Author's response to reviews

Title: Association of Trypanosoma cruzi infection with risk factors and electrocardiographic abnormalities in Northeast Mexico

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Version: 3
Date: 2 December 2013

Author's response to reviews: see over
Philippa Harris
Executive Editor
BMC Infectious Diseases

Ref. #: MS: 5132517210158063

Dear Philippa Harris:

I would like to thank the reviewers for their careful and detailed critique of our manuscript entitled “Association of Trypanosoma cruzi infection with risk factors and electrocardiographic abnormalities in Northeast Mexico”. I have either replied to each specific comment addressed by the reviewers, or have incorporated their suggestions in the revised manuscript. I hope that this revision will answer the thoughtful and significant concerns of the reviewers.

Sincerely,

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In Response to Reviewer Francisco Javier Carod Artal

We want to thank this reviewer for his kind comment to our study, we are answering his questions and suggestions as follows:

MINOR ESSENTIAL REVISION:

- Abstract: Last sentence of conclusion (T. gerstaeckeri) is not indeed a conclusion supported by this research, and should be deleted.

ANSWER: We fully agree with this comment and the last sentence was deleted (page 3, Abstract section of the revised manuscript).

- Methods: authors assessed ECG in volunteers with a positive T. cruzi serology; could this fact have influenced the results? Were there any differences between seropositive volunteers and not volunteers regarding risk factors and ECG abnormalities? Why authors did not random seropositive subjects for the study?

ANSWER: In regard to the first concern, a control group of seronegative volunteers were matched by gender and age from the same localities with seropositives with the purpose to obtain a statistical result with a good confidence level ($P = 0.002$). In regard to the second concern, the second objective of this study was to “analyze risk factors associated with infection by T. cruzi” (page 4, Background section of the revised manuscript), and the dependent variable or main outcome was based on serological status. In addition, this study was performed with volunteers, with no preference for gender, age, occupation, or residency. This epidemiological survey did not include “not volunteers”, because they did not accept to participate. In regard to ECG, both groups were subjected under the same environment of “risk factors”, and the ECG differences were shown in Table 3. The tracing was blind analyzed independently by two cardiologists. Both methodologies were based in Sosa-Jurado et al. 2003 (Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. 98(5): 605-610). This methodology was followed to avoid any influence in our results.
In regard to third concern (Why authors did not random seropositive subjects for the study?), we believed that given the fact that 52 seropositive subjects in 9 different localities, were not expensive to visit, we took the decision to visit all of them, but only 37 were found; the rest of the residents moved to the city or to the United States of America (”Seventeen participants could not be located;... page # 11). However, epidemiological survey was performed by a systematic random household sampling, using information reported by the database of the state of Nuevo Leon.

DISCRETIONARY REVISION:

- Discussion:

A) Chagas disease may affect an important proportion of young women that may potentially transmit the infection to children. This fact should be commented taken into account the high percentage of females infected in this study.

ANSWER: We fully agree with this comment, and a paragraph was added (page 13 of the revised manuscript):

“Women of child-bearing had a seroprevalence rate of 1.31% (data not shown), which suggests that congenital transmission may significantly contribute to the elevated infection rate in children. The INEGI recorded a population of 1,041,600 women of reproductive age and 93,902 births for Nuevo Leon in 2010 [10]. Extrapolating from the highest national seroprevalence reported [4,5], we calculated that up to 61,454 women of child-bearing age may be infected and 5,540 infants congenitally infected in Nuevo Leon. This situation urgently requires the establishment of an active surveillance program to determine the major mechanisms of transmission and the incidence of infection acquired through blood transfusion; programs to screen for congenital Chagas disease transmission are also needed.”

B) Chagas disease seems to be a cause of embolic stroke in a much higher proportion than previously expected. Seropositive patients may be in an increased risk, even they are asymptomatic; and awareness about this fact seems to be lower. Please see: Policy implications of the changing epidemiology of Chagas disease and stroke. Stroke. 2013 Aug;44(8):2356-60; and: Lancet Neurol. 2010 May;9(5):533-42.
ANSWER: I agree with the reviewer’s comments. The existence of a chronic nervous form, of varied neurological symptoms, directly caused by *T. cruzi*, was postulated by Chagas in 1911. (Chagas, C. 1911. Mem Inst Oswaldo Cruz, 3:219-75). Even, the main risk factors for cerebral infarcts are cardiac arrhythmias, and several heart failures (Nussenzweig et al., 1953. Arq Neuropsiquiatr. 11, 386-402; Aras et al., 2003. Arq Bras Cardiol. 81, 414-416; Neiva and Anrade, 1962. Hospital 61: 373-379). Certainly, cerebral infarct may affect chagasic patients without clinical evidence of cardiopathy, such as your kind comments. In fact, questions addressed to epilepsy, decreased level of consciousness, aphasia, neglect, headache, visual-field deficits, parietal syndrome, and seizures are listed in our epidemiological survey, but all volunteers respond negatively, and its variable was deleted in the statistical analyses.
In Response to Reviewer Jennifer Manne

MAJOR COMPULSORY REVISIONS

1. It is unclear what is meant by “systematic random household sampling...according to socioeconomic and cultural level information reported for each municipality in the database of INEGI.” It would be preferable if the authors were clear as to whether they sampled randomly. It at least appears that they initially stratified their sample based on a particular geography in order to represent the urban/suburban/rural populations. However, it is unclear if they also sampled based on particular SES features. If the sample was not completely random, more information should be given on how sampling was performed in the Methods or as an appendix.

ANSWER: In regard to the epidemiological survey, a stratified model was used and the population was randomly-stratified, according to a population of 4,653,458 inhabitants from 51 municipalities, a sample size of 2,688 individuals was estimated for 10 municipalities randomly-selected. This model allows us to represent the rural, suburban and urban localities of Nuevo Leon, according to the socio-economic and cultural level. This paragraph (page 6 of the revised manuscript) describes a detailed description:

“The statistical sample was designed according to a stratified model. Systematic random-stratified household sampling was performed considering the total number of inhabitants distributed in 51 municipalities of the state, and ten localities were random selected. This model allows to represents the rural, suburban and urban regions according to the socioeconomic and cultural level using information reported in the database of the Instituto Nacional de Estadística y Geografía (INEGI) [10]. The urban population was characterized to include houses with complete sanitary services (drinking water, sanitary drainage), commercial centers, hospitals, schools, public transport, etc. Suburban population included individuals living in the peripheral of cities. The rural population was characterized by living in localities with unpaved roads, without street lights and sanitary services, and presence of domestic and peridomestic animals (henhouse, pigpen, stables, etc.). Poor and well building housing were found equally in each region, but in rural and suburban areas were constructed with adobe. A socio-economic index

...
was developed using household characteristic as variables recorded: wall, roof and floor materials, piped water, number of rooms, and were assigned numerical values 0, 1 or 2 representing three economic strata. Using maps [10], between two and ten housing blocks were selected according the density of each population. Selection procedure for the houses to be sampled, involved enumeration of each house in the block, and selection of five houses on each side, with the previous consent of the householder.

And in the next paragraph, a correction was written:

“People participation in the study was random and voluntary to obtain the sample size required by each locality, with no preference for gender...”

2. In the discussion, the statement at the end of paragraph 1, “Thus, it appears that the prevalence of T. cruzi is increasing throughout Mexico” seems like an inappropriate conclusion given the data presented. Even though this study and other recent work may show a greater prevalence of Chagas disease, there may be aspects of study design or the population tested that differ. In addition, the study here is of a single state within Mexico.

ANSWER: We agreed to the reviewer’s comment, in fact, this study was performed only in Nuevo Leon state. Some supporting references on the increasing prevalence of Chagas disease in NL (located at NE of Mexico) follow:

a) Carabarin-Lima et al. (Acta Tropica, 127 (2013) 126–135) reported a similar conclusion: “Nevertheless, the highest prevalence was observed in the northeastern region of the country, which corresponds to the central area of a tropical region that includes the states of Hidalgo, San Luis Potosí, Veracruz, and Tamaulipas named La Huasteca (Guzman-Bracho, 2001). In this region, the prevalence has increased in recent years (Galaviz-Silva et al., 2009)”.

b) The National Seroepidemiological Survey (NSS), conducted by the office of the Secretaría de Salud, reported a seroprevalence of 0.2% in 1992 for Nuevo Leon. (Velazco-Castrejón et al., 1992. Seroepidemiología de la Enfermedad de Chagas en México. Salud Publica Mex. 31:186-196). In this study, a seropositive rate was observed to be 1.93%.
c) Guzmán-Bracho (1998; Rev Panam Salud Publica/Pan Am J Public Health 4: 94-99) reported a seroprevalence of 0.5 in blood donors from Nuevo León, while, Galaviz-Silva et al (2009) found an infection rate of 2.8% “Based on our results, the transmission of *T. cruzi* by blood transfusion may be an emerging risk in Nuevo León, considering that there has been an ~5-fold increase in the incidence of seropositive individuals” (Galaviz-Silva L., et al. 2009. Update on seroprevalence of Anti- *Trypanosoma cruzi* antibodies among blood donors in Northeast Mexico. Am J Trop Med Hyg., 81: 404-406).

Based in these evidences, and according to Reviewer comment, this statement has been changed in page 13 of the revised manuscript to: “Thus, it appears that the prevalence of *T. cruzi* is increasing throughout Nuevo Leon state [8,9].”

3. *The authors do not adequately address the limitations of the study. There should be a more thorough discussion of this aspect of the study.*

As suggested by the reviewer, a new paragraph was included to describe several limitations found in our study (end of discussion, page 15 of the revised manuscript):

“The study has some limitations; first, the prevalence bias could be underestimated, since sample size was obtained from a seroprevalence rate of 0.2% reported by NSS [9] from a IHA/IFI test at titles of 1:32, however, no sensibility data were reported. The sample size taken by NSS was 3,747 inhabitants, but the size population, proportion by gender, age, and origin of the samples (rural, suburban or urban counties) was not reported, which might lead to a slight underestimate of prevalence in the population. Second, sample size has been one of the major limitations to most epidemiological studies. The statistical power of a study to assess epidemiological associations depends on the number of participants, in this case, 2,688 individuals. However was not possible to get a uniformity in the sample size according age, following our study design, and our major deficiency was not sampled individuals under 17 years old, because the samples were obtained by voluntary participation of the population, and in case of children, the consent of parents were legally necessary. Another limitation was the high mobility of the immigrant population, where 17 seropositive participants were lost for prior to the ECG recording, consequently caused a drop out in the number of participants. Finally, the participation of the community members to identify triatomines was low (3 %), only 83
individuals correctly identify specimens of *Triatoma* adults shown to them, but the main difficulty was to recognition of nymphs (often confused with phytophagous insects). In Doctor Arroyo, triatomines were recognized only by older individuals, which was also a limitation in the epidemiological survey”

4. It wasn’t clear whether the authors used multivariate logistic regression for the risk factor analysis. It would likely be advantageous to consider doing this if it was not performed. If it was, perhaps this could be clarified in the methods.

ANSWER: As suggested by reviewer, this paragraph (page 9) was modified to clarify the methodology used to analyses risk factors:

“Chi-square and Fisher’s exact test univariate analysis were used to determine association between seropositivity and age range (years), gender, and ECG alterations with 95% CI (*P* ≤ 0.05). Multivariate logistic regression were subsequently performed to calculate the odds ratios (ORs) between *T. cruzi* seropositivity and risk factors using SPSS software, version 17 (Chicago, IL) and GraphPad Prism version 6.01. (La Joya, CA). Only variables significantly associated in the univariate regression were included.”

MINOR ESSENTIAL REVISIONS

1. The Methods section mentions that house sampling for triatomine infestation was performed by community members. It would be good to address the limitations associated with this approach in the discussion.

ANSWER: In subheading *Triatomine sampling* (page 7), “with the involvement of members of the community” means with the participation of members of community, because triatomine searching inside houses, should be done with the consent and supervision of the householders to avoid us problems. In addition, an explanation on this issue was made in question #3 of “several limitation of the study” (page 15 of the revised manuscript).
2. The concluding sentence states “In Nuevo Leon, Chagas disease remains poorly recognized, and most physicians consider it to be an “exotic disease”, exclusive to South America.” Again, this statement seems unsupported by evidence. It would be preferable to reword this statement and if making this claim, provide supportive citations.

ANSWER: We had experienced most physicians in northeastern Mexico unknown Chagas disease. They are sure it is located mainly in South America and in the south of Mexico, based only in their course-book (Beaver, J. et al. 2003. Parasitología Clínica de Craig Faust. 3° ed. Editorial Masson Doyma México).


[31] Cárdenas Sánchez et al., 2003. Anticuerpos anti-

Both citations were included after that statement (page 15 of the revised manuscript).

“Chagas disease remains poorly recognized, and most physicians consider it to be an “exotic disease”, exclusive to South America [9, 31].”

3. The titled might have been reworked to better describe the various aspects of the study. Perhaps something like “Association of Trypanosoma cruzi infection with risk factors and electrocardiographic abnormalities in Northeast Mexico.”

ANSWER: As suggested by the Reviewer, the title has been reworked and we believed this is most congruent with our findings.