Author's response to reviews

Title: High mosquito burden and malaria transmission in a district of the city of Douala (Cameroon)

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Author's response to reviews: see over
To The Editor in chief of BMC Infectious Diseases

Sir,

I will like to thank the reviewers for their useful comments who substantially improved the quality of the manuscript. I will also like to indicate that this is the first entomological study on malaria transmission ever conducted in the city of Douala. These data are very important for future studies to be conducted in the area. All requested changes by the reviewers were done.

Reviewer N°1
I-Major revision required:
Comment 1&2:
1-Because of the importance of this paper, I suggest the author to re-write the discussion
2- Insecticide resistance in Anopheles gambiae population is not well documented in the discussion section. In fact, the emergence of pyrethroid resistance in Anopheles gambiae has become a serious concern to the future success of malaria control. This has been shown by many African scientists. Please go to Pubmed

Answer 1&2: The discussion section was totally revised.

II-Minor essential revisions
Comment 1
Introduction
1-The second sentence: According to recent reports........such as malaria [1] [2]. I suggest to the author to start this sentence by: Recent reports showed that.....diseases such as malaria [1] [2]
Answer 1: The sentence was not changed because we believe it is much more understandable this way.

Comment 2
2-In the sentence “Although unplanned urbanization characterized by poor housing......is still not well documented” please, add in Cameroon, because in country like Kenya, South Africa, much data exist on this aspect of malaria transmission in urban areas.
Answer 2: done

Comment 3: The city of Douala the largest in the country, is one of ......high mosquito
burden by the population, please add a reference
Answer 3: A reference was added

Comment 4: From indoors residual spraying in the 1950s ..... the use of ITNs, please define firstly ITNs
Answer 4: ITNs was defined

Comment 5: A recent study conducted in the city of Douala, ......mosquito breeding habitats, replace habitats by sites
Answer 5: Done

Comment 6: Methods
1-Study sites: Is it possible to get the map of your study sites? This will be very useful to localize the areas where the collections have done.
Answer 6: A map was not added because we believe it will not add much on the comprehension of the study we provided in the section material and methods sufficient details on the study site.

Comment 7: Field processing of mosquitoes
1-Each anopheline specimen was .......... in Yaoundé. Is it in Yaoundé or in Douala? See the last sentence of your introduction and the key words.
Answer 7: It is not an error because our laboratory is situated in Yaoundé. Field samples collected in Douala were brought back to Yaoundé for processing.

Comment 8: Results
Field sampling section
1-The study was conducted ....... light trap catches. Please, delete this sentence and start from A total.......
Answer 8: Done

Comment 9:
2-The average number ......collected by HLC......54.2 mosquitoes/person/night. Please, you must not calculate the average like that. You must take account the season, so the rainfall. See the paper Yadouleton et al. (2010b): The impact of the expansion of urban vegetable farming on malaria transmission in major cities of Benin Parasites&vectors. (3) p. 118
Answer 9: We added information on the seasonal fluctuation of anopheline densities the following sentence was added: “The seasonal variation of An. gambiae burden was 42.12 and 1.87 bites/human/night during the short (july) and long dry seasons (December to February) respectively and, 99.13 and 68.63 during the short (march – june) and long (August – November) rainy seasons respectively.”

Comment 10: Discussion.
Many good ideas, but not well structured, please, rewrite this section.
Answer 10: Done

Comment 11: Conclusion.
Base on the title and the aims of your work, the last sentence doesn’t bring any information,
**Answer 11:** The phrase was deleted

**Comment 12:** References.
The name of the journal must be uniform everywhere. Maybe you write the full name of the journal or you contract. See reference 19 and 20
**Answer 12:** changes Done

**Reviewer 2**
**Comment 1:** Abstract section of paper
Background section: First sentence – Spelling of Cameroun should be corrected to Cameroon.
**Answer 1:** Done

**Comment 2:** Discussion section of paper
1. Third paragraph in this section: The last sentence in this paragraph - the term “low hygrometry” should read “low humidity” – i.e page 12 of pdf document. Hygromery is a branch of Physics that deals with measurements of humidity of air and gases. I therefore believe authors intended to mean “humidity”.
**Answer 2:** Done

**Reviewer 3**
**Major revision**
**Comment 1:** Methods
Adult mosquito collections
1. In order to make a comparison of both methods it’s important to take for granted some criteria. We can’t work on the HLC outdoor and indoor experiment for the CDC-LT while comparing them. It would be more useful to use both methods indoor and add in the CDC-LT a human stimulus (a sleeping person under mosquito net) since you have mentioned it in the discussion section.
2. How many night men have you used per night capture?
3. Have randomized the CDC-LT
**Answer 1:** In Douala due to the prevailing insecurity household owners didn’t want to let collectors indoors and we were forced to conduct HLC outdoors. Since in the future, most collections in this city may be carried outdoors we estimated necessary to estimate the efficiency of HLC outdoors to CDC LT conducted indoors. The following sentences were added “However the fact that HLC was undertaken outdoors while CDC LT was conducted indoors could have likely overestimated the efficiency of HLC compare to CDC LT. Because of the high insecurity prevailing in the city of Douala, household owners did not permitted indoors HLC this prompted to readjust the study design and to carry HLC exclusively outdoors while CDC LT were placed indoors.”
2) Find the sentence explaining night catches in the method section “Collections were carried out outdoors in four randomly selected sites during two consecutive nights each month (two teams of volunteers collected the mosquitoes during each night one from 19:00 to 01:00 the other from 01:00 to 06:00).”
3) The following information was added “Houses for CDC light trap catches were selected on the basis of the number of occupants varying from 8 to 12 to avoid bias which may result from different population densities per site. Preliminary collections were conducted with light traps only houses with the highest mosquito densities were selected.”
Comment 2: Bioassay analysis
How many « Batches » have you done for the test? How many mosquitoes in global have you tested for each molecular? You should clarify.

Answer 2: The following precision were brought in the section result “A total of 757 An. gambiae females were tested for susceptibility to DDT and pyrethroids. A mortality rate of 33% (82/249), 76% (266/350) and 98% (155/158) was recorded for DDT, permethrin and deltamethrin respectively.”

Comment 3: Field processing of mosquitoes
Parturity rate was not explained. This is very regrettable as trap efficiency between parturity rate vs molecular forms (M and S) may be very different.

Answer 3: We didn’t do ovaries dissections

Comment 4: Data analysis
A log+1 normalization: adding artificially 1 for zero values creates a bias on sampling sensitivity analysis. (Smith T et al. 1995 J Am Mosq Control Ass, 11:377-378). Is a statistical analysis of numbers using the negative binomial regression not more appropriate? What test did you use? The “BonEferroni” (in fact Bonferroni) is this test appropriate?

Answer 4: No we didn’t used the bonferroni test. We used correlation analysis to compare the two collection methods. Although this method is less stringent than binomial or Bayesian methods it is appropriate for this type of comparison.

Please find below the information provided in the data analysis section
“To assess any linear correlation between the two collections methods, the Pearson correlation coefficient was calculated between the average number of mosquitoes collected nightly by LT and HLC. Prior to analysis, the average number in each catches (x) were transformed to Y = log(x+1). To compare methods and test if mosquitoes abundance affected the sampling efficiency of each methods, the ratio of the number of mosquitoes in LT to the number of mosquitoes in HLC (Log(HLC+1)-Log(LT+1)) was plotted against average abundance [Log(HLC+1)+Log(LT+1)]/2 as presented in Overgaard et al. [20].”

The following sentence was added in the discussion section “Although comparison between collection methods commonly used log transformation of direct counts, this method could have little bias following the use of log(x+1) instead of log(x) [20]. The use of negative binomial regression analysis or Bayesian estimations could be more appropriate [20]”.

Comment 5: Result
Field sampling
We also notice that you give an absolute emphasis on the Culex, Mansonia and others. So when considering the number it would have been very interesting if you had mentioned the nuisance that might have been created when put together.

Answer 5: The overall burden is indicated by the following sentence: “The average number of mosquitoes collected by HLC was 54.2 mosquitoes/person/night whereas the average number of mosquito collected by a CDC LT was 10.3 mosquitoes/trap night.”

Comment 6: The sibling species of An.gambiae s.l. identified is very low. Over 1805 An. gambiae s.l only 200 have been identified by PCR, and not reveal the process sampling method. Have you collected them from the CDC-LT ? or in a monthly basis? Please clarify.
**Answer 6:** The number identified by PCR was increased from 200 to 386 and precision on the sample analysis were brought: see the following sentence in the result section “An. gambiae specimens were for the majority (384/386) of the M molecular form the remaining of the S molecular form (2/386) (for molecular identifications of An. gambiae M and S forms, an average of 20 to 40 mosquitoes were randomly selected from mosquitoes collected each month from both HLC and CDC LT).”

**Comment 7:** Infection rate
1. In a total of 1810 anopheles captured only 1765 have been tested by ELISA. Why does that difference?
2. When comparing graphics 2 and 4, we notice that rains are more abundant in the month of July and August while the highest infection rate is absorbed in May, it seems a bit surprising since I was expecting the contrary unless you give further explanations about this new phenomenon.

**Answer 7:**
1) The remaining mosquitoes were kept for further molecular analysis.
2) No study has ever been conducted in Douala before ours and we cannot predict the situation of malaria on the basis of climatic variables only there are many factors influencing malaria transmission pattern and this was highlighted in our study. The following phrases were added in the discussion section “Several parameters were reported to influence parasite prevalence in human and mosquito’s infectivity and include amongst others, the use of protective means, health seeking behaviors, socio-economic status and immunity related factors [41-43].” And “Moreover, the fact that no transmission was detected during certain period of the year does not totally exclude perennial malaria transmission since clinical cases were reported all year long in local health care centre (data not shown). The situation needs further studies for better understanding of parameters influencing malaria transmission in the city of Douala.”

**Comment 8:** Susceptibility assays
This part must be completely reviewed in the light of these points:
1. On what groups have you made the kdr, survivors, dead, or both from both of them?
2. Why you did not calculate the KD50 and the KD95. This is a good indicator of resistance
3. Are you-a relationship between RR and survivors?
4. Why you have not done with the kdr mosquitoes from HLC and CDC-LT and compare it with bioassay. In addition, what is the implication of metabolic resistance in malaria vectors in the study area?
5. Bioassays were conducted without using a well characterized susceptible strain of An. gambiae (eg kisumu as a control). Reference strain is needed to check the quality of treated papers used for the tests (100% mortality of the susceptible strain is expected with insecticides used at the Diagnostic concentration). No data on the control batch (ie untreated paper) was also provided.

**Answer 8**
1) The following precision was included in the result section “A screening of the kdr allele was conducted on 61 specimens selected among survivors to DDT, permethrin and deltamethrin, 38 specimens (62.3%) were found carrying the L1014F kdr allele.”
2&3) We did these type of analyses in an earlier study reporting on the status of mosquito susceptibility to insecticides in the cities of Douala and Yaoundé see “Antonio-Nkondjio et al 2011 Malar J 10, 154”.
4) We are currently undertaking microarray data analysis to assess the implication of metabolic resistance in malaria vector from the city of Douala.

5) The following precision was added in the result section “. The kisumu An. gambiae susceptible strain used as control presented a mortality rate of 100% for both permethrin (100/100) and deltamethrin (100/100) and 94% for 4% DDT (94/100).”

Comment 9: Discussion
1. In paragraph fourth, you talk about the stratification of urban malaria, while you did not specify in materials and methods. This is irrelevant.
2. How can you explain the increase of resistance?

Answer 9
1) I think it must be a misunderstanding the sentence was rephrase for more clarity.
2) The following sentence was included in the discussion “The high prevalence of insecticide resistance in mosquitoes populations in the district of Ndopassi could be attributed to the frequent use of chemical insecticides for both the practice of market gardening and personal protection [34]. Apart of ITNs, insecticide spray and coils were reported regularly use in households [34].”

Comment 10: Minor Essential Revisions
1. You often mention « An. gambiae » throughout the manuscript. Do you mean ss or sl. please specify

Answer 10: I mean An. gambiae ss this precision was included in the text

Comment 11
2. You declare that urban malaria lacks of sufficient documentation so I suggest you to read these two contributions (Gadiaga et al., Malar J. 2011 and Machault et al., Malar 2009; 2010) J.), you will find in the bibliographic section corresponding contributions
3. Please consider the daily temperatures in the Study Site section.
4. Please specify the concentration of glucose in the bioassay section
5. Review graphic 4 specially the error bars, when HBR is null.

Answer 11: 2) Done
3) Daily temperatures were not recorded during the study
4) 10% glucose added in the text

Comment 12
As there’s a bias in the CDC-LT, you do not think it would be better to abandon this method

Answer 12: No because CDC LT is a good alternative for mosquito collection in urban areas it is possible that in the coming years only this method is used for assessing malaria transmission.

Revisions requested by the editor concerning the quality of written English which need to be improved were also addressed.
We look forward to hear from you in due course,
Yours sincerely

Antonio Nkondjio C.