Review of Charlwood and Kampango: A novel experimental hut for the study of entrance and exit behaviour of endophilic malaria vectors

Reviewer: Anonymous

Compulsory Revisions

Please add a section on ethics.

- People were asked to sleep in the huts – is this deemed human subjects research in Mozambique?

  These people were experienced field workers hired to be part time research project staff members.

- How are participants protected from mosquito bites when they are collecting mosquitoes in the middle of the night?

  Participants entered the traps to collect mosquitoes trapped inside. The traps were well fitted to the Experimental hut walls not allowing any outdoor host-seeking vectors reach the collectors while they were aspirating mosquitoes inside the trap.

- Was informed consent, using a format that detailed risks and benefits to the participants given?

  Participants were orally informed about the study importance and the possible risk of acquiring malaria if basic protection measures (such as sleeping under bednets for protection) was not rigorously followed. These participants had also previous experience in several research projects carried out before in the ambit of MozDan Project.

- Is there any way participants could have been bitten while collecting from the nets that might explain the greater number of fed mosquitoes collected from huts (20% versus expected 2%)?
This is, of course, always possible. Nevertheless, they were not stationary during the collection process, which minimises the likelihood of a mosquito being able to feed. Thus we think that there was a negligible chance a participant could have been bitten while collecting from the nets.

**Essential revisions**

**Methods that require clarification**

- It is not clear how mosquitoes enter the huts and if the route made available to the mosquitoes in these experimental huts is similar to that available in local houses. A section in the methods on mosquito entry points is needed and possibly a diagram that allows the reader to easily understand the design.

  The entrance routes were similar to those of local house. The only difference between our hut and local houses was the shape of the house.

- Were there other exits apart from the nets available to mosquitoes? If so can the authors explain how they can be certain that the exit behaviour they are observing is representative of the mosquitoes’ preferred direction of exit, especially as it is contrary to that observed by other authors (where mosquitoes move towards sunrise).

  The other doors were kept closed and although the small gap between the eaves and the roof could have been used by the mosquitoes this is unlikely to have been the main route of exit since this was always relatively dark (being under the overhang of the roof).

- Two collectors were randomly assigned to the huts. It seems likely that the differences between the huts were due to differences in the intrinsic attractiveness of sleepers to mosquitoes. It would have been better to rotate the sleepers as the authors mentioned in the introduction. It would be useful to understand why this was not done (or clarify the text if it was done).

  We agree that this might have reduced the differences between the huts. We were, however, also interested in monitoring at least one of the huts for longitudinal studies. This being the case it was considered that in order to reduce possible effects influencing the longitudinal data set the same
collector should sleep in at least one of the huts. The sleepers would not be likely to affect the number of male, or young female mosquitoes collected in the huts. These differences were as great, if not greater than the differences in mature females collected so we do not believe that host differences were the reason for the differences described.

Reference

• What was the location of hut 1 relative to hut 2? Is there any way that the two huts may have interacted with each other? i.e. mosquitoes exiting on the side of the hut that is closest to other hut.

Huts were separated approximately 120 m apart. Therefore, an interaction between odours from different huts with or without their occupants can be exclude, since the distance falls out the range of many Anopheline and culicine species attraction (Gillies and Wilkes, 1968)

Reference

• What was the location of the huts relative to other resources such as breeding sites or other houses in the village? Are they independent of external sources of kairomones that might have influenced mosquito house exit?

Huts were located approximately 10 m from the nearest putative breeding site (Furvela river). Hut #1 and #2 was approximately located 20 m and 120 m respectively, from one author’s house (old JDC now AK). It is possible that female mosquito exit behaviour was influenced by kairomones emanating from the breeding site or the house occupied by AK.

1. Line 72 state if the mosquito net was treated with insecticide, or not Done
2. References are not all in alphabetical order. **Done**

3. Figure 3 – it is conventional to use N.S. to denote non-significance and asterisks to denote statistical significance. Therefore labelling is confusing. **Done**

4. Figure 5 needs to be better labelled and data displayed in figure 5 is better represented in figure 6. I suggest it is removed. **Done**

5. Text and other figures state that 25 days of data collection were performed, but figure 5 shows 29 days. Is this a mistake? Please clarify

   **This is a graph drawing mistake. Collections were carried in 28 days. New fig has been added.**

6. Figure 7 axes incompletely labelled with units of measurement  
   **Axes now labelled**

**Optional revisions**

- The section on the influence of temperature in Period I is interesting – mosquitoes exit when it is cooler. This was not very easy to see from the text. A sentence explaining the trend shown by the regression equations would be useful, as was done to explain findings in Period II.

   **The effect of predictors on mosquito egress can be described by the equation \( Y = 1.73 + 0.02 \text{HUM} - 0.135 \text{TEM}, r^2 = 0.30; P = 0.04 \)**

- Was there any difference between the huts as measured with CDC LT?

   **No significant differences were found**

- Greater information on how the experimental protocol was disturbing to insects and how this could be improved upon would be helpful in the discussion

   **Programmable suction devices attached to the trap allowing mosquito be sucked into a bag would improve collection**
efficiency at time when it was dark. They would also reduce the possible disturbance while the collector opens the door going outside and move to a different trap to collect mosquitoes. Would also reduce collector exposure to outdoor host-seeking mosquito bites and even the possible influence of collectors of the accuracy of the number collected mainly in the middle of the night, since at this time they are recovering of sleep effect and thereby some mosquito can be left behind.

- The detailed life history measurements made by the authors are a nice feature of the paper. It would be useful to readers if the authors referenced the dissection methods used to classify virgin, newly emerged, mates and parous females.

Mosquito were dissected following the schedule described in Kampango et al., (2011) and Charlwood et al., (2011). Further details about the method were added to the manuscript.