Our comments are found in line with the reviewer comments and are highlighted.

Reviewer 4

This study examines the relationships between mercury exposure using human biomarkers (primarily hair), health status indicators (hemoglobin, anthropometry), and vaccine response to 6 vaccines (immunoglobin levels, specific antibody levels). Both main effects and interactions are being examined. This is a very dense study and the report is quite rich and data perhaps somewhat difficult to follow because of the great amount of information included. The report should be of great interest to the readership of the journal and those working in this field as well. It is well written. The authors find that mercury levels in the hair of children in these areas in Peru, especially those that are from indigenous communities, are elevated and associated with decrements in antibody levels. They also report that malnutrition as a main effect decreases antibody levels and that mercury and malnutrition interact as well as to decrease specific antibody levels. There are also interesting increased levels of antibodies in some cases, for example, an interaction between mercury in hair and lower nutritional status that is associated with an increase in tetanus-specific antibodies. Can the authors speculate a bit more on the mechanism(s) behind an increase in antibody levels that can be attributed to higher levels of mercury exposure and/or greater malnutrition?

Impacts to humoral immunity could occur through many diverse mechanisms. We postulate that the increased antibody levels associated with mercury exposure and malnutrition may relate to a pro-inflammatory response. Interferons are cytokines involved in cell signaling and have been observed to mediate innate and adaptive immune responses ([1]). Interferon gamma (IFN-gamma) is an interferon that is associated with mounting appropriate immune responses against parasitemia ([2]) and humoral immune responses against measles ([3]). Though IFN-gamma may not impact all humoral immune responses similarly, decreases in IFN-gamma could be associated with reduced antibody titers. Malnutrition has commonly been associated with decreases in IFN-gamma ([4, 5]), up to 36% lower production of IFN-gamma was noted in one study ([6]). In young children in this study, lower nutritional status was associated with reduced pertussis, measles, and hepatitis B antibodies. Mercury on the other hand, both methylmercury and inorganic mercury, has consistently been correlated with inflammatory cellular responses that include increased IFN-gamma ([7-9]). With the long-term nature of mercury exposure in our study region, it is possible that elevated cellular IFN-gamma could increase pathways associated with antibody production. However, it is important to note that though increases in antibodies were observed in a few instances in this study, they were for the most part modest in magnitude.

I am not asking the authors to find and include data on actual disease incidence and prevalence in children, but if there is any evidence that some of these select diseases, those for which these vaccines are meant to be protective, are elevated that would be very useful to include. If vaccine efficacy is negatively affected by mercury and/or malnutrition, one would expect their associated diseases to be more of problem where those conditions are more prevalent. Can the authors speculate a bit more on this if such data is not readily available? If such data is readily available and a greater incidence or prevalence of disease is not apparent, that needs to be discussed.

Data on disease prevalence is available, however the quality of the data varies greatly by region. However, even though the reporting system in MDD is poor, greater instances of many diseases are reported. Ministry of Health posts yearly disease summaries by region and of the diseases relevant to this study, data is available for HepB. In 2018, the incidence of HepB was reported to be 3.5/100,000 in Lima and 68.9/100,000 in MDD. The difference in HepB between these regions is likely more related to reduced HepB vaccination rates in MDD. ([http://www.dge.gob.pe/portal/index.php?option=com_content&view=article&id=14&Itemid=154][1]) ([http://www.dge.gob.pe/portal/index.php?option=com_content&view=article&id=647][2]) From our results we would speculate that measles incidence would be greater in communities with higher hair Hg, more impacted by ASGM. Measles was the only antibody where ‘protection’ levels were impacted. We would not expect greater incidence of the other diseases with relation to hair Hg. If measles were higher in these communities, would need follow up studies to determine action to better antibody response, perhaps additional vaccinations.
I recommend publication of this manuscript in IJERPH pending quite minor improvements and considerations.

Specific Comments

Results

Section 3.2.3. (and other relevant sections)-The text in this section (lines 376-379) does not seem to be consistent with what is in Figure 4 panel A. This figure indicates increased antibodies for 4 vaccines and no decreased antibodies. The text states there are decreased measles antibodies. If levels whether apparently increased or decreased are not significantly different, I suggest not reporting those as such. This approach in reporting results should be followed throughout. As clearly stated in the methods sections with respect to levels considered non-responsive, the authors should stay true to their statements.

We thank the review for catching this mistake in reporting our results. We meant to refer to the decreased measles antibodies in older children. We have updated lines 364 to note this distinction. Additionally we have made reference to the age groups more apparent in the legends for Figure 4 and S1.


