Author’s response to reviews

Title: A Prevalence Survey of Enteral Parasites in Preschool Children in the Mangochi District of Malawi

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Author’s response to reviews:

Dear Dr Nausch

Please find attached the response to the reviewers’ comments on our manuscript.

We would like to thank them for their input, and we hope that the responses to each of the points and changes made the manuscript are sufficient. Please do not hesitate to contact us, if any further clarification is required or expansion is necessary.

Response to Reviewer 1

General comments:

1. Explicit hypotheses/aims of the study should be included in the text

We have added the explicit hypothesis to our aim in the introductory section of the manuscript. See line 97-101

2. Outline the specific parasite species that were looked for in stool samples should be added to the methods. It is currently unclear if the absence of parasites other than those listed is
due to their not being looked for or being looked for but not present (e.g. S. mansoni, which could have been readily assessed in stool samples)

The microscopist was trained in the identification of all species of gut enteroparasites. We have detailed that all regional species of protozoa and helminths were assessed for including schistosoma mansoni which was not found in any stool samples. See lines 164-169

3. Please report whether or not stool samples were analysed in sufficient time/storage appropriate to identify unstable eggs (e.g. hookworm?). Was there any evidence that overnight storage compromised sensitivity?

We attempted to mitigate for this issues with the provision of cool boxes to the community and immediate fixation in the laboratory upon arrival. Transport times between the villages and lab were short (2 hours), but it is possible that this did have an effect on hookworm survival. We have added further comment to the discussion to highlight this. We did not have a means to directly assess the impact in our study as the concentration method necessitated the use of a ventilated laboratory and could not be achieved in the villages. See Lines 304-307

4. There have been a number of studies of the impact of schistosomiasis on growth and development during early life and as a result of in utero exposure and also the impact of antibiotics on child growth which are highly relevant to this study and warrant some comment/discussion.

We have made reference to the studies regarding schistosomiasis and have discussed how this may in part be responsible for the high rates of stunting found. See line 375-386

5. I am concerned that the data on azithromycin treatment within the MORDOR trial is not reported. Could a sensitivity analysis be conducted now that the MORDOR study is unblinded?

We have added an additional comment to the results to clarify that there was no significant difference in prevalence of infection with helminths (p=0.776) or protozoal (p=0.113) infections between the villages which had treated with azithromycin and those treated with placebo. We have also reported that on ANOVA of the difference in mean WAZ (p=0.194) HAZ (p=0.08) and WHZ (p=0.753) no evidence for a statistical difference was found between the azithromycin treated villages and those which were treated with placebo. See lines 232-233
6. What was the prevalence of SAM (WHZ<-3) in the cohort? e.g. the minimum reported WHZ is less than -5, indicating that some of the wasted children had severe acute malnutrition, which is a risk factor for infection and more severe symptoms than moderate malnutrition.

Only 2 children met the criteria for severe acute malnutrition, we have added this to the results to further highlight that this was a rare occurrence. See Lines 259-260

7. The authors introduce the study with commentary on the importance of diarrhoeal illness, however information on relevant gastrointestinal symptoms have not been reported here. Please add this data if available

Data was not collected on gastrointestinal symptoms in this study due to the age of the children and the resulting reliability of symptomatology.

8. Reference to OR in text should refer to 'higher/lower odds', not 'higher/lower risk' for accuracy

Changes have been made to correct this.

Abstract: The conclusions in the abstract should be replaced with statements that reflect the data presented. For example, "prevalence of helminth infection was low" … "suggesting current control programs have been reasonably effective" cannot be confirmed by the data from this study.

Abstract: The study was in pre-school children; therefore, the following statement should specify this: "significant proportion of the population"

These changes have been made accordingly.

Line 148: The phrasing suggests that microscopists for 95% of the study were not trained/experienced, please clarify

Both microscopists were trained and experienced in the identification of enteropathogens. The line has been edited to avoid any confusion. See line 161-164
Figure 1: please clarify what is meant by "not linked to stool sample" Figure 1: The opening paragraph of the results repeats what is reported in figure 1, so a reference to the figure is sufficient

In 53 cases anthropometric data could not be found for the samples that had been collected due to miss-assignment of the randomised anthropometric identification numbers. The figure has been edited to explain this more clearly. See Figure 2

Table 2: OR data is reported inconsistently in the text (sometimes OR, sometimes p value), I suggest referring readers to table 2 instead

These changes have been made accordingly.

Line 225: Was this analysis also performed for the other parasites; if so, the full analysis should be reported in a supplementary table and/or the text should indicate that this was done but no other differences found

Analysis was performed for other parasites, however none were significant. This has been clarified in the text.

Lines 209 & 211-213 & 218: Please provide pathogen names in full on first use

These changes have been made accordingly.

Line 253-254: remove this statement as the sample size is underpowered to make this claim: "The overall trend suggested a protective factor of intestinal parasite infection on markers of poor child nutrition."

These changes have been made accordingly.

Lines 250-260: provide relevant details of these analyses, including p-values or include a table to summarise these results

These changes have been made accordingly and p values have been included in the text.
These changes have been made accordingly.

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These changes have been made accordingly.
Line 315 (and throughout): authors should avoid using comparator statements like 'reduced' when referring to prevalence data as they do not have pre-treatment data for comparison

These changes have been made accordingly.

Line 316-317: please include these results in the results section, rather than introducing new data in the discussion

We have added this information to the results section.

Line 331: some reference to other relevant factors associated with stunting is warranted in this paragraph, e.g. reviews of environmental enteric dysfunction, the GEMS study already mentioned in the introduction, etc.

We have expanded in this paragraph on the likely causes of stunting and poor growth.

Line 336-337: this statement lacks specificity and I would suggest removing it

We have altered this statement, to read “Giardia sp. was the most prevalent enteropathogen found in our cohort.”

Line 342: additional limitations should be added to this summary paragraph - most notably, the lack of information on de-worming and antibiotic treatment interventions in the region, absence of data on intestinal and urogenital schistosomes, and the absence of data on diarrhoeal illness

We have added further discussion of these weaknesses in the associated paragraph.

Line 365-369: The following statements are not supported by the study data and should be removed: "It suggests that, at least in this population, control programs introduced as public health interventions have been effective in controlling worm burden. However, the study also highlights that significantly reducing helminth burden in this population has not reduced growth restriction. It therefore adds to the ongoing debate as to the role and benefit of mass drug administration." The authors should focus on their original research questions and highlight how their data address them, rather than extrapolating their data.
The conclusion has been modified taking into account these points to stress the main findings better in line with the aims and hypothesis. It highlights the low prevalence of helminth carriage and finding of protozoal organisms, before highlighting the need for further larger studies in the region.

Line 390: earlier in the text it was indicated that children were not tested for malaria/it was not possible to ascertain their malaria status; please clarify

This part of the ethics approval applied to other sites of the MORDOR trial, it was not relevant in this study and as such we have removed this comment.

Line 393: please report the relative prevalence of symptomatic vs asymptomatic infections identified in this study; according to local guidelines, how many of the infections identified in this study received treatment during the study?

No children were clinically symptomatic from their infection. This has been added to the ethical approval deceleration.

Response to Reviewer 2

Because the children sampled for the paper came from children recruited for another study, which was part of a cluster-randomized placebo controlled trial. It is important that the name of (MORDOR-Malawi) study be reflected in the title. If authors wish not to include MORDOR-Malawi study in the title, then study design and sample size calculation is not appropriate for a prevalence study. Therefore, the results obtained should be interpreted with caution. To determine the burden of disease in epidemiology, requires the use of a cross-sectional study's design. Definitely this will require a large sample size than reported in this study. The author stated that they use a previous prevalence of 8% to calculate the require sample size. There is no reference to support this statement. Was the prevalence of Protozoal diseases or STH or both.

The study did not use the MORDOR Study design and we did not expect, or indeed find, any impact from the treatment intervention in the MORDOR study. We do not therefore feel it is necessary to specify the MORDOR study in the title. We have instead referenced the MORDOR study and attempted to explain all relevant methods.
In reference to the sample size calculation we have discussed this further below an estimated prevalence of 8% for helminths was chosen based upon the prevalence found in the previous studies conducted in Malawi. Please see below for further detail regarding the choice of sample size and power calculations.

ABSTRACT:

The abstract is satisfactory, but the author needs to review the abstract after all the necessary corrections and comments in the manuscript have been worked upon.

The abstract has been modified in relation to comments from both reviewers.

INTRODUCTION:

The introductory section is well drafted, but needs to be improved upon by linking the information's together and more recent references provided.

Line 64 - 67: More recent reference should be provided.

The most recent GBD survey has been now been referenced. More recent papers specific to Malawi are not available this is highlighted in the text.

Line 84: Use soil transmitted helminths instead of Soil based helminths

This change has been made accordingly.

Line 88: With the aim "not an aim"

This change has been made accordingly.

Line 92 - 94: Use "helminths burden" instead of "carriage".

This change has been made accordingly.

METHODOLOGY

Study design:

Line 99: Use "between" instead of "during"

These changes have been made accordingly.
Line 100: What samples were taken?
We have clarified in the text that this relates to stool and blood samples.

Line 101: Was the sample collected in all the villages in the district? If not, provide names of villages.
Samples were not collected from all villages. The names of the villages have been provided, along with a map of their location in the district. See Figure 1 and Table 2.

Line 104: The population was in which year?
This relates to the most recent census take prior to the study in 2008. We have clarified this in the text.

Line 105-108: Need to be revisited. Is this study a hospital based or household based research.
The study setting sections are confusing and not clear, try to revisit this section.
This was household based research. No healthcare facilities were involved. Research teams attended the villages collecting samples on a biannual basis. We have added a comment to make it clear that participants were recruited from households. See lines 117-128.

OUTCOME MEASURES
Line 129-131: rearrange this section to drive home the point you are trying to make.
We have re-written this paragraph with this in mind.
Line 132: Anthropometric data were collected for height "not on the height"
These changes have been made accordingly.

Study Procedure
Line 141-142: "Was the instructions attached to the bottles or what?"
The parents were provided with both an oral explanation by a member of the team as well as written instructions on how to obtain stool samples. We have clarified this in the text. See lines 130-132.
STATISTICAL ANALYSES

Line 166 - 167: You can rewrite these lines like this "Data collected from the study were stored in Microsoft access 2016, exported to Microsoft excel 2016 and analyzed using STATA 14.1."

These changes have been made accordingly.

Comments on methodology

§ The author did not give information's on the method used to sample eligible children.

Children were selected from the biannual census taken by the MODOR-Malawi team. Selection of children was based on computer based randomizer. This part of was previously discussed in lines 117-128. We have rewritten these lines to avoid any confusion about the process.

§ Sample size estimation was not mentioned.

Sample size was based upon calculation using the formula n = (Z2P(1-P))/d2 based upon conservative estimates from previous surveys of helminths in Malawi as no regional data was available. We have added this to the methods to further explain this calculation. This number was corroborated by WHO recommendations as previously discussed on line 138. We have added a reference for this WHO guidance.

§ The author needs to develop a section for questionnaire administration, which should capture the socioeconomic status of the households and how the children were surveyed.

No questionnaires were obtained for the purpose of this, socioeconomic status was not felt to be significantly variable in the rural villages of this region. We have highlighted in the conclusion that no socioeconomic appraisal of children was made. See lines 371-373

§ Map of the study area and study communities should be provided.

We have added a map to the manuscript to show the villages, and their location in the district. See Figure 1

§ No information on ethical clearance before the commencement of the study in the manuscript.

Ethical clearance is discussed as part of the declarations. We have added a comment to direct people to this information.
RESULTS

Line 178: Use demographic status of study participant instead of "the sample demographics".
These changes have been made accordingly.

Line 180: Use for the study instead of "to the study".
These changes have been made accordingly.

Line 181: Use sample bottles instead of "Sample pot"
These changes have been made accordingly.

The information on Line 182 - 188 needs to be revisited and re-presented
We have attempted to explain this further. On advice from the other reviewer we have removed much of the text from this paragraph and updated figure 2 to further explain the recruitment of process.

Line 189 - 191: Is this a subheading or what?
This was not a subheading. This line has been edited in line with above.

Line 191 - 194: The Information is not clear, please rewrite
This line has been edited in line with above.

PREVALENCE OF INFECTION

Line 205 - 206: The author should start this section like this A total of 193 stool samples were collected, 72 (37.3%) were infected with Intestinal parasites.
These changes have been made accordingly.

The information given between line 207 - 219 is not well arranged needs to be re-written.
These changes have been made accordingly.

The information on line 227 - 229 is not shown in table 3 and the P-value is not provided.
The p values have been added for this separate analysis.

Line 250 - 260: There is no table showing the relationship between Nutritional status and parasitic infection in the manuscripts.
In line with comments from the other reviewer we have simplified and removed these lines. As no association was found we did not feel that addition of a further table would add to the data presentation.

Comments

§ Table 3 is not well explained in the manuscript. The Author should link the tables together.

The explanation of table 3 and its link to the test is expanded on in the text.

§ There was no table to show the prevalence of infection in the villages.

This has been added to table 2.

DISCUSSION

Line 263 - 265: What are the criteria? Mention them.

This line has been removed from the manuscript in line with the other reviewers comments.

Line 272: Soil transmitted helminths instead soil based helminths.

These changes have been made accordingly.

I think the information on Line 275 - 289 should have appeared on the methodology.

We have fully explained our use of formol ether concentration in the methods which was chosen in order to examine the stool for protozoa as well as helminths. We have a further line to clarify the reasons for this choice in the methods. See line 159-160. However we feel that it is necessary to discuss the reliability of our methods in these lines on the discussion, as we are comparing our findings to other studies published in Malawi which used different methodology, and discussing reasons for the variation in prevalence between other published studies.

The information on Line 343 on malaria status contradicts the information on line 369 - 390 on malaria treatment. Crosscheck and effect the necessary corrections.

This error has been rectified. Malaria status was not recorded for this study.

We look forward to hearing from you.

Yours Sincerely

Prof K Kalua