Author's response to reviews

Title: Patterns of mixed Plasmodium malaria infections among children six years and under in selected malaria hyper-endemic communities of Zambia: population-based survey observations

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Author's response to reviews:

Reviewer: Wilfred Mbacham

Comment 1: Page 5 Line 21 – The RDT should be stated which of the antigen detection systems are used
Response: The RDTs currently recommended and in use in Zambia are Plasmodium falciparum specific detecting the histidine-rich protein 2 (HRP2) antigens. This information has been included on page 5 line 22.

Comment 2: Page 5 Line 23 – “Determine the burden of mixed Plasmodium species. In this sentence burden should be replaced with proportion
Response: The term ‘burden’ has been replaced by ‘proportion’ page 5 line 25.

Comment 3: Page 6 Line 17 – “Verifiable information” Authors should state - what was checked for!
Response: The verified information was records and actual presence of RDTs results, blood smears and dried blood spots (DBS) for all children below 5 years of age. This information has been clarified on page 6 line 18-20.

Comment 4: Page 8 Line 7 - “Consent “should be changed to “Assent”
Response: Assent in Zambia is only applicable to children of ages between 7 to 17 years and thus no assent was employed as all the children in the survey were below the age of 6 years. The word “consent” on page 8 line 10 and 11 has, therefore, been retained as this study used data collected during the survey were consents were obtained from the parents/guardians of the below 6 years old children.

Comment 5: Page 9 Line 15-20. The authors did not measure burden and so should refrain from using this word. Instead they should use “prevalence” to account for malaria as a leading cause of morbidity or mortality in Zambia.
Response: The term ‘burden’ has been replaced by ‘prevalence’ on page 9 line 20 and line 23

Comment 6: I do not agree with the statement “This suggests that there is need for continued monitoring of the non-falciparum infection prevalence in this population so as to decide when species-specific RDTs should be introduced for diagnostic purposes”

Response: Plasmodium species-specific diagnosis is important in the context of malaria elimination in Zambia. As the disease prevalence declines and as the country moves towards elimination, there is need to detect all species and institute respective specific treatment. For instance, the current national recommended first line drugs, ACTs, are pharmacologically not effective against non-falciparum species that have hypnozotes and this may, in the absence of species-specific diagnosis and treatment, hinder the elimination efforts. A sentence to this effect has been added to further explain the statement in question, on page 10 line 12-15.

Comment 7 Page 10 Line 20-25 - There is no scientific basis for selective protection again one and not the other and might be an effect of exposure than of protection.

Response: We do understand the reviewer’s perception that there is no scientific basis for selective protection against one and not the other with respect to our study observations where less than 2-year-olds children had a relative lower incidence of mixed plasmodia infection compared to their older under five counterparts. This could indeed be an effect of variation in exposure but, ordinarily, one would have expected that the older than 2 years under-fives must have been expected to be more exposed and thus better protected on the basis of acquired immunity. Hence as postulated in the discussion of the current study findings, we thought the well documented maternal passive immunity that, overall, makes infants in malaria endemic areas (Moormann et al, 2009) have less episodes of malaria than their older under five counterparts was the closest plausible explanation. Indeed, the dynamics of immunity in the neonates, infants and toddlers is quite remarkable resulting to variable susceptibility to infection (Doolan et al., 2009 and Moormann, et al, 2009). The limitation in the current study is that, unlike other studies, this was a survey utilising stored sample assessment and only of children under five and not the other age group children. Hence the difficulty to emphatically make conclusions of this passive immunity postulation. To this end, the discussion has now been strengthened on page 11 line 7-11 highlighting these statements.

Comment 8: Figures are not proper labelled or provided with appropriate legends.

Response: The figures have been labelled with appropriately titles.

Comment 9: Spellings should be checked in Fig 1 for P. malariae, or abbreviations cross-checked NMCC or MNCC or Centre for “Canter” in the highlights.

Response: The spelling have been corrected: Page 12 line 6- NMCC, Centre,
Comment 10: Table 1 is standard primers for speciation and published elsewhere and should just be made reference to rather than have it included as a table
Response: The table has been deleted.

Reviewer: Olovia Achonduh-Atijegbe
Comment 1: Abstract: Prevalence of non-falciparum species should be mentioned in the abstract. Specifically state the prevalence of type of mixed infection (e.g. Pf/Pm/Po etc). Not clear in the abstract whereas it is the focus of the study.
Response: A sentence has been added in the results section page 2, line 22-23 that talks about the mixed infections. We could not add more information on the other combination as this will make the abstract longer than specified by journal.

Comment 2: Methods; Page 6 Line 18; what type of RDT was used (specify the antigen(s))
Response: The type of RDT used has been included on page 6 line 20-21.

Comment 3: Page 7 line 5; Delete table 1 since method used is already cited.
Response: The sentence referring to table 1 and the actual table 1 have been deleted.

Comment 4: Page 8 line 11; Include the reference number of the ethical approval
5. Page 9 line & Page 10 line 5: Replace “burden” with prevalence
Response: The reference number for the mixed infection study was 001-01-12 and for the 2012 Malaria Indicator survey was 002-03-12, these have been indicated on page 8 line 9 and line 16

Comment 5: Page 9 line & Page 10 line 5: Replace ‘burden’ with ‘prevalence’.
Response: The word ‘burden’ has been replaced with the word ‘prevalence’ in the discussion page 9 Line 20 and 23.

Comment 6: How will these findings influence malaria treatment in these regions? No clear in the current discussion
Response: The current treatment policy for Zambia recommend the use of coartem that is not effective against hypnozoites. As Zambia moves towards elimination, there will be need to diagnose these species and institute specific treatment. This information has been included on page 10 line 10-15.

Comment 7: What is the difference between figure 2 and table 4? The information in figure 2 can be included in table 4.
Response: Figure 2 shows the prevalence of mixed and mono-infections while table 4 shows the proportion and combinations of the mixed with dominator being only the positive, but we have deleted figure 2.

Comment 8: Page 21 line 9: Key is for figures 1 NOT figure 2.
Response: The legend has been moved to the correct table. Secondly since
Minor comments
Comment 1: Page 27 Line 3: Revise the Notes (legend), the message is not clear.
Response: The legend has been revised, it now reads 'All positives is the n=474'.
Comment 2: Include appropriate legends for all figures and tables
Response: Appropriate legend have been included
Comment 3: Check spelling errors e.g ; Figure 1. P. malariae etc
Response: The spelling have been corrected, figure 1, 'malariea' has been corrected now as written as 'malariae' on page 20