Author's response to reviews

Title: Effect of Ascaris Lumbricoides specific IgE on tuberculin skin test responses in children in a high-burden setting: a cross-sectional community-based study

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Author's response to reviews: see over
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Dear Editor,

This response refers to your communication on the 16th of May 2012 which refers to the peer review of our paper ‘Effect of Ascaris Lumbricoides specific IgE on tuberculin skin test responses in children in a high-burden setting: a cross-sectional community-based study’. We would like to thank both the reviewers and the editor for their valuable comments. We have reviewed the feedback and made the required changes to the manuscript. These changes are summarised below per reviewer report (in blue) and in the revised manuscript that have been uploaded to the website and are attached to this email.

Referee 1: Linda Petrone

Major Compulsory Revisions

This manuscript attempts to assess whether Ascaris specific-IgE positive status affects the tuberculin skin test (TST) responses in children in a setting with a high burden of tuberculosis. The authors demonstrate that an increase in age, a high TB exposure score, previous IPT and TB treatment are associated with having a positive TST. Differently a negative TST is associated with a scar indicating previous BCG-vaccination. In addition, a possible inverse association between being Ascaris-specific IgE and TST positivity was identified, mainly among younger children. This study may be useful in the interpretation of immunological tests of M. tb infection in helminths endemic areas. However there are several issues that should be addressed before recommending this manuscript to be published.

1. The manuscript is hard to be followed, thus the text should be revised to make it more understandable. In addition, I suggest to subdivide the Material and Methods section, as well as the Results section, into paragraphs. Page numbers are missing.

We would like to thank the reviewer for the constructive feedback. We have reviewed the manuscript and made several changes to improve the flow, including the use of shorter sentences, paragraphs and sub-headings (see Appendix 1). Page and line numbers were added to allow easy reference to changes in the text.

2. ABSTRACT, some abbreviations are not defined in the text (e.g. IPT). In addition, the aim is missing in the background section.
The following abbreviations were written out in full in the abstract:

- T helper cell type 1 (Th1) (pg. 3, line 1)
- T helper cell type 2 (Th2) (pg. 3, line 4)
- Tuberculin skin test (TST) (pg. 3, line 6)
- bacillus Calmette-Guérin instead of BCG (pg. 3, line 21)

Abbreviations were removed for the following terms that were only written out in full in the abstract:

- Tuberculosis instead of TB (pg. 3, line 17)
- Isoniazid preventive treatment instead of IPT (pg. 3, line 21)

A sentence was added to the background setting to describe the aim of the study (pg. 3, line 4): ‘Our aim was to investigate whether helminth infection could influence the ability to generate an appropriate Th1 immune response, that is characterized by a positive tuberculin skin test (TST), in M. tuberculosis exposed children.’

3. INTRODUCTION, information regarding T. trichiura seems to me confounding and unhelpful to the interpretation of the study findings. I strongly suggest to eliminate all the sentences concerning this parasite or to introduce, whether available, data on T. trichiura. This could make more understandable the rationale of the work.

Information on T. trichiura was included, as data from other authors suggest possible cross-reaction with the Ascaris IgE test. We therefore feel that the background information on T. trichiura information is an important part of describing the limitations of the proxy test we used for Ascaris IgE. Additionally, research from the study communities has previously shown that as much as 1 in 2 children of school-going age are infected with T. trichiura. The sections that refer to T. trichiurus have therefore not been removed.

4. MATERIAL AND METHODS, the study population is well defined; however the number of enrolled children should be reported in this section. We included the number of enrolled children in this section as follows: ‘…on 271 enrolled participants.’ (pg. 11, line 367)

5. RESULTS, regarding Ascaris-specific IgE evaluation, I suggest to postpone RAST classification data and to declare first the number of Ascaris positive subjects. In addition, in my view, it is interesting to investigate how many Ascaris-positive subjects are also TST positive. Thus, Table 3 should be revised to make it more consistent with these data request.

As suggested by the referee, we have postponed the RAST classification data. We have also adjusted Table 3 to include the description of TST positivity per Ascaris positive/negative classification. The following sentence was also added to the manuscript:
6. DISCUSSION, as in the Introduction section, please eliminate information on T. trichiura. It is more important to focus the reader attention only on Ascaris

We refer to the previous discussion under the Introduction section. We feel that the cross-reactivity information is important for the readers’ interpretation of the study and have therefore not deleted the following sentence:

- ‘Additionally potential cross-reactivity due to Trichuris trichiura infection cannot be excluded[40]’ (pg.14)

We did however delete the sentence describing stool examination method preference per species as it does not add to the interpretation of the paper:

- ‘The Kato-Katz method for stool examination is preferred for Trichuris trichiura diagnosis and the sedimentation method for hookworm and Ascaris detection (26).’ (pg. 14).

**Referee 2: Clemax Sant Anna**

Very interesting paper. This article is complete, succinct, well-organized and thorough in its analysis and the authors recognize most of the potential limitations of the data and analysis. The authors developed a hypothesis that helminth infection may affect Tuberculin response in children in an endemic area for tuberculosis and worming infections. Their data suggest the confirmation of this hypothesis despite this is a cross-sectional study. The methods are well described, as well as the tables. I recommend it for publication.

**Level of interest: An article of outstanding merit and interest in its field**

**Quality of written English: Acceptable**

**Statistical review: No, the manuscript does not need to be seen by a statistician.**

**Declaration of competing interests:**

'I declare that I have no competing interests'

We would like to thank this referee for the encouraging recommendation of this article.

**Editorial requirements:**

-- Copyediting:

After reading through your manuscript, we feel that the quality of written English needs to be improved before the manuscript can be considered further. We advise you to seek the assistance of a fluent English speaking colleague, or to have a professional editing service correct your language. Please ensure that particular attention is paid to the abstract.
We would like to thank the editor for the guidance. We have reviewed the manuscript and made several changes to improve the quality of the written language. Changes have been marked in detail with ‘track changes’ (see Appendix 1).

-- Structure: Please check the instructions for authors on the journal website to ensure that your manuscript follows the correct structure for this journal and article type.

We have confirmed that the manuscript confirms with the journal style requirements. We have added subsections for a conclusion, and list of abbreviations, and have moved the references section, in line with the ‘Overview of manuscript sections for Research articles’. All numbers were rounded to include no decimal places.

The following text were moved from the results section to the conclusion section: ‘Our initial results support our hypothesis that helminth infection may alternate immune responses in children which may play a clinically measureable role in widely used immune diagnostic tests that measures M.tb infection in settings with high prevalence of both tuberculosis and helminths’ and changed to ‘These results support our hypothesis that helminth infection may change immune responses in children. This may play a clinically measureable role in widely used immune diagnostic tests that measures M.tb infection in high prevalence tuberculosis and helminth infection settings.’

The word count for both the abstract and manuscript was updated as well as all the references. We consulted with an additional author on the final version of the manuscript and have updated the author list and author contribution list accordingly. One of the authors (AMM) updated her affiliations that were originally listed.

Please accept this revised manuscript for publication.

Kind Regards

Nelda van Soelen

(on behalf of the authors)