Author’s response to reviews

Title: Latent Tuberculosis Infection and Associated Risk Indicators in Pastoral Communities in Southern Ethiopia: A Community Based Cross-sectional Study

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Author’s response to reviews:

Response to reviewers

We thank the reviewers for their critiques. The comments are helpful and constructive. Accordingly, we incorporate corrections, improvements and additions in our revised manuscript. Language was critically evaluated by native speaker. The changes are highlighted in RED in the revised version of the manuscript. We also describe our corrections and changes point-by-point (see below).

1. Editor Comments:

Dear authors, Thank you for your submission to BMC Public Health. Your paper has been reviewed by three reviewers and I also have some comments about your paper (included below).
Abstract:

Comment-1: Pls include the sampling method in the methods (the introduction can be shorter)

Response: We shortened the Introduction and the sampling method was included in abstract part (see abstract part)

Comnet-2: Line 38- if there was no significant difference pls provide a p value (Did you do a chi square test for this statistic)? I would prefer to see p values in the results

Response: P-values were calculated for all variables, now presented for all results in the text and included in Table-3. Regarding chi-squared test ($\chi^2$), yes we did Pearson's $\chi^2$ for all categorical variables.

Background:

Comment-3: Page 3, Lines 19-29- pls update with statistics from the latest global TB report

Response: Now we used WHO, 2017 as reference. New TB case is the same i.e 10.4 million but death rate is reduced to 1.7 million

Comment-4: Page 3, Line 41 – pls reference this statement:

Response: Now reference is added

Comment-5: Page 3, Line 49- please start the sentence with “The World Health Organization (WHO)” Also, is that statement by WHO really about active TB disease and not latent TB infection? If so pls clarify and or remove this statement.

Response: We clarified The End TB strategy statement and stated that systematic testing and treatment of LTBI in at-risk populations is a critical component in the elimination of TB.

Comment-6: Page 4, line 4- are there any papers that can support this claim? I wonder if there has been research on this previously? Page 4, lines 6-9- I think it may be best to say that for the most part the NTP relies on passive case finding – is that what you mean? I would also be clear
when you are talking about active TB and latent TB infection. We may not expect the NTP to be monitoring latent TB infection. I think in your background we need a better sense of why latent Tb infection is important in this community, i.e. what is the rationale for your study?

Response: For whole page-4 questions see modified rationale part.

Methods:

Comment-7: Pls include reference to whether the interview was face to face (I assume so)

Page 6, line 59- pls correct typo present, not present

Page 7, line 6- pls correct typo bivariate, not bivariate

There is no statement about ethics and there needs to be

Response: All questions from methods part were corrected accordingly (see page 5 paragraph 4, line 4; page-6 paragraph 2 lines 13 and 18; and page 6-7)

Results

Comment-8: Line 28-30 – there is no need to state that blood samples were collected etc. I think there is no need to include every socio-demographic characteristic – you can refer to the table

Response: Corrected accordingly (See page 7, result part first paragraph)

Comment-9: I think if you present IGRA results that relate to quality tests it is best to describe this in your methods

Response: Thank you, now we addressed it in data analysis part in the methods (see page-6 paragraph 2, lines 11-13)

Comment-10: Page 8, line 39- the numbers for the CI come before the abbreviation CI whereas in other parts of the paper it is the other way around – pls correct

Response: Corrected accordingly
Comment-11: Page 9, first line- pls write the results in the present tense, i.e. the results are presented.

Response: Corrected accordingly (see page 9 line 1)

Comment-12: You also don’t seem to present any crude odds ratios in your results section- was there a reason for this? Also, did you consider putting only selected results into your multivariable regression model?

Response: We presented all crude and adjusted odds ratios in Table-3. To address your comment we presented crude odds ratios in text part. Regarding selected results in multivariable regression we included all except Residence and current TB treatment status. In residence case 93.4% were from rural and only 6.6% (33) were come from Urban. In current TB treatment status cases only 18 (3.6%) were used treatment. This is to avoid bias that originates from disproportionate number. We computed multivariable regression in the presence and absence of them but no difference.

Discussion

Comment-13: I think it would be good to say whether results were fed back to individuals and whether they were offered preventive therapy. It might also be good to discuss your findings in the context of TB control in Ethiopia (i.e. is this in the national Tb strategic plan, etc).

Response: In Ethiopia preventive therapy is not common medical practice except for HIV-positive subjects. The goal of this study’s research is to determine the prevalence of LTBI in the pastoralist population in a remote corner of the country and to compare the data with those of other regions in Ethiopia and with other countries. The QuantiFERON tests we used here are recommended by the CDC and WHO to assess LTBI prevalence. Publishing this data, Ethiopian public health authorities will become aware of the LTBI prevalence and make decisions as to whether DOTS preventative treatment is advisable in marginalized pastoralist populations with high rates of LTBI and active TB.

Comment-14: Table 1, I suggest that there is a space between your number and the bracket for your percentages, like this 23 (5.1%)

Response: Corrected accordingly
Comment-15: What does “sick during the survey” mean? Can you describe what this means?
Response: Corrected as sick

Comment-16: Is the imprison variable about a history of imprisonment?
Response: Yes we have variables which indicate type of imprisonment, duration of imprisonment, whether presence of individuals with cough during imprisonment.

Comment-17: Figures 1 and 2 are not good quality – can these images be provided in a higher resolution?
Response: We redesigned all of the images to width of a higher resolution (1200) and dpi of 300 PNG picture to improve the quality of images.

BMC Public Health operates a policy of open peer review, which means that you will be able to see the names of the reviewers who provided the reports via the online peer review system. We encourage you to also view the reports there, via the action links on the left-hand side of the page, to see the names of the reviewers.

2. Responses to Reviewer-1
Reviewer reports:
Judith Bruchfeld (Reviewer 1): This study describes the prevalence of LTBI in a pastoral population in Southern Ethiopia. The study has merits and the subject is pertinent to the WHO plan of TB elimination but due to flaws in study design there are important limitations which preclude some of the conclusions drawn.

Comment-1: One important limitation is the definition of latent TB and how this definition is then used in the study design. The definition of latent TB is given in the background of the manuscript line 34 as "the presence of M. tuberculosis in the body without signs and symptoms, or radiographic or bacteriologic evidence for the presence of TB." This definition of latent TB is somewhat confusing and should be rephrased according to e.g Erkens et al EurRespir J 2010;
"Latent infection with M. tuberculosis" is usually defined as presumptive infection with M. tuberculosis complex, as evidenced by a "positive" tuberculin skin test reaction and/or a positive interferon-gamma release assay (IGRA), without any sign of clinically or radiologically manifest disease."

Response: We clarified the definition of LTBI according to WHO, 2015 which is almost similar to the definition of Erkens et al (see first sentence of second paragraph in introduction part)

Comment-2: Screening for latent TB was performed by IGRA and showing a rather high positivity rate of more than 50%. However positive individuals were screened only for TB symptoms but not further examined with chest radiography to exclude active disease. This is not on line with the definition of latent TB. Could the high IGRA positivity rate be due to concomitant undiagnosed active TB? This should be addressed both regarding the study design, results and discussion sections, i.e. the high IGRA positivity rate could be due to both latent and active TB. Could undiagnose active TB haven been unevenly distributed in the study population e.g. by gender and therefore affecting results by logistic regression?

Response: It is obvious that the drawback of IGRA test is differentiating LTBI from active TB. As indicated in rationale part of the study Pai et al, described that despite limitations, it is believed that IGRA could improve existing information about the global epidemiology of LTBI. The study conducted by Joshi et al., 2007 on Health Care Workers with LTBI indicated nearly two-thirds of HCWs with latent TB infection had abnormal radiographic findings, of which only 3.4% had features suggestive of active TB. In USA, Ronald and colloquies on the evaluation of active and LTBI with imaging concluded that “Universal chest radiography in a large pre-employment TB screening program was of low yield in the detection of active TB or increased LTBI reactivation risk, and it provided no assistance in deciding which individuals to prioritize for LTBI treatment". Combined together we believed that a high role of radiological examination in differentiating LTBI from active in individuals with high IFN-γ value. However, in resource limited setting like South Omo where high TB incidence communities with difficult to reach populations IGRA test that conducted in quality controlled condition with clinical examination for active TB and epidemiological data providing fruitful information on the epidemiology of LTBI.

Regarding unevenly distribution of undiagnose active TB in the study population, we used relatively best sampling technique for multicenter study design seeing that everyone in the target population having an equal chance of being chosen. For example if we take a variable that raised by the reviewer, in our study the amount of males was 50.2% which is almost equal to that of females. In addition, we were checked for the most common individual-level confounders such as gender, age, BMI, health status, any treatment during blood sample collection and vaccination.
with BCG before classifying into LTBI and non-infected. If undiagnosed TB was not equally distributed among those variables, in crude as well as in adjusted odds ratios the IFN-γ response was shown significant differences. As indicated in Table 2 in this study those factors have no confounding effects. From this point of view we believed that undiagnosed TB was evenly distributed.

Comment-3: What was the distribution of positive IGRA results that is what proportion were close to cut-off or well above cut-off?

Response: A. Distribution of IFN-gamma results in study participants are now included in result part (see page 7, highlighted with RED color).

B. There is an increasing debate regarding the variability around cut-off due to other reasons than biological. If values close to cut-off were excluded, were results obtained by logistic regression affected?

Response: To check this we tried by using IFN-γ <1.05 (which is more than three times to manufacture cut off value) as cut off point. The three variables (district, family number and raw meat consumption) which showed significant difference in our original date are also showed significant differences.

Comment-4: Somewhat surprising were the results linking IGRA positivity to the ingestion of raw meat. This should be further elaborated. Could there be confounders not detected? The authors present a hypothesis that”the association of LTBI with raw meat consumption may reflect potential zoonotic transmission”. What transmission do they have in mind? The Mtb specific antigens in the IGRA are not present in M bovis and are only present in a few environmental mycobacteria.

Response: Interferon gamma release assays (IGRAs) are blood tests that detect TB infection by quantifying the patient's IFN-γ response to specific peptides associated with RD-1 region of mycobacterium tuberculosis complex. The peptides used in this IGRA simulate the proteins ESAT 6, CFP 10, and TB 7.7. These proteins are present in Mycobacterium tuberculosis complex organisms including Mycobacterium tuberculosis and Mycobacterium bovis but are absent in Bacille-Calmette-Guérin (BCG) vaccine strains and most nontuberculous mycobacteria. The study conducted by Pong et al, 2012 Journal of the Pediatric Infectious Diseases Society https://doi.org/10.1093/jpids/pis013 showed 100% M.bovis infected children showed positive in QuantiFERON test. CDC (http://www.cdc.gov/tb) also recommend IGRA test for M. bovis in high risk population and individuals who works products from these animals
such as hides, milk, or meat are included under the list of high risk for tuberculosis due to M. bovis.

3. Response to Reviewer-2

Michael Lauzardo (Reviewer 2): The authors of the manuscript, "Latent tuberculosis infection and associated risk indicators in South Omo Pastoral communities, South Ethiopia: Community based cross-sectional study", are to be commended for a fine work that adds to the medical literature. Although in a limited geographic setting that may limit the generalizability of the authors' findings, there is still significant value to the work and it adds to our understanding of latent TB infection (LTBI) in rural and pastoral communities. There are a few points that can be addressed that I believe will strengthen the paper.

General Comments: The study design chosen for the study was appropriate to answer the study question. Appropriate ethical approval was obtained but the lack of ethical board approval for HIV testing is a significant limitation not only of the generalizability of the findings but also potentially of the validity of the findings since a high background rate of HIV may result in a falsely low rate of LTBI due to false negative results of the IGRA among HIV infected. Overall the quality of the writing is very good with only minor errors noted. The charts and figures included are helpful with the exception of Figure 2.

Response: Thank you for your forward comment on study design chosen. Regarding HIV status we didn’t obtained ethical board approval for HIV testing and hence we included it in limitation part of our study. However, we used routine HIV screening data from their respective health facilities on 206 of our study participants. Among 206, three were positive for HIV and two HIV positives were LTBI while one was negative. We cannot talk about the relation between HIV and LTBI from our data since we have only three HIV positives but the three HIV positive among 206 (1.5%) showed low rate of HIV in the study community that may not bring significant change in prevalence of LTBI still HIV positive included naively. To clarify for the reader now we included these 206 HIV tested with their LTBI rate in the result part.

The purpose of Figure-2 was to show the performance of Kit in our specific setup. It was believed that the performance of QuaniFERON in high TB burden countries is compromised.
Here we want to show how much the Kit was specific enough to differentiate real infected individuals from non-infected prior to apply to the whole study.

Specific Comments: In the section labeled as "Level of IFN gamma response to MTBC specific antigens" and references Figure 2, I am not certain why the authors chose to present their data in this manner. It may be due to my ignorance of other ways to assess validity of QUANTIFERON results, but frequently it is presented as the percentage of indeterminant results obtained. The means of the IFN responses could cover up a large number of indeterminant results which is the standard by which most TB programs validate the results of Quantiferon in an operational/clinical setting (Lemp et alPLoS One. 2017 May 17;12(5), Kordy et alPediatr Infect Dis J. 2017 Aug 2.). It would be helpful to know the actual indeterminate rate as calculated by the manufacturer's instructions rather than have the mean IFN gamma responses as the sole means to determine the quality of the results.

Response: In our study we checked quality of Quantiferon test in different directions. First we were checked the performance of the kit by using LTBI infected and negative controls. In this part we measured the response of INF-γ to Nil, TB-antigens and mitogen. Then like your comment we calculated indeterminate value in similar way with Lemp et al PLoS One. 2017 May 17;12(5) using QuantIFERON ®-TB Gold analysis software version 2.62. But to compare with the manufacturer, accepted indeterminate range was not given in manufacturer. Again we tried to compare with Lemp et al PLoS One 2017 May 17;12(5) but in our manufacturer TB antigen minus Nil value of >0.35 IU/ml and <25% of Nil value include under indeterminate but not in Lemp et al. Actually we didn’t reported indeterminate result previously. Now we reported percentage of indeterminate in quality control part in the result section.

Similarly but less importantly, there is no mention of the actual results of the TB antigen minus the nil. Were the positives "low positives" or were the TB antigen minus nil results very high? Likewise, some clarification as to reasoning behind the paragraph of the results section labeled "Assessment of socio-demographic and medical data on study participants in the context of the INF gamma response to MTBC specific antigens" would be helpful. I am not sure what role phenotypic variables have on assessing the quality of the interferon gamma results.

Response: Now we reported actual results of the TB antigen minus the nil in result part. The questions related to what is role of phenotypic variables have on assessing the quality of the interferon gamma results. This is might be our clarification problem. Here we want to say individual-level confounding factors that can affect mycobacterial-specific immune responses which can prone to either false positive or false negative results. For example, the study conducted by Rhodes et al, 2016 (Tuberculosis (Edinb). 2016 Jan;96:37-43. doi: 10.1016/j.tube.2015.10.002. Epub 2015 Nov 11.), showed previous BCG vaccination was
strongly associated with higher IFN-γ response at baseline while being male gender has negative association. Hence we have to check confounder effects of atleast known once in our study population. Indeed our result showed none of these of the individual-level associated variables had a significant effect on the IFN-γ response to Mtb specific antigens in either the crude or adjusted analyses (Table 2). To make clear for the reader now we changed the phrase by individual-level associated factors (see result part)

Once again, I think the authors did a great job with this very well-designed study. Addressing some of the analytical questions above will provide some important clarifications so that this paper can provide further insight into next steps in addressing LTBI in high TB incidence communities with difficult to reach populations.

4. Response to Reveiwer-3:

Shamim M. Islam (Reviewer 3): This is a worthwhile (be it primarily descriptive) analysis on LTBI rates in impoverished global region. Given it utilizes the more specific IGRAs, the results are expected to be more accurate than other surveys (be them in Ethiopia or elsewhere) which may have utilized TSTs in largely BCG vaccinated communities. The QFT positive/LTBI rates of ~ 50% are particularly notable given the lower likelihood of false positive (from either BCG or environmental Mbs - which in a pastoral community is likely to be quite relevant).

A few points to consider and potentially address:

Comment-1: The authors should report on indeterminate rates, which impact interpretation of the overall test interpretations and general study conclusions. Relatedly, the data in Figure 2, assessing test reliability between different groups - may be more relevant if it included mitogen response results, rather than those to MbTB.

Response: Thank you for your direction. Now we included indeterminate rates in result part. Regarding figure-2, the third histogram of figure 2 is mitogen response result. It might be miss label in previous version. Now it is clearly labeled.

Comment-2: The pattern of having somewhat lower IGRA/LTBI positive results in the oldest age group is unexpected (expect steady increase with age/potential exposure), and here where knowing if a significant proportion of Indeterminate results may have impacted results.
Response: Off course we tried to see the age distribution among indeterminate results. Total we have 16 (3.2%) of indeterminate. Of which, 1 (6.25%) was in age <24, 12 (75%) were in age range of 25-44 and 3 (18.75) were in the age range of 45-64. This indicates the distribution of indeterminate rate is almost in line with LTBI and negative in respective to age.

Commnet-3: Relatedly, how were indeterminates considered in total results - were the excluded in the denominator, or included when calculating positive results/negative results? The percentages add up to 100%, suggesting indeterminates were in the negative category?

Response: Objective of the study was to look the prevalence of LTBI. Prevalence of LTBI was estimated by dividing the number of participants with the concentration of IFN-γ > 0.35IU/ml (positive) by the total number of study participants who had undergone the QFT-GIT test (see data analysis part). In such a way interminane was included under denominator. This is the reason why we presented the result in Table 3 as LTBI and without LTBI. However, to avoid confusion now we reported indeterminate rate separately in quality control part.

Comment-4: Respecting the data is collected/subject enrollment complete, but pediatrics data would have been noteworthy, and have definitive disease control ramifications, as younger patients with LTBI (generally) have higher rates of progressing to active TB disease (and sooner), and could target LTBI control efforts.

Response: off course study of LTBI in pediatrics data is noteworth but the problem is feasibility since in pastoralist community youth are mostly keen in cattle raring.

Comment-5: A lack of increased risk with raw milk is somewhat unexpected; the possibility of being confounded/not able to be disentangled by other related factor (raw meat) consumption is possible.

Response: Absolutely our expectation was the same but we didn’t found the association. We used a maximum effort to avoid confounders but still no association.

Commnet-6: Inclusion of the questionnaire/methods of excluding active TB disease would be ideal - such instruments would best be included, at least as supplementary material.

- the high rate of IGRA possibility raises the possibility of active pulmonary and especially non-pul TB in some individuals, as well as individuals who would have lung radiologic findings
Response: This study is a part of the study name “Systems Biology for Molecular Analysis of Tuberculosis in Ethiopia” in the Southern Ethiopian South Omo zone during 2014 and 2016. We used questionnaire for LTBI, KAP and active TB surveillance. To exclude active TB we include clinical sign and symptom of TB in questionnaire as seen in supplement under parts 200 and 300.

Commnet-7: While the use of DOTS or universal LTBI testing would be an ideal disease control strategy in high-burden areas, limited resources truly make this unrealistic. However, these results could lead to increased TB attention/resources at the rates indicate disparity and under-appreciation. For example, more targeted screening of high risk groups, for both infection (contacts of known Tb disease) or active progression (young or older patients) is more practical next step, in this region of Ethiopia, as well as other impoverished, high-burden areas.

Response: Thank you, good direction