

Microbiome responses to anthelmintic treatment depend on pre-treatment helminth infection status in young Ethiopian children

Corresponding Author: Dr Bineyam TAYE

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The dataset has an interesting design with pre- and post-treatment microbiome samples from an STH-endemic region. The analyses are clear and the accessibility of the code used for analysis is a strength of the dataset. While there are limitations to the analysis due to the sample sizes and the use of 16S sequencing, in the revised version these limitations are more directly acknowledged, and the sampling from an underrepresented area provides some additional value.

The efforts to address reviewer concerns have substantially improved the statistical analyses and presentation, and the supplementary tables provide much more accessible and transparent data sharing for future researchers.

Reviewer #2

(Remarks to the Author)

I previously had the pleasure of reviewing this work, which investigates the long-term impact of albendazole treatment on gut bacterial composition. The study leverages a unique cohort of Ethiopian schoolchildren, stratified by baseline helminth infection status.

My previous comments appear to have been addressed as far as feasible, and I appreciate the authors' efforts. I have two remaining points to raise:

Comment 1. The absence of an adequate control group (due to ethical considerations), the combination of helminth species to the umbrella term "STH+" (due to lack in sample size) and the limited taxonomic/functional resolution (due to choice of sequencing platform and assay) restrict the generalizability and impact of the study results. However, the authors transparently acknowledge these limitations in the manuscript.

Comment 2. I appreciate the updated emphasis on the uniqueness of this study cohort and dataset in the discussion (lines 504-523).

Minor comments:

- Throughout the manuscript, inconsistent spellings of *anthelmintic* (and related terms) are used, for example in the title ("anthelmintic"), Figure 1 ("antihelmintic"), the GitHub repository ("antihelminthic"), and the main text (a mixture of "anthelmintic" and "anthelminthic"). Please review and standardize the spelling throughout for consistency.
- Line 75: The statement about schistosomiasis appears to be out of context.
- Line 103: *Optional*. Treat "data" as plural.

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We thank the reviewers for taking the time to review our revised manuscripts and for their helpful suggestions. We respond to each point raised below in blue.

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The dataset has an interesting design with pre- and post-treatment microbiome samples from an STH-endemic region. The analyses are clear and the accessibility of the code used for analysis is a strength of the dataset. While there are limitations to the analysis due to the sample sizes and the use of 16S sequencing, in the revised version these limitations are more directly acknowledged, and the sampling from an underrepresented area provides some additional value.

The efforts to address reviewer concerns have substantially improved the statistical analyses and presentation, and the supplementary tables provide much more accessible and transparent data sharing for future researchers.

We thank the reviewer for these comments and are glad our edits sufficiently addressed their prior concerns.

Reviewer #3 (Remarks to the Author):

I previously had the pleasure of reviewing this work, which investigates the long-term impact of albendazole treatment on gut bacterial composition. The study leverages a unique cohort of Ethiopian schoolchildren, stratified by baseline helminth infection status.

My previous comments appear to have been addressed as far as feasible, and I appreciate the authors' efforts. I have two remaining points to raise:

Comment 1. The absence of an adequate control group (due to ethical considerations), the combination of helminth species to the umbrella term “STH+” (due to lack in sample size) and the limited taxonomic/functional resolution (due to choice of sequencing platform and assay) restrict the generalizability and impact of the study results. However, the authors transparently acknowledge these limitations in the manuscript.

We agree and appreciate the reviewer's previous comments to acknowledge these limitations more clearly in the manuscript.

Comment 2. I appreciate the updated emphasis on the uniqueness of this study cohort and dataset in the discussion (lines 504-523).

We thank the reviewer for the suggestion to include this section. We agree that this addition better highlights the unique attributes of our cohort.

Minor comments:

- Throughout the manuscript, inconsistent spellings of *anthelmintic* (and related terms) are used, for example in the title (“anthelmintic”), Figure 1 (“antihelmintic”), the GitHub repository (“antihelminthic”), and the main text (a mixture of “anthelmintic” and “anthelminthic”). Please review and standardize the spelling throughout for consistency.

We have thoroughly reviewed the manuscript and GitHub repository, ensuring that “anthelmintic” is consistently used throughout. We apologize for this oversight.

- Line 75: The statement about schistosomiasis appears to be out of context.

We thank the reviewer for this helpful comment. We agree that the original phrasing could seem out of context. We have revised the sentence to clarify how water, sanitation, and hygiene (WASH) interventions are broadly relevant to helminth control. The revised text now clearly connects WASH to both soil-transmitted helminths and schistosomiasis, enhancing clarity and conceptual coherence.

- Line 103: *Optional*. Treat “data” as plural.

We have updated the verbs in that sentence and the following to treat data as plural:

“Animal studies identify both short- and long-term alterations in the gut microbiome following anthelmintic administrations (26-28), but data from humans remain much more limited. Data from one epidemiological study in Kenya have shown that anthelmintic drugs, such as albendazole, are associated with an increase in the proportion of Clostridiales and a reduction of Enterobacteriales for at least three months following treatment (29)”

Reviewer #4 replacement for Reviewer 2 (Remarks to the Author):

I have reviewed the revised manuscript and rebuttal. In my view, the authors have addressed the main concerns raised in the previous review to a satisfactory extent. In particular, they updated the taxonomic assignment pipeline to SILVA 138.2, removed the exploratory microbiome “balance score” analysis, removed the weaker DESeq2 component, added clearer discussion of the lack of an untreated control and limited power of the follow-up cohort, and included an additional analysis controlling for helminth infection type.

The manuscript remains largely descriptive, and the mechanistic limitations are not resolved

experimentally, but the authors now acknowledge these points much more clearly and frame the study more appropriately as an observational longitudinal analysis. Overall, I think the revision is substantially improved and that the major concerns have been adequately addressed.

I suggest only minor framing changes: specifically, softening statements that overinterpret the findings or extend them toward policy recommendations, and slightly tempering the wording around “controlling” for seasonal effects.

We appreciate these suggestions about tempering our wording around our approach and findings. We have made several textural changes to address these comments:

First, we toned down the verbiage around policy recommendations to be more suggestive on lines 530 – 533:

“In addition, our findings have public health policy implications, for example suggesting that microbiome-associated actions be considered into the ongoing blanket deworming program.”

Additionally, we tempered the wording around “controlling” for seasonal effects on Lines 509 – 511:

“Moreover, while a period of one year allowed us to minimize the potential effects of seasonal factors, we cannot exclude the influence of the year-to-year variation and their environmental exposures.”