Author's response to reviews

Title: PCR reveals significantly higher rates of Trypanosoma cruzi infection than microscopy in the Chagas vector, Triatoma infestans: High rates found in Chuquisaca, Bolivia

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Version: 2 Date: 7 March 2007

Author's response to reviews: see over
March 7, 2007

Editorial Office
Kinetoplastid Biology and Disease

Dear Editor,

In attachment please find the revised manuscript entitled “PCR reveals significantly higher rates of Trypanosoma cruzi infection than microscopy in the Chagas vector, Triatoma infestans: High rates found in Chuquisaca, Bolivia”. We are including a copy of the revised manuscript as well as our responses to the two reviewers.

Thank you for considering this manuscript. Please do not hesitate to contact me if you require any additional modifications to this manuscript.

Sincerely yours,

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Response to Reviewers.

Reviewer 1.

This is the first time these two methods are applied to obtain information concerning the prevalence of *T. cruzi* infection in *Triatoma infestans*, the main vector of Chagas disease in this part of Bolivia, located in the Andean valleys, the only reported location of sylvatic *T. infestans*. On page 5, in the Background section of the manuscript we underlined the relevance of local ecological and epidemiological conditions in this region of Bolivia that may determine the difference in prevalence observed in this zone with respect to other countries in South America. One of our goals was to validate these techniques for further application in this area looking for a larger number of specimens. The information on *T. cruzi* infection will provide the local epidemiological importance of domestic and peri-domestic insects in the maintenance of active transmission in this area of Bolivia. In addition, this information constitutes a baseline for future studies to be carried out in Chuquisaca.

In the Methods section we have mentioned how insects were captured for analysis: “Nymph and adult insects were collected from inside homes, as well as in their immediate vicinity, by the residents under the community-based anti-Chagas control program. Collection date, locality, and habitat (domestic, peri-domestic, chicken coop) were recorded and live insects, in plastic cups with folded paper, were transported to the vector control laboratory at the Servicio Departamental de Salud (SEDES) de Chuquisaca for microscopic analysis”.

We are confident that the problem of contamination is absent or minimal in our study because of the consistent lack of amplification in all negative controls run along positive control and samples. On pages 13-14 in the Discussion section of the manuscript we have mentioned that: “Water, rather than DNA extracted from uninfected insects, for the negative control has the advantage that it lacks PCR inhibitors that may be present in other substrates, ruling out contamination in a reaction mixture most likely to amplify DNA”, “To further reduce the chances of cross-contamination, only one sample per insect was obtained and the dissection and PCR processing were performed in separate areas of the laboratory. The fragment size of positive samples and positive control reactions were as expected for *T. cruzi*. In addition, PCR negative samples spiked with 0.1 ng of *T. cruzi* DNA showed amplification after a second PCR. Comparison of the sequence of our PCR products to those in GenBank confirmed that the amplified product was *T. cruzi* DNA.”

With respect to the number of specimens included in our study, we analyzed a total of 152 *T. infestans*. We were aware of the insufficient sample size in insects coming from some communities included in the study; however, this potential problem was addressed during the analysis pooling groups and analyzing only insects from localities with a reasonable sample size. In addition, in all results a test of statistical significance was computed.
We believe that the results in this paper are important and relevant to communicate to others who engage in research in this field. Up to this point research regarding the *T. cruzi* infection in *T. infestans* in this part of Bolivia was never envisaged. Our results point to the PCR-based assay as an alternative method to identify the presence of *T. cruzi* in its vector and open avenues to conduct future work in this species that can be utilized to garner a clearer picture of the epidemiology of Chagas disease in Bolivia.

**Reviewer 2.**

**Abstract**

1) As recommended by this reviewer, we included the term “lifecycle stages” and dropped the term “sex” at the end of the first paragraph since sex was not included in the analysis due to a insufficient sample size in adult insects included in the study.

**Background**

2) As suggested by the reviewer, “anti-Chagas programme” is not italicized.

**Results**

3) The reference to Figure 1 is now on page 9 in the Material and Methods (PCR amplification) part and on page 14 in the Discussion section.
4) As suggested by the reviewer, the sentence “Whereas discrepancy… was zero” was changed to clarify the concept as follows: “…discrepancy (PCR negative and microscopy positive) was negative…”
5) We included a paragraph on page 11 under the Results section in the text explaining why the localities of Serrano and Carbajal were excluded from the age effect analysis.

**Discussion**

6) Paragraph 1: To better explain the sensitivity by locality, we included the sentence “Except for Serrano, all other communities showed that PCR was significantly more sensitive than microscopy”.
7) Paragraph 2: We have included the species analyzed in the Paraguayan study: “The high level of infection of insects collected in the field is similar to a study done on *T. infestans* in the Paraguayan endemic zone for Chagas disease.”
8) A possible explanation for the high prevalence of infection in younger nymphs is provided in the fourth paragraph on page 13.

**Discretionary Revisions**
More substantive:

9) Title: The title has been changed as suggested by this reviewer to “PCR reveals significantly higher rates of *Trypanosoma cruzi* infection than microscopy in the Chagas vector, *Triatoma infestans*: High rates found in Chuquisaca, Bolivia.

10) We have decided to divide the analysis into three habitats separating the human habitation from the peri-domicile. In turn, the decision to separate insects from chicken coops from corrals in the peri-domiciliary ecotype was done to have separated birds from mammals other than humans. This is an important distinction since mammals and not birds can transmit the disease. We have provided information about how we defined the habitats on page 8 in the first paragraph of Methods.

11) GPS coordinates of the villages have been included between parentheses after each locality listed on page 7 in the first paragraph of Methods.

Results

12) Concerning the results of the prevalence of infection per locality, we have introduced a comment on the sample size of the localities analyzed in the fourth paragraph of the Discussion on page 13.

Details:

Background

13) Concerning the sentence: “These areas report the highest prevalence…”, we have specified “… the highest prevalence in Bolivia…” on page 5.

14) We tried to retrieve the reference suggested by reviewer 2 so that we could incorporate it into the text. We found and incorporated in the manuscript the reference of Cortez et al. (2006).

15) Concerning the use of more than one reference for certain statements, we have considered that two references from different ecologic and epidemiologic environments will better support the statement.

16) As suggested, we have changed the term “insect vectors” by “vector insects” in the Abstract and in the second paragraph of the Background.

17) Concerning the statement that “conventional microscopy… is best performed in living insects…”, we have changed this statement as suggested by this reviewer to read: “conventional microscopy… is best performed in living insects since once the insect is dead the parasite is nearly impossible to detect in insect feces” including a reference.

18) Concerning the time of 10 minutes for scanning, we have corrected this statement as “10 minutes for extrusion of the abdomen to obtain the sample, mounting the plate and scanning per insect…”
19) The third paragraph has been modified including the suggestion made by this reviewer in the following manner: “Several primers complementary to the conserved region of the kinetoplast-minicircle part of the mitochondrial DNA…”

20) We have introduced the terms “technique using” to the sentence: “…that the technique using the TCZ1 and TCZ2 primers that amplify a 188 bp of a 195-bp repetitive nuclear sequence is the most sensitive…”

21) Concerning the use of mm to report rain precipitation, we have maintained the use of mm to be consistent with the literature and make our data comparable with other publications.