Author's response to reviews

Title: Malaria vectors and transmission dynamics in Goulmoun, a rural city in south-western Chad

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Author's response to reviews: see over
ITEMIZED REPLY TO THE REVIEWER’S COMMENTS

Dear Sir,

We would like to thank you for having considered our manuscript entitled “Malaria vectors and transmission dynamics in Goulmoun, a rural city in south-western Chad” was acceptable for publication in BMC Infectious Diseases. We are extremely grateful for remarks made by the two referees. Therefore, please find enclosed a revised version in which the following changes were made:

Responses to Reviewer 1:

1. Bites of infected anopheline and not infective bites:

“Infective bites” was changed to “bites of infected anophelines” wherever it was needed, including in Figures 4 and 5.

2. Authors explain the population dynamics of both An. arabiensis and An. pharoensis is only linked to the distribution of rainfalls, and not to rice field events. Why not, but quite nothing is exposed concerning rice cultivation calendar (except the indication “twice a year” in the discussion). This information is needed, in details, in the M&M section.

Requested information was added in the Methods section of the revised manuscript.

3. A schematic map of Chad, perhaps with magnification for the study area, would be welcomed:

A schematic map of Chad and magnification of the study area was added. (see response to reviewer2).

4. Summary. Background. If malaria is “by far the leading cause of morbidity and mortality” is not the question in the summary.

Sentences in this paragraph have been revised accordingly.

5. Summary. Methods. Precise the year of the study, and the observations repeated each month

Done.

6. Summary. Results. Do not provide the annual EIR value in a parenthesis; this is the main result which needs some emphasis in its presentation.

Done.
7. Page 4. “Anophelines’ breeding sites consist mostly of temporary and semi-permanent water collections scattered within the village and of more persistent water bodies in the rice fields and along the river banks.” Add a reference or move this sentence in the Result section, or delete.

The sentence was deleted (see our response to point 2 raised by reviewer2).

8. In the study area, precise if human sleep usually inside or outside.

Done.

9. Page 5. This is not clear if ref [20] is valid for Funestus group only or also for An.gambiae complexe.

This sentence and this reference were deleted in response to comments form reviewer 2 (see below).


Requested information has been added.

11. Page 6. Seasonal abundance and biting rate. I don’t like the two sentences after “A first peak of densities was recorded in August…”. I would prefer the manner used later “The highest densities for An.arabiensis were recorded in October (241 b/h/n) and May (20.6 b/h/ n) and the highest densities of An.pharoensis in October (31.9 b/h/n) and May (39.8 b/h/n). The second peak observed simultaneously for both species occur two months after the onset of rainfalls and the transplantation of rice in the irrigated area.”

Changes were made accordingly.

12. Page 7, line 7. The expression “of biting An. pharoensis females…” is puzzling. Delete “females” but indicate somewhere is some male mosquitoes have been caught in HLC.

Done.

13. 2nd §. “(N=1, only in An. arabiensis)”. Delete “only”.

Done.

14. In the Results section, add some indication on the variability between the 3houses (indoors and outdoors) for HLC. Perhaps a new table.

A new table (Table 2) was included and a sentence presenting the main results and trends was added in the Results section of the MS.

15. Discussion. Try to explain (hard task!) why the CSP rate is similar for An.arabiensis and An.funestus, but the HBI is higher for the later.

Some comments on this matter have been included in the discussion, as suggested.
16. Page 10. I don’t like “extrinsic cycle”; “extrinsic development” is better.

Done.

17. Last line of conclusion. Delete “paramount”.

Done.

18. Authors’ contributions. “and gave final approval for the version to be published”. This can not reside in the decision a single author. Delete.

The statement was deleted.

19. Ref 22 and 47. Delete capital.

Done.


Duplication deleted.

21. Figure legends. What does mean [S1] before Figure 3?

This was a typo. It has now been corrected.

Responses to Reviewer 2:

MAJOR COMPULSORY REVISIONS:

1) For the blood meals, only a sample for each species has been analysed. The selection of these samples is not given in “material and methods” part. Readers have no way to understand how these samples have been made. I don’t understand why only seven An pharoensis blood meals on 48, why 52 An funestus blood meals on 286 and 144 An arabiensis blood meals on 1,611 have been analysed. At this time, these results are not consistent. These results have to be completed by the authors or to be removed of the manuscript (material and methods, results and corresponding parts of the discussion).

As mentioned in the Methods section, blood meals were collected only from freshly fed mosquitoes, whereas Table 1 provides the sample sizes collected by PSC over all physiological stages (e.g. unfed, freshly fed, half gravid and gravid females), rooms and collection dates. All suitable samples were processed, although in the case of the abundant An. arabiensis (e.g. morphologically identified as An. gambiae s.l. in the field) no more than 50 freshly fed specimens were dissected per sampling date. Therefore, we believe our results provide relevant information on host feeding preference of (at least the indoor resting fraction) of these anopheline species in our study area. We agree that low sample size for An. pharoensis, resulting in large confidence interval on the estimation of HBI, precludes any inference regarding this species. These points were raised in the Methods, and the results are maintained.
2) There is no data on a field larval sampling in the material and methods part. Please, give some data on this part or delete this sentence “Anopheline’s breeding sites in the village consisted mostly…..or receding periods” and all discussions on breeding sites. Larval data from Bongor could not be extrapolated to Goulmoun.

(see our response to reviewer 1 above). We agree our data do not allow any inference on mosquito larval ecology in our study area. Additional data based on personal observations and casual recording by some of the authors are available but were not considered as “unbiased” and are therefore not provided in the manuscript. Hence, the sentence was deleted. However, in the discussion, we refer to known, typical larval biology traits. Emphasis on larval ecology was reduced and we strongly advocate for such studies to be conducted to guide malaria vector control in Chad.

MINOR ESSENTIAL REVISIONS

3) The authors considered that the high mosquito nuisance from Bongor could be extrapolated to Goulmoun but no data on the culicidae fauna of Bongor and on the culicinae fauna of Goulmoun is given. Without this data, it’s difficult to follow them and to understand the nuisance in Goulmoun. A description of the culicinae fauna collected during HLC and pyrethrum spray collection, and a map of Chad with the localisation of Goulmoun and Bongor will be useful to the readers. The map used in another of their publication (ref 52) with a focus on the study area added could be informative.

We deleted this statement. A map of the study area (Figure 1) is now provided (see response to reviewer 1).

4) The number of person-nights of human landing collection should be added. The number of pyrethrum spray collections (room-night) is not given. Have the bedrooms been randomly selected each collection night or the same bedrooms have been used during all the study? These data should be added.

The requested information is now provided (in Methods and in Table 1 caption).

5) “Results from several studies…… lead to a decrease ….severe malaria and associated anaemia during pregnancy” None of the three references (3,4,5) support the last part of this sentence. Delete this part or give an accurate reference. Actually, the impact of ITNs on malaria pregnancy is still unclear.

We agree and deleted the end of the sentence.

6) The authors have chosen to treat a sample for pcr identification of mosquitoes from An. gambiae complex and from funestus group. But no data is given on the selection of these samples. These data have to be given.

Requested information was provided in the Results section.
7) The authors wrote, “all specimens of the An. funestus group (N=418) were …” but according to table 1, 504 An. funestus have been caught. Please explain the difference or make some changes.

PCR analysis of specimens is costly and we depended upon limited budget. Hence, a random sample of specimens was selected for molecular identification. As now stated in the text (Results), all An. funestus collected by HLC (N=218) and 200 out of the 286 specimens collected by PSC were identified molecularly.

8) The authors give some data on rainfall and discuss its role on anopheline abundance but they don’t give the origin of this data (national weather agency, self-measurement, or satellite measurement). This origin has to be given.

The data reported are monthly means averaged over twenty years (1985-2004). They were indeed obtained from the National Weather Agency. The information has been added in the legend of Figure 2.

9) No information is given in the “Entomological parameters and statistical analysis” part about a statistical analysis but results of a statistical analysis are given in the results section. The statistical test and the statistical software used have to be given here.

Done.

10) Khi² corrected: why corrected and if relevant what correction?

The numbers of infected mosquitoes were <5 for An. ziemanni and An. funestus, requiring a Yates correction for Khi². The information was added in the text.

11) The authors’ team have worked in this area on the insecticide susceptibility of An. arabiensis in Goulmoun (ref 52). It would be interesting to reminds readers of these data in the text. It would improve the conclusion.

Done. Information on insecticide resistance within An arabiensis populations in the study area was included in the study site description (Methods).

**DISCRETIONARY REVISIONS**

12) There is no data on the molecular identification of the five Anopheles nili. This information could be added.

We agree that PCR identification of An. nili s.l. specimens would be relevant. However, this was not done because of the very low densities observed in our collections. It is not possible anymore for us to process these samples but future investigations in Chad will consider this aspect.

13) Reference 13 & 14: reference 14 is not informative. Please delete it.

We believe that both references provide unique information that are necessary for the realization of the ELISA procedure, as reference Wirtz et al., 1987 (former reference 14) proposes several modifications to the protocol described in Burkot et al. 1984 (former reference 13) which pioneered the work. We would like to maintain both references.
14) Reference 18 & 19: Please make a choice

Reference 18 (Koekemer et al., 2002) was deleted.

15) “Dna extraction was carried as…” this sentence is not informative Please delete it and its reference too.

Done.

16) Figure 2: instead of “the main anopheline species”, “the anopheline species involved in malaria transmission”

We do not believe this comment is relevant since at this step of the study, the species are not yet incriminated as vectors. We changed “main anopheline species” by “the most abundant anopheline species biting humans” in the legend of Figure 3.

17) Figure 3: instead of “the 4 vector species”, “the 4 anopheline species involved in malaria transmission”

Done.

18) Figure 2: the diagram is “ornate” perhaps four juxtaposed figures (four histograms) would be more informative

We have tried different ways of showing these data and, for the sake of conciseness and clarity, the actual Figure 3 was thought to provide the relevant information in the most straightforward way.

19) Table 3: The HBR is an average HBR. Perhaps, the authors could give this precision.

Done (heads of column in the new Table 4 have been updated).

20) Table 3: I think that the column “N° of infective bites per man per night” has to be deleted. These data is not coherent with the seasonal transmission described in the text and in figure 3 and is false at least three months in the year.

We believe that, although malaria transmission is seasonal and did not occur from January to March, the average number of bites from infected anophelines received by a person living in Goulmoun during the study period should be given as an indicator of the overall transmission intensity, and of the relative importance of the different vector species in malaria transmission. The head of column was updated to specify that these are average values (see above).

We thank you for your time and consideration, and we are looking forward to hear from you in due course,

Yours sincerely,

Kerah-Hinzoumbé Clément, on behalf of all co-authors.

N'Djamena, March 23\textsuperscript{th}, 2009