Preparatory Work for the Future Development of Scientific Opinions on Animal Health

Annette M. O'Connor
Iowa State University, oconnor@iastate.edu

Rungano Stan Dzikamunhenga
Iowa State University, rsd@iastate.edu

D. Wolfe
University of Guelph

Jan M. Sargeant
University of Guelph

J. Glanville
York Health Economics Consortium

See next page for additional authors

Follow this and additional works at: http://lib.dr.iastate.edu/vdpam_reports

Part of the Comparative and Laboratory Animal Medicine Commons, Laboratory and Basic Science Research Commons, Small or Companion Animal Medicine Commons, and the Veterinary Preventive Medicine, Epidemiology, and Public Health Commons

Recommended Citation
http://lib.dr.iastate.edu/vdpam_reports/4

This Report is brought to you for free and open access by the Veterinary Diagnostic and Production Animal Medicine at Iowa State University Digital Repository. It has been accepted for inclusion in Veterinary Diagnostic and Production Animal Medicine Reports by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Preparatory Work for the Future Development of Scientific Opinions on Animal Health

Abstract
This final report summarizes the results of two reviews and a scoping study related to Canine leishmaniosis (CanL). Three objectives were addressed in this project. Objective 1 was to summarize relative sensitivity and specificity estimates of assays used to detect infection in dogs with Leishmania infantum in studies of naïve dogs in areas where Leishmania infantum infection is endemic. Objective 2 was to summarize data available to estimate the prevalence of parasitological cure (failure to detect organism) after a 12-month follow-up period in animals treated with meglumine antimoniate, miltefosine, and allopurinol or combinations of these drugs for canine leishmaniosis. Objective 3 was to assess diagnostic test characteristics of PCR assays and serological assays (ELISA or IFAT) from studies that use experimental models of Canine leishmaniosis. The same comprehensive search was used for all objectives. The searches yielded 7,405 records. After duplicates were removed, 3,865 records remained. Of these, 243 were broadly identified as diagnostic test evaluation studies and at the 2nd level of screening 18 were considered longitudinal studies. The 18 references were then assessed based on the full text and 7 were considered relevant to the review. The assays assessed by these studies were PCR on skin, buffy coat, bone marrow, blood and conjunctiva, and IFAT and ELISA. For objective 2, 40 potentially relevant records were identified as treatment comparison studies. After full-text screening, 13 studies were included in the review. The treatments varied greatly, and few comparative efficacy estimates were provided. No treatment was associated with 100% cure after 180 days follow-up. For objective 3, 513 citations were identified and the full texts of 169 articles were obtained. Sixty-two articles described at least one of the assays requested (PCR, ELISA or IFAT) and 18 articles described the use of a PCR and either ELISA and/or IFAT.

Keywords
Dogs, diagnostic tests, protocol, scoping review, Leishmania infantum, treatment

Disciplines
Comparative and Laboratory Animal Medicine | Laboratory and Basic Science Research | Small or Companion Animal Medicine | Veterinary Preventive Medicine, Epidemiology, and Public Health

Comments

Authors
Annette M. O’Connor, Rungano Stan Dzikamunhenga, D. Wolfe, Jan M. Sargeant, J. Glanville, and J Wood

This report is available at Iowa State University Digital Repository: http://lib.dr.iastate.edu/vdpam_reports/4
EXTERNAL SCIENTIFIC REPORT

“Preparatory work for the future development of scientific opinions on animal health”

A. O’Connor¹, R S. Dzikamunhenga¹, D. Wolfe², J. Sargeant², J. Glanville³ and H. Wood³

¹Iowa State University, Ames Iowa, USA, ²University of Guelph, Guelph, CA, ³York Health Economics Consortium, University of York, UK

SUMMARY

This final report summarizes the results of two reviews and a scoping study related to Canine leishmaniosis (CanL). Three objectives were addressed in this project. Objective 1 was to summarize relative sensitivity and specificity estimates of assays used to detect infection in dogs with *Leishmania infantum* in studies of naïve dogs in areas where *Leishmania infantum* infection is endemic. Objective 2 was to summarize data available to estimate the prevalence of parasitological cure (failure to detect organism) after a 12-month follow-up period in animals treated with meglumine antimoniate, miltefosine, and allopurinol or combinations of these drugs for canine leishmaniosis. Objective 3 was to assess diagnostic test characteristics of PCR assays and serological assays (ELISA or IFAT) from studies that use experimental models of Canine leishmaniosis. The same comprehensive search was used for all objectives. The searches yielded 7,405 records. After duplicates were removed, 3,865 records remained. Of these, 243 were broadly identified as diagnostic test evaluation studies and at the 2nd level of screening 18 were considered longitudinal studies. The 18 references were then assessed based on the full text and 7 were considered relevant to the review. The assays assessed by these studies were PCR on skin, buffy coat, bone marrow, blood and conjunctiva, and IFAT and ELISA. For objective 2, 40 potentially relevant records were identified as treatment comparison studies. After full-text screening, 13 studies were included in the review. The treatments varied greatly, and few comparative efficacy estimates were provided. No treatment was associated with 100% cure after 180 days follow-up. For objective 3, 513 citations were identified and the full texts of 169 articles were obtained. Sixty-two articles described at least one of the assays requested (PCR, ELISA or IFAT) and 18 articles described the use of a PCR and either ELISA or IFAT.

© This work remains the copyright

KEY WORDS

Dogs, diagnostic tests, protocol, scoping review, Leishmania infantum, treatment
TABLE OF CONTENTS

1 Summary .................................................................................................................................................. 1
2 Table of contents ...................................................................................................................................... 2
3 Background as provided by [requestor] ................................................................................................. 6
4 Terms of reference as provided by [requestor] ...................................................................................... 6
5 Introduction and Objectives .................................................................................................................. 7
6 1. General background and rationale ..................................................................................................... 7
7 2. Objectives ........................................................................................................................................... 7
8 2.1. Objective 1 - Sensitivity and specificity of diagnostic assays in naïve dog populations ............. 7
9 2.2. Objective 2 - Prevalence of parasitological cure after treatment ................................................. 7
10 2.3. Objective 3 - Identifying challenge studies that might report diagnostic test characteristics (PCR, ELISA, IFAT) ........................................................................................................... 7
11 3. Protocol and registration .................................................................................................................. 8
12 4. Eligibility criteria ............................................................................................................................... 8
13 4.1. Objective 1 - Sensitivity and specificity of diagnostic assays in naïve dog populations ............. 8
14 4.1.1. Types of studies ........................................................................................................................... 8
15 4.1.2. Participants ................................................................................................................................... 8
16 4.1.3. Index and comparator tests ........................................................................................................ 8
17 4.1.4. Target conditions ....................................................................................................................... 8
18 4.2. Objective 2 - Prevalence of parasitological cure after treatment ............................................... 8
19 4.2.1. Type of study .............................................................................................................................. 9
20 4.2.2. Participants ................................................................................................................................... 9
21 4.2.3. Intervention ................................................................................................................................... 9
22 4.2.4. Outcome measures .................................................................................................................... 9
23 4.3. Objective 3 - Identifying challenge studies that might report diagnostic test characteristics (PCR, ELISA, IFAT) .................................................................................................................. 9
24 5. Information sources ........................................................................................................................... 9
25 6. Search .................................................................................................................................................. 9
26 7. Study selection ................................................................................................................................... 10
27 7.1. 1st level screening for Objective 1 (Sensitivity and specificity of diagnostic assays in naïve dog populations) and Objective 2 (Prevalence of parasitological cure after treatment). ........................................ 10
28 7.1.1. 2nd level selection steps for Objective 1 (Sensitivity and specificity of diagnostic assays in naïve dog populations) ............................................................................................................. 10
29 7.1.2. 2nd level selection steps for Objective 2 (Prevalence of parasitological cure after treatment) ........................................................................................................................................ 11
30 7.1.3. Selection steps for Objective 3 (Identifying challenge studies that might report diagnostic test characteristics (PCR, ELISA, IFAT)) ................................................................................................. 12
31 8. Data collection process ..................................................................................................................... 13
32 9. Data items .......................................................................................................................................... 13
33 10. Risk of bias in individual studies .................................................................................................... 13
34 11. Summary effect size ......................................................................................................................... 13
35 12. Synthesis of results .......................................................................................................................... 13
36 13. Risk of bias across studies .............................................................................................................. 14
37 14. Additional analyses .......................................................................................................................... 14
38 15. Results ............................................................................................................................................. 14
39 16. Study characteristics for Objective 1 - Sensitivity and specificity of diagnostic assays in naïve dog populations .......................................................................................................................... 14
40 Supporting publications 2015:EN-761.................................................................................................. 2
17. Risk of bias within studies for Objective 1 - Sensitivity and specificity of diagnostic assays in naïve dog populations ................................................................. 14
18. Results of individual studies for Objective 1 - Sensitivity and specificity of diagnostic assays in naïve dog populations ................................................................. 14
19. Risk of bias across studies for Objective 1 - Sensitivity and specificity of diagnostic assays in naïve dog populations ................................................................. 15
20. Additional analyses for Objective 1 - Sensitivity and specificity of diagnostic assays in naïve dog populations ................................................................. 15
21. Study selection for Objective 2 - Prevalence of parasitological cure after treatment ................................................................. 15
22. Study characteristics for Objective 2 - Prevalence of parasitological cure after treatment ................................................................. 15
23. Risk of bias within studies for Objective 2 - Prevalence of parasitological cure after treatment ................................................................. 16
24. Results of individual studies for Objective 2 - Prevalence of parasitological cure after treatment ................................................................. 16
25. Synthesis of results Objective 2 - Prevalence of parasitological cure after treatment ................................................................. 16
26. Risk of bias across studies Objective 2 - Prevalence of parasitological cure after treatment ................................................................. 17
27. Additional analyses for Objective 2 - Prevalence of parasitological cure after treatment ................................................................. 17
28. Study selection for Objective 3 - Identifying challenge studies that might report diagnostic test characteristics (PCR, ELISA, IFAT) ................................................................. 17
29. Summary of evidence for Objective 1 - Sensitivity and specificity of diagnostic assays in naïve dog populations ................................................................. 17
30. Limitations of Objective 1 - Sensitivity and specificity of diagnostic assays in naïve dog populations ................................................................. 17
31. Conclusions for Objective 1 - Sensitivity and specificity of diagnostic assays in naïve dog populations ................................................................. 18
32. Summary of evidence for Objective 2 - Prevalence of parasitological cure after treatment ................................................................. 18
33. Limitations of Objective 2 - Prevalence of parasitological cure after treatment ................................................................. 18
34. Conclusions for Objective 2 - Prevalence of parasitological cure after treatment ................................................................. 18
35. Tables ........................................................................................................ 19
36. Figures ........................................................................................................ 58
37. Appendix/Appendices ........................................................................................................ 66
38. A. Diagnostic Test Evaluation Protocol for the assessment of diagnostic test characteristics of PCR assays and serological assays (ELISA or IFAT) to detect Leishmania infection in naïve dogs longitudinal studies conducted in Europe since 1990 ........................................................................................................ 67
39. 1. Background and Rationale ........................................................................................................ 67
40. 2. Objectives ........................................................................................................ 67
41. 3. Methods ........................................................................................................ 67
42. 3.1. Criteria for considering studies for this review ........................................................................ 67
43. 3.1.1. Types of studies ........................................................................................................ 67
44. 3.1.2. Participants ........................................................................................................ 67
45. 3.1.3. Index and comparator tests ........................................................................................................ 68
46. 3.1.4. Target conditions ........................................................................................................ 68
47. 3.1.5. Search methods for identification of studies ........................................................................ 68
48. 3.1.5.1. Electronic searches ........................................................................................................ 68
49. 3.1.6. Searching other resources ........................................................................................................ 68
50. 3.1.7. Selection of studies ........................................................................................................ 68
51. 3.1.8. Data collection ........................................................................................................ 69
52. 3.1.8.1. Characteristics of the study population to be collected ........................................................................................................ 69
53. 3.1.8.2. Relative sensitivity and specificity data extraction (PCR versus PCR) ........................................................................................................ 70
54. 3.1.8.3. Assessment of methodological quality ........................................................................................................ 70
55. 3.1.8.4. Statistical analysis and data synthesis ........................................................................................................ 72
Scoping review and protocols for
Canine Leishmania

B. Proposed protocol for review for assessment of prevalence of parasitological cure for leishmaniosis........................................... 73
1. Ph(S) Question (PRISMA ITEM 4).................................................................................................................. 73
   1.1. Eligibility criteria (PRISMA ITEM 6)................................................................................................... 73
      1.1.1. Relevant participants................................................................................................................... 73
   1.2. Information Sources (PRISMA ITEM 7).............................................................................................. 73
   1.3. Search strategy (PRISMA ITEM 8)....................................................................................................... 73
   1.4. Study Selection (PRISMA ITEM 9)....................................................................................................... 73
   1.5. Data collection process (PRISMA ITEM 10)....................................................................................... 74
   1.6. Data Items (PRISMA ITEM 11)........................................................................................................... 74
   1.7. General study characteristics and clinical sources of heterogeneity:.................................................. 75
   1.8. Intervention characteristics (only for relevant treatment arms).......................................................... 75
   1.9. Summary measures (PRISMA ITEM 13).............................................................................................. 75
   1.10. Screening for eligibility for meta-analysis ......................................................................................... 75
   1.11. Data synthesis .................................................................................................................................... 76
2. Methods......................................................................................................................................................... 76
   2.1. Approach to presenting the results ...................................................................................................... 76
      2.1.1. Study selection (PRISMA ITEM 17) ............................................................................................ 76
      2.1.2. Study characteristics (PRISMA ITEM 18) ................................................................................ 76
      2.1.3. Risk of bias within studies (PRISMA ITEM 19) ......................................................................... 76
      2.1.4. Results of individual studies (PRISMA ITEM 20) .................................................................. 76
      2.1.5. Synthesis of results (PRISMA ITEM 20) .................................................................................. 76
   2.2. Synthesis of results (PRISMA ITEM 21) ............................................................................................ 76
C. Diagnostic Test Evaluation Protocol for the assessment of diagnostic test characteristics of PCR assays and serological assays (ELISA or IFAT) from studies that use experimental models of Canine leishmaniosis ............................................................................................................................... 77
1. BACKGROUND AND Rationale................................................................. 77
2. Objectives............................................................................................................. 78
3. Methods................................................................................................................ 78
   3.1. Criteria for considering studies for this review................................................................................... 78
      3.1.1. Types of studies ........................................................................................................................... 78
      3.1.2. Participants .................................................................................................................................. 78
      3.1.3. Index and comparator tests ........................................................................................................... 78
      3.1.4. Target conditions ......................................................................................................................... 78
      3.1.5. Search methods for identification of studies ................................................................................. 78
      3.1.6. Electronic searches ....................................................................................................................... 78
      3.1.7. Searching other resources ........................................................................................................... 79
      3.1.8. Data collection ............................................................................................................................ 79
      3.1.9. Form 1: Characteristics of the study population to be collected ................................................ 80
      3.1.10. Form 2: Characteristics of the induced disease model .............................................................. 80
      3.1.11. Form 3: Outcome data form – multiple repeats ....................................................................... 81

Supporting publications 2015:EN-761

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.
3.1.8.4. Assessment of methodological quality ............................................................ 81
3.1.8.5. Statistical analysis and data synthesis .......................................................... 83
3.1.8.6. Investigations of heterogeneity ................................................................. 83
3.1.8.7. Reporting .................................................................................................... 83
D. Search strategies .................................................................................................. 84
E. Risk of bias questions for Objective 1 .................................................................. 88
F. Risk of bias questions for Objective 2 ................................................................. 93

Supporting publications 2015:EN-761

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.
BACKGROUND AS PROVIDED BY [REQUESTOR]

TERMS OF REFERENCE AS PROVIDED BY [REQUESTOR]

This contract/grant was awarded by EFSA to: Iowa State University, Ames, Iowa, 50010, USA

Contract/grant title: Preparatory work for the future development of scientific opinions on Animal Health

Contract/grant number: RC/EFSA/AHAW/2014/01
INTRODUCTION AND OBJECTIVES

1. General background and rationale

Regulation (EC) No 998/2013 of the European Parliament and of the Council on the animal health requirements applicable to the non-commercial movement of pet animals and amending Council Directive 92/65/EEC (the Regulation), provides that the Commission may adopt species-specific preventive health measures for the control of diseases or infections other than rabies likely to spread due to the movement of pet animals, where they are necessary for the protection of public health or the health of pet animals. Those measures shall be scientifically justified and proportionate to the risk of spreading those diseases due to such movement.

Canine leishmaniosis (CanL) caused by infection with *Leishmania infantum* is a major global vector-borne zoonotic disease and is potentially fatal to humans and dogs. The latter are the most important natural reservoir and should therefore be the main target of control measures. Leishmaniosis is known to be endemic in more than 70 countries in the world, including certain regions of southern Europe. The movement of infected dogs from endemic regions, together with the potential expansion of sand fly vector populations in Europe, might represent a risk of introducing the disease into non-endemic countries. For that reason, canine leishmaniosis might fall under the category of diseases other than rabies that require preventive health measures to be adopted by means of a Commission delegated act in accordance with the Regulation, in order to ensure protection of those Member States in which endemic infection with *Leishmania infantum* has not been recorded.

To support the Commission in the possible preparation of such an act, the Commission requested EFSA to assess the available scientific information regarding canine leishmaniosis and to evaluate the relevance of measures aimed at mitigating the risk of introducing the disease through the movement of dogs. Testing dogs that move from endemic areas into disease-free areas for *Leishmania infantum* infections and treating positive dogs could be possible measures of reducing the risk of introduction into free areas in the EU.

2. Objectives

This contract had three objectives, and within each objective, several sub-objectives.

2.1. Objective 1 - Sensitivity and specificity of diagnostic assays in naïve dog populations

Objective 1 was to summarize relative sensitivity and specificity estimates of assays used to detect infection in dogs with *Leishmania infantum* reported in studies of naïve dogs in areas where *Leishmania infantum* infection is endemic.

2.2. Objective 2 - Prevalence of parasitological cure after treatment

Objective 2 was to summarize data available to answer the question “What is the prevalence of parasitological cure (failure to detect organism) after 12 months follow-up period in animals treated with meglumine antimoniate, miltefosine, and allopurinol or combinations of these drugs for canine leishmaniosis?”

2.3. Objective 3 - Identifying challenge studies that might report diagnostic test characteristics (PCR, ELISA, IFAT)

Objective 3 was to conduct an extensive search and study selection process to identify studies relevant to the review designed to “Assess diagnostic test characteristics of PCR assays and...”
serological assays (ELISA or IFAT) from studies that use experimental models of Canine leishmaniosis.”

3. Protocol and registration

The protocols for the three objectives were developed with EFSA staff and the EFSA working group. The process of protocol development is documented in a report delivered to EFSA on the 10th of October 2014. The protocols for each objective are included in Appendix A., Appendix B. and Appendix C. The protocol for the diagnostic test evaluation review was developed using the approach and subheadings recommended in the RevMan software (RevMan, 2012). The protocol for the treatment review was developed using the approach recommended by EFSA (EFSA, 2010) and, although a reporting guideline, the protocol was framed using the PRISMA guidelines for interventions (Liberati et al., 2009).

4. Eligibility criteria

4.1. Objective 1 - Sensitivity and specificity of diagnostic assays in naïve dog populations

For Objective 1, the review about the sensitivity and specificity of diagnostic tests in naïve dogs, the eligibility criteria were:

4.1.1. Types of studies

Studies that enrolled dogs that were naïve for *Leishmania infantum* infection and lived or were introduced to an endemic region were eligible for the review. The studies had to follow the dog over time to detect changes in *Leishmania infantum* infection status.

4.1.2. Participants

The target population of interest were naïve dogs at risk of *Leishmania infantum* in Europe introduced into or living in endemic regions. Naïve dogs were defined as dogs that tested negative for *Leishmania infantum* using both PCR (using any sample) and serology based on ELISA or IFAT.

4.1.3. Index and comparator tests

The aim was to extract sensitivity and specificity data from these studies. The authors may have tested the animals with a PCR or serology test of choice during the study and determined the apparent prevalence of infection. Then the authors may have compared the results of the PCR of choice to other PCR assays, the same PCR assay on a different tissue, or the serological assays ELISA or IFAT. Therefore, the gold standard was the author(s)’ “preferred” PCR and this varied between studies. The comparator test was that reported by the authors, provided it was either a PCR test/tissue or ELISA or IFAT.

4.1.4. Target conditions

The target condition of interest was infection with leishmaniosis caused by infection with *Leishmania infantum* based on the author(s)’ preferred PCR assay and test sample.

4.2. Objective 2 - Prevalence of parasitological cure after treatment

For the 2nd review, about the treatment options for canine infection with *Leishmania infantum*, the eligibility criteria were:
4.2.1. Type of study

Relevant studies were controlled trials that randomly allocated animals to treatment groups.

4.2.2. Participants

The relevant study population was defined as dogs with confirmed cases of canine leishmaniosis in the European region. The European region is defined as member countries of the OIE European region (http://www.oie.int/fileadmin/Home/eng/About_us/docs/pdf/2009_Commission_Europe_A.pdf), and included northern Asia (e.g., Russia, Ukraine, Uzbekistan), Turkey, Israel, and several other non-EU countries.

4.2.3. Intervention

The relevant population was treated with any regime that contained meglumine antimoniate, miltefosine and allopurinol.

4.2.4. Outcome measures

Primary outcome: The primary outcome of interest was a prevalence of parasitological cure at greater than 12 months after the initiation of therapy. This was defined as absence of parasites, assessed using an accurate PCR (based on other review) or xenodiagnosis after confirmation of the presence of the parasites prior to or early in the treatment protocol.

4.3. Objective 3--Identifying challenge studies that might report diagnostic test characteristics (PCR, ELISA, IFAT)

The objective of this task was to identify challenge studies of dogs infected with Leishmania infantum that reported using PCR, IFAT, or ELISA. The aim was to identify such studies, and provide the list of such studies to EFSA. We did not assess if the data needed to determine sensitivity and specificity were explicitly reported.

5. Information sources

A range of information sources indexing published research were searched for studies reporting on canine leishmaniosis (Table 1). Information on on-going or recently completed trials, unpublished research, and research reported in the grey literature was identified by searching databases that index conference proceedings and specialised search engines: Conference Proceedings Citation Index – Science and Open Grey. Where possible, search results were downloaded from the information sources and imported into EndNote® bibliographic management software. De-duplication was undertaken using a number of algorithms including those in EndNote ® and DistillerSR ® (Ottawa, ON, Canada).

6. Search

The search strategy used to identify studies for all three objectives was the same. The area of differentiation was the process of selecting studies, which is described in study selection (Section 7) below. The search strategy used to identify studies indexed in the Science Citation Index (Web of Knowledge; Thompson Reuters) is presented in Figure 1. The strategy comprised three key elements:

- The population: dogs (search line 1)
- The exposure: Leishmania infantum (search line 2)
The setting: The 53 countries that make up the OIE World Organisation for Animal Health Regional Commission for Europe (search lines 4 to 5).

Restricting a search by geographic setting is difficult to achieve sensitively due to the poor reporting of a study’s location in the title, abstract, or other fields of database records. The strategy took the approach of excluding records with a non-eligible country in the address field but not a country belonging to the OIE Regional Commission for Europe. Constructing the strategy in this way ensured that records that contained a European, and a non-European country in the address field were not excluded incorrectly. Although this approach has the potential to miss relevant studies, for example those that took place in a European country but were conducted by an author from the United States, it was necessary to limit by setting in order to increase precision (by removing non-relevant studies from the Americas, Australasia, and Africa) and, therefore, ensure that the volume of records retrieved was manageable within the resource constraints of the project.

Increasing precision by introducing treatment and diagnoses as additional key elements was not feasible as scoping searches indicated the wide range of keywords needed to capture these elements satisfactorily, many of them non-specific, meant that the number of records retrieved was not significantly reduced. The searches were not limited by date, language, or study design.

The search strategy developed for the Science Citation Index was adapted appropriately to perform efficiently in other information sources. Adaptation included a consideration of database interface differences (search syntax) as well as adaptation to different indexing languages. The strategies used to search each information source are presented in Appendix D.

7. Study selection

The study selection process differed slightly for each objective therefore these are described separately. The screening questions were developed in DistillerSR®.

7.1. 1st level screening for Objective 1 (Sensitivity and specificity of diagnostic assays in naïve dog populations) and Objective 2 (Prevalence of parasitological cure after treatment).

The purpose of this step was to identify the body of work likely to be relevant to the topics of interest.

Only one level of screening was used to identify references of potential relevance within the available literature. The following questions were used to screen references:

Question 1: Does the title or abstract describe a primary study in which data may have been collected from which diagnostic test characteristics could be calculated for one or more of the following assays of interest for canine leishmaniosis: PCR, IFAT, or ELISA? Include only studies evaluating tests in dogs (i.e., exclude sand flies, humans, etc.) and exclude studies obviously evaluating *L. Mexicana*, or *L. brasiliensis*.

- Yes—data on assay of interest
- Diagnostic test evaluation (DTE) but assay type not discernible
- No

Question 2: Does the title or abstract describe a primary study comparing treatments or treatment regimens in dogs with naturally occurring or induced canine leishmaniosis, in which at least one of the treatments was one of the following: Meglumine antimoniate (Glucantime), Miltefosine...
Scoping review and protocols for Canine Leishmania

(hexadecyl-phosphocholine), or Allopurinol? Exclude studies obviously evaluating, *L. Mexicana*, or *L. brasiliensis*

- Yes—treatment of interest included
- Treatment comparison but treatment type is not discernible
- No

Question 3: Was the study conducted in a European country based on the OIE definition of a European country?

- Yes (include for data characterization)
- Not discernible (include for data characterization)
- No (reference is excluded)

7.1.1. 2nd level selection steps for Objective 1 (Sensitivity and specificity of diagnostic assays in naïve dog populations)

Studies that responded yes to either of the 1st two items in question 1 of the initial screening were then assessed further to determine if they might be eligible and the assays used. The 2nd level of screening asked:

Does the study describe longitudinal studies in which *Leishmania*-naïve dogs or introduced dogs into an endemic area were followed with PCR (kDNA and/or rRNA) AND serology?

- Yes—proceed to next level
- No—exclude

For the final level of screening, based on the full text, the following question was used:

Did the study use a testing protocol to assess naïve dogs that included both PCR and serology (ELISA or IFAT)?

- Yes—proceed to data extraction
- No—exclude

These series of questions were tested on a test set of abstracts and modified as necessary. When agreement between the two independent reviewers was high (kappa > 90%) screening of all abstracts took place. Conflicts were resolved by discussion.

7.1.2. 2nd level selection steps for Objective 2 (Prevalence of parasitological cure after treatment)

Studies for which reviewers responded yes to either of the 1st two items in question 1 of the initial screening were then assessed further to determine if they might be eligible and the assays used. Note that these questions do represent a deviation from the protocol. The protocol called for inclusion of only randomized studies and studies with 12-month follow-up. As there were so few such studies that met these criteria, the review team modified these criteria to expand the list of included studies. The subsequent screening conducted on the abstract and titles included the following questions:
Does the study describe a controlled trial that assesses the treatment of canine leishmaniasis with one of the drugs of interest (Meglumine antimoniate, Miltefosine, Allopurinol) either alone or in combination with another drug of interest?

1. Yes

2. No

Was the presence of parasites confirmed prior to beginning treatment or early in the treatment protocol?

1. Yes, by PCR or xenodiagnosis

2. Yes, by microscopy/culture

3. Yes, by other testing method

4. No

Was the presence of parasites evaluated at least 6 months after the initiation of therapy?

1. Yes, with PCR or xenodiagnosis, 12 months or more

2. Yes, with PCR or xenodiagnosis, 6–11 months

3. Yes, by microscopy or culture

4. Yes, by other testing method

5. No/Not discernible

7.1.3. Selection steps for Objective 3 (Identifying challenge studies that might report diagnostic test characteristics (PCR, ELISA, IFAT))

For the third objective the approach was to use the full search provided by the York team and use Endnote ® to identify studies that used the term “challenge /induced/ experiment” in the title or abstract from the full search. For this group of studies, the review team then tried to obtain the full text for as many studies as could be found and that were in English. The review team then read the full text of these studies to determine if the authors reported using both PCR (any PCR on any tissue) and any ELISA or IFAT assay. The screening conducted on the full text included the following question:

1. Does the study include data, which may be used to calculate the diagnostic test characteristics of the following assays of interest for canine leishmaniosis: PCR, IFAT, or ELISA? Include studies with data in figures, tables or text. Note the data may be reported as apparent prevalence or prevalence and is unlikely to be reported as sensitivity and specificity.

Yes- provide this reference to EFSA for data extraction

No (exclude)
8. Data collection process

For Objective 1 (Sensitivity and specificity of diagnostic assays in naïve dog populations) and
Objective 2 (Prevalence of parasitological cure after treatment), the study selection process was
conducted by 2 independent reviewers. After initially doing a small subset of studies to ensure
agreement and similar understanding of the eligibility criteria, screening proceeded until all citations
were completed. Conflicts were resolved by discussion. For the data extraction, which is relevant for
Objective 1 and Objective 2 only, a similar process was used and conflicts resolved by discussion. For
Objective 1 data extraction was conducted directly into a MS word ® document, as it was not possible
to design a useful form in Distiller SR®. For Objective 2, data extraction was conducting using forms
in Distiller SR®.

9. Data items

Information was extracted about the study characteristics associated with establishing external
validity: country, year, species etc. For Objective 1 (Sensitivity and specificity of diagnostic assays in
 naïve dog populations) and Objective 2 (Prevalence of parasitological cure after treatment), the
relevant outcome data were extracted and the risk of bias assessed. The proposed data collection forms
are listed in Appendix A. Appendix B. and Appendix C.

10. Risk of bias in individual studies

The risk of bias tool used for Objective 1 is included in Appendix E. The bias assessment tool for
Objective 2 is included in Appendix F. The 3rd Objective did not include risk of bias assessment in its
scope.

11. Summary effect size

For Objective 1 (Sensitivity and specificity of diagnostic assays in naïve dog populations) it was
considered possible but not probable that some studies would use the same reference and index test,
and if so, a summary sensitivity and specificity would be calculated. For Objective 2 (Prevalence of
parasitological cure after treatment), as no pairwise comparison of treatment options was of interest, it
was anticipated from the outset that no effect size would be calculated. Objective 2 (Prevalence of
parasitological cure after treatment) did aim to extract the prevalence of cure from each relevant
treatment arm of interest but as these have different baselines it is not valid to combine into a summary
measure. It would only be valid to extract comparative measures of cure relative to another treatment
option.

12. Synthesis of results

The proposed approach to presentation was to present study-level characteristics, study-level outcomes
and study-level risk of bias. We did not contact authors to obtain missing data for any objectives. This
is a potential limitation of the review. Recently, we conducted a review and around 30% of original
papers did not report measures of variation and by contacting the authors, we were able to obtain
information on numerous papers. However, this was a long process (months). Imputation methods for
studies that did not report measures of variation for the outcomes of interest were not used.

The approach to evidence synthesis depended upon the frequency of the outcomes of interest within
the relevant studies. Tables that described the outcomes used and the associations observed are
reported. We attempted to prepare forest plots for sensitivity and prevalence data when feasible.
13. **Risk of bias across studies**

Given the absence of a specific effect size of interest, we did not anticipate we would be able to assess small study effects for either Objective 1 or Objective 2.

14. **Additional analyses**

No additional analyses were proposed or conducted.

**RESULTS**

From this point forward in the report, the PRISMA items related to each objective are grouped together.

15. **Study selection for Objective 1: Sensitivity and specificity of diagnostic assays in naïve dog populations**

The searches yielded 7,405 records. The source of these records is presented in Table 2. The flow chart of studies is reported in Figure 2. After duplicates were removed, 3,865 records remained. Of the 3,865 references screened, 243 were broadly identified as diagnostic test evaluation studies and at the 2nd level of screening 18 were considered longitudinal studies. The 18 references were then assessed based on the full text and 7 were considered relevant to the review. The citations and rationale for exclusion are included in Table 2.

16. **Study characteristics for Objective 1 -Sensitivity and specificity of diagnostic assays in naïve dog populations**

The characteristics of the study populations are presented in Table 3 and Table 4. Table 3 contains information about the study population while Table 4 contains information about the assays used.

17. **Risk of bias within studies for Objective 1 -Sensitivity and specificity of diagnostic assays in naïve dog populations**

The risk of bias tables for these studies is presented in Table 5. The risk domains and interpretation of these are explained in Appendix E. Generally it was surprising that so few studies reported blinding of the animal’s status to the person running the test. Although tests like PCR are sometimes considered objective, knowledge of the status of the animal may affect the ability to “rerun” an assay or the interpretation of the assays if intermediate results are obtained. There is also concern about spectrum bias with respect to specificity, which would lead to overestimation of specificity, as the populations used to estimate specificity are likely not exposed to common causes of false positives.

18. **Results of individual studies for Objective 1 -Sensitivity and specificity of diagnostic assays in naïve dog populations**

The individual results reported by the authors for the assays assessed are included in Table 6. Synthesis of results for Objective 1. As very few studies used either the same assay or the same sample as the referent, it was not possible to combine the results of the study into a single summary number, such as a summary sensitivity or specificity. Most estimates of sensitivity of a serological assay compared to using the PCR as a gold standard were < 50%. In Table 6, there are 13 estimates of sensitivity that compare a PCR to serology (IFAT or ELISA) and of those only 4 have point estimates for estimates that are equal to or greater than 50%, so the majority of sensitivity estimates (9/13) are less than 50%. The PCR on the conjunctiva was not very sensitive based on the results reported by Gramiccia et al., (2010) and (Otranto et al., 2013). Gramiccia et al. (2010) reported that the sensitivity of PCR using conjunctiva swabs was 0% each time it was compared to PCR buffy coat over 3 testing
periods within 12 months of the start of the study. Gramiccia et al. (2010) also reported that for the 3 animals found positive based on buffy coat PCR after 1 year, the PCR of conjunctiva swabs was 100% sensitive. Another study that assessed conjunctiva swabs reported the sensitivity of 36% when the referent was PCR positive using the skin (4 detected using PCR on conjunctiva compared to 11 detected PCR based on skin) (Otranto et al., 2013). For the same period of time elapsed (~ 12 months) since enrolment, the relative sensitivity of the comparison for PCR based on bone marrow (54.5%) or IFAT (73%) compared to PCR based on skin samples were higher than the conjunctiva PCR versus skin PCR comparison. However Otranto et al. (2013) did not test if these comparative estimates were statistically significantly different i.e., Otranto et al. (2013) did not assess if the estimate of 54.5% sensitivity based on bone marrow PCR was statistically different from the estimate of 36%. Given the small sample sizes it is possible that the extent of random error might mean these estimates do not differ. PCR based on skin was the most commonly used gold standard. There was no comparative assessment of PCR on bone marrow which was used by Olivia et al. (2006). When PCR skin assays were compared to serological based tests, estimates of sensitivity ranged from 13% to 100% depending upon the study (Otranto et al., 2009; Otranto et al., 2013). Specificity had a much narrower range from 87% to 100% (Otranto et al., 2009; Otranto et al., 2010; Otranto et al., 2013).

19. Risk of bias across studies for Objective 1- Sensitivity and specificity of diagnostic assays in naïve dog populations

It was not possible to quantitatively assess the risk of bias across the studies.

20. Additional analyses for Objective 1- Sensitivity and specificity of diagnostic assays in naïve dog populations

No additional analyses were conducted.

21. Study selection for Objective 2 - Prevalence of parasitological cure after treatment

The flow chart is presented in Figure 3. The source of these records is presented in Table 2. After duplicates were removed, 3,865 records remained. Of these 3,865 references screened, 40 were identified as treatment comparison studies. The flow chart of studies is reported in Figure 3. During 2nd level full-text screening, it was discovered that few studies used a 360-day follow-up period; thus, the follow-up period criteria for inclusion was reduced to 180 days. Note that this is a deviation from the original protocol but was done to increase the amount of evidence available. After full-text screening, 13 studies were included in the review; the excluded studies either did not report a treatment of interest or had a follow-up period less than 180 days.

22. Study characteristics for Objective 2 - Prevalence of parasitological cure after treatment

Following full-text screening, 13 references were available for data extraction (Table 7). The manuscripts originated from 6 European countries: Italy (4 studies), Spain (2 studies), France/Italy/Spain (1 multicentre study), Germany (2 studies), France, Greece (2 studies), and the Netherlands (1 study). There was heterogeneity between the studies in each outcome category due to differing initial health statuses of dogs, treatments, treatment protocols, follow-up periods, tissue types sampled at follow-up, test type at follow-up (e.g., PCR or cytology to measure parasite load), and metrics of results reported (e.g., there is no standard metric to report parasite load).
23. Risk of bias within studies for Objective 2 -Prevalence of parasitological cure after treatment

All studies included in this scoping review had a high risk of bias in their results (Tables 8, 9, 10, and 11). As a result, caution should be taken in making inferences regarding treatment efficacy from these studies.

24. Results of individual studies for Objective 2 -Prevalence of parasitological cure after treatment

To improve readability of the findings, the included studies have been categorized by the type of test used at follow-up (outcome). The data from studies reporting PCR outcomes are reported in Table 12 and Figure 4, xenodiagnosis outcomes are reported in Table 13, and parasite load quantification outcomes are reported in Table 14. Using PCR as the detection method, the highest “cure risk” of 33% was observed in dogs that received Meglumine antimoniate: 100 mg/kg SQ BID x 30 days with Allopurinol: 10 mg/kg PO BID x 365 days (Ariti et al., 2013). In that group, 5 of 15 dogs were PCR negative on blood, conjunctiva and lymph node after 720 days. However, this group contained dogs on the treatment for a variable period (some dogs constantly for 720 days while others for 360) and the authors did not differentiate the results by different treatment protocols. Therefore this cure risk is very hard to relate back to the drug protocol because in reality the group contains two protocols. One study that reported the cure risks in the upper range (>90%) used xenodiagnoses for confirmation (Miro et al., 2011). This study used three protocols and the cure risks were 12/12, 8/9 and 5/5 after 180 days (see Table 13 for protocols). Two aspects should be noted when interpreting this finding. First this study used a shorter follow-up period than others (180 days compared to up to 720 days), and the result might be confounded by the assay. The validity of this result is dependent upon the validity of xenodiagnosis as an approach for the detection of chronic Leishmania infection, if a test has low sensitivity then many positive dogs will appear negative. The same can also be said for the study by Neogy et al. (1994), using bone marrow aspirates; this study reported a high cure risk after 180 days. It is unclear if the study had been longer, if this finding would have been reaffirmed, and the sensitivity of direct examination of bone marrow is unclear.

Data was not extracted from four of the 13 included studies because reporting was incomplete (Table 15). These studies were either (1) longitudinal case series, in which dogs may have initially been assigned to a treatment group, but the treatment protocol and/or follow-up time changed throughout the study or (2) did not report useable follow-up data at the dog- or treatment-group level (i.e., data were missing and unexplained). In the longitudinal case series studies, treatment protocols and follow-up periods were often highly variable within groups as individual dog treatment and follow-up varied with response to treatment and dogs often crossed over between groups. Data within these studies often were not reported completely or summarized.

25. Synthesis of results Objective 2 -Prevalence of parasitological cure after treatment

We did not synthesize the data into a summary measure for two reasons. First, the working group did not specify a comparison of interest, instead indicating that they preferred to have results from any treatment used. Second, the working group also indicated that the proportions of cures were of interest, rather than a comparison such as a risk ratio or odds ratio of cure. It might seem sensible therefore to combine the estimates of proportions of cures; however, this was not possible for two reasons, one practical and one theoretical. The practical reason for not calculating summary “proportion of cures” is the extreme heterogeneity meant that it was not sensible to synthesize data and provide a summary estimate of efficacy. The 2nd theoretical reason not to combine these data is that treatments should be
compared relatively to enable control of baseline levels of recovery, so a summary proportion would not be appropriate.

26. Risk of bias across studies Objective 2 -Prevalence of parasitological cure after treatment

It was not possible to quantitatively assess the risk of bias due to small studies, because no single comparison was available for plotting. However, overall we would propose that in the opinion of the review team the risk of bias was low to medium overall for the studies that used-PCR based methods to assess the outcome. For studies that used xenodiagnostic approaches, the risk of bias is high, mainly because of the absence of information about allocation methods. For studies that looked at parasite load, the opinion of the review team was that the failure to ensure methods of assessing the load were blinded to animal status was a major source of bias.

27. Additional analyses for Objective 2 -Prevalence of parasitological cure after treatment

No additional analyses were conducted.

28. Study selection for Objective 3-Identifying challenge studies that might report diagnostic test characteristics (PCR, ELISA, IFAT)

The number of citations available for Objective 3 was 3,865. Based on the protocol, we used text-based searching of the abstract and title in EndNote® to identify challenge studies. The term “challenge” retrieved 73 citations, the term “experiment” retrieved 411 citations and the term “induced” retrieved 98 citations. We combined these groups and after removing duplicates had 513 citations. We then searched for articles that were published after and including 1990 (283 citations). We screened those abstracts, excluding based on the abstract citations that were obviously not relevant, for example murine and hamster models of Leishmania. We also excluded several citations based on the abstract when the language of the full-text publication was not English. We obtained the full text of 169 articles. Sixty-two articles described at least one of the assays requested (PCR, ELISA or IFAT – without specifics of target) and 18 articles described the use of a PCR and either ELISA and/or IFAT. In assessing the PCR and ELISA, we did not exclude articles if the target was not clear. Many articles were not specific about the targets for the PCR, and the working group did not indicate that they were interested in a specific target for the ELISA (i.e., SLA or K 39 and children).

DISCUSSION

29. Summary of evidence for Objective 1 -Sensitivity and specificity of diagnostic assays in naïve dog populations

Overall, the data available from the studies of naïve dogs has limited utility because of the absence of a gold standard diagnostic test for the detection of Canine leishmaniosis (CanL) caused by infection with Leishmania infantum, and all estimates are relative. Also, although described as longitudinal studies, the data are a series of cross sectional studies with changing prevalence. As such, it is not surprising that as apparent prevalence increases so does sensitivity.

30. Limitations of Objective 1 -Sensitivity and specificity of diagnostic assays in naïve dog populations

The absence of a gold standard, latent class methods or a mixed-treatment comparison meta-analysis make it difficult to reach conclusions about the assays. Clearly it would be preferable to conduct a review that used a gold standard or at least had a defined pairwise comparison when no gold standard exists.

Supporting publications 2015:EN-761
31. Conclusions for Objective 1 - Sensitivity and specificity of diagnostic assays in naïve dog populations

There is a large amount of data available for the conduct of a review of diagnostic tests for Canine leishmaniosis (CanL); however, meta-analyses and interpretation is complicated by the absence of a gold standard and consistent use of relative measures of sensitivity and specificity by the primary research. If available, studies that use approaches to assessing non-gold standard settings could be used in a meta-analysis; however, none were identified in the relevant studies. Numerous approaches to defining the scope of the review were discussed based on the time and resources available, and the final decision was made to limit the review to studies of naïve dogs. These studies represent a series of cross-sectional studies with changing prevalence, and although useful for estimating infection pressure, it is unclear to the review team for meta-analysis how the use of naïve dogs solves the issue of relative estimates as compared to studies with estimates obtained from a single point in time. In the review team’s opinion, unless a specific pairwise comparison could be identified that was of interest and a specific referent test agreed upon, it would be necessary to conduct a network meta-analysis of diagnostic tests; however, such approaches are poorly (or not) developed and would need development and validation of the underlying statistical method which is beyond the scope of this project.

32. Summary of evidence for Objective 2 - Prevalence of parasitological cure after treatment

Overall the data suggest that treatment results in less than 50% cure after 180 to 360 days of follow-up. The treatments received were very varied so overall conclusions are limited.

33. Limitations of Objective 2 - Prevalence of parasitological cure after treatment

One of the major limitations of the data available for the review is the absence of studies suited to a specific PICO question that would allow estimation of comparative efficacy. Comparative efficacy is the preferred way to summarize treatment comparisons and is measured by metrics such as risk ratio, rate ratio or mean difference. It had originally been proposed to conduct a PICO format review but during the development of the protocol it was communicated that the working group was concerned that such a format i.e. one that limited the review to specific treatment regimes and comparative efficacy was not feasible. This concern was based on the working groups familiarity with the studies available. Given this expert knowledge, the working group proposed instead collection of the cure risk for every treatment option. The approach therefore was modified to make the cure risk a population characteristic using any regime for the three drugs of interest. Based on this concept, the review protocol was redesigned as a PIT question i.e. a question about the cure risk in treated dogs. The major limitations with respect to inference were discussed but the final decision to use a PIT format was a pragmatic one. Given the final data available, the working group correctly anticipated the type of data available, and if a PICO review had been conducted, every treatment regime would have had one observation. The enormous heterogeneity of populations and interventions would have precluded combining data meaningfully.

34. Conclusions for Objective 2 - Prevalence of parasitological cure after treatment

The data suggest that after long periods of treatment and after long periods of follow-up, several therapies have been associated with being unable to detect the organism in treated dogs. However no studies reported 100% cure risks at the end of the follow-up periods, which varied between 180 and 720 days.
### Table 1  Number of records retrieved by information source

<table>
<thead>
<tr>
<th>Information source</th>
<th>Number of records identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Science Citation Index (SCI)</td>
<td>1,594</td>
</tr>
<tr>
<td>Conference Proceedings Citation Index – Science (CPCI-S)</td>
<td>101</td>
</tr>
<tr>
<td>CAB Abstracts</td>
<td>2,815</td>
</tr>
<tr>
<td>BIOSIS Citation Index</td>
<td>1,352</td>
</tr>
<tr>
<td>MEDLINE and MEDLINE In-Process</td>
<td>1,525</td>
</tr>
<tr>
<td>Open Grey</td>
<td>18</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>7,405</strong></td>
</tr>
<tr>
<td><strong>Total without duplicates</strong></td>
<td><strong>3,865</strong></td>
</tr>
</tbody>
</table>
Table 2  Description of 11 studies identified as potential longitudinal studies but excluded after full-text screening

<table>
<thead>
<tr>
<th>Citation</th>
<th>Rationale for exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Baldelli et al., 2011)</td>
<td>Animals in the study were only evaluated using serology. Some animals were tested using PCR on bone marrow but the authors only tested the serology-positive animals with BM PCR.</td>
</tr>
<tr>
<td>(Dye et al., 1993)</td>
<td>Although the dogs are likely naïve as they were introduced into the kennel area, the testing approaches used during the study consisted only of serology based on IFAT.</td>
</tr>
<tr>
<td>(Fisa et al., 1999)</td>
<td>Not a longitudinal study, rather a series of cross-sectional surveys conducted in an endemic area.</td>
</tr>
<tr>
<td>(Madany et al., 2004)</td>
<td>Only the abstract is available and it does not mention the use of PCR, so excluded.</td>
</tr>
<tr>
<td>(Mansueto et al., 1982)</td>
<td>Excluded because it was published prior to 1990 so not likely to have PCR results.</td>
</tr>
<tr>
<td>(Marty et al., 1988)</td>
<td>Excluded because it was published in 1988 and therefore does not meet the cut-off date of 1990.</td>
</tr>
<tr>
<td>(Mekuzas et al., 2009)</td>
<td>Excluded because this study was published in 1973 and therefore does not have a post-1990 PCR assay.</td>
</tr>
<tr>
<td>(Mitali et al., 1999)</td>
<td>Study does not report the use of PCR as an approach to screening the dogs either at the beginning or during the study.</td>
</tr>
<tr>
<td>(Otranto et al., 2007)</td>
<td>This study uses an immunochromatographic dipstick test and the working group asked only for IFAT and ELISA. Other serological assays were not asked for as per the protocol.</td>
</tr>
<tr>
<td>(Paradies et al., 2010)</td>
<td>Paper not published in English.</td>
</tr>
<tr>
<td>(Vidor et al., 1991)</td>
<td>Study does not appear to use PCR. The full paper is not available in English.</td>
</tr>
</tbody>
</table>
Table 3  Study characteristics of the 7 studies that used naïve dogs in longitudinal studies to evaluate diagnostic tests for the detection of *Leishmania* infection

<table>
<thead>
<tr>
<th>Citation</th>
<th>Study year</th>
<th>Country</th>
<th>Source of dogs</th>
<th>Initial tests for naiveté</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Gramiccia et al., 2010)</td>
<td>2008</td>
<td>Italy</td>
<td>Local strays</td>
<td>PCR, IFAT</td>
</tr>
<tr>
<td>(Hamel et al., 2013)</td>
<td>2009</td>
<td>Germany</td>
<td>Local client-owned</td>
<td>PCR, IFAT</td>
</tr>
<tr>
<td>(Leandro et al., 2001)</td>
<td>NR</td>
<td>NR</td>
<td>Experimental beagles</td>
<td>Laboratory dogs</td>
</tr>
<tr>
<td>(Oliva et al., 2006)</td>
<td>2002</td>
<td>Italy</td>
<td>Imported dogs</td>
<td>PCR, IFAT, Other</td>
</tr>
<tr>
<td>(Otranto et al., 2013)</td>
<td>2011</td>
<td>Italy</td>
<td>Local strays</td>
<td>PCR, IFAT</td>
</tr>
<tr>
<td>(Otranto et al., 2009)</td>
<td>2005</td>
<td>Italy</td>
<td>Local client-owned</td>
<td>PCR, ELISA, IFAT, Other</td>
</tr>
<tr>
<td>(Otranto et al., 2010)</td>
<td>2008</td>
<td>Italy</td>
<td>Local strays, laboratory/experimental</td>
<td>PCR, IFAT, Other</td>
</tr>
</tbody>
</table>
Table 4  Characteristics of diagnostic tests used in 7 longitudinal studies that used naïve dogs to evaluate diagnostic tests for the detection of *Leishmania* infection

<table>
<thead>
<tr>
<th>Citation</th>
<th>PCR description</th>
<th>ELISA description</th>
<th>IFAT description</th>
<th>IFAT cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Gramiccia et al., 2010)</td>
<td>For the n-PCR assay, the first amplification was carried out in a 50 l volume containing 10 l DNA plus 40 l PCR Master Mix (Promega) containing 50 pmol of the kinetoplastid-specific primers R221 and R332 of the small subunit rRNA gene (van Eys et al., 1992). For the second amplification, 3 l of the first PCR product were added to 22 l of PCR Master Mix (Promega) containing 3 pmol of the <em>Leishmania</em>-specific primers R223 and R333 of the same gene (van Eys et al., 1992). In canine assays, 2 negative (no DNA, and buffy coat (BC) DNA from healthy dogs) and 2 positive controls (DNA from <em>L. infantum</em> cultured promastigotes and BC DNA from <em>Leishmania</em>-infected dogs) were employed. Amplification products were analyzed on 1.5% agarose gel and visualized under UV light. Positive samples yielded a predicted n-PCR product of 358 bp. Contamination of amplicons was avoided by using physical separation (rooms and materials) as well as decontamination procedures (UV exposure and bleaching of materials and surfaces). To exclude false-negative results due to scarce sample material, low DNA extraction efficiency, or the presence of PCR inhibitors, a random subset (approximately 10% of the total) of canine DNA samples resulted n-PCR-negative were submitted to conventional PCR for the amplification of the 181 bp fragment of the canine glyceraldehyde-3-phosphate dehydrogenase</td>
<td>The in-house IFAT antigen consisted of cultured promastigotes of the WHO reference strain for <em>L. infantum</em> zymodeme MON-1 (MHOM/TN/80/IPT-1). The assay procedure followed the protocol recommended by the Office International des Epizooties (OIE, 2000). A low cut-off dilution (1/80) was set for the selection of cohort (A) dogs, aiming to include full seronegative animals for follow-up samplings. For all other determinations, the IFAT cut-off titre was set at 1/160 as per standard operational protocol applied for dog examination at kennel entry.</td>
<td>1/80</td>
<td></td>
</tr>
<tr>
<td>Author(s)</td>
<td>Methodology</td>
<td>Result</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamel et al., 2013</td>
<td>Real-time PCRs were used for the detection of <em>Leishmania</em> spp. and <em>E. canis</em>-DNA [15, Silaghi et al., unpublished].</td>
<td>An in-house <em>Leishmania</em> spp.-IFAT was used for the detection of anti-<em>Leishmania</em> spp.-antibodies.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leandro et al., 2001</td>
<td>Parasite’s DNA was detected by polymerase chain reaction (PCR) according to Campion et al. (2000), from popliteal lymph node, bone marrow and skin tissue.</td>
<td>Parasite-specific antibodies were determined by ELISA. ELISA, for total anti-<em>Leishmania</em> antibodies, was performed according to Voller et al. (1976) and modified using the conjugate protein A-HRP (Biorad, Hercules, USA). The optical density of 0.180 was considered the limiting titre.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oliva et al., 2006</td>
<td>Total genomic DNA was extracted from 350 µl of BM sample using the Easy-DNA kit (Invitrogen, San Diego, CA) and was stored at 20°C until use. The first PCR amplification was carried out in a 50-µl volume containing 10 µl BM DNA plus 40 µl PCR Master Mix (Promega) containing 50 pmol of the kinetoplastid-specific primers R221 and R332 of the small-subunit rRNA gene (27). For the second amplification, 3 µl of the first PCR product was added to 22 µl of PCR Master Mix (Promega) containing 3 pmol of the <em>Leishmania</em>-specific primers R223 and R333 of the same gene.</td>
<td>The in-house antigen consisted of promastigotes of <em>L. infantum</em> zymodeme MON-1, and the assay procedure followed the protocol of the Office International des Epizooties (9). The cut-off dilution was set at 1:160.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Otranto et al., 2013</td>
<td>PCR for the amplification of <em>Leishmania</em> DNA was performed on bone marrow, conjunctival swab and skin samples. Total DNA was extracted using the QIAampDNA Micro Kit (Qiagen, GmbH, Hilden, Germany) and the Genomic DNA Purification Kit (Genta Systems, Minnesota, USA), respectively, and a fragment of <em>L. infantum</em> kinetoplast DNA.</td>
<td>An indirect immunofluorescent antibody test (IFAT) was performed using promastigotes of <em>L. infantum</em> zymodeme MON1 as antigen as described elsewhere [19]. Samples were scored positive when a clear cytoplasmic or membrane fluorescence with promastigotes could be observed using a cut-off dilution of 1:80; positive</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
minicircle was amplified using the MC1/MC2 primer set [14]. Amplicons were resolved in ethidium bromide-stained (2%) agarose gels (Gellyphor, Italy) and sized by comparison with markers in the Gene Ruler™ 100 bp DNA Ladder (MBI Fermentas, Lithuania). Gels were photographed using a digital documentation system (Gel Doc 2000, BioRad, UK).  

One skin sample weighing about 30 mg per animal and per collection time was taken from the right shoulder region by using a disposable ophthalmology scalpel after the hair over an area of about 0.5 by 0.5 cm was clipped. The samples were stored at 20°C in Eppendorf tubes containing 1 ml of phosphate-buffered saline. After disruption in liquid nitrogen and pestling (i.e., two freeze-thaw cycles), genomic DNA was extracted from the approximately 30-mg skin samples by using a commercial kit (genomic DNA purification kit; Gentra Systems). An L. infantum kinetoplastid minicircle DNA fragment was amplified with the MC1MC2 primer pair (6). Genomic DNA solution (4 l) was added to the PCR mixture (46 l), which contained 2.5 mM MgCl₂, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 250 M of each deoxynucleoside triphosphate, 50 pmol of each primer, and 1.25 U of Ampli Taq Gold (Applied Biosystems, Milan, Italy). Optimal conditions for PCR amplification were standardized as follows: initial denaturation at 94°C for 12 min; 30 cycles consisting of denaturation at 94°C for 30 s, annealing at 60°C for 20 s, and extension at 72°C for 30 s; and a final extension at 72°C for 5 min. A positive control containing genomic L. infantum DNA and a  

The ELISA was performed by using water-soluble proteins of promastigote forms of L. infantum (zymodeme MON1) as antigens and goat anti-dog IgG antibodies (gamma chain specific) conjugated to alkaline phosphatase (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) as detection antibodies. Samples were considered positive if the absorbance at 405 nm (A 405) was above the arithmetic mean plus 3 standard deviations of the A 405 values for 48 Leishmania control dogs originating from an area in southern Switzerland free of L. infantum, E. canis, and Babesia canis (21). The results were expressed as a system of arbitrary antibody units (AU), where 0 AU corresponds to the threshold value and 100 AU corresponds to the result for the positive standard sample.

IFAT was performed with promastigotes of L. infantum zymodeme MON1 as the antigen. The cells were exposed to sera diluted (1:80) in phosphate-buffered saline in a moist chamber and then to fluoresceinated rabbit anti-dog immunoglobulin G (IgG) serum diluted 1:40 (rabbit anti-dog IgG; lot 125K4752; Sigma-Aldrich Chemie, Germany); each exposure was at 37°C for 30 min. The samples were scored positive when they produced a clear cytoplasmic or membrane fluorescence with promastigotes by use of a cut-off dilution of 1:80. Positive sera were titrated until they gave negative results.
negative control without DNA were included in all the assays. Amplification products (447 bp) were visualized on 2% (wt/vol) agarose gels (Ambion, Milan, Italy) upon staining with ethidium bromide.

DNA was extracted from 100 µl of bone marrow using the QIAamp DNA microkit (Qiagen, Milan, Italy) and from about 50 mg of skin samples using a genomic DNA purification kit (Gentra Systems, Minneapolis, MN), following the producers’ recommendations. A fragment (447 bp) of the *L. infantum* minicircle kinetoplast DNA (kDNA) was amplified using the primers MC1 and MC2 (5).

The sample DNA (4 l) was added to the PCR mix (46 l) containing 2.5 mM MgCl₂, 10 mM Tris-HCl, pH 8.3, and 50 mM KCl, 250 M (each) deoxynucleoside triphosphates (dNTPs), 50 pmol of each primer, and 1.25 U of AmpliTaq Gold polymerase (Applied Biosystems, Milan, Italy).

PCR conditions were standardized as follows: initial denaturation at 94°C for 12 min, 30 cycles consisting of denaturation at 94°C for 30 s, annealing at 60°C for 20 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. Positive (*L. infantum* DNA) and negative (no DNA) controls were included in all the assays. Amplification products were visualized by 2% agarose gel electrophoresis under UV exposure.

An in-house immunofluorescent antibody test (IFAT) was used to detect anti-*Leishmania* IgG antibodies. Promastigotes of *L. infantum* zymodeme MON-1 were used as an antigen, and all procedures were performed as described elsewhere (29). Samples were scored as positive when they produced a clear cytoplasmic or membrane fluorescence with promastigotes using a cut-off dilution of 1:80. Positive sera were titrated until negative.

(Otranto et al., 2010)
### Table 5  Risk of bias in 7 longitudinal studies that used naïve dogs to evaluate diagnostic tests for the detection of *Leishmania* infection (see appendix E. for the risk of bias (ROB) questions and interpretation).

<table>
<thead>
<tr>
<th>Risk of bias domain</th>
<th>(Gramiccia et al., 2010)</th>
<th>(Hamel et al., 2013)</th>
<th>(Leandro et al., 2001)</th>
<th>(Oliva et al., 2006)</th>
<th>(Otranto et al., 2013)</th>
<th>(Otranto et al., 2009)</th>
<th>(Otranto et al., 2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Representative spectrum?</td>
<td>Not discernible</td>
<td>Yes</td>
<td>No</td>
<td>Not discernible</td>
<td>Not discernible</td>
<td>Not discernible</td>
<td>Not discernible</td>
</tr>
<tr>
<td>ROB -- Representative spectrum Notes: representative spectrum</td>
<td>Unclear</td>
<td>Low</td>
<td>High</td>
<td>Unclear</td>
<td>Dogs kept in kennel situation</td>
<td>Kennel/shelter situation</td>
<td>Unclear</td>
</tr>
<tr>
<td>Acceptable reference standard?</td>
<td>No</td>
<td>No</td>
<td>Not discernible</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>ROB -- Acceptable reference standard Notes: acceptable reference standard</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Unclear</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
</tr>
<tr>
<td>Acceptable delay between tests?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No/Not discernible</td>
<td>Yes</td>
</tr>
<tr>
<td>ROB -- Acceptable delay between tests Notes: acceptable delay between tests</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Assumed run concurrently</td>
<td>Assumed run concurrently</td>
<td>Low</td>
</tr>
<tr>
<td>Partial verification avoided</td>
<td>No/Not discernible</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No/Not discernible</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>ROB -- Partial verification avoided Notes: partial verification avoided</td>
<td>Unclear</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>The original sample of dogs were likely not</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
<td>Many withdrawals not tested at</td>
<td>No notes</td>
</tr>
</tbody>
</table>

Notes: representative spectrum no notes

Notes: acceptable reference standard

Note 1: The dogs were from a non-endemic area, so should have been naïve, given they were seronegative on IFAT.

Note 2: The original sample of dogs were likely not...
Scoping review and protocols for Canine Leishmania

<table>
<thead>
<tr>
<th>Differential verification avoided?</th>
<th>Yes</th>
<th>Yes</th>
<th>Yes</th>
<th>Yes</th>
<th>Yes</th>
<th>Yes</th>
<th>Yes</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROB -- Differential verification avoided</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Notes: differential verification avoided</td>
<td>This is assuming the referent was the BM PCR (not the skin PCR)</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Incorporation of tests avoided?</th>
<th>Yes</th>
<th>Yes</th>
<th>No/Not discernible</th>
<th>Yes</th>
<th>Yes</th>
<th>Yes</th>
<th>Yes</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROB -- Incorporation of tests avoided</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Reference testing blinded?</td>
<td>No/Not reported</td>
<td>No/Not reported</td>
<td>No/Not reported</td>
<td>No/Not reported</td>
<td>No/Not reported</td>
<td>No/Not reported</td>
<td>No/Not reported</td>
<td>No/Not reported</td>
</tr>
<tr>
<td>ROB -- Reference testing blinded</td>
<td>Unclear</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Lab personnel blinded to treatment group, but not</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Notes: index test results blinded</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
</tr>
</tbody>
</table>

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.
<table>
<thead>
<tr>
<th>Index testing blinded?</th>
<th>No/Not discernible</th>
<th>No/Not discernible</th>
<th>No/Not discernible</th>
<th>No/Not discernible</th>
<th>necessarily to previous test results</th>
<th>No/Not discernible</th>
<th>No/Not discernible</th>
<th>No/Not discernible</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROB -- Index testing blinded</td>
<td>Unclear</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Unclear</td>
</tr>
<tr>
<td>Notes: reference test results blinded</td>
<td>no notes</td>
<td>no notes</td>
<td>no notes</td>
<td>no notes</td>
<td>Lab personnel blinded to treatment group, but not necessarily to previous test results</td>
<td>no notes</td>
<td>no notes</td>
<td>no notes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intermediate/uninterpretable test results reported?</th>
<th>Not discussed</th>
<th>Not discussed</th>
<th>Not discussed</th>
<th>Not discussed</th>
<th>necessarily to previous test results</th>
<th>Yes</th>
<th>Not discussed</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROB -- Intermediate/uninterpretable test results reported</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Lab personnel blinded to treatment group, but not necessarily to previous test results</td>
<td>Low</td>
<td>Unclear</td>
</tr>
<tr>
<td>Withdrawals explained?</td>
<td>Yes</td>
<td>&gt;10% loss to follow-up</td>
<td>&gt;10% loss to follow-up</td>
<td>Yes</td>
<td>Yes</td>
<td>&gt;10% loss to follow-up</td>
<td>&gt;10% loss to follow-up</td>
</tr>
<tr>
<td>ROB -- Withdrawals explained</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Notes: withdrawals</td>
<td>This is for the 17 group B dogs (there were no losses to follow-up)</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
<td>No notes</td>
</tr>
</tbody>
</table>
Detection of parasite DNA by PCR: 1-2mm³ of the canine tissues were collected and digested overnight at 60°C in 200 ml lysis buffer (50mM NaCl, 50mM Tris-HCl pH 8.0, 10mM EDTA) containing 20% SDS and 200 mg/ml proteinase K. Following phenol/ chloroform extraction and ethanol precipitation, the DNA was re-suspended in 50-ml ultra-pure water. PCR amplification was carried out in 25 ml solution containing 50mM KCl, 10mM Tris-HCl pH 8.0, 4mM MgCl₂, 50 pmol of each primer, 200μmol of deoxynucleotides, 1.25U Taq polymerase and 5 ml of DNA, for 38 cycles in a Perkin–Elmer Cetus 480® thermal cycler. Each cycle consisted of denaturation at 94°C (10-1500), annealing at 60°C (10), and extension at 72°C (20). The PCRs were performed using primers derived from the small sub-unit rRNA sequence (50GGTTCCTTTCCTGATTTACG30 and 50GGCCGGTAAAGGCCGAATAG30) which amplify all types of *Leishmania* (Van Eys et al., 1992). Inhibition of the reaction, or degradation of DNA, was controlled subjecting 5ml of each DNA sample to a PCR using primers from the canine b-actin gene (50TCCTCCCTGGAGAAGGC30 and 50ATCTCCTTCTGCAATCG30) under the same conditions as described above. The PCR products were analyzed by 2% agarose gel electrophoresis, with a 100 bp DNA ladder used as a marker. Samples showing a 600 bp PCR product were scored as positive.
### Table 6  
Individual study results for 7 studies of naïve dogs included in the evaluation of diagnostic tests

#### Longitudinal study on the detection of canine *Leishmania* infections by conjunctival swab analysis and correlation with entomological parameters (Gramiccia et al., 2010)

**Definition of naïve dogs:** Negative on conjunctival swab PCR and IFAT in February 2008

<table>
<thead>
<tr>
<th>Date of follow-up test (Month, year)</th>
<th>Number of dogs tested at time point</th>
<th>Number of dogs test positive with “gold standard” (GS)</th>
<th>PCR1 (Conjunctiva)</th>
<th>PCR2</th>
<th>IFAT</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GS+ and PCR1+ (relative sensitivity)</td>
<td>GS– and PCR− (relative specificity)</td>
<td>GS+ and PCR2+ (relative sensitivity)</td>
<td>GS– and PCR− (relative specificity)</td>
</tr>
<tr>
<td>July 2008</td>
<td>17</td>
<td>17</td>
<td>0 (0%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>September 2008</td>
<td>17</td>
<td>17</td>
<td>0 (0%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>November 2008</td>
<td>17</td>
<td>6</td>
<td>0 (0%)</td>
<td>-</td>
<td>-</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>May 2009</td>
<td>17</td>
<td>3</td>
<td>3 (100%)</td>
<td>-</td>
<td>-</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

#### Prevention of Canine Leishmaniosis in a Hyper-Endemic Area Using a Combination of 10% Imidacloprid/4.5% Flumethrin (Otranto et al., 2013)

**Definition of naïve dogs:** Negative on skin, bone marrow and conjunctival swab PCR and IFAT in March-April 2011

<table>
<thead>
<tr>
<th>Date of follow-up test (Month, year)</th>
<th>Number of dogs tested at time point</th>
<th>Number of dogs test positive with “gold standard” (GS)</th>
<th>PCR1 (Bone marrow)</th>
<th>PCR2 (conjunctiva)</th>
<th>IFAT</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GS+ and PCR1+ (relative sensitivity)</td>
<td>GS– and PCR− (relative specificity)</td>
<td>GS+ and PCR2+ (relative sensitivity)</td>
<td>GS– and PCR− (relative specificity)</td>
</tr>
<tr>
<td>Nov. 2011</td>
<td>21</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>7 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>March 2012</td>
<td>21</td>
<td>11</td>
<td>6 (54.5%)</td>
<td>4 (36%)</td>
<td>8 (73%)</td>
<td>-</td>
</tr>
</tbody>
</table>

#### Arthropod-borne infections in travelled dogs in Europe (Hamel et al., 2013)

**Definition of naïve dogs:** Dogs that lived in Germany and travelled to leishmania-infected areas. Tested negative before travel based on IFAT and blood-based PCR

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.
### Cell-mediated immunity and specific IgG1 and IgG2 antibody response in natural and experimental canine leishmaniosis (Leandro et al., 2001)

**Definition of naïve dogs:** Not specifically stated; however, experimental animals were used and tested by ELISA and lymphocyte proliferation to crude *L. infantum* antigen prior to the start of the study

<table>
<thead>
<tr>
<th>Date of follow-up test (Month, year)</th>
<th>Number of dogs tested at time point</th>
<th>Number of dogs test positive with “gold standard” (GS)</th>
<th>PCR1 (relative sensitivity)</th>
<th>PCR2 (relative sensitivity)</th>
<th>IFAT (relative sensitivity)</th>
<th>ELISA (relative sensitivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; post travel (4-373 days after return (mean 38 days))</td>
<td>106 PCR (blood) 0/106</td>
<td>GS+ and PCR1+</td>
<td>GS– and PCR–</td>
<td>GS+ and PCR2+</td>
<td>GS– and PCR2–</td>
<td>GS+ and IFAT+</td>
</tr>
</tbody>
</table>
| 1-16 months | 3 | Not reported in a manner that enabled assessment. The authors stated “Parasites were detected from lymph nodes or bone marrow and skin biopsies in the three dogs” however it is unclear if this is based on PCR or culture or describes cumulative results of monthly outcomes.

### Incidence and time course of *Leishmania infantum* infections examined by parasitological, serologic, and nested-PCR techniques in a cohort of naïve dogs exposed to three consecutive transmission seasons (Oliva et al., 2006)

**Definition of naïve dogs:** Beagles born in an area of northern Italy where the infection is not endemic and which tested *Leishmania*-negative by serology (immunofluorescent-antibody test [IFAT]), were moved to the study site in July 2002.

<table>
<thead>
<tr>
<th>Date of follow-up test (Month, year)</th>
<th>Number of dogs tested at time point</th>
<th>Number of dogs test positive/negative with “gold standard” (GS)</th>
<th>PCR1 (relative sensitivity)</th>
<th>PCR2 (relative sensitivity)</th>
<th>IFAT (relative sensitivity)</th>
<th>ELISA (relative sensitivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-16 months</td>
<td>3</td>
<td>PCR</td>
<td>GS+ and PCR1+</td>
<td>GS– and PCR–</td>
<td>GS+ and PCR2+</td>
<td>GS– and PCR2–</td>
</tr>
</tbody>
</table>
Toward diagnosing *Leishmania infantum* infection in asymptomatic dogs in an area where leishmaniasis is endemic. (Otranto et al., 2009)

**Definition of naïve dogs:** Negative on IFAT, ELISA, stained lymph node smears and skin PCR in January and February 2005

<table>
<thead>
<tr>
<th>Date of follow-up test (Month, year)</th>
<th>Number of dogs tested at time point</th>
<th>Number of dogs test positive/negative with “gold standard” (GS)</th>
<th>PCR (skin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>November 2005</td>
<td>NR</td>
<td>PCR+ and PCR1+ (relative sensitivity)</td>
<td>14/?</td>
</tr>
<tr>
<td>March 2006</td>
<td>NR</td>
<td>PCR– and PCR2– (relative specificity)</td>
<td>11/?</td>
</tr>
<tr>
<td>March 2007</td>
<td>NR</td>
<td>PCR+ and PCR1+ (relative specificity)</td>
<td>23/?</td>
</tr>
</tbody>
</table>

- * The authors report in Table 4 that the sensitivity is 33%. However, the text says “11 dogs positive on PCR only, and 3 parasitological and serology positive”. The authors do not clarify if the positive serology is ELISA or IFAT. Based on Table 5, it appears that only 1 of those 3 serological/parasitology positive was positive on ELISA therefore our estimate of sensitivity is 1/14 (7%). We were unable to verify the percentages reported in Table 4, as the authors did not report numbers, only percentages. The same issue was repeated for March 2006 and March 2007 data. Note that our approach to verifying the IFAT data did correspond with the authors’ data in Table 4. Also note that Table 4 had a large number of superscripts without any legends or footnotes.
- ^ The number of animals tested at each time point is not clear. The authors do not clarify if all dogs available at the time were used to obtain estimates or if only dogs with complete data were used (173) to estimate the data in Table 4.
- ¶ Dead dog excluded.

Supporting publications 2015:EN-761
### Diagnosis of canine vector-borne diseases in young dogs: a longitudinal study (Otranto et al., 2010)

**Definition of naïve dogs:** Negative on all cytology, IFAT, PCR skin, and PCR bone marrow

<table>
<thead>
<tr>
<th>Date of follow-up test (Month, year)</th>
<th>Number of dogs tested at time point</th>
<th>Number of dogs test positive/negative with “gold standard” (GS)</th>
<th>PCR1 (relative sensitivity)</th>
<th>PCR2 (relative sensitivity)</th>
<th>IFAT (relative sensitivity)</th>
<th>ELISA (relative sensitivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 2008</td>
<td>39</td>
<td>PCR (skin) 0/39</td>
<td>NA</td>
<td>39 (100%)</td>
<td>NR (0%)</td>
<td>NR (87%)</td>
</tr>
<tr>
<td>October 2008</td>
<td>46</td>
<td>1/45</td>
<td>NR (67%)</td>
<td>NR (100%)</td>
<td>NR (100%)</td>
<td>NR (58.5%)</td>
</tr>
<tr>
<td>April 2009</td>
<td>PCR skin 42; PCR bone marrow 44; IFAT 42</td>
<td>3</td>
<td>NR (67%)</td>
<td>NR (100%)</td>
<td>NR (100%)</td>
<td>NR (58.5%)</td>
</tr>
</tbody>
</table>

* EFSA did not define a gold standard so if the study authors defined a gold standard that was used. If the study authors did not define a gold standard, we used the PCR if only one was used, or if multiple PCR assays were used we used the assay most frequently used; if multiple PCR assays were used with equal frequency we selected the gold standard in the following order: bone marrow, blood, skin, lymph node, and conjunctiva.

Supporting publications 2015:EN-761
Table 7 Description of 13 studies included in a review of studies comparing treatment efficacy of meglumine antimoniate, allopurinol, or miltefosine for Leishmania infection in dogs in Europe

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Title</th>
<th>Year published</th>
<th>Country</th>
<th>Source of dogs</th>
<th>Health status of dogs</th>
<th>PCR used</th>
<th>Xenodiagnosis used</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ariti et al., 2013)</td>
<td>Treatment of canine leishmaniasis: long-term molecular and serological observations</td>
<td>2013</td>
<td>Italy</td>
<td>Veterinary hospitals/client-owned dogs</td>
<td>Clinically unwell</td>
<td>See footnote¹</td>
<td>None</td>
</tr>
<tr>
<td>(Guarga et al., 2002)</td>
<td>Evaluation of a specific immunochemotherapy for the treatment of canine visceral leishmaniasis</td>
<td>2002</td>
<td>Spain</td>
<td>Not discernible</td>
<td>Clinically unwell</td>
<td>None</td>
<td>See footnote²</td>
</tr>
<tr>
<td>(Miro et al., 2011)</td>
<td>Infectivity to Phlebotomus perniciosus of dogs naturally parasitized with Leishmania infantum after different treatments</td>
<td>2011</td>
<td>Spain</td>
<td>Veterinary hospitals/client-owned dogs</td>
<td>Clinically unwell</td>
<td>None</td>
<td>See footnote³</td>
</tr>
</tbody>
</table>

¹ “PCR for Leishmania sp. was performed as previously described." Blood, conjunctiva, and lymph node samples used. From supplementary paper: "…DNA hybridization methods through the amplification of the minicircle target sequence. The oligonucleotide primers used are able to direct the amplification of all Leishmania strains tested.”

² “The handling of the animals and the vectors was carried out in an insectarium conditioned according to the recommendations of “The Subcommittee on Arbovirus Laboratory Safety of the American Committee on Arthropod-Borne Viruses, Centre for Disease Control, Atlanta, GA (Anon., 1980). In order to determine the infectiveness of the dogs on the sand flies which fed on it, a colony of Phlebotomus perniciosus kept in laboratory conditions (Molina, 1991) was used. Briefly, each dog was anaesthetised with 0.03 mg/kg of medetomidine hydrochloride (DOMTOR 1, SmithKline Beecham) and its head was introduced for 1 h in a cubic net made of fine gauze and containing a group of 75 females sand flies. The sand flies were dissected 5 days post-breed meal to detect the presence of Leishmania promastigotes in their middle gut. The percentages of infection were estimated with the number of existing females at the moment of dissection.”

³ “The infectivity of dogs was assessed through direct xenodiagnosis. The local colony of P. perniciosus used in this study had been laboratory-reared at the Instituto de Salud Carlos III, Madrid [30]. The colony was kept in a chamber under controlled conditions of temperature (27-28°C), relative humidity (95-100%) and light cycle (17 hours light/7 hours dark). Dogs were sedated by the intravenous injection of 0.5 mg/kg of medetomidine and their heads introduced into individual cube-shaped nets (50 cm wide × 50 cm high × 50 cm deep). Before treatment (Day 0) and 60, 120, and 180 dpt onset, dogs were exposed for one hour to 100 unfed, 7-day-old female sand flies released inside the nets. After one hour of exposure, the sand flies were carefully collected using a mouth aspirator. Fed flies collected from each dog were separated into individual nets (15 × 15 × 15 cm). Next the dogs were removed from their cages and intravenously administered 0.25 mg/kg of atipamezole. Fed sand flies were kept inside the chambers for 5-7 days. Engorged females were dissected and the sand fly midgut was examined under a light microscope to detect L. infantum promastigotes.”
<table>
<thead>
<tr>
<th>Author(s) and Year</th>
<th>Study Description</th>
<th>Year</th>
<th>Country/Region</th>
<th>Treatment Protocol</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mateo et al., 2009</td>
<td>Multicentric, controlled clinical study to evaluate effectiveness and safety of miltefosine and allopurinol for canine leishmaniosis</td>
<td>2009</td>
<td>France, Italy, Spain</td>
<td>Veterinary hospitals/client-owned dogs</td>
<td>Clinically unwell</td>
<td>See footnote 4</td>
</tr>
<tr>
<td>Moritz et al., 1998</td>
<td>Clinical follow-up examination after treatment of canine leishmaniasis</td>
<td>1998</td>
<td>Germany</td>
<td>Veterinary hospitals/client-owned dogs</td>
<td>Clinically unwell</td>
<td>“by PCR method (peripheral blood/bone marrow)”</td>
</tr>
<tr>
<td>Neogy et al., 1994</td>
<td>Exploitation of parasite-derived antigen in therapeutic success against canine visceral leishmaniosis</td>
<td>1994</td>
<td>France</td>
<td>Veterinary hospitals/client-owned dogs</td>
<td>Clinically unwell</td>
<td>None</td>
</tr>
<tr>
<td>Oliva et al., 1998</td>
<td>Comparative efficacy of meglumine antimoniate and aminosidine sulphate, alone or in combination, in canine leishmaniasis</td>
<td>1998</td>
<td>Italy</td>
<td>Not discernible</td>
<td>Clinically unwell</td>
<td>None</td>
</tr>
<tr>
<td>Paradies et al., 2012</td>
<td>Monitoring the reverse to normal of clinico-pathological findings and the disease-free interval time using four different treatment protocols for canine leishmaniasis in an endemic area</td>
<td>2012</td>
<td>Italy</td>
<td>Veterinary hospitals/client-owned dogs</td>
<td>Clinically unwell</td>
<td>None</td>
</tr>
</tbody>
</table>

4 “The RTQ-PCR analysis of bone marrow aspirates was performed by the WHO Collaborating Centre for Leishmaniosis – Instituto de Salud Carlos III (Madrid, Spain). Quantification of leishmanial DNA in the dogs studied was performed using LightCycler high speed thermal cycler and the LightCycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics). PCR primers used for specific amplification of *Leishmania* were R223 and R333. 20 Final volume of the reaction was 20 mL, including 4 mL of target DNA. Quantification was by means of a standard curve which consisted of serial dilutions of *L. infantum* DNA (strain IPC, MCAN/ES/98/LLM-722), ranging from the equivalent to 10⁵ parasites/mL up to 10¹ parasites/mL. Melting peak analysis was performed to check the specificity of the PCR products.”

5 “The PCR technique applied was that described by Reale and others (1999). The target DNA for amplification was a 116 base pair fragment in the constant region of the kDNA minicircle.”
<table>
<thead>
<tr>
<th>Author(s) and Year</th>
<th>Title of the Study</th>
<th>Year</th>
<th>Country</th>
<th>Setting</th>
<th>Status</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plevraki et al., 2006</td>
<td>Effects of allopurinol treatment on the progression of chronic nephritis in canine leishmaniosis (Leishmania infantum)</td>
<td>2006</td>
<td>Greece</td>
<td>Veterinary hospitals/client-owned dogs</td>
<td>Clinically unwell</td>
<td>None</td>
<td>“…bone marrow PCR was performed for the detection of L. infantum rRNA.”</td>
</tr>
<tr>
<td>Saridomichekakis et al., 2005</td>
<td>Periodic administration of allopurinol is not effective for the prevention of canine leishmaniosis (Leishmania infantum) in the endemic areas</td>
<td>2005</td>
<td>Greece</td>
<td>Veterinary hospitals/client-owned dogs</td>
<td>Healthy/no clinical signs</td>
<td>See footnote⁶</td>
<td>None</td>
</tr>
<tr>
<td>Slappendel and Teske, 1997</td>
<td>The effect of intravenous or subcutaneous administration of meglumine antimonate (Glucantime) in dogs with leishmaniasis. A randomized clinical trial</td>
<td>1997</td>
<td>Netherlands</td>
<td>Veterinary hospitals/client-owned dogs</td>
<td>Clinically unwell</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

⁶ “An aliquot of each bone marrow sample served to amplify a 250 bp fragment of the rRNA gene of Leishmania spp. according to a validated PCR protocol (Koutinas et al., 2001; Leontides et al., 2002), where T2 and B4 primers were utilized (Minodier et al., 1997).”
| (Steuber et al., 1998) | PCR follow-up examination after treatment of canine leishmaniosis (CaL) | 1998 | Germany | Veterinary hospitals/client-owned dogs | Not discernible | See footnote 7 | None |

7 “DNA was standardized using the QIAamp Blood Kit® (Qiagen, Germany) for cleanup. For the detection of genomic Leishmania DNA, the PCR procedure described by Piarroux et al. [23] was used. This procedure permitted the identification of a highly repetitive 100 bp sequence of *Leishmania infantum*. For each run, a total volume of 50μL was used consisting of 10μL of extracted specimen DNA, 0.2 mM each of dNTP (dUTP, dATP, dCTP, dGTP, Boehringer, Germany), PCR buffer containing 1.5 mM MgCl₂ (Perkin Elmer, Germany), Taq polymerase Gold (Perkin Elmer, Germany) and 1μM of each primer. The material used was overlaid with 50μL mineral oil in each case. Amplification was performed over 34 cycles using a TRIO-Thermocycler® (Biometra, Germany), denaturing at 94°C (first cycle 3 min, all further cycles 30 sec), annealing at 59 °C (30 sec) and extension at 70 °C (30 sec, in the last cycle 10 min). If the PCR was negative in the first run a second PCR-run has been performed. Detection of amplificates: Amplification products were visualized by submarine minigel electrophoresis in a 4% composite gel (Biozym, Germany) stained with ethidium bromide. Subsequent gel evaluation was performed with the aid of Phoretix 1D GelAnalysis Software (SLG, Germany). After Southern Blot hybridization with the respective peroxidase-labelled probes, the specificity of the amplification products was confirmed using the ECL® direct nucleic acid labelling and detection system in accordance with the manufacturer’s data (Amersham, Germany). The DNA probes needed for this purpose were obtained by sequential amplification of the respective PCR amplificates from *L. infantum* (100 bp products) with subsequent cleanup (Qiagen, Germany). Possible cross contamination was prevented by the use of aerosol-protected filter tips and disposable gloves and strict separation of areas for sample preparation, PCR runs (UV-irradiated laminar flow bench), amplification and gel electrophoresis.”
### Table 8  Risk of bias assessment of studies in which PCR was performed at follow-up.

<table>
<thead>
<tr>
<th>Risk of bias item</th>
<th>(Ariti et al., 2013)</th>
<th>(Miro et al., 2009)</th>
<th>(Pennisi et al., 2005), (Plevraki et al., 2006)</th>
<th>(Saridomichelakis et al., 2005)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selection bias: Was allocation to treatment group randomized?</td>
<td>Yes—Low risk</td>
<td>Yes—Low risk</td>
<td>Yes—Low risk</td>
<td>No—High risk</td>
</tr>
<tr>
<td>Selection bias: Does the study describe the method to conceal allocation?</td>
<td>No—High risk</td>
<td>No—High risk</td>
<td>No—High risk</td>
<td>No—High risk</td>
</tr>
<tr>
<td>Performance bias: Were measures to blind owners/personnel described?</td>
<td>No—Low risk Parasitological cure is an outcome that should not be affected by dog carers’ knowledge of treatment group.</td>
<td>No—Low risk Parasitological cure is an outcome that should not be affected by dog carers’ knowledge of treatment group.</td>
<td>No—Low risk Parasitological cure is an outcome that should not be affected by dog carers’ knowledge of treatment group.</td>
<td>Yes—Low risk</td>
</tr>
<tr>
<td>Detection bias: Do they describe measures to blind outcome assessors?</td>
<td>No—Low risk PCR is an objective test that is unlikely to be affected by assessors’ knowledge of treatment group.</td>
<td>No—Low risk PCR is an objective test that is unlikely to be affected by assessors’ knowledge of treatment group.</td>
<td>No—Low risk PCR is an objective test that is unlikely to be affected by assessors’ knowledge of treatment group.</td>
<td>No—Low risk PCR is an objective test that is unlikely to be affected by assessors’ knowledge of treatment group.</td>
</tr>
<tr>
<td>Attrition bias: Were there incomplete outcome data in the study?</td>
<td>No loss to follow-up—Low risk</td>
<td>Loss to follow-up but explained—Low risk Losses were minimal and balanced between groups.</td>
<td>Loss to follow-up but explained—High risk &gt;20% of dogs in the control group were lost to follow-up. Some withdrawals were related to treatment. A sensitivity analysis was performed.</td>
<td>Loss to follow-up but explained—Unclear risk &gt;20% of dogs lost in some groups; losses were differential between groups and some may have been related to</td>
</tr>
</tbody>
</table>
### Risk of bias item

#### Reporting bias

<table>
<thead>
<tr>
<th>Item</th>
<th>(Ariti et al., 2013)</th>
<th>(Miro et al., 2009)</th>
<th>(Pennisi et al., 2005), analysis was not done to determine potential impact of losses.</th>
<th>(Plevraki et al., 2006)</th>
<th>(Saridomichelakis et al., 2005)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Was there selective reporting of outcomes?</td>
<td>Yes—Unclear risk</td>
<td>Yes—High risk</td>
<td>No—Low risk</td>
<td>Yes—High risk</td>
<td>No—Low risk</td>
</tr>
<tr>
<td>The data collected at day 360 of follow-up were not reported.</td>
<td></td>
<td>The parasitological cure rate for individual dogs was not reported or summarized despite being available. Only reported median parasite load.</td>
<td></td>
<td>The descriptive data for parasite load were not reported, only the p-values for the difference in loads between start and end of treatment were reported in groups in which the change was significant.</td>
<td></td>
</tr>
</tbody>
</table>

#### Other bias

<table>
<thead>
<tr>
<th>Item</th>
<th>(Ariti et al., 2013)</th>
<th>(Miro et al., 2009)</th>
<th>(Pennisi et al., 2005), analysis was not done to determine potential impact of losses.</th>
<th>(Plevraki et al., 2006)</th>
<th>(Saridomichelakis et al., 2005)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Was the sensitivity of the assay(s) used at follow-up reported?</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
</tr>
<tr>
<td>PCR is understood to be a relatively sensitive test for <em>Leishmania</em>.</td>
<td></td>
<td>PCR is understood to be a relatively sensitive test for <em>Leishmania</em>.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>—Unclear risk Funding/conflict of interest: not reported</td>
<td></td>
<td>—Unclear risk</td>
<td>—Unclear risk Funding by the pharmaceutical company making one of the interventions Conflict of interest: not reported Confounding: randomized Reinfecion: possible but not differential between groups</td>
<td>—Unclear risk</td>
<td>Funding/conflict of interest: not reported Confounding: not randomized and health status did not vary between groups Reinfecion: possible but not differential between groups</td>
</tr>
<tr>
<td>Confounding: randomized</td>
<td></td>
<td>—Unclear risk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reinfecion: deltamethrin-impregnated collars used to prevent reinfecion</td>
<td></td>
<td>—Unclear risk</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Other reasons for bias

<table>
<thead>
<tr>
<th>Item</th>
<th>(Ariti et al., 2013)</th>
<th>(Miro et al., 2009)</th>
<th>(Pennisi et al., 2005), analysis was not done to determine potential impact of losses.</th>
<th>(Plevraki et al., 2006)</th>
<th>(Saridomichelakis et al., 2005)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Was funding source reported? Was conflicts of interest disclosed?</td>
<td>No—Unclear risk</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
</tr>
<tr>
<td>Were confounders adequately accounted for through randomization, etc.?</td>
<td></td>
<td>PCR is understood to be a relatively sensitive test for <em>Leishmania</em>.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Could reinfection occur during follow-up differentially between groups?</td>
<td></td>
<td>—Unclear risk</td>
<td>—Unclear risk Funding by the pharmaceutical company making one of the interventions Conflict of interest: not reported Confounding: randomized Reinfecion: possible but not differential between groups</td>
<td>—Unclear risk</td>
<td>Funding/conflict of interest: not reported Confounding: not randomized and health status did not vary between groups Reinfecion: possible but not differential between groups</td>
</tr>
<tr>
<td>Other sources of bias?</td>
<td></td>
<td>—Unclear risk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The number of treatment courses was dependent on the performance of dog. Some dogs received treatment for the entire</td>
<td></td>
<td>—Unclear risk</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.
<table>
<thead>
<tr>
<th>Risk of bias item</th>
<th>(Ariti et al., 2013)</th>
<th>(Miro et al., 2009)</th>
<th>(Pennisi et al., 2005)</th>
<th>(Plevraki et al., 2006)</th>
<th>(Saridomichelakis et al., 2005)</th>
</tr>
</thead>
<tbody>
<tr>
<td>follow-up and some didn’t in the same group.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

676

677
### Table 9  Risk of bias assessment of studies in which xenodiagnosis was performed at follow-up.

<table>
<thead>
<tr>
<th>Risk of bias domain</th>
<th>(Guarga et al., 2002)</th>
<th>(Miro et al., 2011)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Selection bias</strong></td>
<td>Was allocation to treatment group randomized?</td>
<td>No—High risk</td>
</tr>
<tr>
<td>Selection bias</td>
<td>Were measures to blind owners/personnel described?</td>
<td>No—Low risk</td>
</tr>
<tr>
<td>Performance bias</td>
<td>Were measures to blind owners/personnel described?</td>
<td>Parasitological cure is an outcome that should not be affected by dog carers’ knowledge of treatment group.</td>
</tr>
<tr>
<td>Detection bias</td>
<td>Do they describe measures to blind outcome assessors?</td>
<td>No—High risk</td>
</tr>
<tr>
<td>Attrition bias</td>
<td>Were there incomplete outcome data in the study?</td>
<td>Test protocol somewhat subjective and assessor’s knowledge of treatment group could have influenced time spent looking for parasites.</td>
</tr>
<tr>
<td>Reporting bias</td>
<td>Was there selective reporting of outcomes?</td>
<td>Yes—High risk</td>
</tr>
<tr>
<td>Other bias</td>
<td>Was the sensitivity of the assay(s) used at follow-up reported?</td>
<td>No—Unclear risk</td>
</tr>
<tr>
<td>Other bias</td>
<td>Was funding source reported? Were conflicts of interest disclosed?</td>
<td>Funding/conflict of interest: not reported</td>
</tr>
<tr>
<td></td>
<td>Were confounders adequately accounted for through randomization, etc.?</td>
<td>Confounding: unsure if randomized and health status of dogs unclear</td>
</tr>
<tr>
<td></td>
<td>Could reinfection occur during follow-up differentially</td>
<td>Reinfecion: possible but unsure if bias occurred</td>
</tr>
</tbody>
</table>

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.
### Risk of bias domain

**between groups? Other sources of bias?**  
(Guarga et al., 2002)  
(placebo group was untreated throughout follow-up, whereas treatment group was only off treatment for part of the follow-up period)  
(Miro et al., 2011)  
confounders evenly distributed between groups  
Reinfection: possible and potential was differential between groups
Table 10  Risk of bias assessment of studies in which parasite load quantification was performed at follow-up.

<table>
<thead>
<tr>
<th>Risk of bias domain</th>
<th>(Miro et al., 2009)</th>
<th>(Neogy et al., 1994)</th>
<th>(Oliva et al., 1998)</th>
<th>(Plevraki et al., 2006)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selection bias</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was allocation to treatment group randomized?</td>
<td>Yes—Low risk</td>
<td>Yes—Low risk</td>
<td>Yes—Low risk</td>
<td>No—High risk</td>
</tr>
<tr>
<td>Does the study describe the method to conceal allocation?</td>
<td>No—High risk</td>
<td>No—High risk</td>
<td>No—High risk</td>
<td>No—High risk</td>
</tr>
<tr>
<td>Performance bias</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Were measures to blind owners/personnel described?</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
</tr>
<tr>
<td>Detection bias</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do they describe measures to blind outcome assessors?</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
</tr>
<tr>
<td>Cytological parasite load quantification is subjective and assessors’ knowledge of treatment group may have affected time spent looking for parasites.</td>
<td>No—High risk</td>
<td>No—High risk</td>
<td>No—High risk</td>
<td>Yes—Low risk</td>
</tr>
<tr>
<td>Attraction bias</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Were there incomplete outcome data in the study?</td>
<td>Loss to follow-up but explained—Low risk</td>
<td>No loss to follow-up—Low risk</td>
<td>No loss to follow-up—Low risk</td>
<td>Loss to follow-up but explained—Unclear risk</td>
</tr>
<tr>
<td>Reporting bias</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was there selective reporting of outcomes?</td>
<td>Yes—High risk</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
<td>Yes—High risk</td>
</tr>
</tbody>
</table>

Supporting publications 2015:EN-761 43
## Risk of bias domain

### (Miro et al., 2009)
- **Other bias**
  - Was the sensitivity of the assay(s) used at follow-up reported?
    - No—Unclear risk
    - PCR is understood to be a relatively sensitive test for detecting *Leishmania*; however, it is unclear whether it is accurate or precise to measure parasite load.
  
### (Neogy et al., 1994)
- **Other bias**
  - Was the sensitivity of the assay(s) used at follow-up reported?
    - No—High risk
    - Cytological methods are known to be potentially affected by measurement error and sampling error, reducing accuracy and precision.
  
### (Oliva et al., 1998)
- **Other bias**
  - Was the sensitivity of the assay(s) used at follow-up reported?
    - No—Unclear risk
    - Funding by the pharmaceutical company making one of the interventions
    - Conflict of interest: not reported
  
### (Plevraki et al., 2006)
- **Other bias**
  - Was the sensitivity of the assay(s) used at follow-up reported?
    - No—High risk
    - Cytological methods are known to be potentially affected by measurement error and sampling error, reducing accuracy and precision.

---

**Notes:**
- Other bias: The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

---

**Supporting publications 2015:EN-761**
Table 11  Risk of bias assessment of longitudinal case series and studies with poor quality reporting.

<table>
<thead>
<tr>
<th>Risk of bias domain</th>
<th>(Moritz et al., 1998)</th>
<th>(Slappendel and Teske, 1997),</th>
<th>(Steuber et al., 1998)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Selection bias</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was allocation to treatment group</td>
<td>No—High risk</td>
<td>Yes—Low risk</td>
<td>No—High risk</td>
</tr>
<tr>
<td>randomized?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Selection bias</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Does the study describe the method to</td>
<td>No—High risk</td>
<td>No—High risk</td>
<td>No—High risk</td>
</tr>
<tr>
<td>conceal allocation?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Performance bias</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Were measures to blind owners/personnel</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
</tr>
<tr>
<td>described?</td>
<td></td>
<td>Outcome of interest unlikely</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>to have been influenced by</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>dog carers’ knowledge of</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>treatment group.</td>
<td></td>
</tr>
<tr>
<td><strong>Detection bias</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR is an objective test that is unlikely</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
<td>No—Low risk</td>
</tr>
<tr>
<td>to be affected by assessors’ knowledge of</td>
<td></td>
<td>Outcome of interest unlikely</td>
<td></td>
</tr>
<tr>
<td>treatment group.</td>
<td></td>
<td>to have been influenced by</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>dog carers’ knowledge of</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>treatment group.</td>
<td></td>
</tr>
<tr>
<td><strong>Attrition bias</strong></td>
<td></td>
<td>Loss to follow-up not</td>
<td></td>
</tr>
<tr>
<td>Were there incomplete outcome data in the</td>
<td>No—Low risk</td>
<td>explained—Low risk</td>
<td>No—High risk</td>
</tr>
<tr>
<td>study?</td>
<td></td>
<td>Only one dog was lost to</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>follow-up.</td>
<td></td>
</tr>
<tr>
<td><strong>Reporting bias</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was there selective reporting of</td>
<td>Yes—High risk</td>
<td>Yes—High risk</td>
<td>Yes—High risk</td>
</tr>
<tr>
<td>outcomes?</td>
<td></td>
<td>Data not provided for the</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>numbers of dogs receiving</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>multiple courses of</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>treatment or cross-over</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>treatments. Multiple</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>treatments would influence</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>the long-term outcomes.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Data on dogs remaining</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>positive after 365 days not</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>reported (only relapses</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>reported).</td>
<td></td>
</tr>
</tbody>
</table>

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.
<table>
<thead>
<tr>
<th>Risk of bias domain</th>
<th>(Moritz et al., 1998)</th>
<th>(Slappendel and Teske, 1997)</th>
<th>(Steuber et al., 1998)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Other bias</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was the sensitivity of the assay(s) used at follow-up reported?</td>
<td>No—Low risk</td>
<td>No—High risk</td>
<td>No—Low risk</td>
</tr>
<tr>
<td>PCR is understood to be a relatively sensitive test for <em>Leishmania</em>.</td>
<td>—High risk</td>
<td>Microscopy is not highly sensitive due to potential sampling error and measurement error. The microscopy protocol was not strict.</td>
<td>—Unclear risk</td>
</tr>
<tr>
<td><strong>Other bias</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was funding source reported? Were conflicts of interest disclosed? Were confounders adequately accounted for through randomization, etc.? Could reinfection occur during follow-up differentially between groups? Other sources of bias?</td>
<td>Funding/conflict of interest: not reported</td>
<td>Funding: Rhone Merieux provided the intervention</td>
<td>Funding/conflict of interest: not reported</td>
</tr>
<tr>
<td>Confounding: not randomized, no information on potential confounders</td>
<td>Confounding: not reported</td>
<td>Confounding: Differential loss to follow-up may have caused potential confounders to be differentially distributed between groups.</td>
<td>Confounding: not randomized, potential confounders may not have been equally distributed between groups</td>
</tr>
<tr>
<td>Reinfection: Unclear</td>
<td>Reinfection: possibility low (inclusion criteria was that owner would not bring dog to a <em>Leishmania</em>-endemic area)</td>
<td>Reinfection: not possible (dogs never off treatment before end of follow-up)</td>
<td>Reinfection: not possible (dogs never off treatment before end of follow-up)</td>
</tr>
<tr>
<td>Other: Treatment protocol changed according to the performance of the dog. If dog did not respond to treatment, another treatment course was added. Outcome at the end of follow-up would have been biased by number of treatment courses.</td>
<td>Other: Study protocol was not strict and dogs were allowed to receive multiple treatments and cross-over between groups</td>
<td>Other: Treatment protocol changed according to the performance of the dog. If dog did not respond to treatment, another treatment course was added. Outcome at the end of follow-up would have been biased by number of treatment courses.</td>
<td>Other: Treatment protocol changed according to the performance of the dog. If dog did not respond to treatment, another treatment course was added. Outcome at the end of follow-up would have been biased by number of treatment courses.</td>
</tr>
</tbody>
</table>

Paradies (2012) was not assessed as it was an observational study and could not be evaluated with a tool designed to assess the quality of randomized controlled trials.
Table 12  Relevant results reported in 5 included studies in which PCR was performed at follow-up.

<table>
<thead>
<tr>
<th>Author</th>
<th>Treatment protocol</th>
<th>Duration of follow-up (days)</th>
<th>Time not on treatment (days)</th>
<th>Dogs enrolled</th>
<th>Dogs completed treatment</th>
<th>Dogs completed follow-up</th>
<th>Dogs PCR negative at follow-up</th>
<th>PCR samples</th>
<th>High or unclear risk of bias in results?</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ariti et al., 2013)</td>
<td>Allopurinol: 10mg/kg PO BID x 360 days Miltefosine: 2 mg/kg PO SID x 30 days</td>
<td>360 for some dogs and 720 for others</td>
<td>0 for some dogs and 360 for others</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>0</td>
<td>Blood, conjunctiva, lymph node</td>
<td>Yes</td>
</tr>
<tr>
<td>(Ariti et al., 2013)</td>
<td>Difloxacin: 5 mg/kg PO x 30 days Metronidazole: 25 mg/kg PO x 30 days</td>
<td>720</td>
<td>660 for some dogs and 690 for others</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>3</td>
<td>Blood, conjunctiva, lymph node</td>
<td>Yes</td>
</tr>
<tr>
<td>(Ariti et al., 2013)</td>
<td>Meglumine antimoniate: 100 mg/kg SQ BID x 30 days Allopurinol: 10 mg/kg PO BID x 365 days</td>
<td>720</td>
<td>0 for some dogs and 360 for others</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>5</td>
<td>Blood, conjunctiva, lymph node</td>
<td>Yes</td>
</tr>
<tr>
<td>(Miro et al., 2009)</td>
<td>Allopurinol: 10 mg/kg PO BID x 196 days Miltefosine: 2 mg/kg PO SID x 28 days</td>
<td>196</td>
<td>0</td>
<td>37</td>
<td>33</td>
<td>33</td>
<td>NR</td>
<td>Bone marrow</td>
<td>Yes</td>
</tr>
<tr>
<td>(Miro et al., 2009)</td>
<td>Meglumine antimoniate: 50</td>
<td>196</td>
<td>0</td>
<td>36</td>
<td>32</td>
<td>32</td>
<td>NR</td>
<td>Bone marrow</td>
<td>Yes</td>
</tr>
</tbody>
</table>

8At least one bias domain with “High” or “Unclear” risk of bias. (NR = Not reported and excluded from meta-analysis)
Three dogs were PCR negative at some point during follow-up, but all were positive at the end of follow-up.

Four dogs were PCR negative at some point during follow-up. Two of these became PCR positive at the end of follow-up. The two PCR-negative dogs at the end of follow-up had been PCR positive 30 and 60 days before.

Plevraki et al., 2006, these 5 groups were based on the results of urinalysis (protein concentration) and SCr.

**Supporting publications 2015:EN-761**

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s) awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.
<table>
<thead>
<tr>
<th>Year</th>
<th>Duration</th>
<th>Dose</th>
<th>Treatment Details</th>
<th>Treated Body Part</th>
<th>Treated?</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>x 180 days</td>
<td></td>
<td>(Plevraki et al., 2006) ——— 180 0 5 5 5 0</td>
<td>Bone marrow</td>
<td>Yes</td>
</tr>
<tr>
<td>2006</td>
<td>x 180 days</td>
<td></td>
<td>(Plevraki et al., 2006) ——— 180 0 5 4 4 0</td>
<td>Bone marrow</td>
<td>Yes</td>
</tr>
<tr>
<td>2006</td>
<td>20 mg/kg PO SID x 7 days at the beginning of each month for 8 months</td>
<td>365</td>
<td>(Saridomichelakis et al., 2005) 20 mg/kg PO SID x 7 days at the beginning of each month for 8 months</td>
<td>Bone marrow</td>
<td>Yes</td>
</tr>
<tr>
<td>2005</td>
<td>20 mg/kg PO SID x 7 days at the beginning of each month for 8 months</td>
<td>365</td>
<td>(Saridomichelakis et al., 2005) 20 mg/kg PO SID x 7 days at the beginning of each month for 8 months</td>
<td>Bone marrow</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Supporting publications 2015:EN-761

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.
Table 13  Relevant results reported in 2 included studies in which xenodiagnosis was performed at follow-up. (NR = Not reported)

<table>
<thead>
<tr>
<th>Author</th>
<th>Treatment protocol</th>
<th>Duration of follow-up (days)</th>
<th>Time not on treatment (days)</th>
<th>Dogs enrolled</th>
<th>Dogs completed treatment</th>
<th>Dogs completed follow-up</th>
<th>Dogs negative at follow-up</th>
<th>High or unclear risk of bias in results?</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Guarga et al., 2002)</td>
<td>Meglumine antimoniate: 100 mg/kg SQ SID x 21 days Antigenic preparation: 300 µl beginning 7 days before start of antimonial therapy and repeated at intervals of 14 days for 3 treatments</td>
<td>180</td>
<td>159</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>NR</td>
<td>Yes</td>
</tr>
<tr>
<td>(Guarga et al., 2002)</td>
<td>N/A</td>
<td>180</td>
<td>180</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>NR</td>
<td>Yes</td>
</tr>
<tr>
<td>(Miro et al., 2011)</td>
<td>Meglumine antimoniate: 35 mg/kg SQ BID x 28 days Allopurinol: 10 mg/kg PO BID x 180 days</td>
<td>180</td>
<td>0</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>Yes</td>
</tr>
<tr>
<td>(Miro et al., 2011)</td>
<td>Meglumine antimoniate 35 mg/kg SQ BID x 28 days</td>
<td>180</td>
<td>152</td>
<td>11</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>Yes</td>
</tr>
<tr>
<td>(Miro et al., 2011)</td>
<td>Allopurinol 10 mg/kg PO BID x 180 days</td>
<td>180</td>
<td>0</td>
<td>9</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>Yes</td>
</tr>
</tbody>
</table>

12 At least one bias domain with “High” or “Unclear” risk of bias.
### Table 14: Relevant results reported in 4 included studies in which parasite load was estimated before treatment and at follow-up. (NR = Not reported, IQR = interquartile range, SD = standard deviation)

<table>
<thead>
<tr>
<th>Author</th>
<th>Treatment protocol</th>
<th>Duration of follow-up (days)</th>
<th>Time not on treatment (days)</th>
<th>Dogs enrolled</th>
<th>Dogs completed treatment</th>
<th>Dogs completed follow-up</th>
<th>Dogs negative at follow-up</th>
<th>Test &amp; Sample type</th>
<th>Paraside load</th>
<th>High or unclear risk of bias in results?</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Miro et al., 2009)</td>
<td>Allopurinol: 10 mg/kg PO BID x 196 days, Miltefosine: 2 mg/kg PO SID x 28 days</td>
<td>196</td>
<td>0</td>
<td>37</td>
<td>33</td>
<td>33</td>
<td>NR</td>
<td>PCR Bone marrow</td>
<td>Median parasites/ml Pre-treatment: 2312.5 (6.25–27500) (n = 36) Post-treatment: 0 (0–929.25) (n = 33)</td>
<td>Yes</td>
</tr>
<tr>
<td>(Miro et al., 2009)</td>
<td>Meglumine antimoniate: 50 mg/kg SQ BID x 196 days, Allopurinol: 10 mg/kg PO BID x 196 days</td>
<td>196</td>
<td>0</td>
<td>36</td>
<td>32</td>
<td>32</td>
<td>NR</td>
<td>PCR Bone marrow</td>
<td>Median parasites/ml Pre-treatment: 998 (&lt;25–12025) (n = 36) Post-treatment: 0 (0–&lt;25) (n = 32)</td>
<td>Yes</td>
</tr>
<tr>
<td>(Plevra ki et al., 2006)</td>
<td>Allopurinol: 10 mg/kg PO BID x 180 days</td>
<td>180</td>
<td>0</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>PCR Bone marrow</td>
<td>Significant difference between pre- and post-treatment (p = 0.0156)</td>
<td>Yes</td>
</tr>
<tr>
<td>(Plevra ki et al., 2006)</td>
<td>Allopurinol: 10 mg/kg PO BID x 180 days</td>
<td>180</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>PCR Bone marrow</td>
<td>No significant difference between pre- and post-treatment</td>
<td>Yes</td>
</tr>
</tbody>
</table>

13 At least one bias domain with “High” or “Unclear” risk of bias.

Supporting publications 2015:EN-761
Scoping review and protocols for Canine Leishmania

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>Parasite Loads</th>
<th>Method</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Plevra ki et al., 2006)</td>
<td>Allopurinol: 10 mg/kg PO BID x 180 days</td>
<td>0</td>
<td>8</td>
<td>7</td>
<td>PCR Bone marrow</td>
<td>Significant difference between pre- and post-treatment parasite loads (p = 0.0156)</td>
</tr>
<tr>
<td>(Plevra ki et al., 2006)</td>
<td>——</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>PCR Bone marrow</td>
<td>No significant difference between pre- and post-treatment parasitic loads.</td>
</tr>
<tr>
<td>(Plevra ki et al., 2006)</td>
<td>——</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td>PCR Bone marrow</td>
<td>No significant difference between pre- and post-treatment parasitic loads.</td>
</tr>
<tr>
<td>(Neogy et al., 1994)</td>
<td>N-methylglucamine antimonate 300 mg/kg IM q48h x 40 days</td>
<td>140</td>
<td>8</td>
<td>8</td>
<td>Cytology Bone marrow</td>
<td>Pre-treatment: 8 dogs had 1–10 amastigotes/1 oil immersion field (x100)</td>
</tr>
<tr>
<td>(Neogy et al., 1994)</td>
<td>Partially purified antigenic Fraction 2, derived from L. infantum</td>
<td>159</td>
<td>8</td>
<td>8</td>
<td>Cytology Bone marrow</td>
<td>Pre-treatment: 8 dogs had 1–10 amastigotes/1 oil immersion field (x100)</td>
</tr>
</tbody>
</table>

Supporting publications 2015:EN-761
<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Parasite Density Score</th>
<th>Cytology</th>
<th>Bone Marrow</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Neogy et al., 1994)</td>
<td>Meglumine antimoniate: 300 mg/kg IM q48h x 40 days Fraction 2: 50 µg IM q7d x 21 days, started 15 days after antimonial treatment completed</td>
<td>0 (no parasites/1000 micro fields) to 6 (&gt;100 parasites/micro field) (Chulay and Bryson)</td>
<td>Promastigotes 50 µg IM q7d x 21 days</td>
<td>Amastigotes/ 100 oil immersion fields (x100) and 2 dogs had no amastigotes</td>
<td>Pre-treatment: 8 dogs had 1–10 amastigotes/1 oil immersion field (x100) Post-treatment: 0 dogs had any amastigotes in any oil immersion field</td>
<td></td>
</tr>
<tr>
<td>(Oliva et al., 1998)</td>
<td>Aminosidine sulphate 3.5 mg base/kg SQ BID x 21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Oliva et al., 1998)</td>
<td>Meglumine antimoniate 30 mg Sb/kg IM SID x 21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

14 Parasite density score: 0 (no parasites/1000 micro fields) to 6 (>100 parasites/micro field) (Chulay and Bryson)
<table>
<thead>
<tr>
<th>Product</th>
<th>Dose</th>
<th>Route</th>
<th>Duration</th>
<th>Mean (SD) parasite density score</th>
<th>Cytology</th>
<th>Lymph node</th>
<th>Post-treatment:</th>
<th>Pre-treatment:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meglumine antimoniate</td>
<td>20 mg Sb/kg IM SID x 21 days</td>
<td></td>
<td></td>
<td>0.8 (0.9) (n = 10)</td>
<td>Bone marrow, lymph node</td>
<td>0.8</td>
<td>0.7 (0.8) (n = 11)</td>
<td>1.1 (0.7) (n = 11)</td>
</tr>
<tr>
<td>Aminosidine</td>
<td>3.5 mg base/kg SQ BID x 21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.6</td>
<td>0.7 (0.8) (n = 11)</td>
<td>1.5 (1.0) (n = 11)</td>
</tr>
</tbody>
</table>

( Oliva et al., 1998)
### Table 15 Data extractable from longitudinal case series and studies with poor quality reporting. (NR = Not reported)

<table>
<thead>
<tr>
<th>Author</th>
<th>Treatment protocol</th>
<th>Duration of follow-up (days)</th>
<th>Time not on treatment (days)</th>
<th>Dogs enrolled</th>
<th>Dogs completed treatment</th>
<th>Dogs completed follow-up</th>
<th>Dogs negative at follow-up</th>
<th>PCR samples</th>
<th>Parasite load</th>
<th>At least one domain with “High” or “Unclear” risk of bias?</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Moritz et al., 1998)</td>
<td>Meglumine antimoniate: 50 mg/kg x 2 days, then 100 mg/kg x 8 days, then 14 days off, then repeat course of treatment</td>
<td>780–1440</td>
<td>746–1404</td>
<td>6</td>
<td>5</td>
<td>PCR: Unclear</td>
<td>PCR: Unclear</td>
<td>Blood, bone marrow</td>
<td>Not done</td>
<td>Yes</td>
</tr>
<tr>
<td>(Moritz et al., 1998)</td>
<td>Meglumine antimoniate: 50 mg/kg x 2 days, then 100 mg/kg x 8 days, then 14 days off, then repeat course of treatment Allopurinol: 10 mg/kg PO BID x 21–35–630 days</td>
<td>270–1440</td>
<td>Unclear</td>
<td>7</td>
<td>7</td>
<td>PCR: 7</td>
<td>PCR: 2</td>
<td>Blood, bone marrow</td>
<td>Not done</td>
<td>Yes</td>
</tr>
<tr>
<td>(Moritz et al., 1998)</td>
<td>Allopurinol: 10 mg/kg PO BID x 21–35–630 days</td>
<td>270–480</td>
<td>Unclear</td>
<td>3</td>
<td>3</td>
<td>PCR: 3</td>
<td>PCR: 2</td>
<td>Blood, bone marrow</td>
<td>Not done</td>
<td>Yes</td>
</tr>
<tr>
<td>(Paradies et al., 2015)</td>
<td>Meglumine antimoniate: 50</td>
<td>365</td>
<td>245-337</td>
<td>6</td>
<td>6</td>
<td>See footnote15</td>
<td>NR</td>
<td>N/A</td>
<td>Not done</td>
<td>Yes</td>
</tr>
</tbody>
</table>

15 “Very few cases” in this group had lymph node cytology done during follow-up. All that had cytology were positive. Outcome was clinico-pathological relapse = return of abnormal laboratory parameters as well as RBC mass (Hct <35%; reference interval 35–50%), hyperproteinemia (serum total protein, TP >7.5 g/dL; reference interval 5.5–7.5 g/dL), abnormalities of albumin/globulin ratio (A/G ≤0.6; reference interval 0.7–1.3). Separately, cytology on lymph node samples was performed whenever possible.
mg/kg SQ BID until clinical recover (60-120 days for the dogs in the trial)

Meglumine antimoniate: 50 mg/kg SQ BID x 56 days or until clinico-pathological recovery
Allopurinol: 15 mg/kg PO BID x 180 days, begun after antimonial discontinued

---

Within 1 year, relapses had occurred in 15 of 18 dogs in group A and in 11 of 17 dogs in group B (P=0.264). No significant difference (P=0.412) was observed in relapse free-period between group A dogs and group B dogs.

---

16 “The low number of available lymph node cytology tests [in this group] resulted negative in all dogs.”
17 “Most of the lymph node cytology resulted positive throughout the study in this group.”
18 Dose: 100 mg/kg IV SID x 21 days; “In case of partial remission (PR) or no response (NR), the same treatment was given for another 3 weeks (‘prolonged treatment’). If there was a relapse, therapy was resumed but was changed from IV to SC administration or vice versa (cross-over therapy) and evaluated again 3 weeks later.”
19 “Three weeks after the first treatment for 21 days, the overall clinical condition had improved significantly in most dogs in both groups and the cytological examination for parasites was negative in 24 (70.6%) of 34 previously positive dogs. Three weeks after either a normal or a prolonged course of treatment was completed, remission was obtained in 85.4% of 41 patients (partial remission in 3 cases and complete remission in 32). Parasites could be detected in only 2 (5.8%) of 34 initially positive dogs, while no significant difference (P=0.487) between the groups was noted. In addition, mean PCV had increased and proteinuria had decreased in both groups significantly. No significant difference (P=0.671), however, was observed between the groups. Response rates in both groups were not significantly different (P=0.663).”

Supporting publications 2015:EN-761
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Treatment Protocol</th>
<th>Possible Duration</th>
<th>Treatment Course 1</th>
<th>Treatment Course 2</th>
<th>PCR</th>
<th>PCR</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teske, 1997</td>
<td>See footnote 20</td>
<td>Unclear</td>
<td>365 or 730 or for as long as possible</td>
<td>Unclear</td>
<td>22</td>
<td>21</td>
<td>Unclear</td>
</tr>
<tr>
<td>(Slappendel and Teske, 1997)</td>
<td>50 mg/kg IM days 1&amp;2, followed by 100 mg/kg IM days 3-10. If further treatment courses were required, an interval of 10-14 days was left between courses.</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear 21</td>
<td>PCR:</td>
<td>Unclear</td>
<td>PCR:</td>
</tr>
<tr>
<td>(Steuber et al., 1998)</td>
<td>Pretreated with Glucantime (protocol not reported); Allopurinol 10 mg/kg PO BID x 21 days, 35 days, 180 days, or 600 days</td>
<td>Unclear—likely 0 (dogs tested immediately after treatment)</td>
<td>3</td>
<td>3</td>
<td>PCR: 3</td>
<td>PCR: 2</td>
<td>Blood, bone marrow</td>
</tr>
<tr>
<td>(Steuber et al., 1998)</td>
<td>Allopurinol 10 mg/kg PO BID x 21–35 days</td>
<td>Unclear—likely 0 (dogs tested immediately after treatment)</td>
<td>Unclear</td>
<td>PCR: 7</td>
<td>PCR: 7</td>
<td>PCR: 2</td>
<td>Blood, bone marrow</td>
</tr>
</tbody>
</table>

20 Dose: 100 mg/kg SQ SID x 21 days; “In case of partial remission (PR) or no response (NR), the same treatment was given for another 3 weeks (‘prolonged treatment’). If there was a relapse, therapy was resumed but was changed from IV to SC administration or vice versa (cross-over therapy) and evaluated again 3 weeks later.”

21 Some dogs completed 1 to >3 treatment courses; as well, there were unexplained losses between treatment courses. The number of dogs still positive after treatment course 1 did not equal the number of dogs followed in treatment course 2.
Scoping review and protocols for Canine Leishmania

706

FIGURES

Figure 1  Search strategy to identify studies reporting on canine leishmaniosis in Science Citation Index (Web of Knowledge, Thompson Reuters)

#7  #3 NOT #6

#6  #4 NOT #5

#5  AD=(Albania* OR Andorra* OR Armenia* OR Austria* OR Azerbaijan* OR Belarus* OR Belgium* OR Bosnia* OR Bulgaria* OR Croatian* OR Cyprus* OR Cypriot* OR Czech* OR Denmark* OR “Danish” OR Estonia* OR Finland* OR “Finns” OR “Finnish” OR Macedonia* OR France* OR “French” OR Georgia* OR German* OR Greece* OR Greek* OR Hungary* OR Hungarian* OR Iceland* OR Ireland* OR “Irish” OR Italy* OR Italian* OR Israel* OR Kazakhstan* OR Kyrgyzstan* OR Latvia* OR Liechtenstein* OR Lithuania* OR Luxemburg* OR Luxembourger* OR Malta* OR Moldavia* OR Montenegro* OR Netherlands OR Holland* OR “Dutch” OR Norway* OR Norwegian* OR Poland* OR “Polish” OR Portugal* OR “Portuguese” OR Romania* OR Russia* OR “San Marin*” OR Serbia* OR Slovakia* OR Slovenia* OR Spain* OR Sweden* OR “Swedish” OR “Swiss” OR Switzerland* OR Tajikistan* OR Turkey* OR “Turkish” OR Turkmenistan* OR Ukrain* OR “United Kingdom*” OR “UK” OR Britain* OR “British” OR England* OR “English” OR Wales* OR “Welsh” OR Scotland* OR “Scottish” OR Uzbekistan* OR “USSR” OR “Soviet”)

#4  AD=(“united states” OR america* OR “USA” OR “US” OR “canada” OR canadian* OR “mexico” OR mexican* OR brazil* OR colombia* OR argentin* OR peru OR “peruvian” OR venezuela* OR chile* OR ecuador* OR bolivia* OR paragua* OR urugua* OR guyana* OR suriname* OR guiana* OR “cuba” OR “cuban” OR guatemala* OR “haiti” OR “haitian” OR bolivia* OR dominican* OR hondura* OR “el salvador*” OR nicaragua* OR “costa rica*” OR “puerto rie*” OR panama* OR ethiopia* OR sudan* OR kenya* OR africa* OR australia* )3

#3  #2 AND #1

#2  TS=(“canine” OR “canines” OR “dog” OR “dogs” OR “lupus familiaris” OR “l familiaris” OR canidae* OR “canid” OR “canids”)

#1  TS=(“leishmaniosis” OR “leishmaniasis” OR “leishmania” OR “l infantum”)
### Figure 2  Flow diagram showing study identification process for Objective 1-- the review of diagnostic test evaluations in naïve dogs

<table>
<thead>
<tr>
<th>Step</th>
<th>Details</th>
</tr>
</thead>
</table>
| **Identification** | Records identified through database searching ($n = 7405$)  
Additional records identified through other sources ($n = 0$) |
| **Screening** | Records after duplicates removed ($n = 3865$)  
Records after books, theses, and patents removed ($n = 3712$)  
Records after non-European countries removed ($n = 3168$) |
| **Eligibility** | DTE studies included at Level 1 title/abstract screening ($n = 234$)  
Longitudinal DTE studies included at Level 2 full-text screening ($n = 7$) |
| **Included** | Total records excluded Level 1: $n = 2934$  
(non-relevant: $n = 2870$)  
($L. chagasi, mexicana or brasiliensis$: $n = 64$)  
Total records excluded Level 2: $n = 227$  
(not a longitudinal design: $n = 216$)  
(both PCR and serology not used to ID naïve dogs: $n = 11$)  
Studies included in synthesis ($n = 7$) |
Figure 3  Flow diagram showing study identification process for Objective 2, the review of treatment outcomes for Canine leishmaniosis

Records identified through database searching  
(n = 7405)  
Additional records identified through other sources  
(n = 0)  
Records after duplicates removed  
(n = 3865)  
Records after books, theses, and patents removed  
(n = 3712)  
Records after non-European countries removed  
(n = 3168)  
Treatment studies included at Level 1 title/abstract screening  
(n = 40)  
Total records excluded Level 1: n = 2934  
(non-relevant: n = 2830)  
(L. chagasi, mexicana or brasilensis: n = 64)  
Treatment studies included at Level 2 full-text screening  
(n = 13)  
Total records excluded Level 2: n = 27  
Studies included in synthesis  
(n = 13)
**Figure 4**  Forest plot of data about proportion tested with PCR for evidence of *Leishmania* after extended follow-up periods.

<table>
<thead>
<tr>
<th>Study &amp; Protocol</th>
<th>no. negative</th>
<th>no. enrolled</th>
<th>Proportion 95% CI</th>
<th>PCR samples</th>
<th>Follow up days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arlt et al., 2013 Allopurinol: 10mg/kg PO BID x 360 days• Mitoxantrone: 2 mg/kg PO SID x 30 days</td>
<td>0</td>
<td>14</td>
<td>0.00 [0.00, 0.33]</td>
<td>Blood, conjunctiva, lymph nodes</td>
<td>360 / 720</td>
</tr>
<tr>
<td>Arlt et al., 2013 Diflouxacin: 5 mg/kg PO x 30 days• Mitoxantrone: 25 mg/kg PO x 30 days</td>
<td>3</td>
<td>13</td>
<td>0.23 [0.05, 0.54]</td>
<td>Blood, conjunctiva, lymph node</td>
<td>720</td>
</tr>
<tr>
<td>Arlt et al., 2013 Meglumine antimoniate: 100 mg/kg SQ BID x 30 days• Allopurinol: 10 mg/kg PO BID x 365 days</td>
<td>5</td>
<td>15</td>
<td>0.33 [0.12, 0.62]</td>
<td>Blood; conjunctiva; lymph nodes</td>
<td>720</td>
</tr>
<tr>
<td>Perrissi et al., 2005 Metronidazole: 25 mg/kg PO SID x 90 days• Splitycin: 150,000 IU/kg PO SID x 90 days</td>
<td>0</td>
<td>9</td>
<td>0.00 [0.00, 0.34]</td>
<td>Blood; bone marrow; lymph node</td>
<td>210</td>
</tr>
<tr>
<td>Perrissi et al., 2005 Meglumine antimoniate: 55-100 mg/kg SQ BID x 90 days• Allopurinol: 20 mg/kg PO BID x 90 days</td>
<td>2</td>
<td>8</td>
<td>0.25 [0.03, 0.66]</td>
<td>Blood; bone marrow; lymph node</td>
<td>210</td>
</tr>
<tr>
<td>Plevraki et al., 2006 Allopurinol: 10 mg/kg PO BID x 180 days</td>
<td>0</td>
<td>12</td>
<td>0.00 [0.00, 0.26]</td>
<td>Bone marrow</td>
<td>180</td>
</tr>
<tr>
<td>Plevraki et al., 2006 Allopurinol: 10 mg/kg PO BID x 180 days</td>
<td>0</td>
<td>10</td>
<td>0.00 [0.00, 0.31]</td>
<td>Bone marrow</td>
<td>180</td>
</tr>
<tr>
<td>Plevraki et al., 2006 Allopurinol: 10 mg/kg PO BID x 180 days</td>
<td>0</td>
<td>7</td>
<td>0.00 [0.00, 0.41]</td>
<td>Bone marrow</td>
<td>180</td>
</tr>
<tr>
<td>Sarabomichalekis et al., 2005 Allopurinol: 25 mg/kg PO SID x 7 days at the beginning of each month for 8 months</td>
<td>1</td>
<td>19</td>
<td>0.05 [0.00, 0.26]</td>
<td>Bone marrow</td>
<td>365</td>
</tr>
<tr>
<td>Plevraki et al., 2006 No treatment control</td>
<td>0</td>
<td>5</td>
<td>0.00 [0.00, 0.52]</td>
<td>Bone marrow</td>
<td>180</td>
</tr>
<tr>
<td>Plevraki et al., 2006 No treatment control</td>
<td>0</td>
<td>4</td>
<td>0.00 [0.00, 0.60]</td>
<td>Bone marrow</td>
<td>180</td>
</tr>
<tr>
<td>Sarabomichalekis et al., 2005 No treatment control</td>
<td>2</td>
<td>16</td>
<td>0.12 [0.02, 0.38]</td>
<td>Bone marrow</td>
<td>365</td>
</tr>
</tbody>
</table>

Studies that did not report data in a manner that could be used to calculate proportion negative are not included (see Table 12)
 References


Supporting publications 2015:EN-761
The Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position a prejudgment to the rights of the authors.

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.
APPENDIX/APPENDICES
A. DIAGNOSTIC TEST EVALUATION PROTOCOL FOR THE ASSESSMENT OF DIAGNOSTIC TEST CHARACTERISTICS OF PCR ASSAYS AND SEROLOGICAL ASSAYS (ELISA OR IFAT) TO DETECT LEISHMANIA INFECTION IN NAÏVE DOGS LONGITUDINAL STUDIES CONDUCTED IN EUROPE SINCE 1990.

1. BACKGROUND AND RATIONALE

The aim of this review is to evaluate the sensitivity and specificity of assays to detect infection of Leishmania infantum in dogs. Two types of assays are of interest, PCR assays that detect the presence of the organism (antigen) and the serological assays, ELISA and IFAT that detect the presence of antibodies.

Canine leishmaniosis (CanL), caused by infection with Leishmania infantum, is a major global vector-borne zoonotic disease potentially fatal to humans and dogs. The latter are the most important natural reservoir and should therefore be the main target of control measures. Leishmaniosis is known to be endemic in more than 70 countries in the world, including certain regions of southern Europe. The movement of infected dogs from endemic regions, together with the potential expansion of sand fly vector populations in Europe, might represent a risk of introducing the disease into non-endemic countries.

For that reason, canine leishmaniosis might fall under the category of diseases other than rabies that require preventive health measures to be adopted by means of a Commission delegated to act in accordance with the Regulation, in order to ensure protection of those Member States in which an endemic infection with Leishmania infantum has not been recorded.

To support the Commission in the possible preparation of such an act, the Commission requested EFSA to assess the available scientific information regarding canine leishmaniosis and to evaluate the relevance of measures aiming at mitigating the risk of introducing the disease through the movement of dogs. Testing dogs that move from endemic areas into areas free of Leishmania infantum infections and treating positive dogs could be possible measures of reducing the risk of introduction into free areas in the EU.

2. Objectives

The objective of this review is to summarize relative sensitivity and specificity estimates reported in studies of naïve dogs in areas where Leishmania infantum infection is endemic.

3. METHODS

3.1. Criteria for considering studies for this review

3.1.1. Types of studies

Studies that enrol dogs that are naïve for Leishmania infantum infection and live or are introduced to an endemic region are eligible for the review. The studies must also follow the dogs over time to detect changes in Leishmania infantum infection status and test for naïvety and infection with Leishmania infantum using both a PCR assay as a measure of antigen presence and ELISA or IFAT as measures of serological exposure to Leishmania infantum.

3.1.2. Participants

The target population of interest is naïve dogs at risk of Leishmania infantum in Europe introduced into or living in endemic regions. Naïve dogs are defined as dogs that test negative for Leishmania
**infantum** using both PCR (using any sample) and serology based on ELISA or IFAT. Note that this definition may be problematic, as studies that use low sensitivity tests as a definition of naïve animals may not enroll naïve animals.

### 3.1.3. Index and comparator tests

The aim is to extract sensitivity and specificity data from these studies. The authors may test the animals with a PCR or serology test of choice during the study and determine the apparent prevalence of infection. Then the authors may compare the results of the PCR of choice to other assays, other PCR assays, the same PCR assay on a different tissue or the serological assays ELISA or IFAT. Therefore, the gold standard will be the authors’ “preferred” PCR and will change between studies. The comparator test will be that reported by the authors provided it is either a PCR test/tissue or ELISA or IFAT. Apparent prevalence data will not be extracted.

### 3.1.4. Target conditions

The target condition of interest is infection with leishmaniosis caused by infection with *Leishmania infantum* based on the authors’ preferred PCR assay and test sample.

### 3.1.5. Search methods for identification of studies

#### 3.1.5.1. Electronic searches

The searches for this review have already been designed and conducted as part of the scoping review. This approach has been used to make maximum use of the time available for the project. The following databases were searched: Science Citation Index (SCI), Conference Proceedings Citation Index – Science (CPCI-S), CAB Abstracts, BIOSIS Citation Index, MEDLINE and MEDLINE In-Process, and Open Grey. These searches will not be updated as the time frame for execution of the review is limited and the time between the scoping review and the conduct of the review is short.

### 3.1.6. Searching other resources

The only additional resource that will be used for the systematic review will be to evaluate if relevant references are present in the bibliography of papers that are included in the final review. The results of this additional search will be included in the summary of search provided in the report.

### 3.1.7. Selection of studies

Based on the prior screening, we identified all studies for which the reviewers responded yes to the following question:

**Question 1:** Does the title or abstract describe a primary study in which data may have been collected from which diagnostic test characteristics could be calculated for one or more of the following assays of interest for canine leishmaniosis: PCR, IFAT, or ELISA? Include only studies evaluating tests in dogs (i.e., exclude sand flies, humans, etc.) and exclude studies obviously evaluating *L. Mexicana*, or *L. brasiliensi*.

We will now screen these studies to identify studies that meet the more refined eligibility criteria for the review question.

1) Does the abstract describe a study that reports the use of naïve dogs in an endemic region or the introduction of naïve dogs into an endemic region? (yes/no) If no, exclude from the review.
2) Does the abstract or title describe a longitudinal evaluation of infection in an endemic area i.e., apparent prevalence of infection or relative sensitivity and specificity may be reported? (yes/no)- If no, exclude from the review.

For studies that pass Q 1 and 2, the full text will be obtained to assess the following question:

3) Did the study use a testing protocol to assess negative dogs that included both PCR and serology (ELISA or IFAT)? (yes/no)- If no, exclude from the review.

These series of questions will be tested on a test set of abstracts and modified as necessary. When agreement between the two independent reviewers is high (kappa > 90%) screening of all abstracts will take place. Conflicts will be resolved by discussion.

### 3.1.8. Data collection

After identifying the studies eligible for the review, extraction of study characteristics and results will occur and the risk of bias in each study will be assessed. Data collection forms will be developed based on the questions below. The forms will be tested on a subset of studies to ensure high agreement between reviewers. Data extraction will be performed by one reviewer and verified by the 2nd reviewer. Conflicts will be resolved by discussion. This approach is needed because the extraction of text makes resolutions of conflicts very time-consuming. Also as we are extracting a number of comparisons rather than a single pairwise comparison as normally occurs in systematic reviews, it is not possible to design automatic conflict resolution forms in DistillerSR® (i.e., we may need to extract a comparison of PCR on bone marrow compared to PCR on skin, at 2, 5, and 12 months post-introduction to the endemic area, and a comparison of PCR on bone marrow compared to ELISA, at 2, 5, and 12 months post-introduction from the same study). Where ever possible checklists will be used or numerical data will be extracted.

### 3.1.8.1. Characteristics of the study population to be collected

1) Country- list of EU member countries and a text box for others options

2) Year of study conducted (XXXX). If the study doesn’t report the year, this will be scored as not reported rather than using the year of publication.

3) Origin of naïve dog population – local kennel dogs, laboratory dogs, imported from another region,

4) Health status of naïve dog population - clinical (sick) dogs, apparently healthy dogs, mixture, not discernible

5) How was naïvety defined? List all tests conducted (PCR, ELISA, IFAT)

6) PCR-based method of determining infection- extract exact details of assay (text-based answer)

7) Tissues used for PCR test- report all used (skin, bone marrow, lymph node, conjunctival, other)

8) What is the cut-off for a positive PCR to assess naïvety? Text (indicate NR if not reported)
9) Was this PCR assay also used to test animals for infection later? (yes/no)

10) ELISA assay used for determination of infection- extract exact details of assay (text-based answer – if none, leave blank)

11) What is the cut-off for a positive ELISA assay used to assess naïvety? Text (indicate NR if not reported)

12) Was this ELISA assay also used to test animals for infection later? (yes/no)

13) IFAT assay used for determination of infection- extract exact details of assay (text-based answer – if none leave blank)

14) What is the cut-off for a positive IFAT used to assess naïvety? Text (indicate NR if not reported)

15) Was this IFAT assay also used to test animals for infection later? (yes/no)

16) If you answered no to either 9, 12, or 15 provide details of assays used later.

3.1.8.2. Relative sensitivity and specificity data extraction (PCR versus PCR)

1) Referent assay (pick the assay that is used by the authors as a gold standard)-list

2) Index assay (pick the assay that is used by the authors as a comparator)-list

3) Number of dogs enrolled in the study

4) Number of dogs tested using both assays at this time period.

5) Number of positive dogs based on the referent assay at this time period.

6) Number of positive dogs based on index test at this time period

7) Reported sensitivity (express as a proportion)

8) Measure of precision of sensitivity (express as SE- back convert CI by dividing by 4)

9) Reported specificity (express as a proportion)

10) Measure of precision of specificity (express as SE- back convert CI by dividing by 4)

11) Time post-introduction to infection exposure (rounded to nearest month, if 2 weeks use 0.5)

3.1.8.3. Assessment of methodological quality

Assessment of methodological quality will be based upon the QUADAS tool domains for diagnostic test comparisons (Whiting et al., 2003; Whiting et al., 2006; Whiting et al., 2011). Consistent with the recommendations from the Cochrane Working Group on Diagnostic Test Evaluations Systematic Reviews, we will assess the following questions that relate to quality of study execution (even when some are eligibility criteria) (Reitsma et al., 2009). Assessment of methodological bias will be performed by two reviewers independently and conflicts resolved by discussion.
1. Was the spectrum of naïve dogs representative of the dogs who will receive the test in practice? (Representative spectrum). The answer to this is likely to be an unclear risk of bias as it is unclear to the review team that the candidates for testing are likely to be naïve dogs with short-term exposure to infection pressure. The review team suspects that some candidate dogs will also include dogs from endemic regions which may have different exposure to other organisms and long-term exposure.

2. Is the reference standard likely to classify the target condition correctly? (Acceptable reference standard). We will answer yes (low risk of bias) to this question if the reference assay is a bone marrow based kDNA assay, otherwise we will assume no and conclude a high risk of bias.

3. Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests? (Acceptable delay between tests) If the tests are not conducted concurrently we will answer no to this question- i.e., a high risk of bias.

4. Did the whole sample or a random selection of the sample receive verification using the intended reference standard? (Partial verification avoided) If the study does not test all the dogs (except dead dogs) that were originally identified as naïve, we will answer no and assume a high risk of bias.

5. Did dogs receive the same reference standard irrespective of the index test result? (Differential verification avoided) If the study uses a different reference or index test to test a subset of dogs, we will answer no and assume a high risk of bias, i.e. if the study describes only retesting in subsequent months animals that test positive (or negative) based on one assay.

6. Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)? (incorporation avoided) If the authors used the index test as part of the reference test, we will assign a high risk of bias.

7. Were the reference standard results interpreted without knowledge of the results of the index test? (index test results blinded) If the authors do not report blinding of the outcome, we will assess a high risk of bias.

8. Were the index test results interpreted without knowledge of the results of the reference standard? If the authors do not report blinding of the outcome, we will assess a high risk of bias.

9. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice? If the authors describe having access to clinical data i.e., testing sick dogs, then we will assess a high risk of bias, as we assume that candidate dogs will not have clinical signs when tested.

10. Were uninterpretable/intermediate test results reported? If the authors report discarding intermediate test results we will assess a high risk of bias. If the authors do not discuss intermediate results we will report an unclear risk of bias.

11. Were withdrawals from the study explained? If the authors describe > 10% loss to follow-up, we will assign a high risk of bias. If the authors do not report the number of animals that are used in sensitivity and specificity we will assign unclear risk of bias, and if all animals are present and accounted for, we will assess low risk of bias. Note that death is not a reason for loss-to-follow up, rather it is a valid withdrawal.
3.1.8.4. Statistical analysis and data synthesis

As the analysis will to some extent depend upon the type and quantity of data that are located through the literature search, it is not possible to fully specify the analysis at the protocol stage. In general terms we propose to use a hierarchical random-effects model or a generalized linear model as recommended for binary outcome data to model each pairwise comparison (Macaskill et al., 2010). If conducted, preliminary analysis and forest plots will be constructed in RevMan. If conducted, complicated analyses will be conducted either in SAS using Proc GLIMMIX or R (using one of several packages). Given the time available, it may not be possible to use complicated approaches to Diagnostic Test Assessment (DTA) analysis such as those described by (Menten et al., 2013). We will assess publication bias using previously recommended approaches specific to DTA reviews (Deeks et al., 2005; van Enst et al., 2014).

3.1.8.5. Investigations of heterogeneity

It will be very difficult to explore sources of heterogeneity using standard approaches, because there is no pairwise comparison (Macaskill et al., 2010). In most systematic reviews of diagnostic tests there is a comparison of one assay with another assay. However, in this review, we clearly expect heterogeneity because the referent test differs. For example, one study may use a PCR on conjunctiva as the referent test and another may use PCR on bone marrow as the referent assay. Further, one study may use a 0.6 OD as the ELISA cut-off and another may use a 0.4 OD as the cut-off. Such differences clearly explain most of the differences in estimates of sensitivity and specificity. If we do find enough studies that have similar pairwise comparisons, we will assess sources of heterogeneity based on naïve or introduced dogs.

3.1.8.6. Sensitivity analyses

We will explore the impact of individual studies on a summary effect size if one is reported.

3.1.8.7. Reporting

For each study we will provide a table of basic descriptive information. We currently propose to organize the presentation of the results by referent assay. This would mean a series of tables of studies that use PCR as the referent and compare to other PCR assays using the same tissue. Then we would prepare a table for studies that used PCR assays on one tissue as the referent and compared the results of the same PCR on a different tissue. We then propose to create tables that compare PCR to ELISA, PCR to IFAT, and ELISA to IFAT. If possible we will include studies at different time periods in the same table. If it is sensible and possible, we will create paired forest plots. We do not anticipate creating a summary effect size or summary ROC plot. A risk of bias table will be provided.
B. PROPOSED PROTOCOL FOR REVIEW FOR ASSESSMENT OF PREVALENCE OF PARASITOLOGICAL CURE FOR LEISHMANIOSIS

1. PI(S) QUESTION (PRISMA ITEM 4)

- What is the prevalence of parasitological cure (failure to detect organism) after a 12-month follow-up period in animals treated with meglumine antimoniate, miltefosine and allopurinol or combinations of these drugs for canine leishmaniosis?

1.1. Eligibility criteria (PRISMA ITEM 6)

1.1.1. Relevant participants

The relevant study population is defined as dogs with confirmed cases of canine leishmaniosis in the European region. The European region is defined as member countries of the OIE European region (http://www.oie.int/fileadmin/Home/eng/About_us/docs/pdf/2009_Commission_Europe_A.pdf), and includes northern Asia (e.g., Russia, Ukraine, Uzbekistan), Turkey, Israel, and several other non-EU countries. The relevant population is treated with any regime that contains meglumine antimoniate, miltefosine and allopurinol.

Types of outcome measures

The primary outcome of interest is a prevalence of parasitological cure at greater than 12 months after the initiation of therapy. This is defined as absence of parasites assessed using an accurate PCR (based on other review) or xenodiagnosis after confirmation of the presence of the parasite prior to or early in the treatment protocol.

Relevant study designs

Relevant studies will be controlled trials that randomly allocate animals to treatment group.

1.2. Information Sources (PRISMA ITEM 7)

The searches for this review have already been designed and conducted as part of the scoping review. This approach has been used to make maximum use of the time available for the project. The following databases were searched: Science Citation Index (SCI), Conference Proceedings Citation Index – Science (CPCI-S), CAB Abstracts, BIOSIS Citation Index, MEDLINE and MEDLINE In-Process, and Open Grey. These searches will not be updated as the time frame for execution of the review is limited and the time between the scoping review and the conduct of the review is < 2 months.

The only additional resource that will be used for the systematic review will be to evaluate if relevant references are present in the bibliography of papers that are included in the final review. The results of this additional search will be included in the summary of search provided in the report.

1.3. Search strategy (PRISMA ITEM 8)

The search strategy is reported in Appendix D.

1.4. Study Selection (PRISMA ITEM 9)

Two reviewers will independently perform the relevance screening exercise, with disagreements on the inclusion or exclusion of studies resolved by consensus or with the input of the review coordinator.
Screening for eligibility for the review

There will be two levels of screening. The rationale for two levels is that some questions will likely require the full paper to evaluate. The following questions will be used to determine whether a study will be included in the review:

Question 1: Does the study describe a controlled trial that assesses the treatment of Canine leishmaniosis with one of the drugs of interest either alone or in combination with another drug of interest?

- Yes (proceed to next question)
- No (exclude)

Question 2: Were the animals randomized to treatment group?

- Yes (proceed)
- No (exclude)

Question 3: Did the study confirm presence of parasites prior to beginning treatment or early in the treatment protocol?

- Yes (proceed)
- No (exclude)

Question 4: Did the study test for the presence of parasites at least 12 months after the cessation of therapy?

- Yes (proceed)
- No (exclude)

Studies remaining after Question 4 will be considered included in the report. Citations that identify these studies will be provided in an EndNote® library. It is clear from the scoping review that the full text is required to assess Q2 to Q4.

Screening for eligibility for meta-analysis

We do not propose to calculate a summary effect size. Instead we plan to present the findings of each study and provide information about variation in parasitological cure.

1.5. Data collection process (PRISMA ITEM 10)

Two reviewers will extract data independently from studies deemed to be relevant to the review. Data will be compared between the reviewers and any conflicts will be resolved through discussion. Data extraction forms will be designed in DistillerSR®. Initial forms will be designed and piloted on several papers and modified as required for use.

1.6. Data Items (PRISMA ITEM 11)

Information collected from each study will consist of, but is not limited to, the following:
1.7. General study characteristics and clinical sources of heterogeneity:

- Year the study was performed (if not reported, will use the year the study was published)
- Country (must be reported or study will be excluded)
- Population of dogs: hospital, population-based, experimental
- Health status of dogs: clinically unwell, healthy, mixed, not reported (note this is very problematic as this is an important source of heterogeneity at the individual level but the outcome is measured at the group level - different stages of disease have the potential to make the entire review not very useful)
- PCR assay used to confirm infection (antigen) in study dogs

1.8. Intervention characteristics (only for relevant treatment arms)

- Intervention drug protocol
- Number of dogs enrolled
- Number of dogs completing protocol
- Number of dogs completing follow-up period
- Quantity of parasites at beginning of treatment protocol if reported (average)
- Variation of mean parasite level
- Number of dogs included in above
- Units for quantity
- Duration of treatment protocol
- Duration of follow-up (from start of study)
- Duration of time not on protocol
- Number of dogs with parasitological cure (based on study assay) at the end of follow-up

Assessment of risk bias in included studies (PRISMA ITEM 12)

We will use the Cochrane risk of bias tool for intervention studies.

Summary measures (PRISMA ITEM 13)

For each relevant study arm, we will extract the prevalence of parasitological cure.

Dealing with missing data

We will not contact authors to obtain missing data. This is a potential limitation of the review. Recently, we conducted a review and around 30% of original papers did not report measures of variation and by contacting the authors, we were able to obtain information on numerous papers.
However, this was a long process (months). Imputation methods for studies that do not report measures of variation for the outcomes of interest will not be used.

Assessment of heterogeneity

We propose, if the sample size is sufficient, to conduct a meta-regression to determine what factors are associated with the magnitude of parasitological cure. Such a model would require 10 studies per covariate, therefore this may be a series of univariable models. We will initially try to use a log and logit link and determine if either modelling strategy is valid. If this is not possible we will still attempt to present possible sources of variation using tables or subgroup figures so the panel is aware of possible sources of heterogeneity, but formal analysis may not be possible.

Data synthesis

The approach to evidence synthesis will depend upon the frequency of the outcomes of interest within the relevant studies. Tables that describe the outcomes used and the associations observed will be reported. We will attempt to prepare forest plots for prevalence data.

1.9. Approach to presenting the results

1.9.1. Study selection (PRISMA ITEM 17)

We will use a flow chart as recommended by PRISMA to present the number of papers screened, the number of relevant papers, and the number of papers included in the meta-analyses (if conducted).

1.9.2. Study characteristics (PRISMA ITEM 18)

We will provide a table that contains information about the relevant studies and other general characteristics collected.

1.9.3. Risk of bias within studies (PRISMA ITEM 19)

We will provide a table that contains this information about relevant studies.

1.9.4. Results of individual studies (PRISMA ITEM 20)

We will provide a table that contains information about the results of relevant studies. It is possible that there will be several tables, given the potential variety of outcomes. If suitable, we will provide a forest plot(s) that contains individual study data in lieu of a table.

1.9.5. Synthesis of results (PRISMA ITEM 20)

Given that the aim is no longer to evaluate comparative effect, it is unlikely that a meta-analysis will be conducted. It may be possible to provide a forest plot of prevalence estimates but this will depend upon the data and extent of heterogeneity. If a meta-regression is conducted, we will provide the results and interpretation of that analysis.

1.9.6. Risk of bias across studies (PRISMA ITEM 21)

If an analysis to assess small-study effects is possible, we will provide the results of that analysis. If not, we will comment on the potential for small-study effects.

Supporting publications 2015:EN-761
C. Diagnostic Test Evaluation Protocol for the Assessment of Diagnostic Test Characteristics of PCR Assays and Serological Assays (ELISA or IFAT) from Studies that Use Experimental Models of Canine Leishmaniosis.

1. Background and Rationale

The aim of this review is to evaluate the sensitivity and specificity of assays to detect infection of Leishmania infantum in dogs. Two types of assays are of interest, PCR assays that detect the presence of the organism (antigen) and the serological assays, ELISA and IFAT that detect the presence of antibodies.

Routine data about sensitivity and specificity are obtained from observational studies that assess the diagnostic test characteristics compared to a gold standard test. When a gold standard is not available, two general approaches are used. One is to calculate sensitivity and specificity without a gold standard using a host of statistical methods called latent class methods. Canine leishmaniosis has been identified as a candidate disease which would be suitable for application of latent class methods as far back as 1999 when Boelart et al. suggested the approach (Boelaert et al., 1999a; Boelaert et al., 1999b). More recently others have applied this approach; however as the study was not conducted in Europe it was not considered relevant to the review (Solca Mda et al., 2014). Alternatively it is possible to calculate relative sensitivity and specificity, picking one assay to be the referent. This approach has the limitation that it assumes that the index assay being studied cannot be better than the referent. As more sensitive assays are constantly being developed, this is a very major limitation. A third possible source of data for obtaining estimates of sensitivity and specificity may be challenge studies. These studies are unique to veterinary science, and allow deliberate exposure of a naïve animal to an organism. This design is usually used to assess disease mechanisms, disease pathogenesis, and intervention efficacy. It is however theoretically possible to use these to evaluate diagnostic test characteristics. The validity of using challenge study data to obtain sensitivity and specificity information is dependent upon the similarly between the natural mode of infection and the experimental mode of infection. If the approach to introducing the organism into the body differs in a manner that the host’s body reacts differently during induced infection when compared to natural infection, then the data from the challenge study will not be appropriate. The impact of mode of infection likely affects assays that detect antigen and antibody differently. The assessment of validity of the induced model requires content experts.

Most challenge studies do not report sensitivity and specificity data and therefore the ability to obtain such data from a study relies upon the reporting approach used by the authors. Reviewers need to extract the data and calculate the sensitivity and specificity directly. This data is generally reported as apparent prevalence. For example, a study might infect 10 animals and report positive PCR results from conjunctival swabs from 4 of 10 animals 3 months post-infection. If we assume that the infected animal is the gold standard, then the sensitivity of the assay at 3 months post-infection might be reported as 40% (95% confidence interval 28% - 72%). If the study does not include a negative control dog, the specificity cannot be calculated. Also with respect to specificity, as dogs in experimental studies are rarely exposed to possible sources of false positives (i.e., other organism that may have similar antigens), estimates of specificity from such studies will likely suffer from spectrum bias and always be overestimated.

In this document we provide a proposed protocol for extracting, calculating, and summarizing data from challenge studies that could be used to inform the EFSA working group about sensitivity and specificity.
2. Objectives

The objective of this review is to summarize relative diagnostic test sensitivity and specificity estimates reported in experimental studies of dogs infected with *Leishmania infantum* associated with infection in Europe i.e. excluding studies evaluating *L. Mexicana* or *L. brasiliensis* infection.

3. METHODS

3.1. Criteria for considering studies for this review

3.1.1. Types of studies

Studies that enroll dogs that are naïve for *Leishmania infantum* infection and deliberately infect them with *Leishmania infantum* are eligible for inclusion. The studies must also follow the dog over time to detect changes in *Leishmania infantum* infection status using any PCR assay as a measure of antigen presence and ELISA or IFAT as measures of serological exposure to *Leishmania infantum*.

3.1.2. Participants

The target population of interest is naïve dogs at risk of *Leishmania infantum* infection in Europe introduced into or living in endemic regions.

3.1.3. Index and comparator tests

The aim is to extract sensitivity and specificity data from these studies. The authors may test the animals with a PCR or serology test of choice during the study and determine the apparent prevalence of infection. Then the authors may compare the results of the PCR of choice to other PCR assays, the same PCR assay on a different tissue or the serological assays ELISA or IFAT. Therefore, the gold standard will be the authors’ “preferred” PCR and will change between studies. The comparator test will be that reported by the authors, provided it is either a PCR test/tissue or ELISA or IFAT. Apparent prevalence data will not be extracted.

3.1.4. Target conditions

The target condition of interest is infection with leishmaniosis caused by infection with *Leishmania infantum* based on the authors’ preferred PCR assay and test sample.

3.1.5. Search methods for identification of studies

3.1.5.1. Electronic searches

The searches for this review are below.

# 3  #2 AND #1

# 2  canine" OR "canines" OR "dog" OR "dogs" OR "lupus familiaris" OR "l familiaris" OR "canidae* OR "canid" OR "canids")

# 1  TS=("leishmaniosis" OR "leishmaniasis" OR "leishmania" OR "l infantum")

This search differs slightly from the search designed for observational studies; however, as the review is now using induced disease models there is no valid reason for such a limitation because if a challenge study was conducted in either North or South America using the organism of interest it would still be relevant to the review. The sources that should be searched are Science Citation Index (SCI), Conference Proceedings Citation Index – Science (CPCI-S), CAB Abstracts, BIOSIS Citation Index. MEDLINE and MEDLINE In-Process, and Open Grey.

Supporting publications 2015:EN-761 78
3.1.6. **Searching other resources**

We will evaluate if relevant references are present in the bibliographies of papers that are included in the final review. Also reviews identified during the screening process that are likely to contain references to induced disease models of *Leishmania* should be flagged, obtained, and evaluated i.e., pathogenesis reviews and immunology reviews.

3.1.7. **Selection of studies**

**Level 1 Screening**

The review will require two levels of screening, one based on only the abstract and title of the citation. For the 2nd level of screening the full texts will need to be obtained as the required information may not be included in the abstract. Two independent reviewers should review each citation. Citations with yes responses from both reviewers should be passed to the second level of screening and text. Citations with conflicts should be resolved by discussion between the 2 reviewers. Citations with two “no” responses are excluded without further evaluation. Prior to screening at both levels, the screening questions will be tested on a test set of at least 100 abstracts and modified as necessary. When agreement between the two independent reviewers is high (kappa > 90%) screening of all abstracts will take place.

Question 1: Does the title or abstract describe a primary study of dogs that are likely be an induced model of canine leishmaniosis?

Yes - proceed to level 2

No (exclude)

**Level 2 screening**

1) Does the study include canine leishmaniosis relevant to European dog populations?

   Yes - next question

   No (exclude)

2) Does the study include data which may be used to calculate the diagnostic test characteristics of the following assays of interest for canine leishmaniosis: PCR, IFAT, or ELISA? Include studies with data in figures, tables, or text. Note the data may report apparent prevalence or true prevalence and is unlikely to be reported as sensitivity and specificity.

   Yes - proceed to data extraction

   No (exclude)

3.1.8. **Data collection**

After identifying the studies eligible for the review, extraction of study characteristics and results will occur and the risk of bias in each study will be assessed. Data collection forms will be developed based on the questions below. The forms will be tested on a subset of studies to ensure high agreement between reviewers. Data extraction will be performed by two reviewers and conflicts resolved by discussion.
3.1.8.1. Form 1: Characteristics of the study population to be collected:

1) Country- list of EU member countries and a text box for others options

2) Year that study was conducted (XXXX). If the study doesn’t report the year, this will be not reported rather than using the year of publication.

3) Origin of dog population – local kennel dogs, laboratory breed dogs, imported from another region,

4) Breed of dog

5) Central tendency for age of dogs infected (median, mean, not reported)

6) Units for age of dogs (days, weeks, months, years)

7) Number that corresponds to 21, (i.e., if the authors reported the median age was 9 weeks, then report the following - 21 = median, 22 = weeks, 23 = 9

8) Measure of precision used for age of dogs (confidence interval, range, SE, SD)

9) Number that corresponds to 24

10) Central tendency for weight of dogs infected (median, mean, not reported)

11) Number that corresponds to 26 (i.e., if the authors reported the mean weight was 15 lbs, then report the following - 26 = mean, and 27-33 (convert all weights to kg)

12) Measure of precision used for weight of dogs (confidence interval, range, SE, SD)

13) Number that corresponds to 28 (remember to convert correctly if changing SEM from lbs to kgs)

3.1.8.2. Form 2: Characteristics of the induced disease model

(Repeat this form for each arm of the trial). If the study has two doses and one negative control group, this means the study has three arms and therefore this form should be completed three times for each review)

1) Study arm designation (Free text- must match between reviewers)

2) Organism used to infect dogs (text)

3) Lifecycle stage used to infect dogs

4) Route used to infect dogs (intravenous, intraperitoneal, etc.)
### Form 3: Outcome data form – multiple repeats

1. **Study arm**
2. **Method used to assess prevalence** (pick one: PCR, ELISA, IFAT, None)
3. **Authors’ description of the approach to the assay** (text-based)
4. **What is the cut-off for a positive ELISA assay used to assess positive diagnosis?** Text (indicate NR if not reported or leave blank if not applicable)
5. **What is the cut-off for a positive PCR assay used to assess positive diagnosis?** Text (indicate NR if not reported or leave blank if not applicable)
6. **Tissues used for this assay at this time point** (skin, bone marrow, lymph node aspirate, conjunctiva fluids, serum, plasma, other, not reported)
7. **Time post-infection of testing** (enter data as months)
8. **Number of dogs infected**
9. **Number of dogs positive** (if necessary, extract from figures- if reported as percentage – calculate the number positive to obtain the percentage)
10. **Reported sensitivity** (express as a proportion, leave blank if not reported, or a control arm)
11. **Measure of precision of sensitivity** (express as SE- back convert CI by dividing by 4)
12. **Reported specificity** (express as a proportion- if a control arm only - leave these question blank if positive arm). Specificity will be 1 minus proportion of positive dogs.
13. **Measure of precision of specificity** (express as SE- back convert CI by dividing by 4)
14. **Time post-introduction to infection exposure** (rounded to nearest month, if 2 weeks use 0.5)

### 3.1.8.4. Assessment of methodological quality

Assessment of methodological quality will be based upon the QUADAS tool domains for diagnostic test comparisons (Whiting et al., 2003; Whiting et al., 2006; Whiting et al., 2011). Consistent with the...
recommendations from the Cochrane Working Group on Diagnostic Test Evaluations Systematic Reviews. We will assess the following questions that relate to the quality of study execution. (even when some eligibility criteria are involved) (Reitsma et al., 2009). Assessment of methodological bias will be performed by two reviewers independently and conflicts resolved by discussion. This form should be tested prior to extracting data to ensure that all reviewers understand the bias domains. These questions should be completed for each assay, because the potential for bias may differ among PCR, ELISA, and IFAT. In particular, the approach to inducing disease may affect the “validity” of the serology models more than the PCR methods. All questions should be answered as high, low, or unclear risk of bias. More information on how to assess the risk of bias can be found in the Cochrane Handbook; however, that source does not use the same domains of bias as reported here.

1. Was the spectrum of infected dogs representative of the dogs that will receive the test in practice (representative spectrum)? This question asks if the laboratory dogs are biologically similar to the dogs that would be tested. This refers just to the host (not the model). For example, if the study includes only puppies, and the assays will eventually be used in adults and the expected responses are different in pups compared to adults, then the answer would be high risk of bias. Provide a 1-2 sentence rationale for any high or unclear answer.

1b. Was the challenge model likely to induce an assay response that is representative of the real infection? Factors that may be important are the route or dose. This question asks if the disease model is biologically similar to natural infection. Provide a 1-2 sentence rationale for any high or unclear answer.

2. Is the reference standard likely to classify the target condition correctly (acceptable reference standard)? In this situation the reference standard is challenge. The answer here should always be low risk of bias, unless there is a concern that the animals are not actually infected i.e., the model failed to infect animals. This is different from Question 1b, which assumes the model is effective at infecting animals but the approach may mean the animals’ response is dissimilar to natural infection.

3. Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests (acceptable delay between tests)? For challenge studies this question asks if the time that has passed between infection and testing is valid. This refers mainly to PCR assays, and would usually be low risk of bias, provided the experts believe the animals are still infected. If some of animals that were deliberately infected are thought for some reason to have been cured, then the risk of bias would be high if the index test is conducted many months after infection.

4. Did the whole sample or a random selection of the sample receive verification using the intended reference standard (partial verification avoided)? If the study does not test all the dogs (except dead dogs) that were originally infected, assume a high risk of bias.

5. Did dogs receive the same reference standard irrespective of the index test result (differential verification avoided)? Not applicable for challenge studies.

6. Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard) (incorporation avoided)? Not applicable for challenge studies.

7. Were the reference standard results interpreted without knowledge of the results of the index test (index test results blinded)? If the authors do not report blinding of the animals’ status or the study only contains infected animals then assess a high risk of bias.
8. Were the index test results interpreted without knowledge of the results of the reference standard? If the authors do not report blinding of the outcome, we will assess a high risk of bias.

9. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice? If the authors describe having access to clinical data i.e., testing sick dogs, then we will assess a high risk of bias, as we assume that candidate dogs will not have clinical signs when tested.

10. Were uninterpretable/intermediate test results reported? If the authors report discarding intermediate test results we will assess a high risk of bias; if the authors do not discuss intermediate results, we will report an unclear risk of bias.

11. Were withdrawals from the study explained? If the authors describe > 10% loss to follow-up we will assign a high risk of bias. If the authors do not report the number of animals that were used in sensitivity and specificity assessment we will assign an unclear risk of bias, and if all animals are present and accounted for we will assess low risk of bias. Note that death is not a reason for loss-to-follow up, rather it is a valid withdrawal.

After each reviewer has assessed the risk of bias, the reviewers will resolve conflicts and rationales and provide a single summary rationale for each question for each assay for each study.

3.1.8.5. Statistical analysis and data synthesis

As the analysis will to some extent depend upon the type and quantity of data that are located through the literature search, it is not possible to fully specify the analysis at the protocol stage. In general terms we propose to use a hierarchical random-effects model or a generalized linear model as recommended for binary outcome data to model each pairwise comparison (Macaskill et al., 2010). If conducted, preliminary analysis and forest plots can be constructed in RevMan. If conducted, complicated analyses will be conducted either in SAS using Proc GLIMMIX or R (using one of several packages).

3.1.8.6. Investigations of heterogeneity

It will be very difficult to explore sources of heterogeneity across all studies using standard approaches because there is no pairwise comparison (Macaskill et al., 2010). However, the major sources of heterogeneity should only be explored nested within the same assay, for example, within studies that assess PCR on bone marrow. The characteristics of the host (age, weight) and the model (dose, route) are likely sources of heterogeneity and should be explored and the results reported for each assay.

3.1.8.7. Reporting

Tables should be reported that contain 1) the general characteristics, 2) the induced model characteristics, 3) the assays’ characteristics, and 4) results for each study. For the results, raw data and the percentage-based data with confidence intervals should be reported. Risk of bias should be reported for each study. Summaries presented can be pairwise if meta-analyses is conducted for each assay compared to induced infection.
D. SEARCH STRATEGIES

D1. CAB Abstracts (Web of Science – Thomson Reuters) 1910-current. Searched 06/05/14

# 7 2,815 #3 NOT #6

# 6 2,213,258 #4 NOT #5

# 5 2,592,003 AD=(Albania* OR Andorra* OR Armenia* OR Austria* OR Azerbajia* OR Belarus* OR Belgium* OR Bosnia* OR Bulgaria* OR Croatian* OR Cyprus* OR Cypriot* OR Czech* OR Denmark* OR “Danish” OR Estonia* OR Finland* OR “Finns” OR “Finnish” OR Macedonia* OR France* OR “French” OR Georgia* OR German* OR Greece* OR Greek* OR Hungary* OR Hungarian* OR Iceland* OR Ireland* OR “Irish” OR Italy* OR Italian* OR Israel* OR Kazakhstan* OR Kyrgyzstan* OR Latvia* OR Liechtenstein* OR Lithuania* OR Luxemburg* OR Luxembourg* OR Malta* OR Moldavia* OR Montenegro* OR Netherlands OR Holland* OR “Dutch” OR Norway* OR Norwegian* OR Poland* OR “Polish” OR Portugal* OR “Portuguese” OR Romania* OR Russia* OR “San Marin”* OR Serbia* OR Slovak* OR Slovenia* OR Spain* OR Sweden* OR “Swedish” OR “Swiss” OR Switzerland* OR Tajikistan* OR Turkey* OR “Turkish” OR Turkmenistan* OR Ukrain* OR “United Kingdom”* OR “UK” OR Britain* OR “British” OR England* OR “English” OR Wales* OR “Welsh” OR Scotland* OR “Scottish” OR Uzbekistan* OR “USSR” OR “Soviet”)

# 4 2,289,308 AD=(“united states” OR america* OR “USA” OR “US” OR “canada” OR canadian* OR “mexico” OR mexican* OR brazil* OR colombia* OR argent* OR peru OR “peruvian” OR venezuela* OR chile* OR ecuador* OR bolivia* OR paragua* OR uruguay* OR guyana* OR suriname* OR guiana* OR “cuba” OR “cuban” OR guatemala* OR “haiti” OR “haitian” OR bolivia* OR dominican* OR hondura* OR “el salvador”* OR nicaragua* OR “costa rica”* OR “puerto rica”* OR panama* OR ethiopia* OR sudan* OR kenya* OR africa* OR australia*)

# 3 3,959 #2 AND #1

# 2 203,952TS=(“canine” OR “canines” OR “dog” OR “dogs” OR “lupus familiaris” OR “l familiaris” OR canidae* OR “canid” OR “canids”)

# 1 27,913 TS= ("leishmaniosis" OR "leishmaniasis" OR "leishmania" OR "l infantum")

D2. Social Science Citation Index – Expanded (Web of Science, Thomson Reuters) 1900-2014-05-02. Searched 06/05/14

# 7 1,594 #3 NOT #6

# 6 10,670,857 #4 NOT #5

# 5 14,605,415 AD=(Albania* OR Andorra* OR Armenia* OR Austria* OR Azerbajia* OR Belarus* OR Belgium* OR Bosnia* OR Bulgaria* OR Croatian* OR Cyprus* OR Cypriot* OR Czech* OR Denmark* OR “Danish” OR Estonia* OR Finland* OR “Finns” OR “Finnish” OR Macedonia* OR France* OR “French” OR Georgia* OR German* OR Greece* OR Greek* OR Hungary* OR Hungarian* OR Iceland* OR Ireland* OR “Irish” OR Italy* OR Italian* OR Israel* OR Kazakhstan* OR Kyrgyzstan* OR Latvia* OR Liechtenstein* OR Lithuania* OR Luxemburg* OR Luxembourg* OR Malta* OR Moldavia* OR Montenegro* OR Netherlands OR Holland* OR...
Scoping review and protocols for Canine Leishmania

Supporting publications 2015:EN-761

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.
Scoping review and protocols for Canine Leishmania

Supporting publications 2015:EN-761

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

England* OR “English” OR Wales* OR “Welsh” OR Scotland* OR “Scottish” OR Uzbekistan* OR “USSR” OR “Soviet”

# 4 8,409,714 AD=(“united states” OR america* OR "USA" OR "US" OR "canada" OR canadian* OR "mexico" OR mexican* OR brazil* OR colombia* OR argentin* OR peru OR "peruvian" OR venezuela* OR chile* OR ecuador* OR bolivia* OR paragu* OR uruguay* OR guyana* OR suriname* OR guiana* OR "cuba" OR "cuban" OR guatemala* OR "haiti" OR "haitian" OR oríjen* OR "canid" OR "canids")

# 3 2,202 #2 AND #1

# 2 331,977 TS=("canine" OR "canines" OR "dog" OR "dogs" OR "lupus familiaris" OR "I familiaris" OR canidae* OR "canid" OR "canids")

# 1 26,006 TS=("leishmaniosis" OR "leishmaniasis" OR "leishmania" OR "l infantum")

D5. Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1946 to Present> Searched 07/05/14

1 (leishmaniosis or leishmaniasis or leishmania or l infantum).ti,ab. (24460)

2 exp Leishmaniosis/ (17146)

3 exp Leishmania/ (15162)

4 1 or 2 or 3 (27328)

5 (canine or canines or dog or dogs or lupus familiaris or I familiaris or canidae* or canid or canids).ti,ab. (214185)

6 Dogs/ (283536)

7 Dog Diseases/ (44732)

8 5 or 6 or 7 (323510)

9 4 and 8 (2374)

10 exp Europe/ (1093594)

11 exp africa/ or exp americas/ or exp australia/ (1629998)

12 (united states or america* or USA or US or canada or canadian* or mexico or mexican* or brazil* or colombia* or argentin* or peru or peruvian or venezuela* or chile* or ecuador* or bolivia* or paragu* or uruguay* or guyana* or suriname* or guiana* or cuba or cuban or guatemala* or haiti or haitian or bolivia* or dominican* or hondura* or el salvador* or nicaragua* or costa rica* or puerto rico* or panama* or ethiopia* or sudan* or kenya* or africa* or australia*).in. (4474430)

13 (Albania* or Andorra* or Armenia* or Austria* or Azerbaijan* or Belarus* or Belgium* or Bosnia* or Bulgaria* or Croatian* or Cyprus* or Cypriot* or Czech* or Denmark* or Danish or England* OR “English” OR Wales* OR “Welsh” OR Scotland* OR “Scottish” OR Uzbekistan* OR “USSR” OR “Soviet”)

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.
### Scoping review and protocols for Canine Leishmania

| 1657 | Estonia* or Finland* or Finns or Finnish or Macedonia* or France* or French or Georgia* or German* or Greece* or Greek* or Hungary* or Hungarian* or Iceland* or Ireland* or Irish or Italy* or Italian* or Israel* or Kazakhstan* or Kyrgyzstan* or Latvia* or Liechtenstein* or Lithuania* or Luxemburg* or Luxembourger* or Malta* or Moldavia* or Montenegro* or Netherlands or Holland* or Dutch or Norway* or Norwegian* or Poland* or Polish or Portugal* or Portuguese or Romania* or Russia* or San Marin* or Serbia* or Slovakia* or Slovenia* or Spain* or Sweden* or Swedish or Switzerland* or Swiss or Tajikistan* or Turkey* or Turkish or Turkmenistan* or Ukrain* or United Kingdom* or UK or Britain* or British or England* or English or Wales* or Welsh or Scotland* or Scottish or Uzbekistan* or USSR or Soviet).in. (4532270) |
| 1658 | 14 (11 or 12) not (10 or 13) (5175568) |
| 1659 | 15 9 not 14 (1525) |
| 1660 | D6. Open Grey [http://www.opengrey.eu](http://www.opengrey.eu) Searched 07/05/14 |
| 1661 | (canine OR canines OR dog OR dogs OR lupus familiaris OR l familiaris OR canidae* OR canid OR canids) AND (leishmaniosis OR leishmaniasis OR leishmania OR "l infantum") |
| 1662 | 23 results – 18 added to EndNote®, 5 records of epidemiological studies taking place in non-European countries so not downloaded. |
| 1663 | 1664 |
| 1665 | 1666 |
| 1667 | 1668 |
E. RISK OF BIAS QUESTIONS FOR OBJECTIVE 1

Question
1) Spectrum of naïve dogs representative of the dogs who will receive the test in practice?

Yes, they were representative

No, they were not representative

Not discernible

1a) What was the risk of bias due to the representative spectrum of the naive dogs?

Low

High

Unclear

Notes: Aimed at assessing representative spectrum. The answer to this is likely to be an unclear risk of bias as it is unclear to the review team that the candidates for testing are likely to be naïve dogs with short-term exposure to infection pressure. The review team suspects that some candidate dogs will also include dogs from endemic regions which may have different exposure to other organisms and long-term exposure.

2) Is the reference standard likely to classify the target condition correctly?

Yes, the referent was a kDNA PCR used on bone marrow.

No, the referent was not a kDNA PCR used on bone marrow.

Not discernible (a PCR was used on bone marrow but it is unclear whether it was a kDNA PCR)

2a) What was the risk of bias due to the acceptability of the reference standard?

Low

High

Unclear

Notes: Aimed at assessing an acceptable reference standard. We will answer "Yes" (low risk of bias) to this question if the reference assay is a bone marrow-based kDNA PCR assay; otherwise, we will assume the referent could not classify Leishmania status correctly and conclude a high risk of bias.
3) Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests? (acceptable delay between tests)

Yes, the tests were run concurrently.

No, the tests were not run concurrently or the timing of the tests was not discernible.

3a) What was the risk of bias due to the acceptability of delay between tests?

Low

High

Unclear

Notes: Aimed at assessing acceptable delay between tests. If the tests were not conducted concurrently, we answered "No" to this question (high risk of bias).

4) Did the whole sample or a random selection of the sample receive verification using the intended reference standard?

Yes, all originally naïve dogs tested with referent

No, not all originally naïve dogs tested with referent

What was the risk of bias due to avoidance of partial verification?

Low

High

Unclear

Notes: Aimed to assess partial verification. If the study does not test all of the dogs (except dead dogs) that were originally identified as naïve, we will answer "No" and assume a high risk of bias.

5) Did dogs receive the same reference standard irrespective of the index test result?

Yes, all dogs were tested with the same referent test irrespective of the index test result.

No, some dogs were not retested due to results of a single previous test (or this was not discernible).

5a) What was the risk of bias due to avoidance of differential verification?

Low

High
Unclear

Notes: Aimed at assessing differential verification bias. If the study uses a different reference or index test to test a subset of dogs, we will answer "No" and assume a high risk of bias--e.g., if only animals that tested positive (or negative) based on one assay were retested later in the follow-up period.

6) Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?

Yes, the referent test was independent of the index test.

No, the referent test was not independent of the index test (or this was not discernible).

6a) What was the risk of bias due to avoidance of incorporation?

Low

High

Unclear

Notes: Aimed at assessing incorporation avoided. If the authors used the index test as part of the reference test, we will assign a high risk of bias.

7) Were the reference standard results interpreted without knowledge of the results of the index test? (index test results blinded)

Yes, the outcome assessors were blinded.

No, the outcome assessors were not blinded (or this was not reported).

7a) What was the risk of bias due to lack of blinding of index test results during reference standard result interpretation?

Low

High

Unclear

Notes: Aimed at assessing misclassification bias due to index test results blinded: If the authors do not report blinding of the outcome, we will assess a high risk of bias.
8) Were the index test results interpreted without knowledge of the results of the reference standard?

Yes, outcome assessors were blinded.

No, outcome assessors were not blinded (or this was not reported).

8a) What was the risk of bias due to lack of blinding of the referent test results during interpretation of index test results?

Low

High

Unclear

Notes: Aimed at assessing misclassification bias due to reference test results unblinded. If the authors do not report blinding of the outcome we will assess a high risk of bias.

9) Were uninterpretable or intermediate test results reported?

Yes, intermediate test results were reported.

No, intermediate test results were discarded and not included in measures of sensitivity and specificity.

The authors did not discuss intermediate test results.

9a) What was the risk of bias due to intermediate test results?

Low

High

Unclear

Notes: If the authors report discarded intermediate test results, we will assess a high risk of bias; if the authors do not discuss intermediate results, we will report an unclear risk of bias.

10) Were withdrawals from the study explained?

Yes, data from all dogs were presented and losses accounted for.

No, there was >10% loss to follow-up.

The authors did not report the number of animals used to calculate sensitivity and/or specificity.
10a) What was the risk of bias due to loss to follow-up?

Low

High

Unclear

Notes: If the authors describe >10% loss to follow-up, we will assign a high risk of bias. If the authors do not report the number of animals that were used in the calculation of sensitivity and specificity, we will assign unclear risk of bias. If data from all animals are reported, we will assess low risk of bias. Note that death is not considered loss to follow-up, but a valid withdrawal.
F. RISK OF BIAS QUESTIONS FOR OBJECTIVE 2

Bias domain: Selection bias

Was allocation to treatment group randomized?

Random allocation
Non-random allocation
Not reported/Not discernible

What was the risk of bias due to allocation method?

Low
High
Unclear

Does the study describe the method used to conceal allocation?

Yes
No

What was the risk of bias due to allocation concealment?

Low
High
Unclear

Bias domain: Performance bias

Were measures to blind owners/personnel described?

Yes
No

What was the ROB due to knowledge of the allocated interventions by owners/handlers/personnel during the study?

Low
High
Unclear

Bias domain: Detection bias

Do the authors describe measures to blind outcome assessors?

Yes

No

What was the ROB due to knowledge of the allocated interventions by outcome assessors?

Low

High

Unclear

Rationale for ROB due to blinding of outcome assessors

Bias domain: Attrition bias

Were there incomplete outcome data in the study?

No loss to follow-up

Loss to follow-up but explained

Loss to follow-up not explained

What was the ROB due to amount, nature, or handling of incomplete outcome data?

Low

High

Unclear

Rationale for ROB due to incomplete outcome data

Bias domain: Reporting bias

Was there selective reporting of outcomes?

Yes

No
What was the risk of bias due to selective outcome reporting?

Low

High

Unclear

Rationale for ROB due to selective reporting of outcomes

Other bias

Was the sensitivity of the assay(s) used at follow-up reported?

Yes

No

What was the risk of bias due to follow-up diagnostic test sensitivity?

Low

High

Unclear

Rationale for ROB due to follow-up test sensitivity

Other potential sources of bias identified: State any important concerns about bias not covered in the other domains in the tool.

What was the risk of bias due to other potential sources of bias not identified in the preceding questions?

Low

High

Unclear

Rationale for ROB due to other sources of bias

1682