Author’s response to reviews

**Title:** Major reduction of malaria morbidity with combined vitamin A and zinc supplementation in young children in Burkina Faso: A randomized double blind trial

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**Author’s response to reviews:** see over
Dear Editor:

In light of the reviewers’ comments, we have revised the manuscript as described below. Please find as uploaded the revised version. We feel that we have carefully addressed the reviewers’ suggestions and that these have helped to strengthen the paper.

Sincerely yours,

Jean-Bosco Ouedraogo

Response to the reviewer: Amy Webb

Major compulsory revisions

1. We agree with the fact vitamin A (VA) and zinc may help to reduce severe anaemia, and of course severe anaemia is a morbidity factor which probably enhances morbidity due to malaria. When we considered only those who have severe anaemia, there were no differences between the placebo and supplemented groups in term of the mean number of malaria cases: 1.33 vs. 1.41 (p=0.78). We do know that both VA and zinc can help to improve immune function, but not specifically for malaria cases. So we agree with Ms. Webb’s comment but we also want to add a nuance. We are certain that the reduction in malaria and other morbidity markers is not only due to anaemia reduction, but also due to the immunomodulating effect of these two micronutrients. This nuance has been included in the manuscript Discussion section (see paragraph at page 10 lines 10 to 12 lines 19 to 21).

2. We totally agree with the second comment that our study design does not allow us to reach the conclusion that the combination of VA and zinc is synergistic. This limitation has been described in the discussion paragraph at page 8 on lines 7 to 12 and page 9 lines 1 to 3.

Minor essential revision

All the grammatical errors and incorrect figure labels noted by the reviewer have been taken in account as you will see in the manuscript.

Discretionary Revisions

The closing statement in the discussion has been reworked as suggested by the reviewer in the discussion paragraph page 9, lines 24 to 26.
Response to the reviewer: Denis ZOFOU highlighted in green

Major compulsory revisions:

1. Previous works suggesting the beneficial effects against malaria:
   We had already cited in the background two studies from Papua New Guinea that
demonstrated the efficacy of vitamin A and zinc for prevention of malaria (Shankar et al,
1998 and 2000). We have now also added two additional articles on the efficacy of VA
and zinc (Cusick et al. 2005 and Richard et al, 2006). Both studies suggested potential
beneficial effects of VA and zinc against malaria. These are cited in the background
paragraph on page 3, line 15.

2. The haemoglobin measurement was performed using a Coulter counter T540 (Hialeah,
Florida, USA). This correction has been made in the Methods (Laboratory procedures)
page 6, lines 7 to 8.

3. The geometric mean parasite density has been used because parasite density distribution
   was not normally distributed. This was calculated using the software Stata 8.0. This
information has been added into the Data processing and analysis paragraph on page 7
lines 3 to 4.

Discretionary Revisions
Concerning the point of sulphadoxine-pyrimethamine (SP) resistance, we want you to be
informed that when the study was ongoing, resistance to this drug in Burkina Faso was very
low (Tinto et al, 2002). We have added this reference to the section “participants” under study
design, page 4, lines 8.
Response to the reviewer number 3

We greatly appreciate this reviewer’s detailed comments, especially in reference to the potential impact of the imbalance at randomization on the main outcomes. We have attempted to address this issue by adding a paragraph to the Discussion (page 9 lines 10 to 18) that describes some of the potential limitations of our findings.

Major compulsory revisions:

Abstract:
Page2, line 5(C)
- The vitamin A was given as a single megadose to each of the participating children. Both micronutrients were provided for a period of six months These details as suggested by reviewer 3 have been added to the abstract page 2 lines 5 and 9 to 10.

Page 2, line (8)
- The number of enrolled children was 75 in each group but the analysis has been done excluding those who dropped out, i.e. 1 child in each group. This information had already been provided at the beginning of results page 7 and line 9.

Page 2, line 13-15 (C)
- Here it is not the supplementation impact on any specific children that we wanted to show but the potential benefit effect of this supplementation in each group after a 6 month period of supplementation. We have therefore presented the results of the cross sectional studies done at baseline and end of study as the best way to demonstrate the impact of the supplements. We have reworded the abstract as suggested to clarify that the results show a decrease in the prevalence of re-infection. We made again the analysis by 2X2 table for diarrhea and found significant different. This has been added to the manuscript at page 8 lines 11 to 13.

Methods:
Page 4, line 8 (C)
- A study done by Baldet et al. (1999) in the area of the current study found an entomological inoculation rate of 697 infective bites per person per year. This information has been included to the manuscript at page 4, line 8 to 9.
Page 4, line 17 (C)
- Clinical failure with SP was <1%. This information has been provided by the study of Tinto et al, 2002 and is now included in the manuscript on page 4 line 19.

Page 4, line 23 (C)
- The ethical perspectives that justify the randomization of children to vitamin A or placebo was that your IRB did not find any ethical issues with the study design because at the time of study implementation, there was not a national vitamin A supplementation program in place, page 4 lines 24 to 25

Page 5, line 1 (C)
- We have confirmed on page 5, line 5 that both capsules of vitamin A and placebo and also for zinc and placebo were indistinguishable in color, appearance, and other characteristics.

Page 5, line 19 (C)
- The definition of malaria case used in the manuscript was the definition of uncomplicated case of malaria that was in use by the National Malaria Control Program (NMCP) of Burkina and by WHO at the time of study in our settings.

Page 5, line 27 (C)
- Discrepancy criteria that requires slide reading was 1.5 fold difference in parasite density page 6 line 6.

Page 6, line 5 (C)
Based on the local hospital’s data, malaria is responsible for around 50% of visits to the health centre, and a study done by Müller et al, 2003 confirms this assumption, showing 55% prevalence of malaria in rural Burkina Faso. This article has been cited on page 6, line 11.

Page 6, line 20 (C)
When the study was ongoing, the criteria that were in use for in vivo efficacy studies were based on a 14 day period of follow-up to exclude recrudescent malaria in the intensive transmission areas (see WHO/HTM/RBM/2003.50). This definition was subsequently changed to a 28 day period of follow-up but this change was made after completion of the study.
Page 6, line 22 (C)

- We have specified in the Data Processing and Analysis section that the Cox proportional hazards approach was used with Kaplan-Meier curves in the manuscript on page 6, line 27 and at page 7, line 1 to 2.

Results:

Figure 2 (C)

The fact that Kaplan-Meier curve descends to 0 is due to an error during analysis. Children free of malaria cases were considered by the soft as children with malaria case the first day of the study with 0 day as interval. This has been corrected by specifying that those children were free of any case of malaria during the whole time of follow up. The result is showed in figure 2 and is still significant in favour of supplemented children (p=0.016 rather than 0.015).

Table 1 (C)

- The category of “reported children with fever episode in the previous year” as well reported children with mosquito-net use on the previous night” represents the actual proportion of children from whom these data were collected.
  - We enrolled 150 children at baseline but very early during the study 2 children dropped out so we have excluded these two children from analysis. This explains why the baseline n for each group is now 74 rather 75.
  - The data in the table are presented either as the mean +/- SD or percentage with 95% confidence intervals.
  - As suggested, we have provided the mean WHZ and HAZ scores as well as the proportion of children who fall below the <=-2.0 cutoff. The same approach has been taken for the haemoglobin data. We have also modified the category “positive for P. falciparum” so that this now represents the proportion of children with a positive parasitologic examination.
  - The geometric mean parasite density has been used because parasite density was not normally distributed. This was calculated after logarithmic transformation using the software Stata 8.0 for children with parasites

Table 2 (C)

- Since we followed up the same number of children in each group (n = 74) during the 6 month period, we feel that it is not necessary to present the data as total child-years at risk. Therefore we have opted to simply present the actual number of episodes of malaria that occurred during the follow up time. We have noted in a footnote at the bottom of the table the number of child-years of observation as recommended. This
will allow an interested reader to calculate the rates of events per child-years of observation if desired.

- The RR presented here is the risk to have malaria attack if the child is in placebo group.
- We have provided the p values in a column within the table, not as a footnote.

Discussion
- In page 8 line 1 (E); Malaria burden modified to read “burden of clinical attacks of malaria” in page 8 line 17.
- Page 8 lines 16-18 (E); the sentence has been reworded as suggested.

Page9, line 23 (E); This part of the manuscript has been reworded from page 9, lines 10 to 18 and lines 25 to 27 and also in page 10 lines 1 to 4

Minor Essential Revisions

Background
- Page 3, line 21 (E) Zanzibar and Peru studies have been cited in the manuscript at page 3 line 15.

Methods:
- Page6, line15 (E); We mentioned as suggested that it is geometric mean parasite, page 6 line 21.
- Page 8, line 3 (E); We have specified the is was Cox proportional hazards analysis with changed Kaplan Meier curve.

Discretionary Revisions

Results:
- Page7, line 10 (D). We have presented the p value as p <0.001, as recommended.

Discussion:
- Page 9, line 20 (D) and Page 9, line 25; these parts have been reworded in the manuscript.