Author’s response to reviews

Title: Incident Mycobacterium tuberculosis Infection in Household Contacts of Infectious Tuberculosis patients in Brazil

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Author’s response to reviews:

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Dr Straetemans:

Section Editor
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Re: Response to review (INFD-D-17-00428) “Incident Mycobacterium tuberculosis Infection in Household Contacts of Infectious Tuberculosis Cases in Brazil”

Dear Dr. Straetemans,

We thank you for reviewing our manuscript. The reviewers provided several important comments that will significantly strengthen our manuscript. For clarity, we have numbered and bolded comments from reviewers.

Please contact us at your convenience if you have any questions regarding this submission

Sincerely

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Carmen Judith Serrano (Reviewer 1):

1. If available report in the setting of the study the general agreement value between TST and QFT. Compare the general value concordance with the data you found for the agreement TST/quantiferon for the converters

Response (R): The agreement between TST and QFT has been added in the Results section for both the entire study population and for TST converters, as suggested by the reviewer.

2. Do you think the study of the immunological definition threshold for converters is the only way to solve the problem? Discuss a bit how could be approached

R: As discussed in the manuscript, our study provides strong epidemiological evidence of variable “susceptibility” to M. tuberculosis infection among humans. This adds to the large and growing body of evidence showing the importance of both innate and acquired immunity in susceptibility to infection, and the ongoing interest in discovering a phenotype or genotype associated with “resistance to infection”, as indicated by heavily exposed yet uninfected individuals. Further, there is a large amount of experimental data indicating inoculum size is an important determinant of risk of infection and disease progression. Taken together, these data suggest the possible existence of an immunological threshold in the risk of infection in humans that will need dedicated experimental studies with immunological endpoints. Some of these studies may include inoculum dose-escalation studies in different animal models.

Christian Mulder, Ph.D. (Reviewer 2): Major comments:
1. The authors conclude that their study 'suggests the existence of an immunological threshold in certain contacts that become infected only after a sustained or unusually large infectious inoculum resulting from both community and household exposures'. I believe that this is overstated as it is not supported by data from this study.

R: We respectfully disagree with the reviewer on this important point. Our results in this household contact study in a setting with moderate TB shows that some contacts become infected from community exposures, some others become infected “early” (i.e. TST positive as baseline) after a family member develops pulmonary TB and others, a minority, only become infected at a later stage after several months of a sustained infectious exposure (TST converters). Also, a significant proportion of contacts remain without any immunological evidence of M. tuberculosis infection despite multiple opportunities for infection. Importantly, by controlling for index case, contact and environmental confounders, our study design shows none of these factors explain on their own the observed differences in infection status. Furthermore, we found 7% of TST conversion, which is similar to the rates of TST conversion reported in other household contact studies in both high and low TB incidence settings. The similarity in rates of TST conversion across settings is unlikely to be a spurious observation, further suggesting a threshold effect in the risk of TB infection.

As discussed in the manuscript, taken together these results suggest a variable risk of infection in contacts that we hypothesize may be related to an immunological threshold of infection in humans. This hypothesis is consistent with a large and growing body of evidence in both experimental and human studies. We acknowledge that the threshold effect in the risk of infection is very difficult to confirm from an epidemiological standpoint and will require additional immunological studies.

2. Include in the discussion the potential practical implications of this work. Any suggestions on the LTBI-testing policies?

R: We found that QFT did not perform well in TST converters, a population known to be at highest risk of progression to TB disease. The concept of IGRA delay has been proposed and discussed by our group in previous publications. General practitioners should be careful when performing IGRA in close contacts, especially if its performed early after the household contact evaluation is commenced as it could miss some cases of infection. A paragraph has been added in the discussion.
Minor comments:

3. Please use 'patients' instead of 'cases' throughout;

R: We have replaced the term “cases” for “patients” throughout the text as suggested by the reviewer

4. Line 50, page 8: How do the authors define 'the tail end of infections transmitted...'

R: TST converters were likely infected just before the index case was diagnosed, that’s why they were TST negative at enrolment and became TST positive within 8-12 weeks. As index TB patients were started on TB treatment, and hence become non-infectious, contacts with TST conversion represent the last group of close contacts infected by the index patient.

5. Line 29, page 9: Please explain why PLHIV were excluded?

R: When affected by TB, PLHIV have different clinical presentations compared to non-HIV infected persons, and PLHIV are less infectious compared to non-HIV infected TB patients. Also, when infected with TB, PLHIV are frequently anergic to TST complicating the evaluation of LTBI in these individuals. Since the goal of the parent household contact study was to evaluate TB transmission (as measured by TST positivity in exposed contacts), PLHIV were excluded to eliminate a source of uncontrollable bias. Further, Brazil is a setting with low prevalence of HIV-infection (1% in general population and 7% in TB patients).
6. It's not clear if only household contacts are eligible, or also other close contacts, such as colleagues. If the latter, please use the term ‘close contacts’ instead of ‘household contacts’

R: This study only included household contacts. There is a lack of a standard definition for household contacts and different criteria are used across countries and published studies. Guided by the recommendations of the Brazilian National TB program to define household contacts, we used four culturally adapted criteria to define close contact at the household level, as explained in Methods (Page 9: participants section)

7. Line 48, page 9: Contacts were enrolled within the first two weeks after the index case presented. By then, was the diagnostic result already available for all index cases? E.g., I can imagine that bacteriological results were not available yet

R: All index TB patients were sputum AFB-positive at the time of study screening. In most cases, culture results were in process at the time the household contact investigation took place. We only included households with culture-proven TB.

8. Line 4, page 10: Why was CXR performed given that all index cases underwent culture?

R: We obtained CXR because we wanted to evaluate the impact of radiographic variables such as presence of cavitation or advance radiographic disease on transmission, as measured by the proportion of contacts with TST or IGRA evidence of infection.

9. Line 14, page 10: How was individual contact time with the index case measured? Why was decided in Table to use a cut-off of 6 hours?
R: It was measured by direct questioning of the household contact by the following question: “Over the past 3 months, on the days that you had contact with the index case, how much time did you spend with the index patients”? The variable was categorized in:

1. Less than 1 hour
2. Between 1-6 hours
3. Between 7-12 hours
4. Between 13-18 hours
5. >18 hours.

We chose a cut-off value of 6 hours for being the value closest to the median.

10. Please include in the methods section whether BCG-status was collected and how.

R: BCG vaccination status was assessed by direct visualization of a BCG scar in the deltoid area. A sentence has been added to the method section.

11. Line 45, page 10: It's unclear why IGRA-testing had to be done 8-12 weeks before TST testing. Basically you could do IGRA testing followed by TST within the same day without risk for boosting.

R: By study design, eligible contacts had two TSTs: one at enrolment and a second one 8-12 weeks after (if initial TST <10mm). IGRA was done only once at the time of the second TST. We have added a phrase in Methods to clarify this point.
Mengistu Legesse Dadi (Reviewer 3): General comments:

1. While the research idea was interesting, the study was not properly designed, the methods and the results were not clear presented, and it is difficult to follow and understand. The title (Incident Mycobacterium tuberculosis Infection in Household Contacts of Infectious Tuberculosis Cases in Brazil) does not coincide with the results of the study. What is meant by incident M. tuberculosis infection in household contacts?

R: We appreciate Dr. Mengistu’s comments. The present study focused on contacts undergoing TST conversion. Incident infection refers to the fact contacts with TST conversion were initially TST-negative and only became TST-positive during follow-up (i.e. TST conversion). The terminology of “prevalent infection” (e.g. TST-positive at baseline) and “incident infection” (e.g. TST conversion) is widely accepted in the TB literature.

2. Among the 62 TST converters, the majority (69%) were positive by IGRA at baseline and these IGRA positive individuals cannot be considered as incident cases. Moreover, the majority of the contacts who were found positive for Mtb infection by TST either at the baseline (81%) or the converters (87%) had BCG scar. Because of this, the results of TST is not dependable to report incident of Mtb infection in countries where the coverage of BCG vaccination is high.

R: As explained above, eligible contacts had two TSTs one at enrolment and a second one 8-12 weeks after (if initial TST <10mm). IGRA was done only once at 8-12 weeks (at the time the second TST was planted). Therefore, none of the contacts were IGRA-positive at baseline as suggested by the reviewer.

We agree that previous BCG vaccination may influence the TST status in countries with high BCG coverage. However the medical literature supports the concept that the BCG effect is mainly observed in the first 10 years of life. To evaluate this issue, we presented an age stratified analysis (Figure 2) where the proportion of IGRA positivity is high even in children younger than 5 years old, which suggests that most of these cases represent true TB infections rather than BCG-induced false positive results. Further, our definition of TST conversion required an initial negative TST, thus BCG is unlikely to be playing a role in TST conversion. Finally, an initial study from our group in Brazil (reference 22) evaluating TST/IGRA discordance showed NO association between BCG status and TST+/IGRA- results in contacts.
3. IGRA testing was done at 8-12 weeks before TST placement to minimize boosting. It is not clear why the authors could not perform the TST on the same day (at least within a week) after collection of blood for IGRA, and why it essential to wait for about two-three months after performing IGRA?

R: As explained, IGRA testing was only performed at 8-12 weeks. Blood for IGRA was obtained immediately before (same day) a second TST was placed (only if initial TST <10mm)

4. It is also not clear, why the authors could not involve community controls?

R: The absence of community controls is a limitation of our study as it did not allow us to estimate secondary attack rates of infection. This has been stated in the discussion section (page 17).