 3–10 days until a total dose of 5 mg kg⁻¹ (three dogs), 10 mg kg⁻¹ (two dogs), 12 mg kg⁻¹ (five dogs) or 15 mg kg⁻¹ (three dogs) was reached.⁴⁷ A complete or nearly complete clinical remission was observed in all dogs receiving a total of 12–15 mg kg⁻¹, while partial response or lack of response were obtained at lower doses.

Of the animals treated with Fungizone®, 10–18.7% experienced a relapse within the first 8–12 months, and about 80% were culture- or PCR-negative for *Leishmania* organisms after 1–12 months.^{48–50} However, in the study by Cortadellas,⁵⁰ of the PCR-negative animals in the first months following therapy, 14% were found positive after 5 months, suggesting false negative results or re-infection. Ninety-two per cent of dogs treated with Ambizome® experienced a relapse within 8 months of follow-up.

Increased BUN and creatinine values were observed in 23–30% of all animals treated with amphotericin B, regardless of the formulation or protocol used. Vomiting and anorexia within 36 h of therapy onset were observed in about 80% of the animals treated with both lipid Fungizone® emulsions.^{49,50}

Other drugs (Table 6)

Ketoconazole. The antifungal imidazole ketoconazole has been tried in one study in 14 dogs with leishmaniasis⁵¹ at the oral dosage of 7 mg kg⁻¹ day for 7 to 13 weeks. Seventy-one per cent of these animals reached complete or nearly complete clinical remission. There are no data about relapses or follow-up in these animals.

Buparvaquone. Buparvaquone, used for therapy of theileriosis, was used intramuscularly in one study in seven dogs with visceral leishmaniasis⁵² at the dosage of 5 mg kg⁻¹ every 4 days for four doses, with partial or no response in all treated animals.

Pentamidine. Pentamidine is an aromatic diamide compound used intramuscularly that is capable of damaging protozoal DNA. It is associated with several side

effects, such as hypotension, tachycardia and vomiting, as well as injection site irritation.

The use of pentamidine for canine leishmaniasis was investigated in two studies^{53,54} involving a total of 33 dogs, 15 of which were treated with this drug; the others were left untreated. In both studies the drug was administered twice weekly at the dosage of 4 mg kg⁻¹ IM for a course of 4 weeks, and this was repeated after 3 weeks. In all animals, a complete or nearly complete clinical remission was observed, without relapses within 6 months. Unfortunately a longer follow-up was not available. Side effects were not reported in these studies.

Metronidazole, spiramycin, enrofloxacin. Metronidazole was used in two recent studies, in combination with either spiramycin or enrofloxacin.^{32,37} In the first study, 27 animals were enrolled, 14 of which were treated with metronidazole at 25 mg kg⁻¹ and spiramycin at 150 000 UI kg⁻¹ orally once daily for 13 weeks, while the control animals were treated with meglumine antimoniate and allopurinol.³⁷ Fifty-seven per cent of the dogs treated with metronidazole and spiramycin experienced a complete or nearly complete clinical remission; however, none of them tested PCR-negative after treatment. The relapse rate was 16.7% within 4 months. Five dogs developed side effects: one had pemphigus foliaceus, two increased liver enzymes and two, low azotaemia.

In the second study metronidazole at the dosage of 10 mg kg⁻¹ in combination with enrofloxacin at 20 mg kg⁻¹ was administered once daily to 12 of the 36 dogs included.³² Another 12 dogs were treated with enrofloxacin alone at the same dosage and 12 control dogs were treated with meglumine antimoniate. All dogs were treated for 4 weeks. Seventy per cent of the dogs treated with the combination and 50% of the animals receiving enrofloxacin alone showed a complete or nearly complete clinical remission, with a relapse rate of 50% within 3 months for both groups. Side effects were not reported with these drugs.

Repellents (Table 7)

We identified three large field trials investigating the preventive effect of repellent collars or spot on formulations against *Leishmania* infection.^{55–57} These work by reducing the number of sand fly bites, vectors of the disease.

Deltamethrin collars. Two controlled field studies investigated the efficacy of a collar containing 40 mg g⁻¹ deltamethrin.^{55,57} They enrolled altogether a total of 2296 dogs, of which 1118 were treated with the collar for 1 or 2 years, and the remainder were left as untreated controls. The animals treated showed a reduction of infection rate of 50% after 1 year and of 86% after 2 years if compared with untreated dogs. A 0.6% incidence of local erythema and increased salivation was observed in the treated animals in one study.⁵⁵

Permethrin spot on. One controlled field study investigated the repellent efficacy of a 65% permethrin spot on formulation.⁵⁶ In this study the product was administered

Table 6. Trials evaluating the efficacy of miscellaneous molecules for canine visceral leishmaniasis

Citation (reference)	51	52	53	54	37	32	
Name of drug	ketoconazole	buparvaquone	pentamidine	pentamidine	metronidazole/ spiramycine	enrofloxacin, enrofloxacin and metronidazole	
Quality of evidence	C3	C4	B4	B4	B3	B3	
Randomization (allocation generation)	n.a.	n.a.	unclear	unclear	adequate	unclear	
Randomization (allocation concealment)	n.a.	n.a.	unclear	unclear	unclear	unclear	
Masking of outcome assessor	n.a.	n.a.	unclear	unclear	unclear	unclear	
Intention-to-treat analysis	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	
Quality of inclusion of study subjects	well defined in 10 dogs, fairly defined in 4 gods	well defined	well defined	well defined	well defined	well defined	
# Dogs entered in trial (total)	14	7	17	11	27	36	
# Dogs dropped out	0	0	0	0	4	0	
# Dogs treated with studied drug	14	7	8	7	14	12 enrofloxacin	12 enrofloxacin + metronidazole
Treatment of control group	n.a.	n.a.	none	none	meglumine 50–100 mg kg ⁻¹ q12 h SC and allopurinol 20 mg kg ⁻¹ q12 h PO for 90 days	meglumine 50 mg kg ⁻¹ q12 h SC for 30 days	meglumine 50 mg kg ⁻¹ q12 h SC for 30 days
Dose	7 mg kg ⁻¹ q24 h PO	5 mg kg ⁻¹ IM every 4 days for 4 times	4 mg kg ⁻¹ twice weekly IM	4 mg kg ⁻¹ twice weekly IM	spiramycin 150.000 UI kg ⁻¹ and 25 mg kg ⁻¹ metronidazole q24 h PO	enrofloxacin 20 mg kg ⁻¹ q24 h PO	enrofloxacin 20 mg kg ⁻¹ q24 h PO and metronidazole 10 mg kg ⁻¹ q24 h PO
Length of treatment (weeks)	7–13	1.5	course of 4 weeks, repeated after 3 weeks 6	course of 4 weeks, repeated after 3 weeks 6	13	4	4
Length of follow up (months) after treatment withdrawal	0	n.r.			4	3	3
Complete or nearly complete clinical remission	71.4%	0%	100% in treated, 0 = % in controls	100% in treated dogs	57.1%	about 50%	about 70%
Partial clinical remission	n.r.	28.6%	n.r.	n.r.	28.6%	n.r.	n.r.
Clinical failure	28.56%	71.4%	0%	0%	14.3%	50%	30%
Relapse rate	n.r.	n.a.	0 in 6 months	n.r.	16.7% in 4 months	50% in 3 months	50% in 3 months
Survival rate	n.r.	n.a.	min. 6 months in all	n.r.	n.r.	n.r.	n.r.
Parasitologic clearance	71.4%	0%	2/2 tested	n.r.	0% PCR	0%	0%
Method of assessment	microscopy	(cytology)	culture and microscopy				
Side-effects	blood in urine and proteinuria in 2 dogs (14%)	n.r.	n.r.	n.r.	1 dog developed pemphigus foliaceus 2 dogs high liver enzymes, 2 dogs low azotemia	n.r.	n.r.

n.r. not reported, n.d. not done, n.a. not applicable.

Table 7. Trials evaluating the efficacy of repellents against sandflies vectors of canine visceral leishmaniasis

Citation (reference)	55	56	57
Quality of evidence	B1	B1	B1
Type of study	field	field	field
Randomization (allocation generation)	unclear	inadequate	adequate
Randomization (allocation concealment)	unclear	inadequate	inadequate
Masking of outcome assessor	unclear	inadequate	inadequate
Intention-to-treat analysis	unclear	n.d.	n.d.
Quality of inclusion of study subjects	fairly defined	fairly defined	fairly defined
# Dogs entered in trial (total)	1339	390	957
# Dogs dropped out	364	94	137
Type of repellent used	deltamethrin collars 40 mg g ⁻¹	65% permethrin spot on	deltamethrin collars 40 mg g ⁻¹
Residual effect (weeks)	> 26	4	> 26
Dosage	1 collar/dog	0–3 kg: 0.5 mL 4–15 kg: 1 mL > 15 kg: 2 mL	1 collar/dog
# Dogs treated with repellent	704	150, 110, 99	409
Untreated controls	635	160	548
Frequency repellent administration	once	once monthly for 3 months	once
Length of follow up (months)	30	1–3	12
Reduction of infection rate	86% after 2 years	50%	50% after 1 year
Side-effects	0.6% erythema and salivation	none	none

n.r. not reported, n.d. not done, n.a. not applicable.

once monthly for 3 months at the dosage of 0.5 mL in dogs of 0–3 kg in weight, 1 mL in dogs between 4 and 15 kg and 2 mL in dogs over 15 kg in weight. Three hundred and ninety dogs were enrolled, of which 150 were treated once, 110 twice and 99 three times and the remainder were left as untreated controls or excluded if found positive for leishmaniasis before the beginning of the study. At the end of the study, treated dogs exhibited a 50% reduction in the infection rate compared to untreated animals.⁵⁶ Side effects were not observed in any of the dogs treated with 65% permethrin spot on.

DISCUSSION

In this document, 47 trials assessing 14 different interventions with single or multiple molecules were reviewed systematically, and recommendations for or against their use will be proposed in this section. However, the results on the clinical efficacy obtained with this review must be evaluated first for their internal and external validity.

Internal validity

Several biases such as selection, detection, performance and attrition could play a role in reducing the *internal validity* of this analysis.⁵⁸ Selection bias results from inappropriate randomization schemes, while detection bias is the result of lack of a blinded design.⁵⁸ Within this systematic review, there was only one blinded, randomized, controlled trial, while the

remaining studies were either controlled studies lacking blinding and/or randomization (20 trials), or open, uncontrolled studies (26 trials). Therefore, both selection and detection biases are likely to have occurred in the various studies. Conversely, performance bias (e.g. one group of dogs treated preferentially with medications in addition to that being evaluated) was not detected in the assessed trials. Attrition bias can arise both because of *deviations from protocol* and/or because of *loss to follow-up*.⁵⁸ Possible reasons of deviation from protocols include violation of eligibility criteria and nonadherence to treatment.¹¹ In most of the studies assessed in this review, deviations from protocols usually were not discussed comprehensively. Loss to follow-up included drop-outs due to impossibility of re-checking the animals, to adverse events caused by treatments or to other medical concerns.⁵⁸ In order to reduce the bias due to heavy loss to follow-up, intention to treat analyses (ITT) were evaluated for each trial. Unfortunately, they were considered to be adequately performed in only one study.²⁵ Attrition of enrolled subjects was detected in nine of 41 studies dealing prospectively with the treatment of leishmaniasis. However, the attrition of subjects only totaled 33 out of 1101 dogs (3%); therefore, we think that this parameter played a minor role as a source of bias in this review.

In summary, the internal validity of this systematic review is affected by the nonrandomized and/or unmasked designs of most of the trials assessed (46 of 47), by the scarcity of ITT analyses, and by a low attrition rate. Clinical inference from study findings must be evaluated in light of these aspects.

External validity

The selection of subjects enrolled in the trials, the nature and duration of the treatments administered, and the assessed modalities of outcome are all parameters that could decrease the *external validity* of the trials assessed in this review.¹¹

Most of the studies reviewed here were performed in referral university hospitals and some of the patients assessed had failed to respond to previous treatments. Both of these aspects could represent a bias towards selection of dogs with more severe forms of leishmaniasis. Because of the nature of the disease, the quality of the diagnosis of leishmaniasis in the subjects enrolled was thoroughly assessed, and studies with a poorly defined diagnosis were excluded from this review. Among the trials evaluated by this review, the majority reported well-defined grade of diagnostic inclusion criteria (visualization of the parasite or positive PCR in tissue samples) and only a minority a fairly defined quality of inclusion (positive serological titre) of the study subjects.

The type, dosage, frequency, route and length of administration of the drugs used in the trials most of the times reflect the 'standard of care' protocols used in general and specialty veterinary practices.

Evaluation of complete cure was often not reported in early as well as in more recent studies and, although the lack of these results may reduce the significance of the trials, the nature of leishmaniasis is such that making a final statement of complete cure is extremely difficult.

Parasitological clearance based on cytological examination was often reported, while parasitological clearance based on PCR has only recently been described. However, it is felt that cytology does not reliably assess parasitological clearance, and therefore that these results might not be meaningful.

In conclusion, several factors such as enrolment criteria, limited evaluation of outcome measures, predominance of secondary care and second instance treatment may have endangered the external validity of this systematic review. These aspects must be kept in mind before making any therapeutic decision.

Implications for clinical practice and research

The *pentavalent antimony compounds* meglumine antimoniate (combination of pentavalent antimony and N-methyl D-glucamine) and sodium stibogluconate are widely used in the treatment of canine leishmaniasis, and they are currently considered the treatment of choice.^{6,31} Pentavalent antimonials act by selectively inhibiting leishmanial enzymes required for glycolytic and fatty acid oxidation.⁵ Alongside the free form, liposome-encapsulated N-methylglucamine antimoniate is also available, showing higher Sb plasma concentrations, higher volume of distribution and slower elimination.³¹ However, its cost-effectiveness compared to conventional meglumine antimoniate therapy is debatable.⁵ More than 80% of antimony is excreted in the urine within 9 hours of administration, thereby significantly reducing the accumulation and toxicity of these compounds.⁵⁹ Adverse effects are mild and are

represented by lethargy, gastrointestinal signs, and injection site reactions.

From the data scrutinized in our review there is *good evidence for* recommending the use of meglumine antimoniate at a minimum dosage of 100 mg kg⁻¹ daily for a minimum of 3–4 weeks. Better results are usually associated with longer durations of treatment (4–6 weeks).^{15,21,23,26} There is *insufficient evidence for* recommending the use of meglumine antimoniate on an alternate daily basis, the use of stibogluconate and the use of liposomal antimony. However, excellent results were described with a 10-day course of liposomal antimony in one study;³¹ further studies are necessary to confirm these results.

Allopurinol is an hypoxanthine drug metabolized by *Leishmania* organisms into an inactive analogue of inosine. When this analogue is used by leishmanial RNA, faulty protein translation occurs.⁵ It acts as a parasitostatic drug, but it is not a parasitocidal compound. Allopurinol is frequently used alone or in combination with pentavalent antimonials because of its low toxicity, valuable clinical efficacy, convenience of oral administration and low cost.⁵

Unfortunately, there is *insufficient evidence for* recommending the use of allopurinol alone at the dose of 10–30 mg kg⁻¹ daily, divided into two or three oral administrations as a long-term treatment (4–100 weeks) in order to achieve clinical improvement. Also, within a few weeks after treatment cessation, relapses are to be expected in most treated dogs. However, interest in the use of allopurinol, if combined with an initial course of antimonial compounds, lies in the prevention of relapses because of its parasitostatic effects. In fact, there is *good evidence for* recommending the long-term use of allopurinol, after an initial course of meglumine antimoniate therapy, in order to significantly decrease the rate of relapses. The suggested protocol consists of an initial treatment with both drugs for a minimum of 3 weeks followed by long-term allopurinol treatment, at a dosage of 20–40 mg kg⁻¹ daily or intermittently (1 week a month). Side effects should not be life-threatening and should be relatively infrequent.⁶⁰ However, monitoring of hepatic and renal function in long-term patients is advised as xanthinuria and xanthine urolith formation can occur, particularly in dogs with liver disease.⁶⁰

Aminosidine is an aminoglycosidic antibiotic produced by *Streptomyces rimosus*, and it has an identical mechanism of action against bacteria and *Leishmania*: it reaches high intracellular concentrations and is able to inhibit protein synthesis by interfering with binding of the aminoacyl transfer RNAs to the 30 S ribosomal subunit. Five trials, including three controlled studies, albeit with short follow-up,^{21,26,27,44,45} provide *fair evidence for* recommending the use of aminosidine at the dosage of 5 mg kg⁻¹ twice daily for 3–4 weeks to achieve clinical improvement. However, a relapse of clinical signs should be expected within a few months.

Two studies^{44,46} provide *fair evidence against* recommending the use of aminosidine at higher dosages (20–80 mg kg⁻¹ day), due to its side effects. Similar to other

aminoglycosides, aminosidine may be nephrotoxic and ototoxic. It should thus not be used in dogs with impairment of renal function.

Amphotericin B is a broad-spectrum macrolide antibiotic produced by the actinomycete *Streptomyces nodosus*. It is able to bind ergosterol in cell membranes and is thus very active against fungi and some protozoa, including *Leishmania* spp. Unfortunately amphotericin B induces nephrotoxicity through renal vasoconstriction, and may cause fever, vomiting, anorexia and periphlebitis. Amphotericin B administration thus requires close monitoring of renal function during treatment, with temporary withdrawal of therapy if creatinine levels rise above the normal range.

Even though amphotericin B looks like a promising drug for treatment and definitive cure of canine leishmaniasis, there are not enough studies confirming these results and the incidence of (potentially severe) side effects is high. There is therefore *insufficient evidence* for recommending the use of amphotericin B in canine leishmaniasis.

There has always been a drive to find other drugs as an alternative to antimonials in the treatment of canine visceral leishmaniasis. Pentamidine, buparvaquone, enrofloxacin, metronidazole, spiramycin and ketoconazole have been tried,^{32,37,51–54} but as of yet nothing has proven as effective as meglumine antimoniate. In fact, from the data collected in this review, there is only *fair evidence* for recommending the use of pentamidine and *insufficient evidence* for recommending the use of buparvaquone, ketoconazole, enrofloxacin and the combinations metronidazole/spiramycin and metronidazole/enrofloxacin.

In the session dedicated to the the use of *repellents* for prevention of leishmaniasis, we chose to limit our selection of articles to those that reported trials investigating the prevention of leishmaniasis, and not just of sand fly bites. For this reason we only evaluated the three large field studies,^{55–57} and did not include several other laboratory studies, which aimed to evaluate the repellent efficacy of collars, lotions or spot ons against sand fly bites. From the data obtained there is *good evidence* for recommending deltamethrin collars and *fair evidence* for recommending permethrin spot on for prevention of leishmaniasis induced by sand fly bites.

In conclusion, our review confirms the efficacy of traditional therapy based on antimonials, combined with allopurinol for long-term maintenance therapy. However, further studies are needed in order to evaluate alternative drugs, particularly on promising molecules such as liposomal antimonials or on new formulations or protocols for the safe use of amphotericin B.

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Résumé La leishmaniose canine est une maladie systémique due à *Leishmania infantum*. Le but de cette revue systématique est d'identifier et d'évaluer les différentes options thérapeutiques ou préventives proposées pour la leishmaniose canine, et de proposer des recommandations pour ou contre leur utilisation. Quarante sept articles décrivant des essais cliniques permettent de recommander l'utilisation de l'antimoniate de meglumine, à une dose minimale de 100 mg/kg/j pendant au moins 3 à 4 semaines, en combinaison avec l'allopurinol pour obtenir une efficacité clinique satisfaisante et une prévention des récurrences. L'évaluation des articles a également permis de recommander l'utilisation de la pentamidine (4 mg/kg deux fois par semaine) et de l'aminosidine (5 mg/kg deux fois par jour) pour 3 à 4 semaines. Les preuves étaient insuffisantes pour recommander l'utilisation de l'allopurinol seul, de l'amphotéricine B, de la buparvaquone, du ketoconazole, de l'enrofloxacin, et des associations de metronidazole et de spiramycine ou de metronidazole et d'enrofloxacin. Des évidences contre l'utilisation de l'aminosidine à forte dose (20–80 mg/kg par jour) étaient notées à cause des effets secondaires. L'évaluation des articles sur l'utilisation des insecticides répulsifs vis à vis des mouches vecteurs de la leishmaniose permet de recommander les colliers à la deltaméthrine et moins nettement les spot on à la perméthrine.

Resumen La Leishmaniosis visceral canina es una enfermedad sistémica causada por la infección con *Leishmania infantum*. El propósito de esta revisión bibliográfica fue identificar y evaluar evidencias de eficacia de diferentes terapias en el tratamiento o prevención de la leishmaniosis visceral canina, así como establecer recomendaciones a favor o en contra de su uso. Cuarenta y siete artículos en los que se describen ensayos clínicos realizados entre 1980 y 2004 cumplieron los criterios de selección. La evaluación de los ensayos clínicos produjo datos positivos para recomendar el uso de antimonio de meglumina a una dosis mínima de 100 mg/kg al día durante al menos 3–4 semanas, combinado con alopurinol para obtener una eficacia satisfactoria y reducir el índice de recidivas. Asimismo, el examen de los artículos proporcionó cierta evidencia para recomendar el uso de pentamidina (4 mg/kg dos veces por semana) y aminosidina (5 mg/kg dos veces al día) durante 3–4 semanas. No hubo pruebas suficientes para recomendar el uso de alopurinol como terapia única, anfotericina B, buparvacona, ketoconazol, enrofloxacin, ni las combinaciones metronidazol con espiramicina o metronidazol con enrofloxacin. También encontramos suficientes datos para contraindicar el uso de aminosidina en dosis elevadas (20–80 mg/kg al día) debido a los efectos secundarios. En cuanto a la evaluación de sustancias repelentes contra el vector mosca de la arena (flebotomo), se encontraron buenos resultados para recomendar el uso de collares de deltametrina y para el uso de permetrina tópica.

Zusammenfassung Die canine viszerale Leishmaniose ist eine systemische Erkrankung, die von *Leishmania infantum* verursacht wird. Das Ziel dieses systematischen Überblicks war es, Evidenz für die Effektivität der therapeutischen und prophylaktischen Eingriffe bei caniner viszeraler Leishmaniose zu evaluieren, und Empfehlungen zu geben für oder gegen ihren Einsatz. Siebenundvierzig Artikel, die klinische Studien beschrieben, welche zwischen 1980 und 2004 publiziert worden waren, erfüllten die Selektionskriterien. Die Evaluierung der klinischen Studien ergab deutliche Hinweise für eine Therapieempfehlung mit Meglumine Antimonat bei einer Mindestdosierung von 100 mg/kg täglich für mindestens 3 bis 4 Wochen, in Kombination mit Allopurinol, um eine gute klinische Wirksamkeit und eine reduzierte Rückfallsquote zu erreichen. Die Evaluierung der klinischen Studien ergab auch ausreichende Hinweise für eine Therapieempfehlung mit Pentamidin (4 mg/kg zweimal wöchentlich) und Aminosidin (5 mg/kg zweimal täglich) für 3 bis 4 Wochen. Unzureichende Hinweise wurden gefunden für eine Therapieempfehlung mit Allopurinol alleine, Amphotericin B,

Buparvaquone, Ketokonazol, Enrofloxacin, und die Kombinationen von Metronidazol mit Spiramycin oder Metronidazol mit Enrofloxacin. Ausreichende Hinweise gegen die Verwendung von hochdosiertem Aminosidin (20–80 mg/kg pro Tag) aufgrund seiner Nebenwirkungen wurden präsentiert. Die Evaluierung der Publikationen über Massnahmen zur Abwehr der Sandfliegen als Vektoren für Leishmaniose ergab deutliche Hinweise für eine Empfehlung von Deltamethrin Halsbändern und ausreichende Hinweise für die Empfehlung der Spot-on Behandlung mit Permethrin.