Author's response to reviews

Title: Plasmodium falciparum parasitaemia and malaria in pregnant women at first clinic visit in the mount Cameroon Area

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Author's response to reviews: see over
The Editor,
BMC Infectious Diseases.

Dear Editor,

We are re-submitting for review and consideration for publication, our revised manuscript entitled:

*Plasmodium falciparum parasitaemia and malaria in pregnant women at first clinic visit in the mount Cameroon Area*  Anchang-Kimbi JK, Nkweti VN, Ntonifor HN, Apinjoh TO, Tata RB, Chi HF and Achidi EA. We thank the BMC Infectious Diseases journal for giving us the opportunity to revise our manuscript and it is obvious to us that the revision has greatly improved on the quality of the manuscript.

We have examined, made corrections and replied to comments suggested by the reviewer in the forwarded ms text attached to this mail. As requested, we have responded point by point to the reviewer’s comments as indicated below. I wish to inform the Editor that all the authors have read and approved the revised version of the manuscript and authorized me to submit it on their behalf.

We look forward to a fruitful review of the article.

Thank you.

Yours sincerely

Anchang-Kimbi JK
This study focused mainly on *P. falciparum* parasitaemia and malaria in pregnant women at first clinic visit in the Mount Cameroon area. For clarity, the title of this work is revised as follows: *P. falciparum* parasitaemia and malaria in pregnant women at first clinic visit in the mount Cameroon Area.

1. We note here that the mount Cameroon area is a hyperendemic for malaria thus malaria parasite infection may account for the majority of febrile illness as shown in the results (shown in point 2). Previous studies carried out in Mt Cameroon area by Kimbi *et al*. Malaria Journal 2013, 12:193 showed a significant association between malaria parasite infection and fever in school-aged children and malaria clinical infection status (symptomatic vs asymptomatic) differed with haematological parameters. After data verification mean WBC counts between febrile ($2.79 \times 10^9$/L) and afebrile ($2.80 \times 10^9$/L) cases were similar. The absence of a significance difference in haemoglobin levels, WBC counts or platelet counts between symptomatic and asymptomatic women can be explained the fact that the malaria cases were probably mild disease cases or limited sample size for comparative analysis. Finding have been reported and discussed accordingly (lines 224-225, 284-286).

The authors do agree that other infections beside malaria parasite infection, cause febrile illness among the pregnant women. In this study, the prevalence of HIV infection was 5.9%. Further analysis showed that HIV positive women (10.9%) were more likely (OR = 3.26; 95% CI= 1.1 – 9.7; P = 0.026) to present with fever than without fever (3.6%). However, a majority (71.4%) of the febrile HIV positive women were co-infected with the malaria parasite (lines 218 -221).

Fokam *et al*. East African Medical Journal 2010: 87 (7) reported a seroprevalence of 5.1% and 2.5% for Dengue virus-2 and Dengue virus-1 respectively in the mount Cameroon area. It is possible that HIV, Dengue and others may not be major contributors of fever illness among pregnant women in this area. Nonetheless, it will be interesting for future studies to address other possible causes of fever illness among pregnant women.
2. Lines 206-213: The proportion of women with a febrile illness who were parasitaemic has been clarified and evidence indicating that parasitaemia is significantly associated with fever has been revised in the manuscript (lines 208 - 218) as per the response below.

Temperature was recorded for 257 women out of whom, 24.9% (64/257) had fever (temperature >37.5°C). Malaria parasitaemia was significantly ($\chi^2 = 62.34; P < 0.001$) associated with febrile status where a significantly higher proportion of women with febrile illness (64.1%; 41/64) were parasitaemic for *P. falciparum* infection when compared with aparasitaemic febrile cases (35.9%; 23/64). The 41 parasitaemic febrile cases correspond to the symptomatic (malaria) cases involved in the analysis. After data verification, 27/68 had asymptomatic infection and not 16/68 as previously indicated. Since temperature was recorded for 257 women, therefore, the recalculated overall prevalence of symptomatic and asymptomatic infection is 16.0% (41)(95%CI = 11-20%) and 10.5% (27) (95%CI = 7.3-15%) respectively and not 13.5% (41/303) and 8.9% (27/303). A total of 189 (73.5%) women were uninfected. Majority of the malaria cases (61% 25/41) were women who reported at the clinic during unscheduled clinic days meanwhile most of the asymptomatic parasitaemic cases (92.8%; 25/27) were recorded during scheduled visits.

3. Lines 269-271: Section has been revised and effected in the manuscript as per the response of comments above (Lines 269-281).

4. A brief summary of the relevant entomological literature (noting the local vector populations proven in earlier research) specific to this setting has been provided (see relevant literature below) to strengthen the contention that water source and vegetation proximity to households was associated with increased risk of malaria. Thus the discussion section (line 299-311) has been revised for better understanding. Also, the section on the study area of the manuscript (Lines 110-123) has been re-worded as per relevant entomological literature on the local vector populations from previous studies in the study area.

A study by Tanga *et al.*, 2010; Transactions of the Royal Society of Tropical Medicine and Hygiene (2010) revealed that anopheline mosquitoes are predominant (82.73%) and diverse (*Anopheles gambiae* s.l. (56.86%), *An. funestus* s.l. (32.57%), *An. hancocki* (9.38%), and *An.
nili (1.18%) compared with Aedes with two species (Culex and Mansonia). An. gambiae is proportionately more abundant throughout the year and show peak of abundance towards the rainy season. Anopheline species are highly anthropophilic and exophilic (particularly An. gambiae) with a human blood index (HBI) of 99.05%. The high survival rates of the malaria vectors (mean probability of daily survival of 0.92, annual mean life expectancy of 21.9 days and expected mean infective life of 7.4 days) suggest a high vector potential for the species.

Wanji et al. J Vector Borne Dis. 2009 46(1):75-80. characterised Anopheles breeding sites in the mount Cameroon area during peak to late rainy season period (August-November) The study revealed that about 94% of the breeding sites were temporary water bodies. Of these temporary water bodies, 80.8% were productive breeding sites (contained Anopheles larvae) and majority (85.7%) found within 20 m from the nearest inhabited house while 21.4% breeding sites were located between 20 and 50 m from the nearest inhabited houses. None were found beyond a distance of 50 m. Furthermore, Tanga et al., 2010 reported a high parous (an indication of haematophagy) rate (≤ 70%) for all anopheline species suggesting availability of potential breeding sites close to human residences.

5. Lines 105-111: has been removed as requested.
6. Figure 1: The ‘clinical malaria status’ has been changed and title read thus: ‘Prevalence of malaria and asymptomatic P. falciparum infection in pregnant women at scheduled and unscheduled clinic visit in the Mutengene and Muea Medical Centres.’

**Minor Essential Revisions**

1. Line 23: has been re-worded to read thus ‘Pregnant women in malaria endemic areas are at high risk of P. falciparum infection and its complications.’
3. Line 76: adverse consequences of malaria in pregnancy have been outline in lines 54-56.
4. Line 91: clarified as ‘malaria has been shown to alter haematological parameters’ (line 81-82)
5. Line 94: Relevance to pregnant women has been alighted. (lines 81-87)
6. Line 102: ‘risk factors that predisposed… effected as risk factors associated with’. (line 95)
7. Lines 117-118: Word ‘starts in’ has been replaced with ‘runs from’ (lines 105 – 107)
8. Line 123: ‘infectivity bites corrected as infective bites’ (line 118)
9. Line 146: word axillary removed
10. Line 201: FCFA means franc des Communautés Financières d’Afrique. (line 200-201). The income stratification used was based on self-reported monthly income. (line 157)
Major comments

1. Lines 105-111: has been removed from introduction as requested.

2. Line 137 – Malaria treatment and prophylaxis during pregnancy have been explained and added to the introduction section of the manuscript. Regardless of symptoms, in areas of stable malaria transmission of sub-Saharan Africa, intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) (IPTp-SP) is recommended for all pregnant women at each scheduled antenatal care visit (at least one month apart) up to the time of delivery. Treatment of falciparum malaria involves administration of quinine plus clindamycin (if available) and if treatment fails, Artesunate combined therapy (ACT) is recommended (lines 62 - 67).

3. The authors do agree that other infections beside malaria parasite infection cause febrile illness among the pregnant women. This study focused mainly on *P. falciparum* parasitaemia and malaria in pregnant women at first clinic visit in the Mount Cameroon. We also note here that the mount Cameroon area is a hyperendemic for malaria thus malaria parasite infection may account for the majority of febrile illness.

After data verification, no significant difference in WBC counts was observed between febrile (2.79 x 10^9/L) and afebrile cases (2.80 x 10^9/L). Lymphocyte percentage was the only leucocytic change associated with malaria infection and fever in the present study. The absence of a significance difference in haemoglobin levels, WBC and platelet counts between symptomatic and asymptomatic women may be that most of the symptomatic women probably had mild disease. It is also possible that the sample size was small for comparative analysis. (lines 224-225, 284-286).

In the study area, antenatal clinics have the capacity to carry out the following routine tests for malaria, HIV and urinary tract infections at first ANC clinic visit. We did not record the prevalence of urinary tract infections. However, it will be interesting for future studies to address other possible causes of fever illness among this vulnerable group in our setting.

Previous studies in the study area show that most standing water around houses are breeding sites for the malaria parasite vectors. Wanji *et al.* *J Vector Borne Dis.* 2009 46(1):75-80 characterised
Anopheles breeding sites in the mount Cameroon area during peak to late rainy season period (August-November). The study revealed that about 94% of the breeding sites were temporary water bodies. Of these temporary water bodies, 80.8% were productive breeding sites (contained Anopheles larvae) and majority (85.7%) found within 20 m from the nearest inhabited house while 21.4% breeding sites were located between 20 and 50 m from the nearest inhabited houses. None were found beyond a distance of 50 m. Furthermore, Tanga et al., 2010 *Trans R Soc Trop Med Hyg* reported a high parous (an indication of haematophagy) rate (≤ 70%) for all anopheline species suggesting availability of potential breeding sites close to human residences (lines 301 - 313).

In this setting anopheline mosquitoes are predominant (82.73%) and diverse compared with *Aedes* with two species (*Culex* and *Mansonia*) (Tanga et al., 2010 *Trans R Soc Trop Med Hyg*).

4. The relation of HIV to fever and to malaria has been clarified as indicated below and added to the results section of the manuscript (lines 218 -221). The prevalence of HIV infection in the study population was 5.9% (18/303). HIV positive women (10.9%; 7/64) were more likely (OR = 3.26: 95% CI= 1.1 – 9.7; P = 0.026) to present with fever compared with no fever (3.6%; 7/193). However, a majority (71.4%; 5/7) of the febrile HIV positive women were co-infected with the malaria parasite. The prevalence of syphilis among pregnant women is low in the study area.

5. The relationship between IPTp-SP and malaria has been analysed, reported (lines 243 -249) and discussed (lines 330 – 340) in the revised manuscript. Reported IPTp-SP uptake was significantly associated with malaria at first clinic visit where women who had taken SP (32.3%; 10/31) frequently ($\chi^2 = 9.67; P = 0.008$) presented malaria than those who had not taken SP (13.8%; 31/225). In addition, the women who took SP and had malaria frequently (70%; 7/10) reported for ANC at the medical centre during unscheduled clinic days. It is important to note that, women who took SP (26.9%; 14/52) were more likely (OR = 3.93: 95% CI= 1.8 – 8.39; P < 0.001) to report history of fever that those who did not take SP (8.6%; 21/245).

Fitting IPTp-SP usage into binary and multinominal regression models did not alter the risk factors for *P. falciparum* infection and malaria earlier reported.
6. The reported low IPT-SP coverage has been justified and discussed as recommended (lines 330 – 240).

Reported IPTp-SP uptake was low. This is because the study population involved women reporting for their first clinic visit. Typically, in the study area, the first SP dose is administered after first ANC consultation during which about 90% coverage of at least one SP during pregnancy is achieved (Anchang-Kimbi et al Malar J 2014). SP uptake correlated with malaria at ANC enrolment. In peri-urban and rural communities, pregnant women with malaria often prefer to self medicate through drug store and herbs. Seeking treatment in health facilities is generally viewed as a last resort, usually when the disease poses a major threat to life. SP is an inappropriate treatment for malaria and over use of anti-malarial drugs especially SP may have implications on resistance against SP for malaria prevention in pregnancy. There is urgent need to evaluate SP efficacy as IPTp in this setting and to encourage pregnant women to seek appropriate diagnosis and treatment of febrile illness at the medical centre.

7. Other factors associated with being single were analysed and reported (lines 255 – 260) and discussed accordingly (lines 342– 363). Age and gravidity were significantly associated with marital status. A majority of the single women (60.9%; 56/92) were found in the younger age group (≤ 20 years) while most of the married women (89%; 97/109) were in the older age group (>25 years). The difference was statistically significant ($\chi^2 = 57.73; P < 0.001$). Similarly, there was a significant association ($\chi^2 = 55.33; P < 0.001$) between marital and gravidity status where the majority of unmarried women (60%; 51/85) were primigravidae and the married women were predominantly (89.6%; 108/115) multigravidae. Increased risk of *P. falciparum* among single women could be due to that fact they were significantly younger and majority were primigravidae.

In the study area, all pregnant women irrespective of their marital status are welcome at ANC and there no prejudice towards single women presenting on time. Also, no significance difference in mean gestational age at first ANC was noted between single and married women.

**MINOR COMMENTS**

8. Line 24: First sentence of the abstract has been revised as follows: Pregnant women in malaria endemic areas are at high risk of *P. falciparum* infection and its complications.

9. Line 85 – 86: Sentence has been re-worded (lines 75-76).

10. Line 105-7: font size adjusted to 12

11. Line 146: fever defined as temperature > 37.5°C (line 159)
12. Line 199: ‘A higher proportion of the women’ changed to the majority of the women (line 197 - 198).
Major comments

1. The association between knowledge on MiP and malaria has been clearly presented (lines 264 – 268) and discussed accordingly (lines 334 – 329). Knowledge of MiP did not differ with type of visit (scheduled or unscheduled visits) or ITN use. On the contrary, Women with knowledge on MiP (GA = 23.7 ± 5.6weeks), on the contrary had their first ANC later (t = 2.78; P = 0.006) than those without knowledge on MiP (GA = 21.9 ± 5.1weeks). We note that knowledge of MiP varied significantly ($\chi^2 = 13.48; P = 0.001$) among women of different gravidity status with a higher proportion of multigravid women (43.3%; n = 91) being more informed about MiP than secundigravidae (34.8%; n = 73) and primigravidae (21.9%, n = 46). The relationship observed between knowledge of MiP and malaria can be explained by the fact that the majority of the women who had some knowledge of MiP were multigravid and seek ANC later than women of lower gravidity. It is known that multigravid women have acquired gravidity-dependent pregnancy associated immunity to malaria and as such less likely to be susceptible to malaria in pregnancy.

No relationship was observed between IPTp-SP and malaria parasitaemia. Rather reported IPTp-SP uptake was significantly associated with malaria at first clinic visit where women who had taken SP (32.3%; 10/31) frequently ($\chi^2 = 9.67; P = 0.008$) presented with malaria than those who had not taken SP (13.8%; 31/225). In addition, the women who took SP and had malaria, frequently (70%; n=7/10) reported for ANC during unscheduled clinic days. It is important to note that, women who took SP (26.9%; 14/52) were also more likely (OR = 3.93: 95% CI= 1.8 – 8.39; P < 0.001) to report history of fever that those who did not take SP (8.6%; 21/245) (lines 241-247).

2. Sixty-eight out of 303 (22.4%) women enrolled were positive for malaria parasitaemia. Temperature was recorded for 257 women out of whom, 24.9% (64/257) had febrile illness (temperature >37.5°C). Malaria parasitaemia was significantly ($\chi^2 = 62.34; P < 0.001$) associated with febrile status where a significantly higher percentage of women with febrile illness (64.1%; 41/64) were parasitaemic for P. falciparum infection when compared with aparasitaemic febrile cases (35.9%; 23/64). The 41/68 parasitaemic febrile cases correspond to the symptomatic (malaria) cases involved in the analysis. After data verification 27/68 had asymptomatic infection and not 16/68 as previously indicated.
Since temperature was recorded for 257 women, the recalculated overall prevalence of symptomatic and asymptomatic infection were 16.0% (41)(95%CI = 11-20%) and 10.5% (27) (95%CI = 7.3-15%) respectively and not 13.5% (41/303) and 8.9% (27/303). A total of 189 (73.5%) women were uninfected. Majority of the malaria cases (61% 25/41) were women who reported at the clinic during unscheduled clinic days meanwhile most of the asymptomatic parasitaemic cases (92.8%; 25/27) were recorded during scheduled visits (lines 208 – 220).

3. After data verification and analysis, there was no significant difference in WBC counts between febrile and afebril cases and its discussion in the manuscript removed. Equally, Table 3 in the manuscript is redundant and have been removed.

4. After data verification and analysis, primgravidity was a significant factor associated with malaria (Table 2) which is in conformity with previous findings. However, this association was only significant in univariate analysis.

5. Data in Table 4 has been presented as individual variables with associated odds ratios

MINOR COMMENTS:

1. 30,000 FCFA have been converted to USD for reference
2. Identified minor typographical errors in text have been effected
3. Design used for Tables 1, 2 and 3 clearly delineate the different types of data presented.
4. Figure 1 is maintained and duplication of data avoided.