Author's response to reviews

Title: Determinants of vitamin A deficiency in children between 6 months and 2 years of age in Guinea-Bissau

Authors:

Niels Danneskiold-Samsøe (nds@bio.ku.dk)
Ane B Fisker (a.fisker@bandim.org)
Mathias J Jørgensen (mathiasjul@gmail.com)
Henrik Ravn (HJN@ssi.dk)
Andreas Andersen (a.andersen@bandim.org)
Ibraima D Balde (i.balde@bandim.org)
Christian Leo-Hansen (chr_leohansen@hotmail.com)
Amabelia Rodrigues (a.rodrigues@bandim.org)
Peter Aaby (p.aaby@bandim.org)
Christine S Benn (cb@ssi.dk)

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Author's response to reviews: see over
Dear Dr. Dizon

Thank you for the opportunity to submit a revised paper. Please find below a point to point response to the comments raised by the reviewers.

I hope the paper is now acceptable.

Sincerely,

Niels Danneskiold-Samsøe

Reviewer: Sam K Newton
Kintampo Health Research Centre, P. O. Box 200, Kintampo, Ghana

The authors have clearly stated that they enrolled children who “had missed at least one routine vaccination”. It is my opinion that those who miss immunizations are different from the general population and the results obtained from using such a sample is very different from what pertains in the general population. This point has even been acknowledged by the authors themselves under the section “statistical analyses’ that the “prevalence of VAD may not entirely reflect the vitamin A status in the population” While I commend the authors for their honestly in acknowledging this, that very point is what makes the selection of subject for this study problematic. It is not a representative sample upon which to make conclusions about the prevalence of VAD.

NDS response to reviewer:

We clearly understand the reviewers’ comments, particularly since the adherence to the vaccination programme is very high in Ghana. We have clearly not emphasised sufficiently that the vaccination coverage in rural Guinea-Bissau is low. In a recent study from the Bandim Health Project we found that only 50% of children were fully vaccinated by one year of age. Hence, the present study group is representative of a large part of the children in Guinea-Bissau. Furthermore, we specifically analysed the data for children aged 9-11 months, the age group in which children are due to receive measles vaccine. 67% of children in this age group were eligible for the trial, i.e. they had not yet received measles vaccine when our team visited their village. In this subgroup we found no difference in the risk of VAD between the children who had received all the prior vaccines in a timely manner, and children who had not. We have edited the paper to emphasise these points:

1) In the text a ‘may’ was added to our main findings on the prevalence on VAD:
“Based on the collected blood samples young children in rural Guinea-Bissau may have a major VAD problem.” [page 14]

2) We have inserted the following paragraph: “In the present study we took advantage of the infrastructure of a trial and only children eligible for the trial were bled. Thus, children were between 6-23 months of age and missed at least one routine vaccination, and no children were included in the first month after VAS provided in a campaign. Children with incomplete vaccination history have been shown to have a higher risk of VAD in other settings [18]. However, in our study population rural Guinea-Bissau it is common to be missing one or more vaccines; in a recent study from the Bandim Health Project we
found that half of the children at one year of age in Guinea-Bissau are missing at least one vaccine [19]. Furthermore, 67% of children in the age group from 9-11 months of age had not yet received a measles vaccine at the time of the home visit, and we did not find any statistical difference in the risk of VAD between the children who had received all the prior vaccines timely and children who had not. Hence, though we cannot exclude a slight overestimation of VAD due to the focus on children missing one or more vaccines, we believe that the study reflects the situation in rural Guinea-Bissau. “[Page 15]

3) The conclusion also discusses the inclusion criteria and their potential influence on the conclusions: “We found that around two-thirds of children between 6 months and 2 years of age have RBP levels indicating VAD. Though we only included children who were not fully vaccinated, this accounts for a large proportion of the children in rural Guinea-Bissau, and there was no indication that children vaccinated timely had significantly lower risk of VAD. Though we may not have been able to control fully for the effect of infections, the results still point to VAD as a major public health problem in rural Guinea-Bissau.” [Page 19]

4) Furthermore, we have emphasised that the main focus of our paper is to identify the major risk factors for vitamin A deficiency in Guinea-Bissau. Hence, we have changed the title of the paper to: “Determinants of vitamin A deficiency in children between 6 months and 2 years of age in Guinea-Bissau”. [Page 1]

In the last but one paragraph under the methods section, the author explained that they initially intended to collect blood samples from 600 children but because enrollment proceeded faster than expected they continued to collect some more samples. The authors have not stated the objectives of the initial study which required a lower sample size of 600 children.

NDS response to reviewer: The samples were collected to describe baseline vitamin A status among children enrolled in a randomised trial of vitamin A supplementation. This information has been added to the manuscript:

“We originally planned to collect blood samples from 600 children at enrolment, prior to randomization, to describe baseline vitamin A status among the children and study risk factors for VAD. The study was observational and no sample size calculations were made. In the beginning of the trial all eligible children in a village were included in the study. However, enrolment proceeded faster than expected and to describe the full seasonal and geographical variation we continued sampling five children from each village until samples from both the rainy (June-November) and the dry (December-May) season had been obtained in all regions. Hence, a total of 1254 samples were collected.” [Page 5-6]

Statistical analyses
Apart from the earlier point which is the acknowledgement of the fact that the prevalence of VAD may not entirely reflect the vitamin A status in the population, the authors went on to further say that they had conducted a sub group analyses of VAD for children = 9 and <12 months for comparison with those who had been timely vaccinated. I am of the opinion that subgroup analyses does not solve the problem because the numbers in the subgroup are likely to be small and not enough to make any meaningful conclusions about the prevalence of VAD. The authors have not shown any sample size calculations to show what numbers are actually required for the determination of VAD. While I acknowledge that due to the fact that no surveys on VAD have actually been conducted prior to this study, using the assumptions from the
WHO in the background section, a sample size calculation could have been made for readers to determine what conclusions could be made from the numbers which are being reported for this study.

NDS response to reviewer: The subgroup aged 9-11 month was selected for good reasons. All children are supposed to receive measles vaccine between 9 and 11 months of age and 67% of children in this age group were missing a measles vaccine and were eligible to be enrolled in the trial when we visited the villages. Within this age group (n=380) we distinguished between children were timely (n=124, 33%) or not timely vaccinated (67%) and found that there were no differences in the prevalence of VAD between the two groups. We do not think that 380 children can be considered a small sample, also reflected by the fact that the confidence intervals are quite narrow and exclude large differences between those who were and those who were not timely vaccinated.

Results:
Child factors: The authors state that twins had a higher risk of VAD but we are not told the numbers of sets of twins in the sample and what conclusions can be drawn from them.

NDS response to reviewer: There were only 13 pairs of twins among the 36 twins included in the study. We acknowledge that there are limitations to which conclusions can be drawn. The following sentence has been added to the manuscript:

“The increased risk of VAD amongst twins could possibly be explained by a lower nutritional status since twins are more often born low-birth-weight and run a higher risk of being stunted [37]. However, due to the low number of twins (n=36) the result should be interpreted with caution.” [page 19]

Subgroup analysis
Lots of subgroup analyses have been done even with respect to the different ethnic groups but we are not told how many numbers those conclusions are based on and perhaps that explains why there are such large confidence intervals from the analyses.

NDS response to reviewer: “The number of inclusions for different major ethnicities was: Balanta (282), Fula (215), Mandinga (123), Pepel (235) – now mentioned in table 1 alongside the number of inclusions in other subgroups. We are not quite sure what the reviewer means by large confidence intervals; the differences between these groups were clearly significantly different as indicated in the table.

Discussions
The authors conclude that the prevalence of VAD is higher than the WHO estimate. But that is to be expected from the kind of sample which was chosen. The authors conclude under the section of “strengths and weaknesses” that the present study is the first to describe the prevalence of VAD. Unfortunately this study does not do that. The authors report that “may have overestimated the prevalence of VAD slightly”. I beg to differ. I think they have greatly overestimated the prevalence of VAD based on the reasons above and makes the results of this study debatable. This study does not have the power to say that “timely vaccinated children did not have significantly better vitamin A status.

NDS response to reviewer: As discussed above, we disagree that the 380 children in the age group 9-11 months of age should be insufficient to assess whether timely and not timely vaccinated children had different prevalence of VAD. The narrow confidence intervals exclude large differences. As mentioned above
we have rephrased the discussion to emphasise that though the study was not a random sample of the total population in the 6-24 months age group, a large proportion of the rural Guinean children are not timely vaccinated, and the data did not suggest major differences between timely and not timely vaccinated children; hence it is therefore probable that the degree of VAD reported in the present study is likely to be representative for the country.

Summary and Conclusion
The manuscript requires a major revision and some of the points raised cannot be corrected with editorial revisions due to the methodology which was adopted in selecting subjects. What the authors have presented is the prevalence of VAD in a group of children who are not representative of the general population. The conclusions to the paper are not valid.

NDS response to reviewer: Despite the limitations of the study due to the inclusion criteria in the trial, major benefits were gained by using its infrastructure. As argued above we believe that the study provides a reasonable estimate of VAD among young children in rural Guinea-Bissau. Furthermore, it identifies subgroups at particular risk for whom future interventions may be targeted.
Given the objective of assessing vitamin A status (and the lack of a nationally representative vitamin A survey) it would be useful to have a more explicit discussion of the population that the survey does and does not represent, and the potential biases that might exist if one were to try to generalize the results to the entire country. Specifically it would be useful to address: (1) Was the study designed to be statistically representative of this age group on a national level or not? Were all areas of the country eligible for inclusion in the HDDS prior to sampling? (2) Were any geographic areas or population groups not represented that might lead to bias when trying to generalize results to the country? (3) Given seasonal patterns in VAD shown in Figure 3 (and the fact that the sample size contributing to the overall sample varied by month) what are the potential implications for the estimate of prevalence?

NDS response to reviewer: Ad. 1) The study took place within village clusters selected by the EPI sampling method in the Bandim Health Projects HDSS (ref Kristensen BMJ 2000). Ad 2) The villages in the study represent all 9 health regions of Guinea-Bissau and with minor exemptions (the infrastructure did not allow access to some of the islands) all geographic areas were represented in the sample (Figure 1). However, the included populations did not completely correspond to the respective population sizes in the various regions according to the 2009 census (Review table 1). Since the risk of VAD varies with region this might have had an impact on the estimate of prevalence. However, calculating a weighted average yielded a national prevalence of 67%. All major ethnic groups and most minor groups were included in the study. All socio-economic groups were also included. 3) The four months with the lowest prevalence of VAD corresponded to 48.2% of inclusions. This would tend to underestimate the prevalence of VAD.

**Figure 1. Map of Guinea-Bissau with borders between health regions and villages included in the study (black dots).**

**Review table 1, proportional population size by region from government census and the HDSS.**

<table>
<thead>
<tr>
<th>Region</th>
<th>%0-14 year olds (2009)</th>
<th>% of inclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=615622)</td>
<td>(n=1102)</td>
</tr>
<tr>
<td>Biombo</td>
<td>6.5</td>
<td>26.0</td>
</tr>
<tr>
<td>Gabu</td>
<td>15.7</td>
<td>13.4</td>
</tr>
<tr>
<td>Cacheu</td>
<td>12.6</td>
<td>4.8</td>
</tr>
<tr>
<td>Bafatã</td>
<td>15.1</td>
<td>11.0</td>
</tr>
</tbody>
</table>
The following text *was changed in the manuscript methods and discussion:*

“The Bandim Health Project (BHP) runs a Health and Demographic Surveillance System (HDSS) covering 182 randomly selected clusters of 100 women and their children below 5 years of age in the 9 rural health regions of Guinea-Bissau (Figure 1).” [page 4]

“All geographic areas were represented in the sample. However, the included populations did not completely correspond to the respective population sizes in the various regions. Due to the inter-regional variation in risk of VAD this could have affected the overall estimate of VAD prevalence. However, calculating a weighted average the combined prevalence from all regions was 67%. The four months with the lowest prevalence of VAD corresponded to 48.2% of the study population, which will tend to underestimate the prevalence of VAD.” [page 16]

“In conclusion, we may not have obtained a precise estimate of the true prevalence of VAD in the children in Guinea-Bissau, but neither setting the cut-off for VAD lower, nor exclusion of infected children or estimating the prevalence of VAS in timely vaccinated children resulted in a prevalence below 57.5% as estimated by the WHO. The analysis of determinants of VAD was also robust in the sensitivity analyses.” [page 16]

Discussion: “Vitamin A status might have been slightly better in older children”? This statement seems to expand the implications of these findings to a group that was not included in the study and I wonder whether there is data available to support such a statement. Is there any data on age (especially for over vs. under 2 year olds) as a risk factor related to vitamin A status in Guinea Bissau or other relevant settings that might be brought into this discussion? What is known about coverage rates of the biannual VAS program in Guinea Bissau by age and how might this affect such a statement? (as a side-note it would be good to also bring the latter point into the background/discussion as I don’t see any mention of coverage of this program.)

NDS response to reviewer: *The other VAD studies on children referred to in the manuscript also included older pre-school children. Since they were not essential we have deleted the following sentences from the manuscript text:*

“Vitamin A status may have been slightly better in the older children. Hence, we may have overestimated the prevalence of VAD slightly by not including children up to 5 years of age” [page 8, old manuscript]

Results/Discussion: For the vitamin A supplementation, what approach and recall period was used to get information about receipt of vitamin A capsules? There is suggestion in some of the literature that biannual supplementation might not be frequent enough in some settings for children to reach sufficient vitamin A
status and that more frequent dosage (every 4 months) may be needed. For this reason I think that it might also be useful to explore potential associations between VAS in the past 4 months in addition to 6.

NDS response to reviewer: Most recent vitamin A supplementation was noted on the health card of the children as a date if the date was within 6 months prior to inclusion. The number of children receiving VAS within 4 months was 493 compared to 589 within 6 months prior to inclusion. Having received VAS within 4 months was not associated with a significant lower risk of VAD. We also investigated the effect of having received VAS within 2 months prior to inclusion (379 children). This did not have any effect either. These results have been added in the manuscript:

“Having received a live vaccine as the most recent vaccine rather than an inactivated vaccine was associated with a lower risk of VAD (PR= 0.84 (0.74-0.96) (Table 1)). We did not find an effect on the risk of VAD of having received VAS within the previous 6 months (Table 1). VAS within 2 or 4 was not associated with significantly lower risk of VAD either (Supplementary table S2).” [page 13]

“Several trials have evaluated the temporal effect of supplementation on VAD, but none including children within the study age group. We found only two studies investigating the effect of VAS on vitamin A status in the present age group. An observational study from Ethiopia reported increased VAD in children who had missed one or more doses of VAS within the last year [18]. The other observational study collected paired blood samples before and after supplementation and found a significant increase in plasma retinol 4 months after VAS [27]. We could not document any effect of missing VAS within the preceding 2, 4 or 6 months. Possible explanations could be geographical or methodological differences between the studies.” [page 17]

Given importance of season as a variable, what was the timing of vitamin A supplementation campaigns relative to season in the years covered in this study? Were there any interactions between VAS and season?

NDS response to reviewer: Of the 358 children receiving VAS within 6 months prior to inclusion in the study, 215 received it in the dry season and 143 received it in the rainy season. Given the lack of effect of VAS on the risk of deficiency we did not initially test interaction between VAS and season or any other variable. However, we have conducted the analysis on the reviewers’ suggestion: There was no interaction between receiving VAS within 6 months prior to inclusion and season.

I am also not clear on the description of the subgroup analysis of “timely vaccinated” children in light of the statement that “only children who had missed at least one routine vaccination” were included. Perhaps I missed something but it would help to clarify whether this sub-group was or was not included in the multivariate analysis for risk factors of VAD and to provide the sample size of this group. I am also wondering whether timely vaccination was considered as a variable for inclusion in the models?

NDS response to reviewer: According to the Expanded Program on Immunization the measles vaccine is supposed to be given at 9 months of age but can be given this vaccine up to 12 months of age without being considered as a late vaccination. To assess the potential bias introduced by our inclusion criterion: “missing one or more vaccines” we therefore considered children between 9-11 months of age timely vaccinated for measles vaccine, and subdivided them into children who had had all their prior vaccines in time or not. A total of 380 children were 9 to 11 months of age. Of these, 124 children were timely vaccinated. This
variable was not considered as a variable for inclusion in the models as it only concerned a subgroup of participants.

The following changes were made in the methods section:

“Since only children missing a vaccine according to the vaccination schedule were included in the study, the estimates of the prevalence of VAD may not entirely reflect vitamin A status in the population. The measles vaccine is supposed to be given at 9 months of age but children can be given this vaccine up to 12 months of age without this being considered as a late vaccination. We therefore defined a subgroup of children as being “timely vaccinated” if they were 9-11 months of age, had received the third dose of DTP/Pentavalent vaccine before 7 months of age and were missing only MV (Figure 2). Next, we studied the prevalence of VAD within this subgroup and included it in our simple model for risk factors for VAD (see below).” [page 9]

P.7 “Reception” (receipt?) of VAS within the previous 6 months had no effect on VAD”. I would be cautious not to make conclusions about effects given that this is an observational study. Also, this statement appears to be made based only on the simple model as I don’t see VAS in the larger (multivariate) model. I think it would be useful to undertake a sensitivity analysis including VAS in the multivariate adjusted (large) model to be able to make a more conclusive statement here about associations between VAS and VAD in this study. Also, it is important to note that children who had received VAS within the previous month were excluded from the study—is it possible that excluding these children might have led to an attenuation of the effect of VAS on vitamin A status? Presumably this point could also be addressed through a sensitivity analysis and/or could be brought into the discussion.

NDS response to reviewer: To accommodate the reviewers’ comment we have tried to include children having received VAS 2, 4, or 6 months prior to inclusion in both the simple and the large multivariate model respectively. In neither of these analyses did we find an effect of VAS on VAD. An attenuation of the effect of VAS within 1 month on vitamin A status is possible. The following changes were made in the manuscript:

“To assess whether there was a temporal effect of previous VAS on vitamin A status we also tested the effect of including children who had received VAS within 2, 4, and 6 months prior to inclusion into the trial in the simple and the large model.” [page 10]

“VAS within 2 or 4 was not associated with significantly lower risk of VAD either (Supplementary table S2).” [page 13]

“Since study children did not receive VAS within the preceding month, a short-lived increase in serum RBP after supplementation in a campaign is not likely to have affected the results [20]. On the other hand, we might have overestimated the prevalence of VAD by not including this group. However, we were not able to document any effect of receiving VAS from 1 to 2 months before inclusion.” [page 16]

Table 1. Could you please clarify in the table what statistical tests were used for each analysis? What does the p-value for categorical variables (with more than one category, such as birth facility) represent?

NDS response to reviewer: The statistical test used in the columns termed ‘Simple model PR for VAD’ and ‘Large model PR for VAD’ was the Poisson multivariable regression model using a robust sandwich estimator (Stata command: poisson VAD VAR2 VAR2…etc, irr robust). The statistical test used in the columns termed
‘Simple model p’ and ‘Large model p’ was Wald tests of the parameters of the Poisson regression models (Stata command: testparm VAR). The following text was added to the text and the table 1 legend of the manuscript:

“Wald tests were used to test for overall equality of categories within variable.” [page 10]

“Wald test for overall equality of categories within variable” [Table 1 legend]

Minor Essential Revisions.

p.6. Results: how many people had missing covariates <2% and were therefore not included in the analysis?

NDS response to reviewer: The number of missing data in variables not included in the simple models and large model have now been listed in supplementary table S1, also inserted below.

Supplementary table S1. Overview of missing data in included variables

<table>
<thead>
<tr>
<th>Variable included in the large model</th>
<th>Missing data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight for age a</td>
<td>8</td>
</tr>
<tr>
<td>Arm circumference for age</td>
<td>1</td>
</tr>
<tr>
<td>Cough at the day of inclusion</td>
<td>3</td>
</tr>
<tr>
<td>Maternal ethnicity b</td>
<td>13</td>
</tr>
<tr>
<td>Most recent vaccination type a</td>
<td>19</td>
</tr>
<tr>
<td>Child use of bed net b</td>
<td>10</td>
</tr>
<tr>
<td>Final inclusions into larger model</td>
<td>1050</td>
</tr>
</tbody>
</table>

*one inclusion with data missing in both variables: weight for age and most recent vaccination type, b one inclusion with data missing in both variables: maternal ethnicity and child use of bed net.

We have also inserted the following sentence:

“Hence, 1102 samples were included in the study. Due to missing information on a variable (Supplementary table S1) the final number of children included in the large model was 1050.” [page 11]

Discretionary Revisions. These are recommendations for improvement which the author can choose to ignore. For example clarifications, data that would be useful but not essential.

Abstract: It’s not clear what ‘indicator variables’ are: rephrasing for clarity would be useful.

NDS response to reviewer: These ‘indicator’ or proxy variables for biochemical measurement of VAD are mentioned in the background section and are not essential for the abstract. The sentence has been deleted.
Description of backward selection: it would be useful to mention the rationale for removing variables from the final multivariate model as well as the order/method used to remove variables from the model...presumably it was stepwise with removal by descending p value?

NDS response to reviewer: We removed variables from the final model if the p-value was above 0.20, to make the model simpler. We used automatic stepwise backwards selection to make the selection process transparent. This has been clarified:

“All variables were tested one by one in a simple model, controlling only for infection. In a larger model we included the obligatory variables as well as all variable from the simple model, and used automated stepwise backwards selection to exclude variables to a descending value of p<0.20.” [page 10]

Methods: For discussion of approaches to missing data it sounds like you used the complete case approach where covariates were missing for <2% of data and the missing indicator approach where covariates were missing for >2%. You might state this directly.

NDS response to reviewer: We are sorry, but we are not familiar with “complete case approach”. Hence, we have not followed this advice.

p. 5 Statistical analysis: Do the + 10% cutoffs refer to a change in threshold used for RBP analyses? (you might just mention the values actually used to make this clear.)

NDS response to reviewer: Yes. The threshold of 0.749 for VAD measured in DBS corresponding to plasma RBP of 0.83 was used for the ±10% sensitivity analysis. We have clarified:

“The Youden index [14] was used to find the DBS cut-off value of 0.749 which corresponded to the highest combined sensitivity and specificity with regard to identifying individuals with VAD defined as plasma RBP < 0.83.” [page 8]

“Next, in a sensitivity analysis, we assessed the prevalence of VAD using a 10% lower or 10% higher cut-off (0.749 ± 0.0749) in order to test the importance of the cut-off for VAD.” [page 9]

P. 8 Sensitivity analysis: “retaining only the most deficient children”: I think I know what you mean by this, but you might clarify this point to make it more clear in the text.

NDS response to reviewer: By setting the cut-off for VAD lower than 0.83 (by setting the corresponding value for DBS at 0.6741) only the most deficient children will be held in the VAD group. The following text was changed in the manuscript:

“Using the low cut-off for VAD thereby retaining only the most deficient children by setting the cut-off for VAD lower than 0.83, identified the same main risk factors for VAD in the large model with a few exceptions.” [page 14]

P. 9 “We found surprisingly few studies investigating the effect of VAS on vitamin A status” Could you cite those that you found here? Do you mean observational studies only or are you talking about trials as well?

NDS response to reviewer: We found two observational studies investigating the temporal effect of VAS. One from Indonesia (see reference 1 at the end of this response) and one from Ethiopia (cited in
manuscript). However, only the one from Ethiopia had registered VAS data prior to inclusion in the study. We have found a number of clinical trials testing the effect of VAS on vitamin A status but none within the study age group (see reference 2-8 at the end of this response). The following text was changed in the manuscript:

“In countries where VAD is a public health problem, the WHO recommends VAS every 4-6 months [26]. Several trials have evaluated the temporal effect of supplementation on VAD, but none including children within the study age group. We found only two studies investigating the effect of VAS on vitamin A status in the present age group. An observational study from Ethiopia reported increased VAD in children who had missed one or more doses of VAS within the last year [18]. The other observational study collected paired blood samples before and after supplementation and found a significant increase in plasma retinol 4 months after VAS [27]. We could not document any effect of missing VAS within the preceding 2, 4 or 6 months. Possible explanations could be geographical or methodological differences between the studies.”

Studies investigating the temporal effect of VAS:

Observational study


Clinical trials


