RESEARCH NOTE

Is outdoor-resting behaviour in malaria vectors consistent?

Short report from northern Ghana [version 1; peer review: 2 approved with reservations]

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Abstract

Background: Recent studies have observed vectors resting predominantly outdoors in settings where anti-vector tools are extensively deployed, attributed to selection pressure from use of control tools. This present study examined if the outdoor resting behaviour in the vector population is random or indicative of a consistent preference of one resting site over the other.

Methods: Mark-release-recapture experiments were conducted with outdoor-resting *Anopheles gambiae* and *An. funestus* mosquitoes collected from two villages in northern Ghana during rainy and dry seasons. Mosquitoes were marked with fluorescent dyes and released indoors. The experiments were controlled with indoor-resting mosquitoes, which were marked and released outdoors. Species of all recaptured mosquitoes were identified and assessed for consistency in their resting behaviour.

Results: A total of 4,460 outdoor-resting mosquitoes comprising *An. gambiae sensu lato* (s.l.) (2,636, 59%) and *An. funestus* complex (1,824, 41%) were marked and released. Overall, 31 (0.7%) mosquitoes were recaptured mostly from outdoor location comprising 25 (81%) *An. gambiae* s.l. and 6 (19%) *An. funestus* complex. Only 3 (10%) of the recaptured mosquitoes were found resting indoors where they were released. The majority of the outdoor-recaptured mosquitoes were *An. arabiensis* (11, 39%), followed by *An. coluzzii* (7, 25%); whereas all indoor-recaptured mosquitoes were *An. coluzzii*. For the control experiment, 324 indoor-resting mosquitoes constituting 313 (97%) *An. gambiae* s.l. and 11 (3%) *An. funestus* complex were marked and released. However, none of these was recaptured neither indoors nor outdoors. More mosquitoes were captured and recaptured during

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rainy season, but this was not statistically significant ($Z=0.79$, $P=0.21$).

Conclusions: These results suggested the tendency for the mosquitoes to retain their outdoor-resting behaviour. Further investigations are required to ascertain if emerging preference for outdoor resting behaviour in malaria vector populations is consistent or a random occurrence.

Keywords

Mark-release-recapture, resting behaviour, outdoor resting, malaria vectors, northern Ghana.

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Africa

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Introduction

Vector control with indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) has contributed largely to reduction in malaria incidence and mortality¹. These tools are both insecticide and indoor-based and target vectors that feed indoors (endophagic) and rest indoors (endophilic)²⁻⁴. However, recent reports are indicating that vector control tools are inducing behavioural changes in mosquitoes such that some vector species have shifted from indoor-resting behaviour to resting outdoors (exophilic). This was as documented from studies in Ethiopia⁵, Ghana^{6,7}, Kenya^{8,9}, Libreville¹⁰ and Tanzania^{11,12}. Such behavioural diversification in vector population can be detrimental to vector control^{13,14}, which mainly target vectors resting indoors. Therefore, efforts to understand emergence of such behaviour are highly essential for the elimination agenda.

Outdoor resting behaviour in vector populations may be triggered by heavy use of insecticide-based indoor intervention in settings where control tools are widely implemented^{11,15,16}. Vectors in these settings avoid contact with insecticides and rest outdoors where no insecticide is used^{11,15-17}. Such behaviour promotes outdoor transmission as vectors can bite unprotected humans outdoors^{11,18}.

Mark-release-recapture (MRR) studies are a standard experimental method extensively employed to investigate mosquito biology, ecology, life history and behaviour, measuring parameters including dispersal and flight distance; survival, population size and density, as well as blood feeding, host seeking and reproductive behaviour¹⁹⁻²². MRR experiments have been used particularly for field and laboratory studies involving mosquito species such as *Aedes*^{23,24}, *Culex*^{25,26} and *Anopheles*^{19,20,22}; to collect data highly relevant in understanding pathogen transmission, gene flow and development and optimization of vector intervention tools^{20,22,27}.

Northern Ghana has documented outdoor resting in *An. gambiae sensu lato* (*s.l.*) population^{6,7} but it is not clear whether this behaviour only occurs randomly in the vector population. This present study examined if the preference for outdoor resting in the vector population was consistent or not. MRR experiments were conducted using field-collected mosquitoes of unknown age in two villages in northern Ghana, where coverage of IRS and LLINs are high^{7,28,29}.

Methods

Study sites

The MRR experiments were conducted in two villages in northern Ghana, Kpalsogu (9.33° N, 1.02° W) and Libga (9.35° N, 0.51° W) during the rainy season between July-November 2017 and dry season in December 2017-January 2018. The region has a unimodal rainfall pattern with monthly density between 150-250 mm and a forest vegetation zone with mean daily temperature ranging between 25-30°C; and an average relative humidity between 65-75%³⁰.

This region has a previously documented malaria incidence rate of about 40% in under-five children^{29,31} and LLINs coverage

is >70% in both villages^{7,29}. Kpalsogu has been under IRS implementation since 2008⁷. Extensive farming is practiced in both villages, occurring throughout the year as the villages are close to irrigation dams.

Mosquito collection

Live outdoor-resting adult female *Anopheles* mosquitoes were collected from pit shelters, granaries and animal resting shelters. One granary and one animal shelter each were selected per compound. Six pit shelters were also dug, which were positioned about 5-10m outside six randomly selected compounds in each village. These compounds were selected if they were 50m apart and provided good shade for mosquitoes to hide. The mosquitoes were carefully collected using mouth and Prokopack aspirators (Model 419, John W. Hock Company) so that they remain alive.

To compare the trend in behaviour of mosquitoes when resting indoors and also as a control for the experiments, indoor-resting mosquitoes were also collected from sleeping rooms in the compounds where the pit shelters were situated, using the same collection methods as described above. Collection was done once weekly during the early mornings (05.00-07.00) for four months

Mosquito processing, marking and subsequent release

Captured mosquitoes were immediately transported to the insectary for morphological identification as female *An. gambiae s.l.* and *An. funestus* complex³². Following identification, indoor and outdoor mosquitoes were carefully kept in separate cages in the insectary, irrespective of their abdominal status. The mosquitoes were kept in the insectary for about 7-8 hours during which they were fed with 10% glucose until experiment time at dusk of the same day between 18:00 – 19:00.

The marking of mosquitoes was carried out 3 hours prior to release time. Batches of 15-20 mosquitoes from the same compounds were put into the same paper cup. The outdoor-collected mosquitoes, designated as 'test' mosquitoes, were dusted with red fluorescent dye (BioQuip Products, Inc. California, USA) while the indoor mosquitoes, serving as control, were similarly marked with green dye (BioQuip Products, Inc. California, USA). All mosquitoes were left in the cup to rest after marking, until when transported to the study sites for release.

Two random compounds were selected near the pit traps for each release event. Between 200-250 test mosquitoes per species complex were released in five sleeping rooms (indoors) in each of these compounds. The mosquitoes were released indoors to assess if they would remain indoors to rest or return outdoors where they were originally collected from.

The control mosquitoes were released outdoors; outside one of the selected compounds. Two release events were conducted for the control mosquitoes where approx. 150 specimens were released in each release event. These mosquitoes were released outdoors to ascertain if an alternate trend in the resting behaviour would be demonstrated.

Recapture of released mosquitoes

The mosquitoes were left for 48 hours before recapture was initiated. This offered sufficient time for the mosquitoes to redistribute in their new environment and enabled us to assess if the mosquitoes opted to return to their initial collection location.

To recapture, mosquitoes were sought from sleeping rooms, animal houses, clay pots, granaries and pit traps deploying light traps, exit traps, pyrethrum spray catches as well as mouth and Prokopak aspirations. All rooms and compounds in each of the study sites were sampled to recapture the released mosquitoes. Recapturing was done for up to 7 consecutive days before the next release event.

In all, twelve (12) rounds of MRR experiments were conducted for the test mosquitoes within the study period, with six experiments performed per study site. Most of the experiments during the rainy season were done at Kpalsogu, whereas most of those done in the dry season took place at Libga. Two control MRR experiments were also conducted, one each during rainy and dry season at Kpalsogu.

Processing and identification of recaptured mosquitoes

All recaptured mosquitoes were morphologically identified as marked and unmarked. Only the marked *Anopheles* mosquitoes were selected for analyses and assessed for change in resting behaviour. All unmarked mosquitoes were only counted and recorded.

The marked recaptured mosquitoes were processed to discriminate the sibling members of *An. gambiae s.l.* and *An. funestus* complex. DNA was extracted from these mosquitoes and molecular species were genotyped using the protocols previously described^{33,34}.

Ethical considerations

Ethical approval was obtained from the Ghana Institutional Review Boards (IRB) of Noguchi Memorial Institute for Medical Research (NMIMR-Study #106/16-17). Village heads were also engaged in discussions where concerns on release of mosquitoes into sleeping rooms were clarified. A series of meetings with the village heads, household heads and village health workers during which the nature, benefits, and absence of risks from this study was explained. Subsequently, verbal informed consent was obtained from household heads before mosquitoes were released into their rooms. The IRB approved this type of consent taking. MRR experiments present no potential risks as mosquitoes were released in the compounds where they were initially captured and a proportion was to be recaptured.

Data analysis

The number of recaptured mosquitoes was counted and proportion determined. The different release events were considered as replicates. Data from both study sites were merged together as there was no significant difference in the results obtained from the different replicates. Z-test was used to compare the difference in proportion of the recaptured mosquitoes

between the rainy and dry season. Stata/IC 15.0 (2017 StataCorp LP) was used for all statistical analyses.

Results

Recaptured mosquitoes and their species

Overall, a total of 4,460 outdoor-resting test mosquitoes were released to indoor space, while 324 indoor-resting control mosquitoes were released outdoors. The morphological identification of the released (test) mosquitoes revealed 2,636 (59%) were *An. gambiae s.l.*, while 1,824 (41%) were *An. funestus* complex (Figure 1). Whereas the control mosquitoes comprised 313 (97%) *An. gambiae s.l.* and 11 (3%) *An. funestus* complex.

Following all the 12 MRR experiments conducted, a total of 3,950 mosquitoes were collected from outdoor location, and 141 from indoor location. Of the outdoor-caught mosquitoes, 31 (0.8%) were marked and 3,919 (99.2%) were unmarked including males and non-Anophelines. Subsequent analyses of the outcome of the MRR experiments were focused only on the marked mosquitoes, which were identified to have been recaptured after being released.

Recapture of the test mosquitoes was achieved from nine (9) out of twelve release experiments. Thirty-one (31) marked test mosquitoes were recaptured from 4,460 released. This indicates a recapture rate of 0.7%. More mosquitoes (17, 55%) were recaptured from Kpalsogu during the rainy season than from Libga (14, 45%), where MRR was mostly done during the dry season. The difference in recapture rate between the study sites, as well as the rainy and dry seasons was not statistically significant ($Z=0.79$, $P=0.21$).

The breakdown of the recaptured test mosquitoes comprised 25 (81%) *An. gambiae s.l.* and 6 (19%) *An. funestus* complex (Figure 1), from which 28 (90%) were resting outdoors, and 3 (10%) indoors. The majority of the mosquitoes recaptured outdoors were *An. arabiensis* (11, 39%), followed by *An. coluzzii* (7, 25%) and *An. funestus s.s.* (6, 21%). *An. gambiae s.s.* constituted the least outdoor-recaptured species (4, 15%) (Table 1). The three mosquitoes recaptured indoors were all identified as *An. coluzzii*. None of the control samples that were released outdoors was recaptured from any location, indoor or outdoor.

Discussion

Behavioural shift from predominantly indoor to outdoor-resting is becoming widespread in vector populations in settings where anti-vector interventions are extensively deployed^{6,15,16,35,36}. The aim of this study was to examine if outdoor resting behaviour of the malaria vector populations described in the study settings^{6,7} was consistent or it was just a random occurrence. The study observed that among the test mosquitoes recaptured, the majority was collected outdoor. This suggested that these mosquitoes returned outdoors to rest after being released indoors. This pattern of mosquitoes returning outdoors was observed from nine out of the twelve experiments, despite a low recapture rate. As outdoor resting behaviour in

Figure 1. Composition of released and recaptured marked test and control mosquitoes. “Test” (left) are mosquitoes collected from outdoor and released indoors, whereas “Control” (right) are those mosquitoes collected from indoor and released outdoors. “Test” mosquitoes are the mosquitoes being investigated for consistency or randomness in resting behaviour. “Control” mosquitoes are the counterpart used to confirm alternate trend in the behaviour of the “test” mosquitoes.

Table 1. Molecular species of the recaptured test mosquitoes. These were the marked test mosquitoes only, 28 of which were recaptured outdoors and 3 indoors.

Mosquito species	# Recaptured	
	Indoor	Outdoor
<i>An. arabiensis</i>	0	11
<i>An. coluzzii</i>	3	7
<i>An. gambiae s.s.</i>	0	4
<i>An. funestus s.s.</i>	0	6

vector population could counteract control efforts^{18,37,38}, research assessing the extent of this behaviour is well-timed and necessary to guide decision making in malaria control programmes.

Switch to outdoor-resting behaviour in the vectors may be as a result of avoidance behaviour from indoor interventions^{38,39}, which were heavily used in the study sites. Most sleeping rooms had LLINs and they were also sprayed with pirimiphos methyl, the insecticide that was being used for IRS at the time of the experiments⁷. These mosquitoes might be avoiding contact with the insecticide indoors and chose to rest outdoors, where they may be safe, as there was no outdoor intervention in the

villages. This could have also accounted for the predominant outdoor resting preference documented in the vectors in a previous study from this setting⁶ and others all over Africa^{6,8,12,36,37}, which prompted this investigation.

The highly endophilic vectors, *An. gambiae s.s.*, *An. coluzzii* and *An. funestus s.s.* were recaptured outdoors after being released indoors. This suggests a likely switch to outdoor resting behaviour because these vector species may be avoiding contact with insecticide indoors and exiting to rest outdoors^{40–42}. Change from endophily to exophily in response to control intervention is increasingly being documented in these highly endophilic vectors^{8,12,16,35,43,44}. This could have negative impact on malaria control as it can promote outdoor and residual transmission in these settings^{11,38,45}.

Anopheles arabiensis was found to predominate the mosquito species found resting outdoors from the recaptured test mosquitoes. This may be because *An. arabiensis* is known to be highly exophilic and zoophilic (preference for animal blood meal)^{46–48}, where it tends to stay outdoors to rest and feed on animals when there is intervention indoors⁴⁹. This vector species has also been observed to display insecticide avoidance and early-exiting behaviour^{12,18,42}, which make them difficult to control¹¹.

The study recorded a much lower recapture rate than most MRR studies reported. Several reasons could have accounted for this. One is that the intervention indoors could have could have

killed the mosquitoes while attempting to rest post-feeding^{50,51}. Another reason is the age of mosquitoes^{20,22,52}, which was unknown in this study. Plausibly, if the mosquitoes were old, they may have died within the period of recapture. Indeed, senescence is a factor associated with reduction of mosquito daily survival in the wild^{53,54}. Age was also previously suggested to be responsible for the low recapture rate in a similar experiment²⁰. Moreover, predators such as spiders were particularly common in the study areas, and were found inside animal houses that provide favourable environment for the mosquitoes to rest. Likewise, as some of the released mosquitoes were blood fed as well as semi-gravid and gravid, they might divert to seeking favourable oviposition spots which naturally occurs in the night. These mosquitoes may not return to rest within the same locality or might have been exposed to predators or died naturally. Other factors including emigration from the study area, climate condition, stress and negative effect of the experimental procedures could have also contributed to this low recapture success as previously documented in other MRR experiments^{20,22,27}.

The study also recaptured more mosquitoes during the rainy season relative to the dry season. This might be because more mosquitoes were released during this rainy season as expected. However, none of the control mosquitoes was recaptured throughout the experiments. The possible explanations could be that the mosquitoes might have died due to the residual effect of insecticides^{50,51} from their initial contact before collection. It may also be due to predator attack and other factors as suggested above.

The study acknowledges the limitations in the sample size used in this study. Also, due to cost and logistic reasons, more MRR experiments could not be conducted equally at the study sites during each season.

Conclusions

This study suggests that preference for outdoor resting in malaria vectors may be an emerging possibly consistent behaviour. There is a need for further studies to establish this observation in settings where interventions are extensively deployed. Furthermore, a probe into the genetic basis underlying this behavioural change will also be highly essential. This is important as malaria control moves to the elimination phase in sub-Saharan African countries.

Data availability

Underlying data

Open Science Framework: Is outdoor-resting behaviour in malaria vectors consistent? Short report from northern Ghana, <https://doi.org/10.17605/OSF.IO/HFRVW>⁵⁵.

This project contains the following underlying data:

- Raw datasheet.xlsx
- Species Identification_Raw.xlsx

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

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Ibrahima Dia

Medical Zoology Pole, Pasteur Institute of Dakar, Dakar, Senegal

The paper by Hamid-Adiamoh *et al.* deals with the problem of outdoor-resting behaviour of malaria vectors as observed in several contexts in Africa following the implementation of control strategies. In fact, even if this phenomenon is confirmed in many places in tropical Africa, including in northern Ghana, there is no evidence of its consistency or if it is a phenomenon specific to populations usually found outside human dwellings. This study was carried out in two villages in northern Ghana during the rainy season and dry season. It consisted of releasing inside the human rooms mosquitoes collected resting outside and endophilic mosquitoes outside. The major results showed a tendency for mosquitoes to retain their exophilic behavior.

The paper is well presented and the results seem conclusive but it has some limitations:

- The two villages selected do not have the same status concerning the control measures implemented (IRS in the village of Kpasolgu since 2008), a presentation of the results by village is necessary to understand their possible effect on the exophilic behavior.
- In some parts, the authors should highlight the outdoor-resting behavior rather than residual transmission since no data on the mosquito aggressiveness to humans has been presented knowing that it is this fraction which is studied for the estimation of transmission. In addition, there is often no definite link between the resting place and the biting site, as mosquitoes can have different resting and biting behaviors.
- The lack of data on the physiological status of mosquitoes does not allow a better appreciation of the outdoor-resting behavior. Indeed, resting mosquitoes are often those that have already taken their blood meal and are looking for an appropriate site for blood digestion. For example, if exophagous mosquitoes are collected outdoors before and then released indoors, they will tend to return to their preferred biting site and vice versa. As data on the physiological state of mosquitoes are lacking, this should be discussed.
- If previous data on the vectors are available, it would be interesting to present them to allow a better understanding of vector behavior changes, especially in the village of

Kpasolgu where the IRS have been carried out since 2008.

Specific remarks:

- Page 3, column 1, "Introduction section", line 10, replace "Libreville" by Gabon to homogenize with the other countries.
- Page 3, column 1, "Introduction section", last paragraph of the introduction section. Even if the age is unknown, please provide the physiological age if known.
- Page 3, column 1, "Methods section", line 5, replace "density" by "amount".
- Page 3, column 2, "Mosquito processing, marking and subsequent release":
In this part, we understand that the mosquitoes collected without determining their physiological age, have digested the bloodmeal they have taken and are looking for a suitable breeding site to lay their eggs (case of gravid mosquitoes) or for a host (case of unfed mosquitoes) to take a blood meal. If these data are not available, this issue should be discussed as a limitation of the study.
- Page 4, column 1, paragraph 3, line 3, remove "Most of" and give the exact number of experiments carried out in each of the two villages.
- Page 4, column 2, "Discussion section", line 3, remove the word "vector".
- Page 5, column 2, second paragraph, if you have existing data on vector behaviour, please provide them to better understand the switch to outdoor resting behaviour.
- Page 5, column 2, last line, the words "could have" were repeated.
- Page 5, column 1, first paragraph, the authors talk about the physiological status, while no data is given in the paper. If these data are available, they should be presented in the paper, to support the idea raised in this part.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical Entomology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 17 Feb 2022

Majidah Hamid-Adiamoh, MRC Unit The Gambia at the London School of Hygiene & Tropical Medicine, Banjul, The Gambia

The authors are very grateful to the reviewer for his suggestions and critiques that have improved this manuscript. Please kindly see our responses to each of the comments below.

General

The paper by Hamid-Adiamoh et al. deals with the problem of outdoor-resting behaviour of malaria vectors as observed in several contexts in Africa following the implementation of control strategies. In fact, even if this phenomenon is confirmed in many places in tropical Africa, including in northern Ghana, there is no evidence of its consistency or if it is a phenomenon specific to populations usually found outside human dwellings. This study was carried out in two villages in northern Ghana during the rainy season and dry season. It consisted of releasing inside the human rooms mosquitoes collected resting outside and endophilic mosquitoes outside. The major results showed a tendency for mosquitoes to retain their exophilic behavior.

The paper is well presented and the results seem conclusive but it has some limitations:

Comments: The two villages selected do not have the same status concerning the control measures implemented (IRS in the village of Kpasolgu since 2008), a presentation of the results by village is necessary to understand their possible effect on the exophilic behavior.

Response: Two new tables have been generated to present results by study sites.

Comments: In some parts, the authors should highlight the outdoor-resting behavior rather than residual transmission since no data on the mosquito aggressiveness to humans has been presented knowing that it is this fraction which is studied for the estimation of transmission. In addition, there is often no definite link between the resting place and the biting site, as mosquitoes can have different resting and biting behaviors.

Response: We have addressed this observation. Outdoor resting has been focused on throughout the manuscript. Residual transmission was only inferred as a consequence of outdoor feeding.

Comment: The lack of data on the physiological status of mosquitoes does not allow a better appreciation of the outdoor-resting behavior. Indeed, resting mosquitoes are often those that have already taken their blood meal and are looking for an appropriate site for

blood digestion. For example, if exophagous mosquitoes are collected outdoors before and then released indoors, they will tend to return to their preferred biting site and vice versa. As data on the physiological state of mosquitoes are lacking, this should be discussed.

Response: We agree with the reviewer on this comment. We have included the limitation in determining the physiological status of the mosquitoes in this study, despite efforts to do so.

Comment: If previous data on the vectors are available, it would be interesting to present them to allow a better understanding of vector behavior changes, especially in the village of Kpasolgu where the IRS have been carried out since 2008.

Response: Unfortunately, this is not available. Only the high exophilic behaviour of mosquito population known to be highly more endophilic are available and presented in the text.

Specific remarks

Comment: Page 3, column 1, "Introduction section", line 10, replace "Libreville" by Gabon to homogenize with the other countries.

Response: We have replaced Libreville with Gabon as corrected.

Comment: Page 3, column 1, "Introduction section", last paragraph of the introduction section. Even if the age is unknown, please provide the physiological age if known.

Response: This suggestion has been incorporated. We now identify the mosquitoes released as adults.

Comment: Page 3, column 1, "Methods section", line 5, replace "density" by "amount".

Response: We have replaced density with amount as corrected.

Comment: Page 3, column 2, "Mosquito processing, marking and subsequent release": In this part, we understand that the mosquitoes collected without determining their physiological age, have digested the bloodmeal they have taken and are looking for a suitable breeding site to lay their eggs (case of gravid mosquitoes) or for a host (case of unfed mosquitoes) to take a blood meal. If these data are not available, this issue should be discussed as a limitation of the study.

Response: This has been discussed as a limitation.

Comment: Page 4, column 1, paragraph 3, line 3, remove "Most of" and give the exact number of experiments carried out in each of the two villages.

Response: The exact numbers are now provided as suggested.

Comment: Page 4, column 2, "Discussion section", line 3, remove the word "vector".

Response: Vector is now removed.

Comment: Page 5, column 2, second paragraph, if you have existing data on vector behaviour, please provide them to better understand the switch to outdoor resting behaviour.

Response: The existing data we have is the predominant outdoor resting in the vector

population in the same study sites and this has been cited.

Comment: Page 5, column 2, last line, the words “could have” were repeated.

Response: The second 'could' is now removed.

Comment: Page 5, column 1, first paragraph, the authors talk about the physiological status, while no data is given in the paper. If these data are available, they should be presented in the paper, to support the idea raised in this part.

Response: This data were not recorded as the study decided to release all mosquitoes irrespective of their abdominal status because of the small number collected. However, the study did observe that some of the mosquitoes were blood fed, half-gravid and gravid.

Competing Interests: The authors disclose that there are no competing interests.

Reviewer Report 10 February 2022

<https://doi.org/10.21956/aasopenres.14444.r29099>

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Collins Kalwale Mweresa

Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya

In general: The final version of the revised manuscript should be proof-read and improved by a native English speaker to settle grammatical issues and provide concise and precise statements.

Title:

- Needs to be revised so that it can capture the study accurately. This was an experimental study.

Background:

- Sentence 1 and 2 should be revised to enhance logical flow.

Methods:

- Specify the numbers of mosquitoes' releases and numbers of replicates involved. Why didn't you release equal numbers of *Anopheles gambiae* and *An. funestus*?

Introduction:

- Provide published evidence/data on the contribution of outdoor transmission to changes in malaria prevalence. Quantify the magnitude of malaria reduction due to IRS and LLLIN. Sentence two should be revised. Collect more literature on causes and mechanisms of mosquito resistance to insecticides, what was the aim and specific objectives of this study? This needs to be captured in the last paragraph.

Methods:

- Why was this particular study area preferred? Insert period by months in brackets for each season, what agricultural activities are supported by irrigation in the study area?
- Specify whether indoor and outdoor mosquito collections were done in the same site/compound, simultaneously, involved equal numbers of sites/compounds and state the time intervals over which the experiments were conducted.
- Specify the time interval between marking and release of the mosquitoes?
- How many mosquitoes of each species were released daily and at what frequency? How come that equal numbers of mosquito species were not released during the study? How did you deal with the variation associated with the release of unequal numbers of mosquitoes per species?
- Thus, accurate study and experimental design should be provided. How come that mosquitoes of the same age/colony were not used in this study?

Ethical review:

- Why were children allowed to sleep in the rooms and why was this not mentioned in the section above? And what was the inclusion and exclusion criteria used to select children for this activity? Did any of the children fall sick during the study? If so, how were they handled?
- Do not begin sentences with abbreviations e.g. MRRR or numerical values. Instead write in full/words if used at the start of the sentence. How did you confirm whether the captured and released mosquitoes were not infective?

Results:

- Sentence three is incomplete and needs to be revised, rephrase the legend for figure one.
- Description of test and control mosquitoes should be done accurately and transferred from the results to the methods section. Sentence six should be revised. What was the proportions of *An. gambiae* s.s and *An. Arabiensis*? And that *An. gambiae* s.s is not a species but sub-species. Table 1 needs to be revised by changing from molecular species to molecular identification of captured (...), the information provided in the same table is equally weak and should be deleted.
- Generally, the results provided in this section are not sufficient enough to make a valid and reliable conclusion.

Discussion:

- This section needs to be strengthened. What was the time interval between insecticide application and the onset of experiments? Account for such time interval. What new/novel information has this study generated towards malaria elimination and eradication? What next?

Reference:

- Ensure that all references are in conformity with the format recommended by this particular journal.

Conclusion:

- Suggestions made hereby should be incorporated and entire document proof-read by a native English speaker prior to indexing of this write-up.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical Entomology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 17 Feb 2022

Majidah Hamid-Adiamoh, MRC Unit The Gambia at the London School of Hygiene & Tropical Medicine, Banjul, The Gambia

The authors are very grateful to the reviewer for his suggestions and critiques that have improved this manuscripts. Please kindly see our responses to each of the comments below.

In general

Comment: The final version of the revised manuscript should be proof-read and improved by a native English speaker to settle grammatical issues and provide concise and precise statements.

Response: We hope the manuscript now reads well with the changes incorporated.

Title

Comment: Needs to be revised so that it can capture the study accurately. This was an experimental study.

Response: The title has been revised and it now reads: "Is outdoor-resting behaviour in malaria vectors consistent? Short experimental report from northern Ghana".

Background

Comment: Sentence 1 and 2 should be revised to enhance logical flow.

Response: We have revised these sentences as suggested.

Methods

Comment: Specify the numbers of mosquitoes' releases and numbers of replicates involved.

Response: We have indicated the numbers of mosquitoes and replicates appropriately.

Comment: Why didn't you release equal numbers of *Anopheles gambiae* and *An. funestus*?

Response: We released all the female mosquitoes collected each. We did not control the number to be released, regardless of the species. We have also discussed this as a limitation.

Introduction

Comment: Provide published evidence/data on the contribution of outdoor transmission to changes in malaria prevalence.

Response: These have been provided.

Comment: Quantify the magnitude of malaria reduction due to IRS and LLLIN.

Response: These have been provided.

Comment : Sentence two should be revised.

Response: This sentence has been revised.

Comment: Collect more literature on causes and mechanisms of mosquito resistance to insecticides,

Response: We have included literatures on behavioural resistance to insecticide.

Comment: What was the aim and specific objectives of this study? This needs to be captured in the last paragraph.

Response: The aim of the study is now clearly indicated in the text.

Methods

Comment: Why was this particular study area preferred?

Response: We have included the reasons for the choice of the study area, which were because of the observation of predominant outdoor resting previously documented in the vectors; as well as the area being under coverage of anti-vector interventions.

Comment: Insert period by months in brackets for each season

Response: This is done accordingly.

Comment: What agricultural activities are supported by irrigation in the study area?

Response: This has been indicated as rice and tomatoes

Comment: Specify whether indoor and outdoor mosquito collections were done in the same site/compound, simultaneously, involved equal numbers of sites/compounds and state the time intervals over which the experiments were conducted.

Response: Yes, this was done simultaneously and it has been indicated. Other suggestions have also been incorporated.

Comment: Specify the time interval between marking and release of the mosquitoes?

Response: This is already indicated in the manuscript to be within 3 hours.

Comment: How many mosquitoes of each species were released daily and at what frequency?

Response: This information is already provided in the text. Again, in this revision, we have included two tables to indicate the number released and recaptured by species by study site

Comments: How come that equal numbers of mosquito species were not released during the study? How did you deal with the variation associated with the release of unequal numbers of mosquitoes per species? Thus, accurate study and experimental design should be provided. How come that mosquitoes of the same age/colony were not used in this study?

Response: We released all the female mosquitoes collected each. We did not control the number to be released, regardless of the species. We were unable to due to technical error and failed alternative arrangements. We have also discussed these as a limitation.

Ethical review

Comments: Why were children allowed to sleep in the rooms and why was this not mentioned in the section above? And what was the inclusion and exclusion criteria used to select children for this activity? Did any of the children fall sick during the study? If so, how were they handled?

Response: The study did not release mosquitoes in rooms where children were sleeping. There was no children in any of the rooms used in the study.

Comments: Do not begin sentences with abbreviations e.g. MRRR or numerical values. Instead write in full/words if used at the start of the sentence.

Response: This has been duly corrected.

Comments: How did you confirm whether the captured and released mosquitoes were not infective?

Response: This was not done.

Results

Comments: Sentence three is incomplete and needs to be revised, rephrase the legend for

figure one.

Response : This sentence has been revised.

Comments: Description of test and control mosquitoes should be done accurately and transferred from the results to the methods section.

Response: This is already done at the methods section.

Comments: Sentence six should be revised.

Response: This sentence has been revised.

Comments: What was the proportions of *An. gambiae* s.s and *An. Arabiensis*?

Response : Details of percentage of *An. gambiae* s.s. and *An. arabiensis* are also already in the manuscript. This can be seen in the text below and specifically highlighted:

"The breakdown of the recaptured test mosquitoes comprised 25 (81%) *An. gambiae* s.l. and 6 (19%) *An. funestus* complex (Figure 1), from which 28 (90%) were resting outdoors, and 3 (10%) indoors. The majority of the mosquitoes recaptured outdoors were *An. arabiensis* (11, 39%), followed by *An. coluzzii* (7, 25%) and *An. funestus* s.s. (6, 21%). *An. gambiae* s.s. constituted the least outdoor-recaptured species (4, 15%) (Table 3)".

Comments: And that *An. gambiae* s.s is not a species but sub-species.

Response: We will like to confirm that *An. gambiae* s.s. is a species not a sub-species.

Comments: Table 1 needs to be revised by changing from molecular species to molecular identification of captured (...), the information provided in the same table is equally weak and should be deleted.

Response: Authors think that molecular identification is more relevant under a method than result. We will like to retain the Table 1 as it currently is indicating the species of the mosquitoes recaptured.

Comments: Generally, the results provided in this section are not sufficient enough to make a valid and reliable conclusion.

Response: The authors appreciate the reviewer's comment. We believe the data we present here adequately support our conclusion. We do hope that the changes we have incorporated have improved the manuscript.

Discussion

Comments This section needs to be strengthened. What was the time interval between insecticide application and the onset of experiments? Account for such time interval.

Response : This time interval is now indicated in the text.

Comments: What new/novel information has this study generated towards malaria elimination and eradication? What next?

Response: This study acknowledges that the findings indicate a possible consistent outdoor-resting behaviour in a setting with high indoor intervention only. The study thus recommends a parallel outdoor intervention to be implemented in such settings to complement the existing tools. Further investigations are also encouraged to establish the

findings.

Reference

Comments: Ensure that all references are in conformity with the format recommended by this particular journal.

Response: These have been done appropriately.

Conclusion

Comments: Suggestions made hereby should be incorporated and entire document proof-read by a native English speaker prior to indexing of this write-up.

Response: All suggestions have been accepted and incorporated.

Competing Interests: We disclose that there were no competing interests