**Author’s response to reviews**

**Title:** Use of saliva to monitor meningococcal vaccine responses: proposing a threshold in saliva as surrogate of protection

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**Author’s response to reviews:**

Responses for Reviewer 1:

1. You refer to the independent variables as 'predictors' of stunting and 'predictors' of anemia. However, your analysis and interpretation is that of the association of several independent variables with either stunting or anemia. If your interest was to predict the occurrence of the outcomes, I was looking for 'r-square' in your analysis to explain how well the data fits the model. Unfortunately, I did not see r-square values presented in your paper.

Response: comment accepted; to make it clear, our intention was to assess association of several independent variables with either stunting or anemia. The word “predictors” here was to say independent variables that has association with either anemia or stunting rather than comparing them. But we accepted a comment.

2. Although the analysis for prediction and for association will essentially be the same, the analysis of predictor variables would necessitate 'testing' the fitted regression line with a fresh set of data to see if indeed the prediction is good. However, based on your discussion, I think your interest is to determine the association of the independent variables with the outcome/s.
Response: yes, of course our interest was to determine the association of independent variables with the outcome/s, i.e. for anemia and stunting separately. Variables were first checked by bivariate analysis independently with either stunting or anemia then candidate variables were entered into multivariable logistic regression to isolate independent variables by adjusting for other variables, model fitness was checked by Hosmer - Lemeshow.

3. I also have concerns about the temporal relationship between anemia and stunting. I understand that stunting is the result of long-term or chronic malnutrition. Thus, anemia (or the hemoglobin status) could affect height over a long period of time especially if anemia is a chronic problem. However, stunting cannot affect current anemia status, which is the one you measured, although stunted children would likely be also anemic. In other words, LAZ cannot be a predictor of anemia; they are most likely associated in cross-sectional studies.

Response: here we are reporting significant variables based on statistical outputs, another issue is that whether stunting has effect on anemia status of those stunted children in long run or not repeated measurement is needed in different times (prospective cohort study), that is the limitation of cross-sectional studies and we accept this as a limitation of our study and we will incorporate in our manuscript.

4. I am not sure if you did stratified analysis before you did the multivariate analysis. It will be good to see how the associations of interest behave when strong confounders are controlled for in the analysis using stratified analysis.

Response: it was not stratified but multivariable logistic regression analysis controls confounders by itself by adjusting for other variables.

5. Many independent variables were investigated in the study --- 19 for stunting and 19 for anemia. However, the authors failed to mention what variables were included in the full models before the final models (Tables 4 and 5) were obtained. At this point, I can only guess that the variables that were included in the full model were those which had p values less than 0.25 as explained in the report. Even if this is so, it will be good to explain what the full models contained (at the bottom of Tables 4 & 5) to help the readers understand better.

Response: comment accepted. To make it clear, there are a total of 19 variables were checked with stunting and then for anemia in bivariate logistic regression model. Among these variables those which had p<0.25 were entered in to multivariable logistic regression and those reported
variables in Table 4 and 5 were variables which had p<0.05. In Table 2 and 3 we put a strike (*) on those variables which had p<0.25 and there is also a foot note under each Tables.

6. I also have concerns about the definition and categories of introduction of complementary feeding -- in Table 1, the categories are 'earlier than 6 months' and 'just at 6 months' and the total is 477. This does not coincide with the categories in Table 2 which shows categories of 'before or after 6 months' versus 'at 6 months' and the total is also 477. It will be difficult to conclude what is the optimal time to give complementary feeding if the analysis includes a mix of children who were given CF before 6 months and those given CF after 6 months. This also goes against the desired practice of initiating CF at 6 months for mothers who exclusively breastfeed up to 6 months.

Response: comment accepted. It was earlier than 6 months. The one in Table 2, 3 and other where were typing error. Introduction time to CF was asked whether the child started feeding at any time before six month or just at six month you can see also in our questionnaire.

7. Some typographical errors need to be corrected. For instance, the age of the child cannot be 13.19 years. Although the text says anemia 6 months status is categorized as mild, moderate and severe, Table 3 only shows two categories -- anemic and not anemic.

Response: comment accepted.

8. Finally, the sample size computed was 498 but only 477 were included in the analysis. It will be good to explain the reasons for non-participation so that possible selection biases could assessed by the readers.

Response: comment accepted and we incorporated in the manuscript.

Responses for Reviewer 2:

Abstract:

Not all of these details are necessary in the methods section; for example, use of WHO anthro program and SPSS can be added as one line later in the methods section of the manuscript. I am
more interested in which specific outcomes were used (height-for-age z-scores, weight-for-age z-scores) and why. It is not relevant at this point of the paper which software program was used.

I assume binary models were run on anemia status, stunting, and wasting, but this is not mentioned in the methods section of the abstract. The author should include the exact measures in this section.

The first sentence of the abstract conclusion is not clear; does this mean higher relative to the national average in Ethiopia? Does this mean higher prevalence? The abstract conclusion refers to solutions oriented toward breastfeeding. What about solutions oriented toward some of the other findings, such as dietary diversity?

Responses:

- Comment accepted regarding limiting the details from the abstract.

- The specific outcome which we used was length-for-age z-score, since children in our study were under two years of age; length for age was used rather than using height-for-age z-score. In addition to this, our intention was to assess long term malnutrition, i.e. stunting rather than focusing on other types of malnutrition and anemia.

- The measures which were used were anemia and stunting, but there was no analysis done for wasting. Comment accepted regarding additional clarifications on measures; as the analyses were done for each outcomes separately and the exact measures used in our study.

- To mean higher prevalence was in relation to WHO categories/classification of public health importance.

- Comment accepted regarding conclusion part of abstract and incorporated within the manuscript.

Background

Line 56: parasites affect not just blood loss, but also ability to absorb nutrients. This is currently worded as if parasites only cause blood loss.

Response: comment accepted and incorporated
Line 61: Is this the underlying logic for including this age group in the model? If complementary foods are the main concern, perhaps breastfeeding patterns and frequency should be included in the analysis.

Response: yes, this was the underlying logic for including this age group

In our study, we made a consideration to the issue of breastfeeding practice by asking questions; frequency of breast feeding, appropriateness of baby position during breastfeeding, and/or whether switching to the next breast without finishing or not.

Lines 72-83 do not seem like they should be a series of one-line paragraphs. If this is a formatting issue, resolve.

Response: comment accepted.

Line 70: Is there any explanation for this observed increase in anemia within the literature for the period 2011 and 2016?

Response: the 2016 report of Ethiopian demographic health survey was a key indicators report and it have no details.

Line 77: why is stunting so much lower in Kemba Woreda, and what is the significance of this (or not?)

Response: the reason which was described on that paper was due to the narrowing of age of children (that was 6-23 months) in the study making the magnitude of stunting declined as compared to studies having wide target children <5 years of age and institution based. Significance of this Kemba Woreda’s study is that, it is a nearby district to our study area which makes it suitable for comparison and to compare the discrepancy whether it varies or not.

Sample Size and Sampling Procedure

Line 87: Is there a village-level fixed effect to account for differences in villages? Are there enough observations per village to do this? I see no area-level controls in the attached tables.

Response: “Fixed effect”……not clear for me what to mean. Regarding selection of children, villages were selected first by lottery method, which gives equal chance for each villages to be selected, then children were selected from selected villages in proportion to population size. At
the beginning we considered villages as homogeneous and our intension is to show district level prevalence and its associated factors.

Line 94: What was the actual prevalence of anemia in this area at the end (66.6% seems like a high prevalence to base sample size estimation on). Consider referring to the observed prevalence in line 95. If the condition was suspected to be more prevalent than it actually was observed to be, you may have under sampled.

Response: until this study’s proposal had been written there was only one research published in this age category, except the one with a prevalence of 66.6% in Ethiopia to consider for anemia, of course, there were many for stunting and key reports of national data of EDHS 2016, which was 72.3% prevalence, after comparing for these decision was made with 66.6% prevalence of anemia. And similarly for stunting.

Line 101: The phrase 'family folder' is unclear.

Response: It is a government prepared data that contains list of individuals within a households. It also have data on date of birth, sex, age, immunization history of children, family planning history of mothers, ANC, delivery and PNC. Each house hold have their own family folder with a code and these folders were shelved in health posts throughout the country. This method of tracking is said to be Community Health Information System (CHIS).

Data Collectors and Measurements

Line 122: It is not necessary to spell out the degrees of the individuals taking measurements.

Response: comment accepted.

Laboratory investigations

Line 135: Why was adjustment for smoking within the household made? Is there a citation saying that a different hemoglobin level should be used if an individual in the home smokes?

Response: this was because if there is an individual living in a household that the sample was taken the residing child was considered as a passive smoker and if a person smokes it is recommended by WHO to adjust for smoking.
Data Management

Line 163: What proportion of anthropometrics were in implausible ranges? What is the attrition rate? It will be difficult to make inferences about the generalizability of your results without a clear understanding of how much of the sample is dropped.

Response: a total of 21 samples were dropped due to incompleteness, which was either for lack of information for hemoglobin count or length or date of birth. Those flagged by WHO Anthro were dropped. Among dropped ten were because they were flagged which accounts 2% the total sampled children 498. The rest 11 were because of lack in information on hemoglobin count.

Line 160: Eliminate this wording from the initial abstract. There is no need to present this part twice.

Response: comment accepted.

Statistical analyses

Lines 167-171. If the authors mean what I suspect, they should substitute 'bivariate' for 'simple' in this case. If there is only one explanatory variable and one outcome, it is a binary regression - it then seems the authors used this for model building, which is fine, as long as any non-statistically-significant but theoretically important predictors were still included in the multiple regression.

Response: comment accepted.

Operational Definitions

Line 174: this is < -2 standard deviations from the reference median. This is where you should discuss the origin of the WHO Multicenter Growth Study.

Response: yes it was from WHO Multicenter Growth Study.

Line 177: Ensure the first time you mention the World Health Organization it is completely spelled out and then the acronym is applied.

Response: comment accepted.
Lines 180, 182: Definitions do not seem mutually exclusive; please clarify each of them. Are they both meant to be 8 hours or 8 times?

Response: comment accepted. It was frequency of breast feeding and it was “times”.

Results

Line 187: This needs to be clarified much sooner, response rate does not belong in the results section.

Response: as per my understanding response rate is reported in result part. Regarding clarifications of for those dropped we accepted a comment.

Line 187: Should this be 13.69 months? The way the sentence is written it seems as if both quantities given are in years.

Response: comment accepted.

Line 195: The sample size estimate was based on a stunting rate of ~60%, correct? What are the implications of this?

The question is Not Clear for me ….I didn’t get the line and … “a stunting rate of ~60%”

Line 202: Again, why adjust for smoking? Is this a common practice? Is this due to policy concerns regarding smoking?

Response: adjustment is recommended by WHO for altitude and smoking. This is because at very high altitudes, where the quantity of oxygen in the air is greatly decreased, insufficient oxygen is transported to the tissues and RBC (red blood cells) production is greatly increased. In this case, it is not the concentration of RBCs in the blood that controls RBC production but the amount of oxygen transported to the tissues in relation to tissue demand for oxygen. Similarly, as a person smokes an oxygen transport from lung to blood vessels decreases and this increases hemoglobin in circulating bloods, thus WHO recommends adjustment? The principle why we adjusted was, if any a person in a household smoke, the children would be passive smoker and that’s why it needs adjustment (Ref. 11).

Line 213: Previously it seemed anemia was one of the outcomes; should anemia be fit as an interaction with drinking water from an unsafe source to attempt to understand the role of parasites in this sample?
Response: lack of parasite investigation in this study is one of the limitation.

Line 221: Again, is there a village-level fixed effect? Relationships between exposures and outcomes are bound to vary by residence, especially if some drinking water sources are safer than others for instance. (For example, if a well is contaminated in one village and not another, this will affect the water source coefficient).

Response: the analysis was done equally without considering for any village level variations.

All sub villages are considered as homogeneous.

Discussion

Lines 226-234: A map of supplementary figure illustrating regions' prevalence might be most useful for this portion of the discussion.

There is no detailed report of each regions on this age category (6-23 months) rather there is only 6-59 month’s report even which is the cumulative total of the country. There was no anemia report even in 2014 mini EDHS report.

The other thing is using 2011 EDHS report is not appropriate because it is old enough to use and there are a signs of changes in trends in prevalence of anemia.

Let you see these figures and comment…..

Figure 2: 6-59 months prevalence of stunting across regions of Ethiopia, according to 2016 EDHS key indicators report.

N.B: there is no 6-23 months regional prevalence of stunting across regions in 2016 EDHS key indicators report.

Conclusion
Line 277: This section needs to be expanded. What are some of the long-term implications of the results? What are some of the priorities that should be set, and how does this tie back in to the regional variation discussed earlier in the manuscript?

Response: Comment accepted;

Line 280: There are no limitations discussed or listed of this study; first, the power calculations seem problematic given the higher anticipated prevalence of stunting. Second, why are height and other nutritional statuses (height for weight) not considered? Rather than listing prevalence of stunting across regions, why might this region have had less than anticipated? The meaning of the findings needs to be explored in more depth.

Response: Comment accepted