



# From evidence to guidelines: the GSLC experience

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# What is 'GSLC'?

*GSLC = Gruppo di Studio sulla Leishmaniosi Canina* (Canine Leishmaniasis Working Group, CLWG) is an expert panel established in November 2005 in collaboration with the Italian Society of Veterinarians of Companion Animals (SCIVAC).

The aim of the CLWG is to provide a scientific-based consensus approach for the management of CanL with regards to **diagnosis** and **clinical classification of disease, therapy** and **prevention**. The main outcome - but not unique - is the production of **guidelines** intended to assist veterinary practitioners



<http://www.gruppoleishmania.org/>

# Published or 'in press' guidelines

*Veterinaria*, Anno 21, n. 3, Giugno 2007 19

LINEE GUIDA LEISHMANIOSI

## LEISHMANIOSI CANINA: LINEE GUIDA SU DIAGNOSI, STADIAZIONE, TERAPIA, MONITORAGGIO E PREVENZIONE

### Parte I: Approccio diagnostico e classificazione del paziente leishmaniotico e gestione del paziente proteinurico

CANINE LEISHMANIASIS: GUIDELINES FOR DIAGNOSIS, STAGING, THERAPY,  
MONITORING AND PREVENTION.

Part I: Diagnostic approach and classification of the patient affected by leishmaniasis  
and management of dogs with proteinuria

*A CURA DEL GRUPPO DI STUDIO SULLA LEISHMANIOSI CANINA (G.S.L.C.)*

# Published or 'in press' guidelines

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Journal of the American Veterinary Medical Association  
(accepted for publication)

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1 **Canine leishmaniasis: guidelines for diagnosis and clinical classification**

2

3 **Authors\*:** Saverio Paltrinieri (DVM, Prof, DECVCP), Laia Solano-Gallego (DVM, PhD,  
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Published or 'in press' guidelines

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**LEISHMANIOSI CANINA:  
LINEE GUIDA SU DIAGNOSI, STADIAZIONE,  
TERAPIA, MONITORAGGIO E PREVENZIONE  
Parte II: Approccio terapeutico**

**CANINE LEISHMANIASIS: GUIDELINES FOR DIAGNOSIS, STAGING,  
THERAPY, MONITORING AND PREVENTION  
Part II: Therapeutic approach**

*A CURA DEL GRUPPO DI STUDIO SULLA LEISHMANIOSI CANINA (G.S.L.C.)*

## DIAGNOSIS (I)

### Search strategy for identification of studies

- ✓ A search strategy for MEDLINE following Cochrane Reviewers' Handbook (Alderson 2004) was attempted (however this is most relevant for drug/vaccine intervention trials)
- ✓ Search for relevant citations in international conference proceedings
- ✓ Where inadequate or incomplete, information was supplemented with the experience of CLWG members

## DIAGNOSIS (II)

### Limitations of EBVM approach in literature review and guideline generation

- ✓ Almost no 'ring trials among diagnostic centres' were available in literature for any of the diagnosis tools employed in canine leishmaniasis
- ✓ A large variety of methods were developed in the categories of 'serology' and 'molecular methods', however only a few studies were available on comparative diagnostic performances within each category

One serological technique (IFAT) is universally taken as reference method because of ...  
tradition?

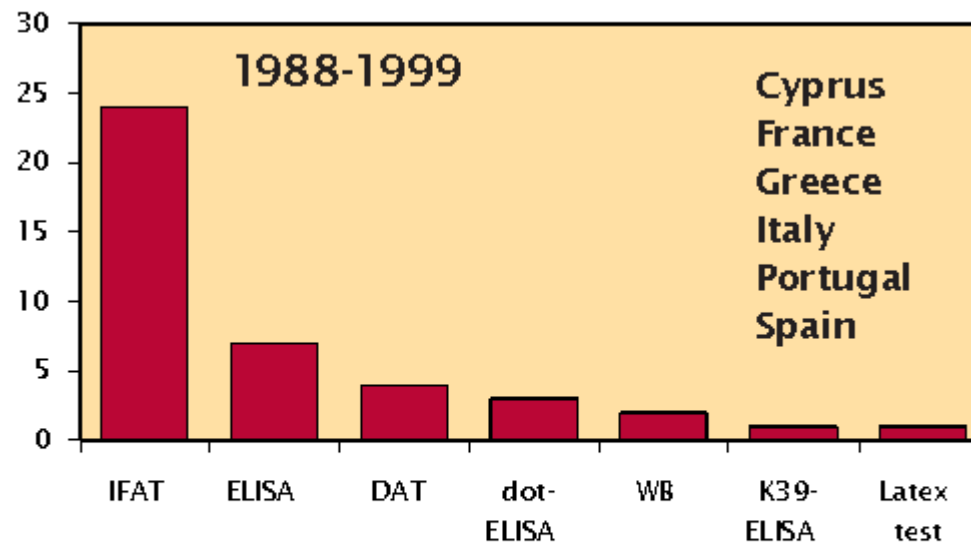


Fig 2. Serological investigations on canine leishmaniasis reported by European research groups in the past 12 years and the techniques used (Gradoni, 1999)

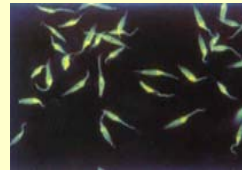
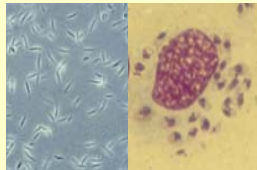
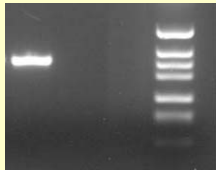
## DIAGNOSIS (III)

### Limitations of EBVM approach in literature review and guideline generation

- ✓ Very few studies were available on prospective comparative evaluation of diagnostic tools in naturally infected dogs at well-defined infection stages
- ✓ Conversely, most of the studies were performed using cross-sectional samples from dogs at different (unknown) infection stages

In a chronic progressive infection like leishmaniasis, after a long pre-patent period (**4-7 months**) the diagnostic markers convert to positive according to the following sequence:

**PCR** → **Culture/Microscopy** → **Serology** → **Clinical evidence**



0-22 months  
(m: 8.5)

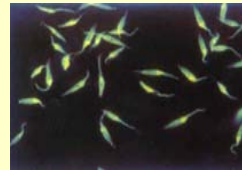
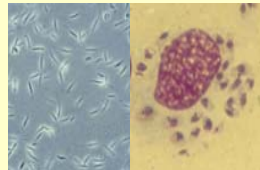
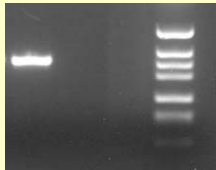
0-2 months  
(m: 1.5)

3-14 months  
(m: 7.0)

(m= median)

# Ideally, an EB approach in laboratory diagnosis methods should include the following:

PCR → Culture/Microscopy → Serology → Clinical evidence



Compare:

- Tissues sampled
- Genome targets
- Techniques (Conv., Nested, Real-time)

Compare:

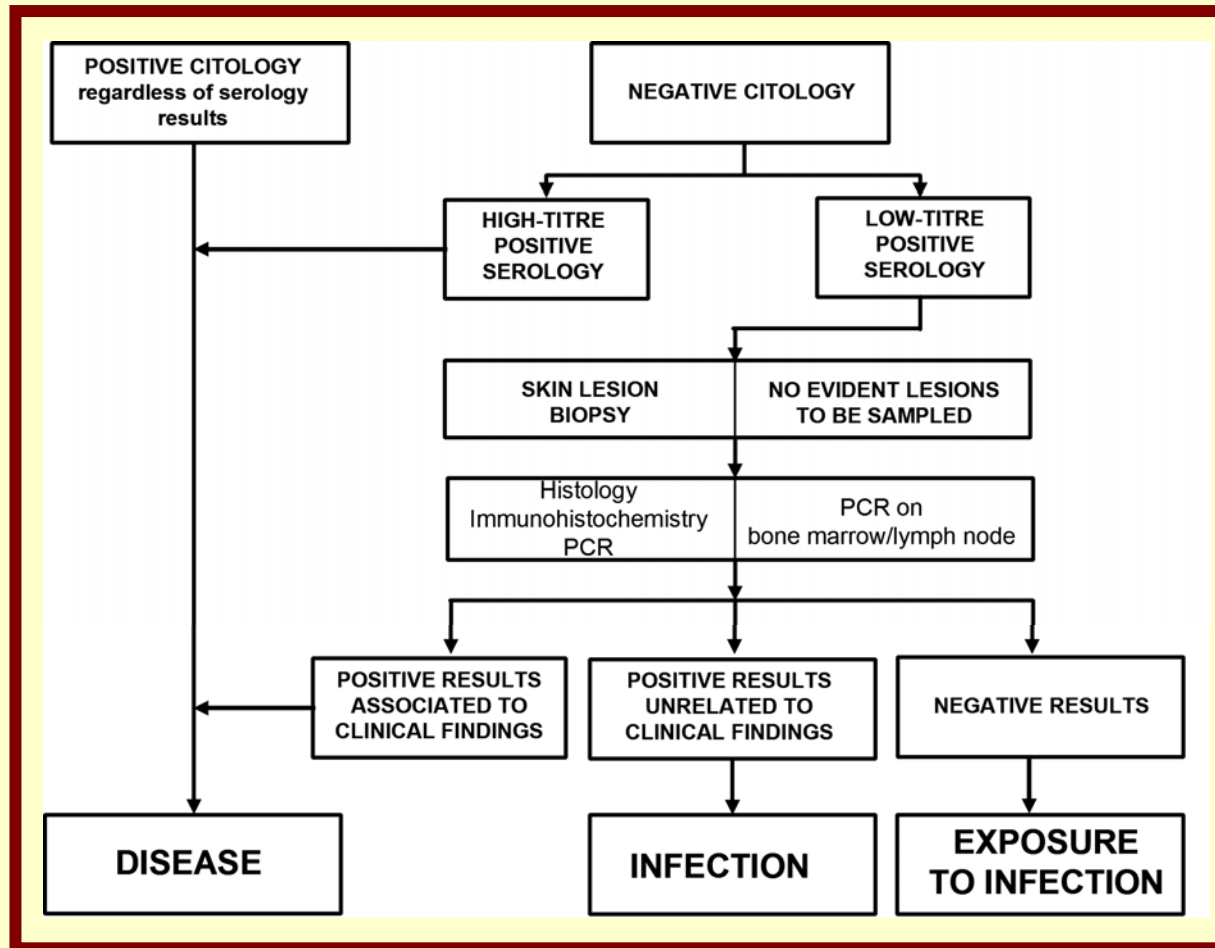
- Tissues sampled
- Staining/Immuno-
- Medium (Blood-agar, Liquid, Nutrients)

Compare:

- Antigens
- Techniques (IFAT, ELISAs, WB, DAT, Latex)
- Cut-off, Se, Sp

- Early/late lab changes
- Early/late signs
- Common to species/breed
- Individual

# A GSLC consensus was reached about staging the infection/disease according to conversion of the main diagnostic markers



## DRUG THERAPY (I)

### Search strategy for identification of studies and assessment of methodological quality

- ✓ A search strategy for MEDLINE following Cochrane Reviewers' Handbook (Alderson 2004)
- ✓ Search for relevant citations in international conference proceedings
- ✓ Studies were rated as 'adequate' or 'inadequate' depending on the quality of methodology
- ✓ Where inadequate, information was supplemented with the experience of CLWG members

## DRUG THERAPY (II)

Drugs evaluated for anti-leishmanial activity were reported in 62 studies

Pentavalent antimony:	34
Allopurinol:	19
Aminosidine:	4
Amphotericin B:	3
Miltefosine:	3
Pentamidine:	1
Spiramicin/metronidazole:	1
Enrofloxacin:	1
Marbofloxacin:	1
Domperidone:	1

## DRUG THERAPY (III)

### Limitations of EBVM approach in literature review and guideline generation

- ✓ Despite their (relative) high number, the large majority of studies were considered 'inadequate'
- ✓ Main limitations were:
  - Non-randomised/non-controlled trials
  - Unmasked allocations for observer/owner
  - Variable criteria for initial diagnosis and efficacy evaluation
  - Insufficient follow-up period
  - Low number of enrolled patients leading to low/no significance

## DRUG THERAPY (IV)

Basically, only one drug combination reached EBVM standards to be considered adequate for recommendation:

Meglumine antimoniate (MA) administered sc for at least 4 weeks at the dose of 100 mg/kg/d, plus oral allopurinol at the dose of 10 mg/kg bid for 6 months

The following variations, although suggested in guidelines, were not validated by EB criteria:

- 100 mg MA/kg/d vs 50 mg MA/kg/bid
- MA administered for 4 weeks vs 8 weeks
- Allopurinol administered for 6 months vs 12 months, or vs 'for life'

# What about the EB approach in human visceral leishmaniasis?

## Example of a single-drug dose-ranging trial: Liposomal amphotericin B

### Short-Course Treatment of Visceral Leishmaniasis with Liposomal Amphotericin B (AmBisome)

R. N. Davidson, L. di Martino, L. Gradoni, R. Giacchino,  
G. B. Gaeta, R. Pempinello, S. Scotti, A. Cascio,  
E. Castagnola, A. Maisto, M. Gramiccia, D. di Caprio,  
R. J. Wilkinson, and A. D. M. Bryceson

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We evaluated liposomal amphotericin B (AmBisome; Vestar, San Dimas, CA) administered to 88 immunocompetent patients (56 children) with visceral leishmaniasis (VL) caused by *Leishmania infantum*. Thirteen patients received 4 mg/kg on days 1–5 and 10 (total dose, 24 mg/kg), and all were cured; 42 received 3 mg/kg on days 1–5 and 10 (18 mg/kg), and 41 were cured; 32 received 3 mg/kg on days 1–4 and 10 (15 mg/kg), and 29 were cured (amastigotes were not cleared from 1 child, and 2 relapsed). One adult was cured with a total dose of 12 mg/kg. The four children who were not cured received 3 mg/kg for 10 days; none had further relapses. There were no significant adverse events. For VL due to *L. infantum*, we recommend a total dose of AmBisome of  $\geq 20$  mg/kg, given in  $\geq 5$  doses of 3–4 mg/kg over  $\geq 10$  days.

# Example of a single-drug dose-ranging trial: Miltefosine

## Trial of oral miltefosine for visceral leishmaniasis

*Shyam Sundar, Frank Rosenkaimer, Manoj K Makharia, Ashish K Goyal, Ashim K Mandal, Andreas Voss, Peter Hilgard, Henry W Murray*

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**Methods** Oral doses of miltefosine were given to six groups of five Indian men for 28 days: 50 mg every second day (group 1), 100 mg every second day (group 2), 100 mg/day (group 3), 150 mg/day (group 4), 200 mg/day (group 5), and 250 mg/day (group 6). Assessment for apparent cure—taken as an afebrile state with decreased spleen size and a splenic-aspirate parasite-density score of 0—was done on days 14 and 28. Definitive cure at 8 months required a parasite-free bone-marrow aspirate and no clinical evidence of relapse.

**Findings** 21 of 30 patients were apparently cured on day 14. Transient episodes of vomiting and diarrhoea, were common during weeks 1–2 and were seen in 22 patients. Four other patients in groups 5 and 6 had miltefosine withdrawn after 7–10 days because of vomiting. One patient in group 6 developed renal insufficiency and severe diarrhoea and died on day 21. On day 28, all 29 remaining patients were apparently cured. By 8 months, seven of ten patients in groups 1 and 2 had relapsed; however, 18 of 19 patients treated daily (groups 3–6) appeared to be cured. Among the 21 definitive cures were the four patients treated for 10 days or less and 12 for whom previous therapy with pentavalent antimony had failed.

**Interpretation** Treatment with miltefosine at 100–150 mg/day for 4 weeks has promise as an effective oral treatment of visceral leishmaniasis including antimony-resistant infection.

# Example of a combined-drug dose-ranging trial: Liposomal amphotericin B plus Miltefosine

## New Treatment Approach in Indian Visceral Leishmaniasis: Single-Dose Liposomal Amphotericin B Followed by Short-Course Oral Miltefosine

Shyam Sundar,<sup>1</sup> M. Rai,<sup>1</sup> J. Chakravarty,<sup>1</sup> D. Agarwal,<sup>1</sup> N. Agrawal,<sup>2</sup> Michel Vaillant,<sup>3</sup> Piero Olliaro,<sup>4,5</sup>  
and Henry W. Murray<sup>6</sup>

**Background.** In Bihar, India, home to nearly one-half of the world's burden of visceral leishmaniasis, drug resistance has ended the usefulness of pentavalent antimony, which is the traditional first-line treatment. Although monotherapy with other agents is available, the use of 2 drugs with different modes of action might increase efficacy, shorten treatment duration, enhance compliance, and/or reduce the risk of parasite resistance. To test the feasibility of a new approach to combination therapy in visceral leishmaniasis (also known as kala-azar), we treated Indian patients with a single infusion of liposomal amphotericin B (L-AmB), followed 1 day later by short-course oral miltefosine.

**Methods.** We used a randomized, noncomparative, group-sequential, triangular design and assigned 181 subjects to treatment with 5 mg/kg of L-AmB alone (group A; 45 subjects), 5 mg/kg of L-AmB followed by miltefosine for 10 days (group B; 46 subjects) or 14 days (group C; 45 subjects), or 3.75 mg/kg of L-AmB followed by miltefosine for 14 days (group D; 45 subjects). When it became apparent that all regimens were effective, 45 additional, nonrandomized patients were assigned to receive 5 mg/kg of L-AmB followed by miltefosine for 7 days (group E).

**Results.** Each regimen was satisfactorily tolerated, and all 226 subjects showed initial apparent cure responses. Nine months after treatment, final cure rates were similar: group A, 91% (95% confidence interval [CI], 78%–97%); group B, 98% (95% CI, 87%–100%); group C, 96% (95% CI, 84%–99%); group D, 96% (95% CI, 84%–99%); and group E, 98% (95% CI, 87%–100%).

**Conclusions.** These results suggest that treatment with single-dose L-AmB followed by 7–14 days of miltefosine is active against Indian kala-azar. This short-course, sequential regimen warrants additional testing in India and in those regions of endemicity where visceral leishmaniasis may be more difficult to treat.

**Trial registration.** ClinicalTrials.gov identifier: NCT00370825.

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DOI: 10.1086/591972

# Example of a single-drug dose/frequency-ranging trial: Amphotericin B 1mg/kg vs 0.75 mg/kg, and 15d vs 30d on alternate days

## Amphotericin B Treatment for Indian Visceral Leishmaniasis: Response to 15 Daily versus Alternate-Day Infusions

Shvam Sunder<sup>1</sup>, J. Chakravarty<sup>1</sup>, V. K. Rai<sup>1</sup>, N. Anrao<sup>2</sup>, S. P. Singh<sup>1</sup>, V. Chauhan<sup>1</sup> and Henry W. Murray<sup>3</sup>

**Background.** For patients with Indian visceral leishmaniasis, amphotericin B deoxycholate is usually given as 15 alternate-day infusions of 1 mg/kg over 30 days (total dose, 15 mg/kg); daily treatment with 1 mg/kg for 20 days (total dose, 20 mg/kg) is also used. This study was done to address the unsettled therapeutic questions of administration schedule (alternate-day vs. daily administration) and dose (1 vs. 0.75 mg/kg) and to determine whether the duration of amphotericin B treatment in Bihar, India, can be shortened to 15 days.

**Methods.** To compare alternate-day versus daily administration and 1-mg/kg versus 0.75-mg/kg doses and to determine whether the duration of treatment could be abbreviated, Indian subjects randomly received 15 infusions of 1 mg/kg (group A; 245 patients) or 0.75 mg/kg (group B; 244 patients) on alternate days or 1 mg/kg (group C; 500 patients) or 0.75 mg/kg (group D; 496 patients) daily. Noninferiority testing compared 6-month cure rates using a 5% margin.

**Results.** Overall, 1439 of the 1485 subjects completed treatment and responded. Treatment interruptions (nephrotoxicity) but not infusion-associated reactions or study removals were more common with daily administration. Final cure rates at 6 months were similar: group A, 234 patients (96%; 95% confidence interval [CI], 92%–98%); group B, 225 patients (92%; 95% CI, 88%–95%); group C, 483 patients (97%; 95% CI, 95%–98%); and group D, 476 patients (96%; 95% CI, 94%–97%;  $P > .05$ ).

**Conclusions.** Provided that the serum creatinine level is repeated once, daily treatment with amphotericin B, 0.75 mg/kg for 15 days (total dose, 11.25 mg/kg), is efficient and effective for visceral leishmaniasis in India.

**Trial registration.** ClinicalTrials.gov identifier: NCT00310505.

**Clinical Infectious Diseases** 2007;45:556–61

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1058-4838/2007/4505-0005\$15.00

DOI: 10.1086/520665

# Example of a randomized comparative trial: Amphotericin B vs Antimony

*Journal of Antimicrobial Chemotherapy* (2003) **52**, 464–468

DOI: 10.1093/jac/dkg356

Advance Access publication 29 July 2003

JAC

## Amphotericin B lipid complex versus meglumine antimoniate in the treatment of visceral leishmaniasis in patients infected with HIV: a randomized pilot study

Fernando Laguna<sup>1\*</sup>, Sebastián Videla<sup>2</sup>, Manuel E. Jiménez-Mejías<sup>3</sup>, Guillem Sirera<sup>4</sup>, Julián Torre-Cisneros<sup>5</sup>, Esteban Ribera<sup>6</sup>, Dolores Prados<sup>3</sup>, Bonaventura Clotet<sup>4</sup>, Mariano Sust<sup>2</sup>, Rogelio López-Vélez<sup>7</sup> and Jorge Alvar<sup>8</sup> on behalf of the Spanish HIV–Leishmania Study Group†

Optimal treatment for HIV-related visceral leishmaniasis (VL) has still to be established. A pilot clinical trial was carried out in 57 HIV–VL coinfecting patients to compare the efficacy and safety of amphotericin B lipid complex (ABLC) versus meglumine antimoniate. The patients were randomized to receive either ABLC 3 mg/kg/day for 5 days (ABLC-5, 18 patients), ABLC 3 mg/kg/day for 10 days (ABLC-10, 20 patients) or meglumine antimoniate 20 mg Sb<sup>v</sup>/kg/day for 28 days (19 patients). Treatment was considered successful if parasites were not detected in a bone marrow aspirate after treatment. Parasitological cure was attained in 33% (95% CI: 13%–59%) of the ABLC-5 group, in 42% (95% CI: 16%–62%) of the ABLC-10 group and in 37% (95% CI: 16%–62%) of the meglumine antimoniate group ( $P = 0.94$ ). Eight out of 19 patients administered antimoniate discontinued treatment prematurely following serious adverse events, compared with one in the ABLC groups ( $P = 0.0006$ ). The efficacy of ABLC is similar to meglumine antimoniate, but the severity of toxicity in the treatment of HIV–VL is lower with ABLC.

## CONCLUSIONS

- ✓ Because approaches to canine leishmaniasis management are still very heterogeneous in clinical practice, generation of Guidelines is urgently needed
- ✓ Unfortunately, available studies are largely inadequate to meet EBVM quality standards, so that most information requires to be supplemented by individual experiences of expert panels
- ✓ High-quality studies meeting EBVM standards, like those developed in the field of human visceral leishmaniasis, should be strongly encouraged