Reviewer’s report

Title: Evaluation of PCR procedures for detecting and quantifying Leishmania donovani DNA in large numbers of dried human blood samples from a visceral leishmaniasis focus in Northern Ethiopia.

Version: 1 Date: 9 January 2013

Reviewer: Peter Melby

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This is a methods-focused short report of the use of qPCR for detection of L. donovani in dried blood samples from Ethiopian patients with visceral leishmaniasis (VL). The work nicely shows the utility of the approach, which has significance for both clinical diagnosis and large-scale epidemiological studies. The quantitation of number of parasites using the standard curve is a strength of the work. For the most part the study was well-conceived and the manuscript well-written. I have the following specific comments/criticisms:

Major compulsory revisions:

1. I realize that the focus of the paper is on the methods but still more detail needs to be presented related to the cohort of subjects to be able to understand the context of the study. Were these subjects symptomatic or did they have signs or symptoms of VL? Were they assessed prospectively in the community or were they sampled because they sought care at a health centre. Were the lab investigators blinded to the clinical status of the subject?

2. The leading sentence in para. 3, pg 3 that indicates only 30-50% sensitivity of parasitological and serological diagnostic methods is incorrect (and inconsistent with the data discussed in the subsequent sentences).

3. What preventative and quality control measures were taken to ensure that positive PCR results were not due to amplification of contaminating parasite DNA?

4. Given the differences in protocol from the original study (cited as reference 12) where the JW11 and JW12 primers were used, representative melting curves should be shown to confirm that there were not non-specific products of amplification that would be quantified by the SYBR green detection method.

5. The sequence results should be presented more thoroughly. How large a fragment was sequenced? How different were the putative L. major sequences from the L. donovani sequence (perhaps they should be shown), and how much variability is there in the ITS1 sequence within and across the Leishmania spp.? Which polymerase was used for the PCR amplification and was its expected level of fidelity sufficient to ensure the sequence differences were not introduced in the amplification? Was L. major cultured in the laboratory where the processing and PCR amplification of the dried blood samples took place (i.e. could it be a contaminant that led to erroneous amplification of L.maj or DNA in
the two dried blood samples)?

Minor essential revisions:
6. Line 4, para 3, pg 3 refers to three tests but only two are listed.
7. The reference to the lower limit of detection in para. 2, pg 10 should indicate that it is per mL of blood.
8. The last sentence in para. 2 on pg 11 raises the question of why the estimation of errors and rate of infection was not done.
9. The manuscript would benefit from careful editing because there are several typographical errors and awkward sentences.
10. The authors refer to the filter paper punches as 6 mm (presumably diameter) and r=3 mm which might be confusing.
11. Are the bands at the bottom of the gels in Fig. 3 primer-dimer?

Discretionary revisions:
12. The Results and Discussion sections could be combined.

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

Quality of written English: Needs some language corrections before being published

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

Declaration of competing interests:
I declare that I have no competing interests