June 5, 2007

Editorial Office
BMC Infectious Diseases
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 the formatting requirements suggested.

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 this method is applied to obtain information concerning the blood meal source of *Triatoma infestans*, the main vector of Chagas disease in this part of South America. Our goal was to validate this technique for further application looking for a larger number of species. During the surveillance phase of control programs aimed to eradicate *T. infestans*, identification of the source of re-colonizers of human dwellings after spraying with residual insecticides is fundamental to improve strategies. To understand the patterns of vector dispersal and transmission of *T. cruzi* infection to humans, one mean is the identification of domestic and peri-domestics hosts that serve as food for this species. Environmental conditions and human behavior and beliefs differ markedly from one site to another in South America. Thus, the information on host preferences will provide the local epidemiological importance of domestic and peri-domestic animals in the maintenance of active transmission as well to contribute to the identification of trends of insect movement.

On page 9 we have mentioned as an advantage of this method over the serology the availability of reagents to perform these assays. On page 10 we also emphasized that “having reliable and inexpensive primers already developed” will be more feasible that a medium equipped laboratory may reproduce the same methodology.

The sensitivity of the technique was indirectly measured by its ability to detect the blood meal source from 1 to 40 hours in the experimental study and after two month in the field collected specimens.

On pages 4 and 5 we underlined the relevance of local ecological and epidemiological conditions in this region of Bolivia that may determine the difference in human prevalence and house infestation observed in this zone with respect to other countries in the Southern Cone of South America.

We have included the information about the developmental stage of specimens included on the methods part of the manuscript (p 13) and results by stage and habitat on page 8.

Concerning the replicates, three insects (different nymphal instars or a mix of one adult and nymphs) per time were included in the experimental study. On page 8, under the results we mention that we did not find differences in amplification by stage or habitat.

As recommended by this reviewer, we introduced updated reference on the burden and trends of Chagas disease.

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 feeding preferences of *T. infestans* in this part of Bolivia was never envisaged. Our results points to the PCR-based assay as an alternative method to identify at the species level the content of complex blood meals and open avenues to conduct future work to
discern the complete pattern of feeding preferences in this species that can be utilized to garner a clearer picture of movements of *T. infestans*.

**Reviewer 2.**

**General comments**

Concerning the confirmation of guinea pig DNA, we have compared the sequences of our PCR products to those in Gene Bank that confirmed the DNA amplified as guinea pig (p 7-8 and p11).

To discard lack of amplification due to inhibition of PCR, we “spiked” the negative samples with guinea pig DNA, repeating the amplification (p 8, 10). In addition, the same extracted DNA has been used in subsequent experiments observing always constant amplification.

As recommenden by reviewer 2, we have included general information about SINEs and specific to the amplified SINE in guinea pig along with Gene Bank accession number, estimated number of copies and references about SINEs in other blood sources for triatomines.

In Abstract-Results: the statement “in as little as” was corrected.

**Title:** We changed the title to be more specifics as “guinea pig blood meal”.

**Background**

We tried to retrieve the reference suggested by reviewer 2 so that we could incorporate them into the text. We found and incorporated in the manuscript the reference of Morel and Lazdins (2003).

We have modified our statement concerning the elapsed time of asymtomatic conditions stating “post-infection, ten to 20 years of asymptomatic conditions lead...”(p 4).

As suggested, we have included information about the effectiveness of the treatment past the acute phase specially in school children (Reference 3).

We have change the phrase “reinforcing domestic foundations” by “plastering adobe walls and using tile roofs to reduce vector habitat in homes..” (p 4).

As recommended, we changed the statement “only mammals can be infected” by “only mammals maintain an infection” (p 4).

There are multiple mammalian hosts and we have indeed mentioned *T. infestans* as the main vector in Bolivia (p4) but also we stated that geographic characteristics and local
domestic vertebrate populations, peri-domestic, as well as possible zoonotic or “wild” vertebrate hosts in conjunction with the existence of silvatic populations of *T. infestans* in this area may complicate the success of the program against this species.

In the same page we modified the phrase “ecology of the parasite life cycle” by “persistance of *T. cruzi* among hosts,..” to clarify this statement.

We have changed the expression: “these heterogeneities are a major driver of local triatomine populations...” by a more specific statement that says “Sylvatic populations of *T. infestans* are only known to occur in the Andean highlands and probably are a major factor in local triatomine population dynamics and reinfection patterns” (p 5).

The reference for the statement that Chagas disease is the most serious human parasitic disease of the Americas in terms of social and economic impact is provided in:


We have provided the information about the developmental stage of specimens included (p 13). Under the Results section, we also have stated that there was no difference in the likelihood of amplification for insects from different habitats or life stages (p 8).

We have mentioned that the guinea pig DNA was “still present after 40 hours” (p7).

Discussion

We have modified this paragraph: “Previous studies using PCR to detect *T. cruzi* in Triatomine vectors found that the stomach was more often negative whereas intestine and rectum samples showed much less inhibition to PCR, reflecting either differences in the distribution of *T. cruzi* or PCR inhibition [21] (p10).

This paragraph has been also modified including the suggestion made by this reviewer in the following manner: “Although a positive result indicates past feeding on a guinea pig, for a negative result further study is needed to determine whether guinea pig DNA is absent or is due to the loss of signal over time and the rate at which, a blood meal is eliminated or degraded to the point it can no longer be detected” (p11).

In the methods section is stated that the “cut was made as close to the posterior as possible to avoid the stomach” (p14).

We have included under sample collection the description of different insects used in Assay 1 (p13).
A reference for primers used [14] as well as the Gen Bank accession number is given on page 13.

References

We have inserted all the recommendations made by reviewer 2 concerning this part of the article.

Figures

We have made the suggested changes in Figure 1. The ladder has been replaced to the left of the figure and the 71 bp has been clearly signaled with an arrow.

Other comments

We have followed all the suggestions made by the reviewer concerning these points across the manuscript.