Author's response to reviews

Title: The impact of different doses of vitamin A supplementation on male and female mortality. A randomised trial from Guinea-Bissau.

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Author's response to reviews: see over
Dear Editor
Many thanks for the invitation to submit a revised manuscript. Below, please find a point-to-point response to the reviewers’ comments. We have furthermore revised the language as suggested. We hope the paper is now acceptable for publication.
Best wishes,
Dorthe Yakymenko

Reviewer: Aamer Imdad

Reviewer's report:
This paper addresses an important question related to effectiveness of vitamin A supplementation in reducing mortality in children 6 months to 5 years. Overall the paper reads well but revisions in certain sections of the paper can improve authenticity of findings and therefore that of conclusions. I have few major queries about the hypothesis and the conclusions of the paper and then certain suggestions to improve methods, results and discussion section.

Major Comments
• The literature described in the background focuses on proposed increased effectiveness of vitamin A supplementation in children who had recent vaccination (especially DTP) and that the low dose can be as effective as higher dose and then the hypothesis that “a low dose of vitamin A compared with the recommended dose is associated with lower mortality in girls. We also hypothesised that the lower dose would be particularly beneficial in girls who had DTP vaccine as their most recent vaccine.” I would like that the authors clarify the following questions

  1. Are they testing the hypothesis that low dose is as effective as higher recommended dose irrespective of immunization status/history or the hypothesis that low dose vitamin A will be enough/equally effective in children who have been immunized or they testing both of the above hypothesis togather?

DY: We acknowledge that the hypothesis was not very precisely formulated. We hypothesised that the low dose is better than the recommended dose for girls. We furthermore hypothesised that this would be most pronounced in girls who had DTP as their most recent vaccine compared with girls who had other vaccines as their most recent vaccines. The wording has been changed to:
“We aimed to test the observation made in our former trial by testing the a priori hypothesis that a low dose of vitamin A compared with the recommended dose is associated with lower mortality in girls. We furthermore hypothesised that the lower dose would be particularly beneficial in girls who had DTP vaccine at their most recent vaccination contact compared with girls who had received other vaccines.” [Page 4]. Accordingly we have added an estimate for the effect of the low versus the recommended dose in children who had BCG or measles vaccine as their last vaccine.

• In conclusions, authors claim that they didn’t find conclusive evidence in favor of low dose vitamin A supplementation for reducing mortality (in girls). Can the results be interpreted as “There was no differential effect of low or high dose vitamin A supplementation for reducing mortality (in girls, who have been immunized)?

DY: The trial was not designed as an equivalence trial. The mortality was low and the confidence of the effect estimates very wide that it is not possible to interpret the data as suggested.

Other Comments
Abstract:
• Result’s section: Please clearly state that the results were statistically insignificant. A “Tendency” in favor/against of an intervention is not conclusive evidence.

DY: This point was also raised by reviewer 2. The wording has been changed according to the precise suggestions from reviewer 2.

Full text
Introduction:
• 1st paragraph last line, please give the recommended frequency of supplementation (i.e. every 4-6 months)

DY: This has been added [Page 3].

• Third paragraph, please also refer to other studies that investigated low dose (more frequent) vitamin A supplementation for reducing mortality (Rahmathullah, L., B. A. Underwood, et al. (1990). "Reduced mortality among children in southern India receiving a small weekly dose of vitamin A." N Engl J Med 323(14): 929-35)

DY: This references has been added [Page 3]

• Comments on hypothesis as above

Methods:
Study settings
• What was the baseline mortality rate in the study area

DY: This has now been provided [Page 4]

• A brief description of health facilities in the area, were there hospitals around and sick got immediate health care etc?

DY: This has now been provided [Page 4]

• Baseline stunting rates?

DY: This has now been provided [Page 4]

Enrollment and randomization:
• How was the sequence generated?
• How was allocation concealed?
• Blinding?
• Who delivered the intervention and who collected the data?
• Were the data analysts blinded to allocation?

DY: This information has now been provided [Page 6+8].

Outcomes
• What were the secondary outcomes? Did authors look at morbidity outcomes? Were any side effects noted as it is important to investigate that were there less side effects (especially vomiting) with low dose of vitamin A?
Results:

- Please give data according to age groups, as it has been shown previously that there is no effect of vitamin A supplementation in children 6 months to 1 year of age


DY: The reference which the reviewer is quoting actually gives a mortality estimate of 0.69 for 6-11-month-old children. However, we are grateful for the suggestion to conduct this analysis. It revealed a tendency for a better effect of the lower dose in the youngest children. The analysis is presented in the supplementary table (former table 3) and commented upon in the results and discussion sections. In the process we decided that it was optimal to adjust all analyses for age as a continuous variable rather than a categorical variable. This has been implemented throughout the paper, resulting in minor changes in the effect estimates.

- State clearly that results were not statistically significant

DY: We have changed the results section according to the reviewers’ suggestion.

- Please show data for cause specific mortality (may be as webtable). When the authors have done verbal autopsies, then why the data have not been presented in complete details?

DY: There are too few deaths to allow for a meaningful comparison of causes of deaths between the randomisation groups. The data was mainly used to exclude deaths due to accidents (N=2). This has been added [Page 9]. We shortened the paragraph on the verbal autopsy methodology accordingly [Page 7].

Discussion

- Please also site and defend your hypothesis/findings against the literature where scientists don’t agree with the hypothesis that effect of vitamin A supplementation may be mediated through immunization and there is a differential effect of vitamin A on girls. Please see the following references as an example

DY: In the present paper we are testing the main hypothesis that a small dose may be better than the recommended dose for females. This was done to test previous findings, made by us and others, that a low dose may be better than the recommended dose. We have speculated and tested as a secondary hypothesis whether this could be related to vaccination status. This was not the case. As there was no data to support the main hypothesis we find it a bit beyond the scope of the paper to discuss the potential arguments against VAS-vaccine interactions. We have included the two meta-analyses and a discussion of the evidence of sex-differences in the response to VAS as suggested. See specific comments in relation to each of the papers below.

DY: This paper addresses the effect on supplementation of neonates in relation to maternal prevalence of vitamin A deficiency. The paper does not contradict a possible interaction between VAS and vaccines. Furthermore, the paper has been heavily criticized (Sachdev et al, 2010).


DY: The paper concludes that VAS did not affect the antibody affinity of Hib vaccine and Hepatitis B vaccine. This finding does not exclude that there may be important VAS-vaccine interactions. In fact, the two studies made by Newton et al, providing VAS with DTP/pentavalent vaccine, yielded a MH-estimate of an almost 3-fold significantly increased mortality among children who received VAS with DTP compared with placebo with DTP/pentavalent vaccine (Newton 2008).


DY: This meta-analysis of sex differential effects on mortality after neonatal VAS concludes that based on current literature nothing can be concluded.


DY: The study did not include the Ghana VAST trial. We have added the reference.[Page 13]


DY: This reference has also been added.[Page 13]

Conclusion:
• The statement “The trial confirmed that the impact of VAS may differ by sex” is not supported by the findings of the study. Please rephrase

DY: We have omitted the sentence [Page 14].
Reviewer: Siddhartha Gogia

Reviewer's report:
Major Compulsory revisions

1. Abstract
   “Children who had received the low dose tended to have higher mortality, the MRR being 1.20
   (0.58 to 2.47) after 6 months and 1.15 (0.72 to 1.84) after 12 months. This tendency was similar
   in boys and girls. As hypothesised the low dose tended to be associated with lower mortality in girls
   if the most recent vaccine was DTP (MRR=0.56 (0.13 to 2.35) after 6 months).”

   Change to
   “There was no significant difference in mortality at 6 months and 12 months of follow up between
   the high dose VAS group and the low dose VAS group. The MRRs were 1.20 (0.58 to 2.47) after 6
   months and 1.15 (0.72 to 1.84) after 12 months. This tendency was similar in boys and girls. There
   was no evidence of low dose VAS being associated with lower mortality in girls if the most recent
   vaccine was DTP, MRR=0.56 (0.13 to 2.35) after 6 months.”

   DY: This has been changed almost as suggested (The MRRs are presented as the low versus the
   recommended dose, hence we have swopped low and high, and furthermore changed “high” to
   “recommended” as that is the wording we use throughout the paper).[Page 2]

2. “We found that the low dose was associated with a significant reduction in mortality at 6 and 9
   months of follow-up[10].”

   The abstract of the concerned paper reads as “(mortality rate ratio 0.69, 95% confidence interval
   0.36 to 1.35) and nine months (0.62, 0.36 to 1.06) of follow-up. There was a significant interaction
   between sex and dose, the lower dose being associated with significantly reduced mortality in girls
   (0.19, 0.06 to 0.66) but not in boys (1.98, 0.74 to 5.29). The lower dose of vitamin A was
   consistently associated with lower hospital case fatality in girls (0.19, 0.02 to 1.45). Paradoxically,
   in children aged 6-18 months, the low dose was associated with slightly higher morbidity.”

   Apart from mortality in girls, all other effects are not significant.

   DY: We are grateful that the reviewer pointed that out. We have changed the wording to:
   “We found that among girls the low dose was associated with a significant reduction in mortality at
   6 and 9 months of follow-up[10]. This difference was mainly seen in girls 18 month of age…”
   [Page 4]

3. Main analyses of mortality
   “Mortality tended to be slightly higher for those who received the low dose, the MRR being 1.20
   (0.58-2.47) after 6 months and 1.15 (0.71-1.84) after 12 months.”

   Change to
   “There was no significant difference in mortality at 6 months and 12 months of follow up between
   the high dose VAS group and the low dose VAS group; the MRR being 1.23 (0.60-2.54) after 6
   months and 1.17 (0.73-1.87) after 12 months.”

   DY: Changed as suggested by the reviewer [Page 10]

4.
“We found a tendency for girls to benefit more from the low dose if the most recent vaccine before the campaign was DTP, the MRR being 0.56 (0.13-2.35) after 6 months of follow-up and 0.72 (0.27-1.94) after 12 months. There was no difference for boys.”

Change to
“There was no evidence of low dose VAS being associated with lower mortality in girls if the most recent vaccine was DTP, MRR=0.56 (0.13 to 2.35) after 6 months.”

DY: We have changed the wording to:
“The low dose of VAS in girls was not significantly different depending on the most recent vaccination. After 6 months of follow-up, the MRR was 0.60 (0.14-2.50) if the most recent vaccination was DTP and 3.06 (0.63-15) if the most recent vaccine was BCG vaccine or measles vaccine (p for same effect=0.12). After 12 months the estimates were 0.77 (0.29-2.07) and 1.21 (0.45-3.26) respectively (p for same effect=0.55).” [Page 10]

5.
Post hoc analyses
This section should be deleted as statistically the data is not robust at all.
“If a child received VAS for the first time in this trial the low dose tended to be more beneficial, the MRR comparing the low dose versus the high dose being 0.61 (0.24-1.58) after 6 months of follow-up.”
There is actually no significant difference between the high and low dose groups.
“However, if a child had received VAS before, the high dose tended to be more beneficial (4.46 (0.97-20) (p for interaction=0.09)).”
Again, the difference is not statistically significant.

DY: We have omitted most of these post hoc analyses; however, a recent paper of ours (accepted for publication with PLoS ONE, and enclosed with this resubmission for your information) has shown an interesting interaction between VAS at birth and subsequent vitamin A supplementation. Hence, we found it indicated to assess the effect of a lower versus the recommended dose in the subgroup of children who had been randomised to VAS or placebo at birth, and have kept that analysis in the paper, albeit only in a few lines of text [Page 11+13] and the supplementary table.

6.
Comparison of participants and non-participants
Its desirable to compare the population characteristics of these 2 groups but there is no need to compare mortality rates between these 2 groups as a) this is not among the mentioned objectives. b) the results are speculative at best in the presence of a strong selection bias

DY: We have clearly described the differences between participants and non-participants and emphasised that the comparison should be interpreted carefully due to strong selection bias. However, selection bias would not explain the observed sex-differences [Page 13]. We think it is an interesting finding which deserves to be mentioned. However, should the editor wish so we would be willing to omit the analysis.

7.
Discussion
Please delete
‘Though none of our findings were significant we found the predicted pattern of a low dose being relatively more beneficial for girls with DTP as their most recent vaccination before enrolment. A post hoc analysis showed that girls who had received VAS previously tended to benefit more from receiving a high dose.

6
Compared with non-participants, it was only boys and children who had received VAS previously, who benefited from participating in the campaign.”

**DY:** *We have modified the statements [Page 12].*

8. A priming effect of VAS?
As this section is completely based on the above mentioned non-robust post hoc analysis, it should be deleted.
Please consider the following statements
1. from reference 10, the reference which is the basis for the present study and has been the most widely cited in this article
   “There was a significant interaction between sex and dose, the lower dose being associated with significantly reduced mortality in girls (0.19, 0.06 to 0.66) but not in boys (1.98, 0.74 to 5.29). The lower dose of vitamin A was consistently associated with lower hospital case fatality in girls (0.19, 0.02 to 1.45).”
2. From present article
   “former VAS makes children more likely to benefit from another high-dose supplementation, and that especially girls might benefit even more from a high dose of vitamin A during childhood if supplemented previously.”
3. “If a child received VAS for the first time in this trial the low dose tended to be more beneficial, the MRR comparing the low dose versus the high dose being 0.61 (0.24-1.58) after 6 months of follow-up. However, if a child had received VAS before, the high dose tended to be more beneficial (4.46 (0.97-20) (p for interaction=0.09)).”
What I understand from this is that if a subject gets a low dose before, another low dose does not benefit. A higher subsequent dose is needed for benefit.
Programmatically, this would mean that the girls get a low dose initially and a high dose subsequently. “**Priming refers to a increased sensitivity to certain stimuli due to prior experience.”**
Although, there is negative priming as well, the one described in psychology. It is the negative priming that is working here i.e. increase in the required dose for subsequent benefit. As the authors themselves admit that “However, this pattern of a sex-differential response to repeated VAS is not consistent in all vitamin A trials.” and “We have no biological explanation for a possible priming effect of VAS on subsequent VAS.;” this portion is best omitted, especially when it is based on data which suggest otherwise. And the conclusion “Hence, it may be speculated that children with sufficient vitamin A levels benefit more from subsequent VAS.” is biologically implausible.

**DY:** *We have shortened this section considerably; however, we have kept a few lines as a possible explanation for the contrasting results between this trial and our previous trial [Page 13].*

9. Please delete
   “We have no biological explanation for a possible priming effect of VAS on subsequent VAS. One small study reported a priming dose of vitamin A to be associated with a higher increase in vitamin A concentration after VAS[22].
   Hence, it may be speculated that children with sufficient vitamin A levels benefit more from subsequent VAS.
   Sex-differences in the response to VAS
   The present trial confirmed previous observations of sex-differences in response to VAS[8,10,19,23-28]. First, as seen in the previous trial, girls tended to benefit from a lower dose of VAS if DTP was the most recent vaccine. Furthermore, boys seemed to benefit more than girls from
participating in the campaign. This sex-difference may be due to a negative interaction between VAS and DTP in girls[26-28].”

DY: Changed to:
“The present trial did not clearly confirm previous observations of sex-differences in response to VAS. However, though the results were by no way significant, as seen in the previous trial, girls tended to benefit more from a lower dose of VAS if DTP was the most recent vaccine than if the most recent vaccine was BCG or measles vaccine. Furthermore, only boys benefitted significantly from participating in the campaign. These sex-difference may be due to a negative interaction between VAS and DTP in girls[23-25]” [Page 12+13]

10. Conclusions
“The trial confirmed that the impact of VAS may differ by sex and routine vaccinations and adds the observation that it may further be primed by previous VAS.”
Change to
“There was no evidence of low dose VAS being associated with lower mortality in girls if the most recent vaccine was DTP, MRR=0.56 (0.13 to 2.35) after 6 months.”

DY: The conclusion has been changed to “In conclusion, we did not confirm the previously observed effect of a lower dose of vitamin A being more beneficial for girls at either 6 or 12 months of follow-up.” [Page 14]

Minor Essential Revisions
1. Background
“Vitamin A supplementation (VAS) given to children above 6 months of age may reduce overall mortality in low-income countries by 23-30%[1,2]. The World Health organisation (WHO) currently recommends high-dose VAS at immunisation contacts after 6 months of age. Children between 6 and 11 months should receive 100,000 IU, children aged 12 months and older 200,00 IU[3].”
Change to
“Vitamin A supplementation (VAS) given to children above 6 months of age reduces overall mortality in low-income countries by 23-30%[1,2]. The World Health organisation (WHO) currently recommends high-dose VAS at immunisation contacts after 6 months of age. Children between 6 to 12 months are advised a dose of 100,000 IU and children older than 12 months, a dose of 200,00 IU; every 4 to 6 months [3].”

DY: Changed as suggested [Page 3]

2. “Routine vaccines during childhood have been shown to have non-specific effects on overall mortality[4-7]. Our group hypothesised that the effect of VAS on mortality may depend on an amplification of the non-specific effects on the vaccines[8].”
Change to
“Routine vaccines during childhood have been shown to have additional non-specific beneficial effects in reducing overall mortality[4-7]. Our group hypothesised that the effect of VAS on mortality may depend on an amplification of the non-specific effects of the vaccines[8].”

DY: It is widely accepted that measles vaccine and BCG vaccine has additional beneficial effects on overall mortality. However, observations suggest that this is not the case for DTP, which is
associated with increased mortality in populations with herd immunity to pertussis and a high infectious disease burden (see for instance references below). We have specifically hypothesised that VAS may amplify the negative effects of DTP. This has been clarified in the background [Page 3]

Further references on DTP:


4. “Previous trials have indicated that a low dose might be more beneficial than a higher dose on mortality[9,10] and morbidity[11].”

   Change to

   “Previous trials have indicated that a lower dose of vitamin A might be more beneficial than a higher dose in reducing mortality[9,10] and morbidity[11].”

   DY: changed as suggested [Page 3].

5. Samples size considerations

   “With an expected mortality of 1.2 % at 6 months of follow-up it would be possible to detect a 55% reduction in female mortality among girls …”

   Change to

   “With an expected mortality of 1.2 % at 6 months of follow-up it would be possible to detect a 55% reduction in mortality among girls….”

   DY: Changed as suggested [Page 8].

6. “In the previous trial we had found the low dose to be associated with a 72% reduction in mortality after 9 months[10].”

   Change to

   “In the previous trial we had found the low dose to be associated with a 72% reduction in female mortality after 9 months[10].”

   DY: Changed as suggested [Page 8].

6. Data analysis and statistical methods

   “Statistical analysis was conducted using Stata/SE 9.2.” (please give reference)

   DY: A reference has been provided [Page 8].