

Reviewer's report

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

Version: 1 **Date:** 30 June 2007

Reviewer: Anuraj Shankar

Reviewer's report:

General

The authors present the results of a double-blind randomized controlled trial of supplementation with either vitamin A and zinc combined or placebo in 6 to 72 month old children in Burkina Faso. The primary outcomes were clinical malaria episodes and prevalence of infection.

The question of the effects of combined supplementation is an important one to address and the authors give good justification for this. The data presented appear to indicate a protective effect and the findings may be worthy of publication. However, there are numerous details that need to be clarified with regard to methodology, data analysis, and data presentation. These are detailed below. A shortcoming of the study is an imbalance at randomization such that there were greater *P falciparum* episodes, previously reported fever episodes, and anemia in the VA/Zn group. One could speculate that the greater exposure to malaria in the intervention group effectively led to a greater acquired immunity in this group that could be seen in the slower re-infection rate after radical cure with SP. Alternatively, one could speculate that the VA/Zn effect is in fact an underestimate of the true effect because that group was perhaps subjected to ongoing greater transmission of malaria. In any event, the authors should address the implications of this imbalance in the discussion. In addition, they could carry out additional statistical analyses to address or control for the imbalance. The other important issue is the clarification of the specificity of the case definition. If it has low specificity in this high transmission area then the effects could be due to effects of VA/Zn on other morbidities that are simply being misclassified as malaria. The authors should address and discuss this.

The following encoding has been used to classify the revisions requested:

C: Major Compulsory Revision

E: Minor Essential Revision

D: Discretionary Revision

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Abstract:

Page 2, line 5 (C): clarify how often vitamin A was given and also mention the duration of the study.

Page 2, line 8 (C): Is the number enrolled 75 or 74.

Page 2, line 13-15 (C): it is not really correct that there was a decrease in prevalence due to the micronutrient supplementation. In this trial all children were given radical cure with SP at the beginning of the study. Any differences in parasite prevalence at the end of the study would be due to differences in re-infection, not reductions in prevalence. The difference in the re-infection prevalence is what should be analyzed and presented. Also, only the difference in the number of cases (or incidence density) should be reported. The main effects seen in Table 2 would seem to be significant reductions in total fever episodes, malaria episodes, and also diarrhea episodes (a simple 2x2 table indicates the difference to be significant at $P < 0.05$).

Methods:

Page 4, line 8 (C): Give more background information on the epidemiology of malaria in the area such as the biting rate, attacks per year, nutritional status of the population

Page 4, line 17 (C): It is mentioned that SP was given as radical treatment. Please mention the efficacy of this in the study population and area.

Page 4, line 23 (C): Please review briefly the ethical perspective that justified the randomization of children to vitamin A or placebo. This may help the reader understand how this decision was considered.

Page 5, line 1 (C): Please confirm that the capsules for both placebo and VA and placebo and Zn were indistinguishable by appearance, color, or other characteristics.

Page 5, line 19 (C): An episode of *P. falciparum* malaria was defined as axillary temperature $\geq 37.5^{\circ}\text{C}$ accompanied by the presence of asexual forms of the parasite and no other obvious cause for illness. Could the authors please give the specificity of this case definition in the study area. This is important because vitamin A and zinc may result in reduced morbidity from other causes and a low specificity case definition could potentially lead to spurious findings of reduced malaria morbidity.

Page 5, line 27 (C): Please give the criteria for a discrepancy that required slide readings to be checked by a third investigator.

Page 6, line 5 (C): The authors assumed a clinical attack rate of 50% in the placebo group. What data was this based on? Likewise they estimated an effect size of 50% and it would be useful to mention how that figure was chosen. In general the study is somewhat underpowered due, in part perhaps, to the large presumed effect size.

Page 6, line 20 (C): why was 14 days used to exclude recrudescence malaria episodes rather than 28 days as is generally used to exclude low level drug resistance.

Page 6, line 22 (C): specify that the test as the Wilcoxon Rank Sum test and the Cox Proportional Hazards analysis (Kaplan-Meier curve).

Results:

In general please see the discussion of the results given in Abstract section above (C).

Figure 2 (C): The Kaplan-Meier curves for both placebo and VA/Zn descends all the way to 0 indicating that all the children had at least one malaria episode. But in Table 2 the proportion of children free from any pathological episode is 17% in the placebo and 31% in the VA/Zn group. Also, please clarify if the analysis is by Cox Proportional Hazards.

Table 1 (C): The category of “reported children with fever episode in the previous year” and “reported children with mosquito-net use on the previous night” would both be counts (i.e. integers) but are reported as real numbers. The authors should explain this. The authors have stated in Figure 1 that there were 150 total children enrolled or 75 per group and that one in each group was lost to follow-up. Thus, the baseline N for each group in Table 1 should be 75 not 74. The authors should give the mean WHZ score and HAZ score, as they have, but the number below the <-2.0 cut-off should be given and the percentage can be in parentheses next to it. The same would be true for the anemia. The “Positive for P. falciparum” should also be a count and not include a decimal. The authors should also clarify that the geometric mean parasite density refers only to those who were positive and does not include those who were negative in the denominator for that calculation.

Table 2 (C): Mean fever and malaria episodes should be deleted and the data presented more simply as indicated below. The children (denominator) could be either as counts or preferably as total child-years at risk. This will enable calculation, and comparison of, incidence density. In the event that all the Child-yrs are the same then those columns could be eliminated and the number of child-yrs at risk could be indicated as a footnote at the bottom of the table. Also, all relative risks should be presented as risk in the intervention group as compared to the placebo group. If the numeric value for the p-value is given then it is not necessary to indicate in a separate footnote if it is less than 0.05 or not.

Category	Placebo, n=75	VA/Zn, n=75	RR (95% CI)	p
events	Child-yrs	events	Child-yrs	
Fever episodes	153	119	0.78	
Malaria episodes	106	74	0.70	
Malaria free	13	23	1.77	

Cough 38 34 0.89

Diarrhea 39 21 0.54

Discussion:

Page 8, line 9 (E): The authors refer to the “burden of malaria”, and could be more specific what is meant by this (i.e. clinical attacks, anemia...).

Page 8, lines 16-18 (E): These two sentences are very awkward and confusing. What is meant by duration of supplementation having an effect? Has this been demonstrated in the data? Also, do the authors mean that “Mean parasite density observed during the follow-up period tended to be higher in the placebo group.” This sentence in the paper should be reworded.

Page 9, line 23 (E): The authors make reference to the study disclosing a role of micronutrients on mortality. This seems inappropriate because there are no findings on mortality presented.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Background:

Page 3, line 21 (E): it may be useful to cite the results of recently published zinc trials in Zanzibar (*P. falciparum*) and in Peru (*P. vivax*).

Methods:

Page 6, line 15 (E): mention that parasite density was the geometric mean parasite density

Results:

Page 8, line 3 (E): Change Kaplan Meier analysis to Cox Proportional Hazards analysis.

Discretionary Revisions (which the author can choose to ignore)

Results:

Page 7, line 10 (D): for the p value use $p < 0.001$

Discussion:

Page 9, line 20 (D): What is meant by “timelessness”? Do the authors mean less time consuming, or perhaps more practical?

Page 9, line 25 (D): The authors state that vitamin A and zinc may play an important role in malaria control strategies in African children. It may be worth mentioning that the data may also be applicable to the use of micronutrients elsewhere as well.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.