Reviewer's report

Title: Cryptic Leishmania infantum infection in Italian HIV infected patients

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Reviewer: Roser Fisa

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General comments:
The paper is interesting as it emphasise the importance of asymptomatic L. infantum infection of HIV infected patients in the Mediterranean area. The high prevalence observed is in agreement with the few studies performed in the Mediterranean area, and highlights the increased risk of suffering clinical VL that such patients are under. The authors studied a large number of HIV patients and used methods appropriate for humoral immune response studies and parasitemia studies.

Major Compulsory Revisions:

- The major criticism of the work is that although the methods may be theoretically adequate, they lack a correct description. This leads to serious problems for the interpretation of the results and the discussion.

- It is also important to revise the patients that could be considered cryptic after clinical history or clinical examination. Inclusion of patients that do not comply with this criterion may invalidate the results and percentages. Such patients must be considered separately.

Specific comments. Some items need to be revised:

1.- In Methodologies:

1.1. Serological analysis
The authors must explain the method used or provide suitable references. References 8 and 9 do not adequately describe the IFAT or ELISA used in this paper for leishmaniasis diagnosis. In the text, the authors indicated the antigens used in detail, but they fail to explain anything about the conjugate, the dilutions used, or the optical densities, among other important details. For the IFAT, the high cut-off selected (1/100) must be explained, as in asymptomatic patients and in co-infected patients lower values, are recommended. Also, in Discussion, the authors must explain the reason for their selection as it could explain the low positivity observed.

1.2. DNA extraction
The authors must indicate what quantity of blood was used for DNA extraction and indicate whether they used whole blood, or some more concentrated system,
such as buffy-coat or peripheral mononuclear cells. This is important if they want to extrapolate their results to parasites/ml of blood, as they indicate on page 6 (line 10: Results were expressed as parasite charge per ml of blood).

1.3. TaqMan PCR

- The authors indicate on page 6, line 10: “Results were expressed as parasite charge for ml of blood”. On page 6, line 19 they say: “All samples with a concentration of 1 parasite per ml or more were considered positive”. Does this second concentration refer to ml of blood or ml of buffer calculated from the standard curve of parasites? This point must be clarified thoroughly. How do the authors make the correlation?

- In Methods, the authors explain the reproducibility assay of the TaqMan PCR but not the results obtained.

2. In Results:

2.1 Patient 15 with a previous history of VL could be included in the study but must be excluded when percentages are calculated, because we would expect that this patient had specific IgG and even parasitemia. This patient introduces a bias in the variables studied in ELISA, IFAT and Real-time PCR, and thus the parasitemia observed. The same applies to patient 13, who was symptomatic with severe anaemia and splenomegaly, and thus cannot considered a cryptic patient.

2.2. The number of subjects included in the ELISA and IFAT study must be changed, as no serological results are available for a patient 5, (Table 1).

2.3. Page 7, line 14. Parasitemia ranged between 1 and 8000 parasites/ml. These values must be revised (see 1.3. TaqMan PCR), those data don’t sound. They are really extremely high if asymptomatic patients are being studied. Revised results must be almost compared with Ref. 16: Mary et al., and Ref. 18: Bossolasco et al., who studied VL patients by L. infantum during diagnosis and after treatment using different real-time PCRs, and others references.

3. In Discussion

An extended discussion of the results is needed to take into account the necessary revision of the results.

Minor Essential Revisions

4. References

The references need to be revised
Page 3, reference 2. Desjeux et al. 2003 speaks about AIDS not HIV + PCR in urine samples in HIV+ references, such as Fisa et al., 2008, could be included.
Reference for ELISA is needed. References in Discussion of parasitemia are also needed.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.