Reviewer's report

Title: Cryptic Leishmania infantum infection in Italian HIV infected patients

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Reviewer: Joaquina Martin-Sanchez

Reviewer's report:

The authors wish to determine the prevalence of asymptomatic Leishmania infantum infection in Italian HIV infected patients and to study a possible correlation between Leishmania parasitemia and HIV infection markers. The objectives are well defined and the diagnostic techniques used – immunological and PCR – are a priori appropriate for the first objective. The results obtained are interesting as they corroborate those contributed by other authors in other endemic geographical areas. It also contributes to a greater understanding of the epidemiology of leishmaniasis due to L. infantum in endemic areas, as these high prevalences found in HIV+ individuals concur more closely with the elevated prevalences of canine leishmaniasis. However, some deficiencies were found which should be rectified (major compulsory revisions):

1. As this is a prevalence study, it is essential to have an accurately defined study population. The authors fail to address this issue at any time, with the exception of the title, which refers to them as “Italian HIV infected patients”, clearly a description offering insufficient information. In the patients and methods section the authors should indicate the geographical area which the patients come from and the time period in which the study was carried out.

2. Where it states the diagnostic methods used, PCR (the technique on which the authors base their results) is not adequately described. Even though it is made clear that the PCR technique described by Manna et al., 2008 was used, a brief description of the same should still be provided, indicating primer and probe sequences and their concentration. They should clarify why the threshold has been set at 1 parasite/ml, despite the sensitivity of the technique allowing for the detection of 0.001 parasites/ml – are there doubts regarding the specificity of PCR with these levels of parasites?

3. The serological methods used have not been adequately described: a. It is unclear whether the serological techniques have been used on the 145 individuals or only on the 25 with parasitemia demonstrated by PCR. b. It is not clear what dilutions of serum are used with these techniques.

4. With regard to the second objective, that of studying the possible correlation between Leishmania parasitemia and HIV infection markers, I do not believe that the statistical methods used are the most appropriate, nor have they been adequately explained. I would recommend carrying out a uni- and multivariate regression analysis. The HIV infection markers that were investigated (variables
investigated) should be expressly indicated, as should the p and Odds Ratio values.

Minor essential revisions:

The threshold used in the serological techniques (1/100) is excessively high for those individuals with compromised immunity – have the authors not considered the possibility of lowering it to 1/50? They would surely find that a greater number of individuals have anti-Leishmania antibodies at low titres.

Discretionary revisions:

In both the serological analysis and the DNA extraction sections, the information provided on how the parasites are washed and recounted appears somewhat excessive due to its obvious nature.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests