Author’s response to reviews

Title: Effect of praziquantel treatment of Schistosoma mansoni during pregnancy on intensity of infection and antibody responses to schistosome antigens: results of a randomised, placebo-controlled trial.

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Author’s response to reviews: see over
Dear Sir/Madam,

RE: Cover Letter for the revised manuscript: Effect of praziquantel treatment of Schistosoma mansoni during pregnancy on intensity of infection and antibody responses to schistosome antigens: results of a randomised, placebo-controlled trial.

We are very grateful to the reviewers and below are our responses to their comments

Reviewer: Evan Secor

Comment 1: The first sentence of the results section of the abstract (page 2) does not make much sense. It should be rewritten for better clarity. This section would also be improved if overall percentages of people in the different infection intensity groups were included here

Response: Thank you for this important observation. The section has been rewritten to cater for this concern. Additional information has been added as follows:

‘At enrolment S. mansoni infection intensity among the women was as follows; 252 (65.1%) had light infection (median (IQR) epg: 35 (11, 59)), 75 (19.3%) moderate (median (IQR) epg: 179(131, 227)) and 60 (15.5%) had heavy infection (median (IQR) epg: 749 (521, 1169)). At six weeks after praziquantel treatment during pregnancy S. mansoni infection was not detectable in 81.9% of the women and prevalence and intensity had decreased to 11.8% light, 4.7% moderate and 1.6% heavy a similar reduction when compared with those first treated after delivery (undetected (88.5%), light (10.6%), moderate (0.9%) and heavy (0%), p=0.16).’

Comment 2: Is the bar for praziquantel group 6wks PE SEA IgG4 level (Fig 3B) drawn correctly? The author states that this value is significantly differently lower than the placebo group 6wks PE SEA IgG4 level (page 11, lines 14-15 and the praziquantel group enrolment SEA IgG4 level (page 13 lines 20-21) but the levels look quite similar. While it is clearly possible that there is a significant difference, most of the other significant changes in the manuscript are clearly visible. The plot may be correct but it may benefit the author to check it.

Response: We are very grateful to the reviewer for highlighting this. The positioning of the sentence would have otherwise misled the reader and the p value reported had a typographical error

i) Have cross-checked the bars and the statistics
ii) The bars were drawn correctly
iii) The comparison reported in that particular sentence (“On the other hand, the level of IgG4 against SEA showed significant decline (p=0.002) at six weeks post-treatment”.) was a signed rank comparison between enrolment and six weeks post-treatment for the praziquantel group and not between placebo and praziquantel group.

iv) The p-value should be 0.02 instead of 0.002

These omissions have been addressed thus:

‘On the other hand, the level of IgG4 against SEA showed significant decline between enrolment and six weeks post-treatment (p=0.02) and at six weeks post-enrolment the levels were not significantly different between the placebo and praziquantel group.’

As correctly pointed out by the reviewer, the bars may not show this significant difference as clearly as for other significant differences, however, the statistical test does reveal a significant difference. This should be expected considering signed rank comparisons were used and the antibodies data had high variability.

**Reviewer: Ishag Adam**

Comment 1. In the introduction page 4 lines 5 and 4 from below the sentence the However, immune responses are normally suppressed during pregnancy..............Suggest the sentence be reworded to be as However, immune responses are modified, or altered during pregnancy to allow........

Response: The sentence is meant to show the specific immune alterations or modifications that occur during pregnancy, that is suppression, in order to provide the readers with an indication of the direction of the alterations being reported in this paper. However we are also aware that not all elements of the immunity are suppressed Thus we have accepted the suggestion and made the change in the text by replacing “suppressed” with “altered”

Comment 2: In mid page 8 statistical tests. The statistical tests used here are tests used for the data which is not normally distributed, so the first step to see if data are normally distributed or not. If data are normally distributed other tests should be used.

Response : A sentence has been included to clarify this

‘Since the data was not normally distributed, non parametric statistical tests were used, except when comparing the boosts in antibody data that approached a normal distribution, when regression analysis was applied.’

Comment 3. Here authors used correlation between two variables, although applied perfectly but this was not mentioned in the methods (statistic section)

Response: This comment did not specify the section in question but we guess it refers to the associations between levels and infection intensity (page 12). This was not included since we only indicated statistical analyses for the data addressing the major objectives of the study. To address the concern raised by the reviewer, an additional statement has been included in the statistical analysis 3, as follows

‘Initially the influence of infection intensity at enrolment and pre-treatment antibody levels on the boost in the antibody levels were explored using spearman’s correlation analysis.’

Comment 4: In the same area the word significant should be inserted
Response: The word significant was inserted in the last sentence of this paragraph (page 12)

“....treated after delivery, particularly significant for IgG3, IgG4 and IgM against SWA and for IgG1 and IgG2 against SEA (table 2).”

Comment 5: In page 13 in the discussion, this can influence the drug levels and hence the efficacy.

Response: We are very grateful to this information. However, inclusion of the suggested information may greatly take away from the message at is central to this manuscript. It directly touches on the factors that may influence the levels of praziquantel in the circulation, which is not a focus of this manuscript. We would not be comfortable including it as suggested.

Comment 6: At the end of the discussion, I suggest mentioning The use of placebo during pregnancy now in 2009 would be questioned.

Response: Again we are grateful for this observation. We understand the reviewer’s suggestion in relation to the benefits of treatment to the women in this study. However, it would be contradictory to our recommendation that more studies may be necessary. This recommendation is based on careful consideration of the following points:

i) This study was carried out in a low transmission area and in a largely low infection intensity population. We are not certain what the situation would be in a high transmission, largely high infection intensity, situation

ii) WHO (2006) recommended that placebo controlled trials need be carried out in areas that have different levels of transmission.

Thus, at this stage, we would rather not include the recommendation as suggested.

Reviewer: Paulo Marcos Coelho

Comment: There are two articles showing clearly the limitation of the Kato-Katz method in order to evaluate the prevalence in areas of low endemicity.............. suggested to add two references in the text

Response: We are grateful for the update on this area
The reference Enk et al 2008 is indeed a recent and relevant paper. This has now been added in the text and the reference list.

Comment: in page 10: levels of antibodies...... Second sentence ...(Fig. 3A) showed slight decline between enrolment and six weeks post-enrolment but this effect was not statistically significant. If it was not statistically significant, was not possible to establish a slight decline. The reviewer suggest the sentence be removed

Response: We accept the suggestion and have removed the sentence. We adjusted the section to read starting thus:
“Among the placebo group, levels of antibodies against SWA (figure 2A) and SEA (figures 3A), at enrolment and six weeks post-enrolment during pregnancy were consistently lower than at six weeks after delivery; and this effect was strong at enrolment for IgG1........”

Again we are grateful to the reviewers for their helpful comments, which we hope we have addressed satisfactorily

Yours sincerely

Robert Tweyongyere