Author’s response to reviews

Title: Cryptic Leishmania infantum infection in Italian HIV infected patients

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Author’s response to reviews: see over
Dear Editor,

please find enclosed the revised version of the manuscript “Cryptic *Leishmania infantum* infection in Italian HIV infected patients” (MS 1320126427220823).

All the comments of the two reviewers have been taken into due consideration and the text has been corrected accordingly.

Regarding the specific comments of the **reviewer #1**: 

**Major revisions:**

1. The study population has been accurately defined in the Patients and Methods section.
2. The PCR technique has been described more extensively.
3. The serological methods used have been described more accurately. Serology was used on all the 145 patients.
4. Statistical analysis was performed carrying out a uni- and multivariate regression analysis.

**Minor revisions:**

Serum samples from all 145 patients have been retested lowering the threshold to 1:50. The number of patients who had anti-Leishmania antibodies did not change.

Regarding the specific comments of the **reviewer #2**: 

**Major revisions:**

1. Serological and molecular techniques have been explained more extensively. Serum samples from all 145 patients have been retested lowering the threshold to 1:50. The sensitivity and specificity of laboratory techniques depend on a good antigenic preparation, so we felt it was more useful to focus on recovery and purification of antigen. Referee 2: The dilution of serum was 1:100, the result was identical even further concentrating the serum 1:50.
2. Results were revised as suggested. In particular, patient 13, who was symptomatic with severe anaemia and splenomegaly, was excluded from the analysis. Patient 15, who had a history of previous visceral leishmaniasis, was not excluded because he continued to be asymptomatic despite laboratory findings, so we considered him a cryptic patient. Serum samples from all 145 patients have been retested lowering the threshold to 1:50.

3. Discussion was revised taking into account the revision of results.

   Minor revisions:

4. References were revised as suggested.

In addiction:

The approval of the ethics committee was not necessary because visceral leishmaniasis is a common infection in our geographic area, so the screening of immunocompromised patients is habitual. Nevertheless, patients were verbally informed about the study and after giving their oral consent demographic and clinical data were collected. Research was carried out in compliance with the Helsinki Declaration

Finally, table and figure 1 have been modified including only 24 patients, and figure 2 has been added.

The other authors and I thank you and the reviewers for the attention given to the manuscript.

I hope to have given exhaustive responses and I look forward to your decision.

Regards,

Claudia Colomba