PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Impact of iron fortification on the geo-spatial patterns of malaria and non-malaria infection risk among young children: a secondary spatial analysis of clinical trial data from Ghana
AUTHORS	Aimone, Ashley; Brown, Patrick; Owusu-Agyei, Seth; Zlotkin, Stanley H.; Cole, Donald

VERSION 1 - REVIEW

REVIEWER	Bonnie Mappin University of Queensland, Australia
REVIEW RETURNED	19-Sep-2016

GENERAL COMMENTS	This is detailed and well written manuscript however there is no clear conclusion from the research or actionable suggestions. Therefore, I think further work is required to be able to address the impact of iron fortification on the geo-spatial patterns on the risk of infection. Here are my detailed suggestions: 1. Title should be updated to '…geo-spatial patterns of malaria and non-malaria infection risk…' 2. The conclusion in the abstract is not validated in the paper. Until further work is completed I don't think it is demonstrated that there is a predictable graphical variation from iron fortification. 3. The first reference should be updated from 2014 to 2016 as it has been updated. The content of the reference has not changed. 4. How long was the study population under the trial? This should be clear in the methods. I have looked up the reference and I think its for 6 months, yet in table 2 September-November 2010 is listed, so was it only 3 months? 5. Land cover data is available annually – I am not sure from your reference if you are using the land cover data from the year of the trial. 6. Reference 21 says that NDVI is a proxy for moisture not soil moisture. Please remove soil. 7. Additionally, I think it worth mentioning that NDVI is a vegetation index and that it is useful in characterising the vector habitat quality with a references such as Guerra C,

	Snow R, Hay S. A global assessment of closed forests, deforestation and malaria risk. Ann Trop Med Parasitol. 2006 8. The subtitle independent variable preparation, I think is not accurate. Land cover, elevation and NDVI are often used in predicting malaria prevalence. 9. Given none of the spatial variables demonstrated significant associations with endline infection status and that the selection of the three used is not exhaustive. I think your analysis may benefit from expanding these spatial datasets. Consider Weiss et al Re-examining environmental correlates of Plasmodium falciparum malaria endemicity: a data-intensive variable selection approach – explores all spatial environamental datasets that have been used in malaria mapping and develops variables that best explain the variance. 10. There is very little analysis comparing the results of the with iron to without iron groups (including not including their result tables in the main manuscript). There needs to be more analysis to show the impact of the iron on the infection risk – not on the baseline to endline but in reducing infection risk. 11. Table 2 should have combined added before Iron and No-iron groups. 12. The discussion needs to address whether the conclusions of this study is conclusive enough of overwrite the conclusions of the reference 8 and make a clear conclusion if the authors think it is deemed safe to provide iron supplements to children that may be iron replete. 13. In your conclusions you say 'future research should include biomarkers of non-malaria infection…'. Can you be more specific than future research? What sort of research are you referring to? Malaria or non-malaria based? In what context?

REVIEWER	Anna Chmielewska Department of Clinical Sciences, Umeå University, Sweden
REVIEW RETURNED	03-Oct-2016

GENERAL COMMENTS	This is a report of geospacial patterns - related risk of infection in children who received iron supplementation vs placebo for 5 months in rural Ghana (malaria endemic region). Iron deficiency is very common in Africa and iron supplementation policies are of great importance. However, due to a considerable concern of increased risk of malaria and the related mortality esp. in iron replete children, iron fortification programs in Africa had been limited after the WHO and UNICEF joint recommendation (2006). As for a primary outcome of this RCT, the Authors have shown that iron supplementation did not increase the risk of malaria in this endemic region if the bed nets treated with insecticides and a proper malaria treatment were available. Recently, they have also published the analysis of the geo-spacial patterns associated with infection risk in this cohort at baseline . (Malar J. 2016 Jul 8;15:349) The current analysis accounts for the impact of receiving iron supplementation or not. Conclusions are very much similar to those from the Malaria J. paper: the further the distance to the nearest health care centre and lower the altitude, the higher the risk of infection.

	The primary RCT was a study of high methodological quality and extreme clinical importance. The current paper is very well written and both reviewer- and reader-friendly. All items required by the CONSORT Statement are provided and the check list is attached to the manuscript. Tables are well organized, self-explanatory and easy to follow. However, the impression I had when reading the paper was that is does not add very much when one is familiar with the proviously reported primary results from this important and well-conducted trial. Similar risk of malaria in iron-supplemented and unsupplemented children was a clear massage: is spite of a high rates of parsitemia at baseline, iron administration did not increase the risk of subsequent parasitic infection. Since the aim of the current analysis was to study the geo-spacial factors related to infection status after the intervention, then I feel it would be valuable if the link between the geo-spacial factors at baseline and after the intervention was made. Did they change and does it matter? Also, a short description of how these results could be implemented in iron fortification programes for those readers who are not familiar with this kind of health initiatives. Line 24: please rephrase „of a change to support problem“ to other expresion to make it more clear Line 40: delete „at“ after „baseline“

VERSION 1 – AUTHOR RESPONSE

Reviewer #1:

1. Title should be updated to "…geo-spatial patterns of malaria and non-malaria infection risk…"
Authors' response: The title has been changed accordingly (page 1, top).

2. The conclusion in the abstract is not validated in the paper. Until further work is completed I don't think it is demonstrated that there is a predictable graphical variation from iron fortification.
Authors' response: The word "predictable" has been removed from the conclusion of the abstract (Page 2, bottom).

3. The first reference should be updated from 2014 to 2016 as it has been updated. The content of the reference has not changed.
Authors' response: Reference 1 has been updated (page 3, bottom).

4. How long was the study population under the trial? This should be clear in the methods. I have looked up the reference and I think it's for 6 months, yet in table 2 September-November 2010 is listed, so was it only 3 months?
Authors' response: Each study participant was under trial for 6 months; however, not all subjects started the intervention at the same time. Therefore, endline infection measures were collected over a course of approximately 2 months (Sep-Nov). The authors agree that this is not clearly explained in the Methods section or in Table 2, and thus both have been revised accordingly (page 5, top and title of Table 2, page 11).

5. Land cover data is available annually – I am not sure from your reference if you are using the land cover data from the year of the trial.
Authors' response: Reference 20 has been revised to better reflect data product used and year of

collection (page 6, top).

6. Reference 21 say that NDVI is a proxy for moisture not soil moisture. Please remove soil.
Authors' response: The word ‘soil’ has been removed in the Methods section (page 6, top).

7. Additionally, I think it worth mentioning that NDVI is a vegetation index and that it is useful in characterizing the vector habitat quality with a reference such as Guerra C, Snow R, Hay S. A global assessment of closed forests, deforestation and malaria risk. Ann Trop Med Parasitol. 2006.
Authors' response: Thank you for this suggestion and very useful reference. Both have been added to the text in the Methods section (page 6, top).

8. The subtitle ‘independent variable preparation’ I think is not accurate. Land cover, elevation and NDVI are often used in predicting malaria prevalence.
Authors' response: The sub-heading in the Methods section has been changed to “Variable preparation” (page 5, bottom).

9. Given none of the spatial variables demonstrated significant associations with endline infection status and that the selection of the three used is not exhaustive, I think your analysis may benefit from expanding these spatial datasets. Consider Weiss et al. Re-examining environmental correlates of Plasmodium falciparum malaria endemicity: a data-intensive variable selection approach – explores all spatial environmental datasets that have been used in malaria mapping and develops variables that best explain the variance.
Authors' response: Thank you for this suggestion and helpful resource. The potential benefits of conducting future research to expand the spatial datasets have been described in the Discussion section (page 16, top).

10. There is very little analysis comparing the results of the with iron to without iron groups (including not including their result tables in the main manuscript). There needs to be more analysis to show the impact of the iron on the infection risk – not on the baseline to endline but in reducing infection risk.
Authors' response: Added analysis and discussion around the potential impact of the intervention on infection risk in the Results and Discussion sections (page 9, bottom - Results; and page 13-14 - Discussion).

11. Table 2 should have combined added before the No-iron groups.
Authors' response: The word “combined” has been inserted into the title for Table 2 (page 11).

12. The discussion needs to address whether the conclusions of this study is conclusive enough to overwrite the conclusions of the reference 8 and make a clear conclusion if the authors think it is deemed safe to provide iron supplements to children that may be iron replete.
Authors' response: Thank you for this suggestion. Overwriting the conclusions from the Pemba study is a complex issue because, unlike the trial in Ghana, the study participants in Zanzibar were not provided with malaria prophylaxis or treatment. The results of the current study supported the main findings from the Ghana trial (i.e. did not demonstrate an adverse effect of the iron intervention on clinical malaria risk in the context of universal provision of bed nets and malaria treatment when indicated); however, they are not conclusive enough to refute the findings of more recent research (in mice) suggesting that other pathogenic infections are also of concern when it comes to potential adverse interactions with supplemental iron. What we can say at this point is that geographical location likely plays a role in infection risk, and thus may provide a complementary means of assessing or predicting the safety of iron interventions in malaria endemic areas, as well as the need for concurrent infection control measures (pages 13-16).

13. In our conclusion you say ‘future research should include biomarkers of non-malaria infection…’

Can you be more specific that future research? What sort of research are you referring to? Malaria or non-malaria based? In what context?
Authors' response: We agree that this statement is not very clear or specific as written, nor does it necessarily contribute to the fluency of the concluding paragraph. It has therefore been removed (page 17, bottom).

Reviewer #2:

14. Line 24: please rephrase „of a change to support problem“ to other expression to make it more clear
Authors' response: A “change of support problem” is also referred to in the literature as “ecological inference”, which is perhaps more accessible to a broad readership. This term has thus been added to the corresponding sentence in the “Strengths and limitations” section (page 3, middle).

15. Line 40: delete „at“ after „baseline“
Authors' response: This typo has been removed from the “Measures from trial data” sub-section (page 5, bottom).

16. The impression I had when reading the paper was that is does not add very much when one is familiar with the previously reported primary results Since the aim of the current analysis was to study the geo-spatial factors related to infection status after the intervention, then I feel it would be valuable if the link between the geo-spatial factors at baseline and after the intervention was made. Did they change and does it matter? Also, a short description of how these results could be implemented in iron fortification programs for those readers who are not familiar with this kind of health initiatives.
Authors' response: Included the reference for our published baseline analyses (Malaria Journal), as well as additional phrasing in the Discussion section comparing baseline and endline findings with respect to geo-spatial factors. Results from these analyses could be applied in the assessment or planning stages of micronutrient programs in order to determine where child populations may be at greatest risk of infection (malaria and/or non-malaria), and thus where caution should be used when implementing a population iron intervention (page 15-16 - Discussion).

VERSION 2 – REVIEW

REVIEWER	Bonnie Mappin University of Queensland, Australia
REVIEW RETURNED	03-Jan-2017

GENERAL COMMENTS	Thank you for the updates and the changes made. I have one additional comment: Given your response to my comment 4 about the length of trial and listing of 6-month in methods, please check if the 5-month description in the abstract needs to be updated to be consistent?

VERSION 2 – AUTHOR RESPONSE

Reviewer: Given your response to my comment 4 about the length of trial and listing of 6-month in methods, please check if the 5-month description in the abstract needs to be updated to be consistent?

Authors' response: Thank you very much for drawing our attention to this inconsistency. The 6-month

and 5-month trial durations are in fact both correct, as each participant received the iron intervention (or control) for 5 months and then were followed up for an additional 1 month after the intervention was discontinued. Therefore, while the total length of the trial was 6 months, only data from the 5-month intervention period were used in the present analysis. We have, adjusted the methods section (rather than the abstract) to reflect this (pages 5 and 7).