RESEARCH ARTICLE

Characterisation of human exposure to nocturnal biting by malaria and arbovirus vectors in a rural community in Chókwè district, southern Mozambique [version 1; peer review: 1 approved, 4 approved with reservations]

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Abstract

Background: Understanding the magnitude of human exposure to mosquito biting is fundamental to reduce pathogen transmission. Here we report on a study quantifying the levels of mosquitoes attacking humans throughout the night in a rural area of Southern Mozambique.

Methods: Surveys were carried out in Massavasse village, southern Mozambique. The abundance and composition of host-seeking mosquito communities at night were assessed by human-landing catches (HLC) at one-hour intervals. Periods when people were located predominantly outdoors or indoors were used to estimate the amount of residents' exposure to mosquito bites in either location, to explore the potential impact a bed net could have had in reducing biting by each vector species.

Results: A total of 69,758 host-seeking female mosquitoes comprising 23 species in four genera were collected. The exposure to biting by virtually all vector species was consistently high outdoors, typically at early evening and morning, with exception of *An. gambiae s.l* which was likely of biting a person with nearly same intensity indoors and outdoors throughout the night. Bed nets use could have reduced biting by *An. gambiae s.l* (dominated by *An. arabiensis*), *Ma. africana*, *Ma. uniformis*, *Cx. pipiens*, *Cx. antennatus*, and *Cx. poicilipes* by 53%, 47%, 46%, 38%, 31%, and 28% respectively, compared to non-users. Conversely, a bed net user would have had little protection against *An.*

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pharoensis, *An. ziemanni*, *An. tenebrosus*, and *Cx. tritaeniorhynchus* biting exposures.

Conclusions: This study showed that Massavasse residents were exposed to high levels of outdoor biting by malaria and arbovirus vectors that abound in the village. The findings help to identify entomological drivers of persistent malaria transmission in Mozambique and identify a wide range of arbovirus vectors nocturnally active in rural areas, many with outbreak potential. The study highlights the need for a surveillance system for monitoring arboviral diseases vectors in Mozambique.

Keywords

Malaria, arboviruses, mosquito vectors, exophagy, endophily, man-biting exposure, Massavasse village, Mozambique

article can be found at the end of the article.

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Introduction

Mosquitoes are responsible for transmitting some of the deadliest and most debilitating diseases to humans. Today, more than half of the global population is at risk from mosquito-borne diseases such as malaria, dengue, chikungunya, yellow fever, Japanese encephalitis and West Nile fever[1]. These diseases have caused enormous suffering and remain a lasting impediment to societal development of endemic countries, particularly those in sub-Saharan Africa[2]. Collectively, mosquito transmitted arboviral diseases, notably, dengue, chikungunya, Japanese encephalitis, and West Nile fever have been steadily increasing in geographic range, and in the frequency and magnitude of outbreaks[3–6]. Dengue incidence has increased more than 30 fold over the last three decades, becoming one of the world's most common and rapidly widespread arboviral disease primarily, but not only, due to the expansion of its main vectors[3,7–10].

Historical and recent evidence indicate that it is highly likely that Mozambique is endemic for at least some arboviruses, most notably dengue, chikungunya, and Rift-valley fever[11–15]. However, the burden and geographic distribution of these and other arboviral diseases remain largely unknown as are the identities of the vectors apart from the major species.

Mozambique accounts for over 6% of global malaria deaths and 4.1% of malaria cases[16], and the high rate of malaria transmission is the basis for classification of malaria as a major public health threat in Mozambique. While undoubtedly true, this has also served to divert attention away from other important vector-borne diseases. For example, a recent study reported that in 22 health units across 11 provinces of Mozambique, nearly 72% of febrile cases classed as malaria-negative had received treatment for malaria[17], without consideration of other possible causes, including arbovirus infections. However, the recent dengue outbreaks in the northern provinces of Nampula and Cabo Delgado[18] have come as a sharp reminder of the presence of mosquito-borne diseases other than malaria in many areas of Mozambique and of the need for even basic studies on the vectors ecology.

More than a hundred mosquito species have been recorded in Mozambique, including many known malaria and arbovirus vector species[19–22]. However, the public health importance of local mosquito fauna remains poorly determined, though a great proportion of them have been found infected with pathogenic viruses[21]. The historic interest in malaria and the focus on controlling transmission is the most likely explanation why most entomological studies focused on the main malaria vectors in the *Anopheles gambiae* complex and the *An. funestus* group, neglecting the possibility of secondary malaria vectors or the primary arbovirus vectors occurring in the same areas.

The diversity of mosquito vector species reported from Mozambique suggests complexity in the transmission ecology of mosquito-borne diseases with unknown, yet potentially imminent, risks of outbreaks. Effective vaccines are few, and vector control interventions remain the most reliable and cost-effective control measures against virtually all known mosquito-borne arboviruses[23]. Therefore, successful elimination or control of a vector relies on understanding the dynamics of its behaviour and, variations of key transmission indicators, notably the incidence of mosquito contact with a human, also known as the human-biting rate (HBR)[24,25]. When calculated correctly, the HBR gives the most realistic estimation of the magnitude of human exposure to a mosquito vector over time and space and, in combination with information on the human host's nocturnal and diurnal behavioural habits, it can indicate the likely success of protection methods directed at individuals or the community[26,27]. Apart from information on the dominant malaria vectors *An. funestus* and *An. arabiensis* in a few regions[28–32], detailed patterns of nightly human exposure to host-seeking mosquito vectors in Mozambique is scant. The timing and diurnal cycles of vector activity are dynamic, often changing according to geographic regions[26,33], local environmental and climatic conditions[28,34,35], host availability[36], or vector control pressure[37–39].

In-depth and site-specific characterization of human exposure to mosquitoes is essential when designing and deploying accurate vector control to optimally target host habits. As such, the overreaching goal of this study was to investigate patterns of human exposure to potential vectors of malaria and arboviruses in Massavasse village, an irrigated rice ecosystem located in the Chókwè district in southern Mozambique.

Methods

Study site

Entomological surveys were carried out in 2016 in Massavasse village (-24.624839°S; 33.111787°E). Massavasse village is in Lionde Administrative Post, southeast Gaza province, southern Mozambique (Figure 1). The village has one of the highest malaria prevalence compared to other regions in southern Mozambique, and a well characterized mosquito population[31]. It comprises one of the most intensively irrigated areas of Mozambique (Figure 1). The hot and rainy season in the village ranges from October to April, whereas the dry and cold season ranges from May to September. The mean air temperature during the hot/rainy season ranges between 25°C and 34°C, and 22°C and 16°C during the winter/dry season; the maximum average annual rainfall is 600 mm. The village has been inhabited by at least 4,711 individuals divided in, at least 989 households[40]. The villagers grow rice in the irrigated fields surrounding the village, and at the lowlands of Limpopo River, which delineates the village from north to east (Figure 1). Four main irrigations channels bordering the village provide temporary and permanent breeding sites for many mosquito species all year round. Known malaria vector species include members of *Anopheles gambiae* complex and *An. funestus* group and several other species including *An. pharoensis*, *An. tenebrosus*, *An. ziemanni*. More than 15 species of culicines, including known arbovirus vectors, such as *Culex tritaeniorhynchus*, *Cx. poicilipes*, *Cx. Antennatus*. *Mansonia uniformis*, *Man. africana*, *Aedes sudanensis*, among others, have also been found in the village.

Figure 1. Map of south Mozambique, showing the location of area studied at Massavasse village in the Lionde administrative post, Chókwè district, Gaza province. Mozambique administrative border polygons and roads, and the polygons of river lines and water areas were obtained from the Humanitarian Data Exchange (https://data.humdata.org/). Polygons of cropland areas around Massavasse village were made by authors with Google Earth Pro, using global croplands data obtained from the Global Land Analysis & Discovery (https://glad.umd.edu/dataset/croplands?s=03).

Mosquito collection

Mosquito surveys were carried out from February to April 2016. Paired indoor and outdoor human landing catches were conducted in neighbourhoods two to six of Massavasse village (Figure 1). In each neighbourhood, on each sampling date, two sampling locations were randomly selected and two purpose-built sentinel huts (Figure 2) were placed at least fifty metres apart at each location. Mosquito collections were conducted hourly from 19:00 to 05:00 with pairs of collectors, one pair seated inside and other outside the sentinel huts, with legs uncovered. Mosquitoes were collected after alighting but before biting the collectors' legs. Collectors were randomly assigned to the sentinel hut positions and undertook landing collections for a period of one hour after which they were rotated between positions in subsequent collection periods, allowing collectors to alternate between sentinel points and allow one hour's rest between collections periods avoiding unnecessary interruptions and reducing the potential influence of relative attractiveness[41] and collector fatigue on mosquito catches. The time of sunset and sunrise was obtained with a hand-held GPS unit (Garmin e-Trex® H; Garmin Ltd, Southampton, U.K.).

Description of sentinel huts

The portable sentinel huts were built from polyethylene shade cloth walls on aluminium frames measuring 290 cm x 264 cm x 270 cm (Figure 2). The roof was made of 100% waterproof canvas. The walls had no windows and were composed of a high-density polyethylene shade cloth locally obtained, that purportedly blocks 98% of ultraviolet rays and

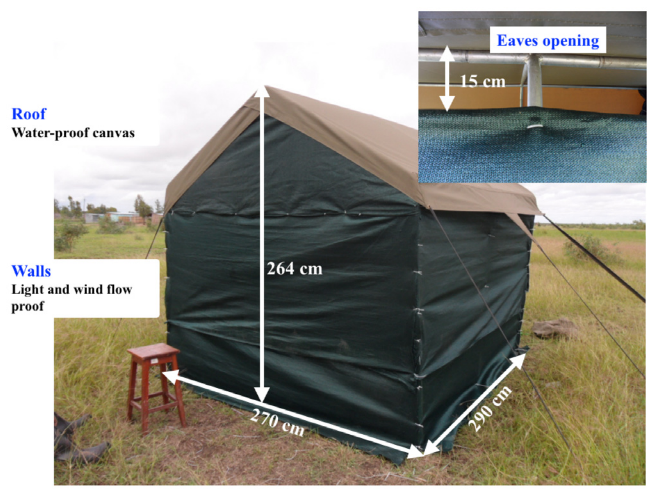

Figure 2. Details of the experimental hut used for indoor host-seeking mosquito collections.

95% of visible light (manufacturer's claim). Previously, the same material had been shown to prevent 99% of indoor draughts or air currents[42]. On this basis, we assumed that the only possible route for the cues from the hut interior would be through the 15 cm 'eave' apertures between the walls and the roof (Figure 2S). The walls were fastened to the frame with plastic cable ties to facilitate rapid assembly and deconstruction. When complete, the roof was secured with guy lines, to minimise movement. During periods when it was in use, the average temperature inside the hut was 27°C (range 25°C - 28°C).

Sleep survey
The start and end times of sleep periods and the time spent indoors and outdoors by inhabitants were determined from data collected at 312 of the 989 households that existed in Massavasse village at the time. At least 60 to 62 randomly chosen households were visited in each of the five neighbourhoods studied. A household was defined as a house or compound occupied by a group of individuals during the study. Only adult individuals (≥ 18 years old) were interviewed based on their willingness to participate. Permission to interview the participate was sought from the head of the household. Everybody that accepted to be interviewed, including the head of the household, signed an informed consent form.

Participants were administrated a questionnaire containing questions to understand where they slept at night, what time they slept at night and woke up in the morning. In those cases where a household had more than one adult, individuals were interviewed separately to prevent them from influencing each other in their responses.

The survey was undertaken from afternoon to near sunset when most of the people were expected to have returned home from their daily duties. A copy of the questionnaire used can be found in Extended data.

Sample processing and analysis
Collected mosquito samples were transported to the insectary and euthanized by putting paper cups with specimens inside a refrigerator for 20 to 30 minutes. Female and male *Anopheles* mosquitoes were morphologically identified to species according to taxonomic keys from Gillies & De Meillon[43] and Gillies & Coetzee[44], whereas non-*Anopheles* mosquitoes were identified using taxonomic keys proposed by Edwards[45]; Jupp[46]; Harbach[47] and Service[48]. Mosquito samples that could not be morphologically identified, such as sibling species members of *An. gambiae* complex and *An. funestus* group, were further identified by molecular analysis (PCR) using the protocols proposed by Scott *et al.*,[49] and Koekemoer *et al.*,[50], respectively.

Data analysis
Estimation of human-biting rates
Estimation of human exposure to mosquito bites was only carried out for species where the total catch was equal to or greater than 1% of all mosquitoes caught.

Crude hourly mosquito biting density was determined as the average number of mosquitoes landing on a person/site/night. The hourly man-biting density of a given mosquito species was expressed in terms of Williams's mean (Mw), described as follows: $\mathrm{M_w} = exp\left(\frac{\sum ln(n_i+1)}{N}\right) - 1$, where n_i is the total number of species *i,* and *N* is the number of sampling days. The Williams´ mean is a more appropriate measure of central tendency than the arithmetic mean, since the estimate is less affected by unusually small or very large samples[51], not an uncommon event when sampling mosquito populations[52].

Behaviour-adjusted hourly total number of bites experienced by unprotected individuals (B_u) were calculated based on data from both human and mosquito behaviours, as suggested by Killeen *et al*[27]. As such, the biting rate of a mosquito species *i*, experienced by unprotected individuals at time (*t*) was estimated based on the proportion of people reported to have stayed indoors (S_t) multiplied by crude indoor biting rate by species *i* at time *t* (B_{Iit}) plus the proportion of people reported to have stayed outdoors ($1\text{-}S_t$) multiplied by the crude outdoor biting rate (B_{Oit}):

$$B_{uit} = B_{Iit} S_t + B_{Oit}(1 - S_t) \quad (1)$$

Thus, the overall nightly biting rate by mosquito species *i* experienced by an unprotected individual was calculated by summing biting rates for each observation hour, that is:

$$B_{ui} = \sum_{t=1}^{n} B_{I,t} S_t + B_{O,t}(1 - S_t) \quad (2)$$

Where t = 1 corresponded to period from 19:00 – 20:00, t = 2 period 20:00 – 21:00, and continues up to time t = n, which in our case corresponded to period from 05:00 hrs – 06:00. We defined an unprotected individual as someone lacking any type of insecticide treated bed nets (ITNs). The biting exposure by species *i* at time *t* for a potentially protected individual was estimated by combining mosquito biting rate over time (*t*), the sleeping behaviour of humans, and the protective efficacy of ITNs (*P*), assumed to be constant, which was, as in Killeen *et al.*,[27]. The effective adherence of ITNs use at a given time of the night was assumed to be equivalent to the proportion of people sleeping at that time (S_t).

At the time of writing, there have been no investigations in Mozambique of how human habits and behaviours might compromise or enhance the protective efficacy of insecticide treated nets ITNs). Hence, we explored the extent to which an ITN would potentially protect an individual human against mosquito bites, assuming a conservative average minimum protective efficacy level of 80% (*P* = 0.8) for ITNs, based on studies conducted elsewhere in Africa[53–56]. When an individual is sleeping under an ITN, this is equivalent to a relative exposure to bites of 20% (i.e., 100 – 80)[27]. The biting exposure experienced by protected individuals (*Bp*) was estimated, according to Killeen *et al.*,[27] as follows:

$$B_{pit} = B_{Iit} S_t (1 - P) + B_{Oit}(1 - S_t) \quad (3)$$

Residents were assumed to have remained outdoors after emerging from indoor in the morning, and thus to have been exposed to bites by mosquitoes seeking hosts outdoors. Residents' sleeping hours were assumed to be spent indoors and under an ITN.

The proportion of mosquito bites experienced indoors for an unprotected individual (π_I) was estimated by dividing the total number of bites received indoors (B_I) by the total number of bites received by unprotected individuals, as follows:

$$\pi_{Iit} = \frac{B_{Iit} S_t}{B_{Iit} S_t + B_{Oit}(1 - S_t)} \quad (4)$$

The proportion of bites experienced outdoors for an unprotected human (π_O) was estimated as π_{Oit} = 1 - π_{Iit}. Similarly, the relative proportion of biting for protected (π_{pi}) individual was estimated as:

$$\pi_{pi} = \frac{B_{pit}}{B_{uit}} = \frac{B_{Iit} S_t (1-P) + B_{Oit}(1-S_t)}{B_{Iit} S_t + B_{Oit}(1-S_t)} \quad (5)$$

Statistical analysis

The significance of differences in magnitude between indoor bite exposure vs outdoor bite exposure was estimated using generalized linear models and assuming negative binomial distribution of mosquito counts with log link to explanatory variables. Response variables were mosquito counts, and predictor variables were collection site (indoor = 1, outdoor = 2), studied neighbourhood and day of survey. For the binary explanatory variables, collection site (indoor = 1, outdoor = 2), indoor was arbitrarily considered as the reference level in the regression analysis. Neighbourhood and day of survey were considered random factors to account for unmeasured variability of mosquito counts, and site was considered fixed factor. All data analysis were performed using the software R v. 4.2.2[57].

Ethical clearance

This study received ethical approval from the Comité Nacional de Bioética para Saúde de Moçambique (CNBS) of the Ministry of Health of Mozambique (MISAU), reference 208/CNBS/15. Collectors were provided with Fansidar® (Sulfadoxine-Pyrimethamine) to reduce the likelihood of malaria infection or transmission, in accordance with the recommendations of the Mozambique National Malaria Control Program. This type of preventive measure has been shown to reduce the risk of malaria in HLC volunteers by 96.6% in similar studies carried out elsewhere[58].

Consent

Written informed consents were obtained from all individuals that accepted to participate in the study.

Results

Relative abundance of nocturnally active anthropophagic mosquitoes

A total of 69,758 host-seeking female mosquitoes belonging to twenty-three species and four genera were caught during 35 nights of paired human landing collections indoor (n=19,752) and outdoor (n= 50,006). The genera *Anopheles*, *Culex* and *Mansonia* were the most common (Table 1). Among species in the genus *Anopheles*, *An. gambiae s.l* were the most common [63.2% (7,840/12,401)], followed by *An. pharoensis* [16.0% (1,978/12,401)], *An. ziemanni* [11.8% (1,466/12,401)] and *An. tenebrosus* [8.5% (1,048/12,401)]. The genus *Culex* was mostly represented by *Cx. tritaeniorhynchus* [38.5% (8,511/22,078)], followed *Cx. pipiens s.l* [27.1% (5,990/22,078)], *Cx. poicilipes* [25.6% (5,663/22,078)], and *Cx. antennatus* [8.4% (1,854/22,078)]. The genus *Mansonia* comprised *Ma. uniformis* [88.3% (31,068/35,183)] and *Ma. africana* [11.7% (4,115/35,183)]. The genus *Aedes*, comprised three species, of which *Ae.* (*Coetzeemyia*) *fryeri* was the most predominant species caught [89.5% (77/86)], followed by *Ae*. (*Muscidus*) *sudanensis* [5.8% (5/86)] and *Ae*. (*Stegomyia*) *subargenteus* [4.7% (4/86)]. *Coquillettidia aurites* was the most common of the *Coquillettidia* collected (Table 1).

Table 1. Relative abundance of nocturnally active mosquito species collected by human landing catches in Massavasse village, southern Mozambique from February to April 2016.

Species	Indoor	Outdoor	Total
Anopheles gambiae s.l.	3403	4437	7840
Anopheles pharoensis	28	1950	1978
Anopheles ziemanni	53	1413	1466
Anopheles tenebrosus	25	1023	1048
Anopheles funestus	40	29	69
Aedes fryeri	4	73	77
Aedes subargenteus	0	4	4
Aedes sudanensis	1	4	5
Coquillettidia aurites	1	8	9
Coquillettidia versicolor	0	1	1
Culex tritaeniorhynchus	1007	7504	8511
Culex pipiens s.l.	1019	4971	5990
Culex poicilipes	1219	4444	5663
Culex antennatus	229	1625	1854
Culex quinquefasciatus	43	9	52
Culex bitaeniorhynchus	2	3	5
Culex sitiens	1	2	3
Mansonia uniformis	11156	19912	31068
Mansonia africana	1521	2594	4115

The results of molecular identification carried on a subsample of 806 and 49 mosquito specimens identified morphologically as members of *An. gambiae* complex and *An. funestus* group, respectively are depicted in Table 2. Successful amplification was achieved with 379/806 and 320/806 specimens of *An. gambiae* complex obtained from indoor and outdoor subsamples, respectively. Only 17/49 and 13/49 specimens of *An. funestus* from indoor and outdoor successfully amplified (Table 2). *Anopheles arabiensis* was the most common member of the *Anopheles gambiae* complex, followed by *An. merus*, *An. quadriannulatus*, and *An. gambiae s.s.* *An. gambiae s.s* was only detected in the indoor collections, but *An. arabiensis* was caught biting indoors and outdoors. Of *An. funestus* group, two species were recorded: *An. funestus s.s* was found in indoor and outdoor samples, whereas *An. parensis* was only found in the indoor catches (Table 2).

Nocturnal biting rhythms

The crude mean biting densities of the most common mosquito species already known to be malaria or arbovirus vectors are depicted in Figure 3. Indoor and outdoor activity of *An. gambiae s.l* showed similar patterns, with the biting peak time ranging from around 22:00 to 03:00. The outdoor

Table 2. Result of PCR analyses for molecular identification of sibling species members of *Anopheles gambiae* complex and *An. funestus* group.

Species complex/ group	Molecular ID	Total	
		Indoor	Outdoor
Anopheles gambiae	*Anopheles gambiae s.s.*	2	0
	Anopheles arabiensis	334	270
	Anopheles merus	40	28
	Anopheles quadriannulatus	3	22
Anopheles funestus	*Anopheles funestus s.s.*	10	13
	Anopheles parensis	7	0
Anopheles gambiae	Not amplified	24	83
Anopheles funestus		9	10

biting peak in the biting rhythms of *An. tenebrosus* and *Cx. tritaeniorhynchus* resembled that of *An. gambiae s.l.* Conversely, the outdoor activity of *An. pharoensis* showed a bimodal pattern with two distinct peaks, the larger peak occurring around 22:00, followed by a second small peak in the early morning (02:00). The outdoor biting activity of *An. ziemmani*, *Cx antennatus* and *Cx. poicilipes* peaked later in the morning, around 04:00. These vector species, together with *An. pharoensis*, *An. tenebrosus* and *Cx. tritaeniorhynchus* were actively seeking hosts at a lower intensity indoor throughout the night, but without an obvious peak. Indoor and outdoor biting activity of *Cx. pipiens s.l* and *Ma. africana* peaked around 01:00 to 03:00 (*Cx. pipiens s.l*) and 04:00 (*Ma. africana*) (Figure 3). *Man. uniformis* outdoor biting peaked at 04.00, two hours behind its activity peak indoors (Figure 3).

Negative binomial regression model analyses indicated that a typical person was likely to be bitten by *An. gambiae s.l* with nearly same intensity indoors and outdoors throughout the night (Table 3), whereas biting by all other vector species were far more likely to occur outdoors (Table 3).

Sleep survey data (underlying data https://doi.org/10.17605/OSF.IO/AYFM6) indicated that all inhabitants typically retired indoors by 22:00 and emerged in the morning from 03:00. Estimates of human-biting exposures, adjusted by multiplying the crude biting rate by the proportion of people indoors and outdoors at night, indicated that an unprotected individual (i.e., a non-user of ITNs) would receive an average of 85.93 bites/night by *An. gambiae s.l*, of which 66% were indoors and 34% outdoors (Table 4). For an LLIN user, biting exposure would be reduced by 53% *(i.e.,* 40.5 bites/night), assuming an ITN protective efficacy of 80%. Results also indicated that both protected and non-protected individuals were exposed to similar numbers of *An. pharoensis*, *An. tenebrosus*, *An. ziemanni*, and *Cx. tritaeniorhynchus* bites because using an ITN may reduce biting exposure to these vector species by only 2%, 5%, 9% and 14%, respectively (Table 4). Modest reductions in biting exposure would also be expected for *Cx. pipiens s.l* (38%), *Cx. poicilipes* (28%), *Cx. antennatus* (31%), *Ma. africana* (47%) and *Ma. uniformis* (46%). The dynamics of mosquito biting exposure for non-user individuals are depicted in Figure 4. For nearly all species, a high number of bites on a non-protected individual occurred before bedtime, followed by a small increase of bites in the early morning. Most biting would be expected during late evening before bedtime.

Discussion

Understanding the behavioural preferences and habits of local populations of disease vectors is crucial for optimising control measures and enhancing epidemic preparedness against emergent mosquito-borne diseases. Although a wealth of studies exist on the malaria vectors of the region[31,59], this study is the first to provide a description of the nocturnal biting rhythm of *Anopheles* mosquitoes frequently found in the malaria - endemic area around Massavasse village and to provide direct measures of human exposure to biting by arbovirus vector species that are frequently found in rural settings of Mozambique[21].

Biting activity increased in all mosquito species during early evening. In *An. gambiae s.l.,* biting activity extended from early evening to early morning, biting indoors and outdoors at nearly the same intensity. This pattern of *An. gambiae s.l* biting activity differed from other studies which reported peak activity at around 04:00 - 06:00[33,60–65]. However, the discrepancy may be explained by the fact that the vector population in the present study was not homogeneous. Molecular analyses confirmed the presence of *An. arabiensis*, *An. merus* and *An. quadriannulatus* and *An. gambiae s.s*, in descending order of abundance. *An. arabiensis*, *An. merus* and *An. quadriannulatus* are well known exophagic malaria vectors which can blood-feed both indoors and outdoors. *An. gambiae s.s*, on the other hand, is a strongly endophilic vector[66]. In fact, the biting pattern exhibited by *An. gambiae s.l* in this study resembles that of *An. arabiensis* reported in Matola city, also in southern Mozambique[32], and by *An. merus* in Kenya[67,68]. Marked spatial and temporal variation in host-seeking rhythms among members of the *An. gambiae* complex have also been reported elsewhere[33]. The variabilities may reflect geographic differences in environmental factors modulating host-seeking activity[37,69–71], or the eco-evolutionary and behavioural adaptation of mosquito vectors to the human population's habits, including the control measures used by different host populations. Evidence that shifts in mosquito behavioural preferences can occur following introduction of insecticide-based control interventions comes from studies on *An. farauti* in Solomon Island[37], *An. funestus* in Benin[38,72], and *An. gambiae* in Burkina Faso and Equatorial Guinea[73,74]. All have reported changes from indoor feeding behaviour to a predominantly exophagic habit following the intense insecticide pressure of scaled up indoor residual spraying and ITN coverage.

Host-seeking activity of *An. pharoensis* occurred almost entirely outdoors. The bimodal biting rhythm of *An. pharoensis* reported here, with a peak of activity around 22:00 hrs and a

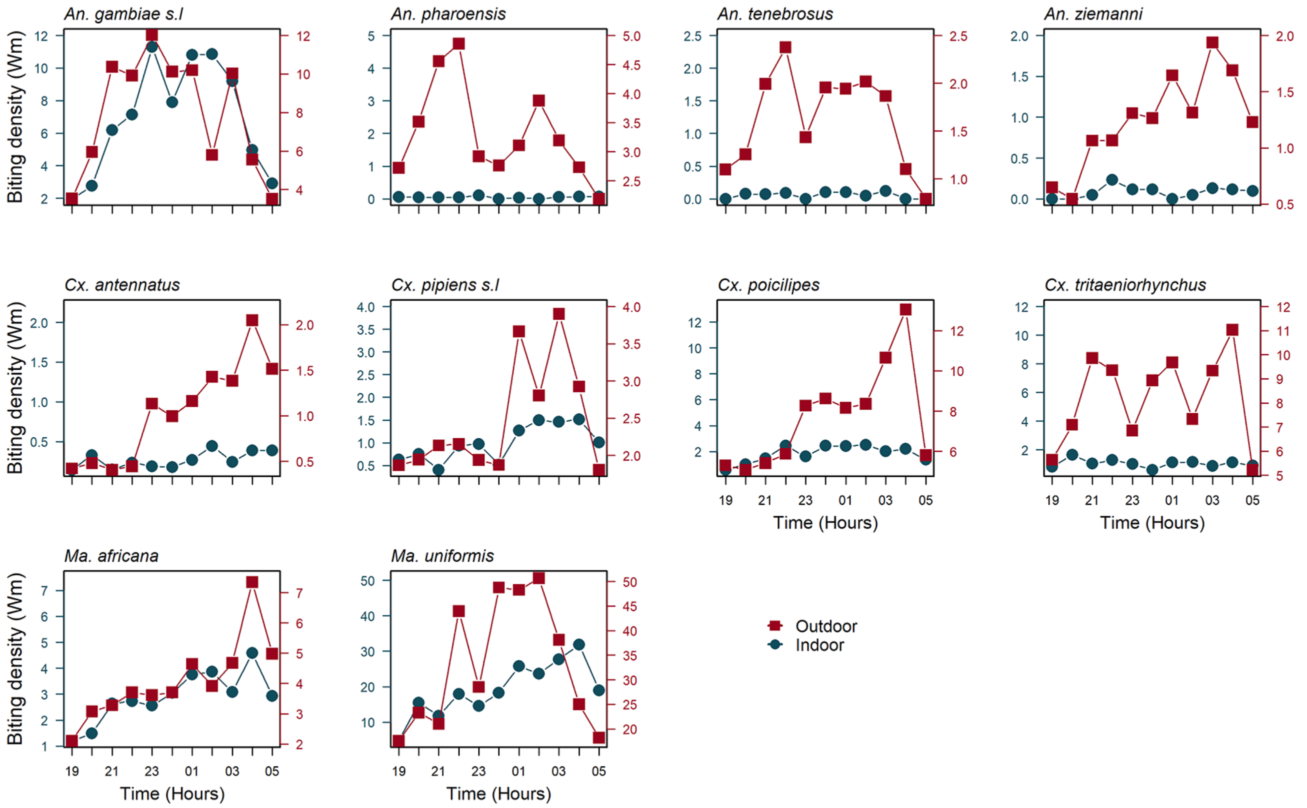

Figure 3. Crude indoor and outdoor hourly (19:00 to 05:00) mean biting density per person/site/night by the predominant mosquito vector species in Massavasse village from February to April 2016. Biting densities were determined by human landing catches and, are expressed in term of Williams mean (Wm) (see methods).

Table 3. Abundance and crude nocturnal human-biting rates (# bites/person/night) by the predominant mosquito taxa in Massavasse village, February to April 2016.

Species	Total collected		Biting rate*		IRR (± 95%CI)**
	Indoor	Outdoor	Indoor	Outdoor	
An. gambiae s.l.	3,403	4,437	87.00	75.99	1.10 (0.97 - 1.26)
An. pharoensis	28	1,950	0.54	36.41	3.18 (1.91 - 5.28)
An. ziemanni	53	1,413	0.90	13.71	4.89 (2.89 - 8.30)
An. tenebrosus	25	1,023	0.06	1.62	4.87 (2.91 - 8.14)
Cx. tritaeniorhynchus	1,007	7,504	11.47	90.33	4.33 (3.39 - 5.53)
Cx. pipiens s.l.	1,019	4,971	10.96	26.99	2.91 (2.12 - 3.99)
Cx. poicilipes	1,219	4,444	20.06	84.58	2.52 (2.12 - 2.99)
Cx. antennatus	229	1,625	0.27	1.04	4.82 (3.34 - 6.96)
Ma. africana	1,521	2,594	31.89	45.03	1.40 (1.19 - 1.64)
Ma. uniformis	11,156	19,912	210.68	363.33	2.05 (1.77 - 2.38)

*Crude biting rate expressed as Williams mean (Wm).

**Indoor collections were considered as reference in GLMM models.

IRR – Incidence Risk Ratio estimates from negative binomial GLMM models.

Table 4. Estimates of mosquito biting exposure (bites/person/night) in non-protected (non-user and protected (ITN users) individuals in Massavasse village (February to April 2016); exposure values determined by multiplying the crude biting rate by the proportion of people indoors or outdoors at night.

Species	Total biting exposure		Biting reduction (%)	Biting exposure (non-user)		Proportion of bites (non-user)	
	Non-users	ITN-users	ITN-users	Indoor	Outdoor	Indoor	Outdoor
An. gambiae s.l.	85.93	40.50	52.87	56.79	29.14	0.66	0.34
An. pharoensis	15.20	14.94	1.71	0.33	14.87	0.02	0.98
An. ziemanni	4.60	4.19	9.06	0.52	4.08	0.11	0.89
An. tenebrosus	6.81	6.48	4.74	0.40	6.40	0.06	0.94
Cx. tritaeniorhynchus	39.65	34.17	13.82	6.85	32.80	0.17	0.83
Cx. pipiens s.l.	16.12	9.97	38.16	7.69	8.43	0.48	0.52
Cx. poicilipes	38.65	27.69	28.36	13.70	24.95	0.35	0.65
Cx. antennatus	4.94	3.40	31.19	1.93	3.01	0.39	0.61
Ma. africana	37.16	19.77	46.81	21.75	15.42	0.59	0.41
Ma. uniformis	252.96	136.06	46.21	146.13	106.83	0.58	0.42

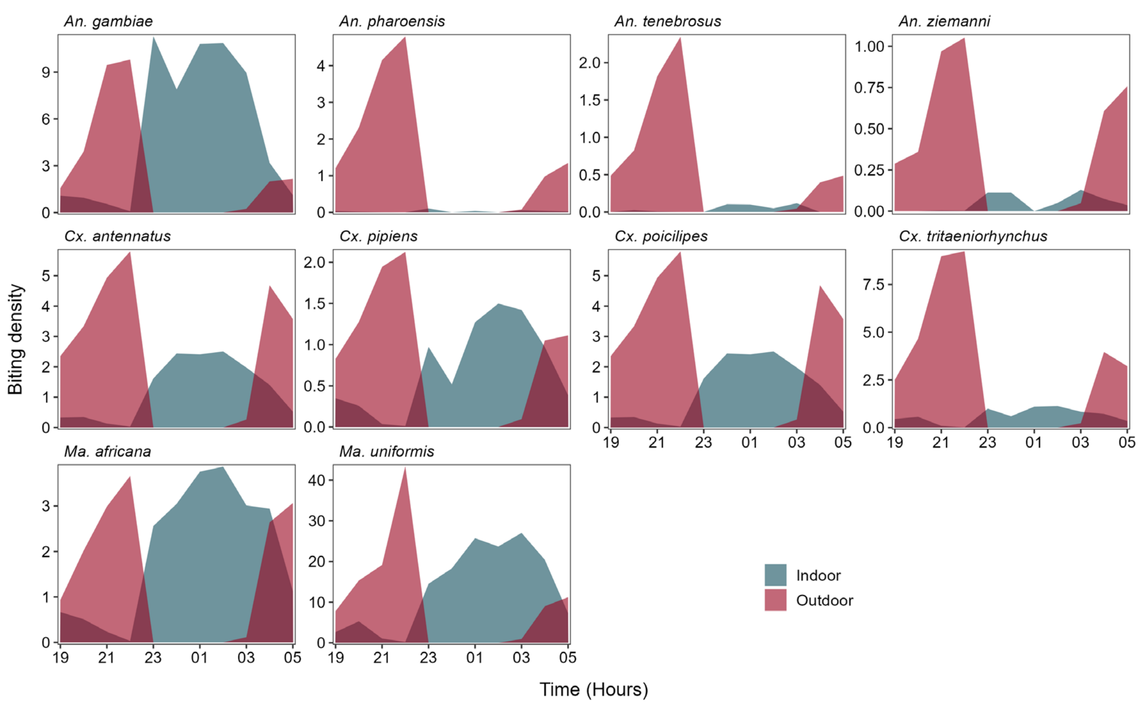

Figure 4. Mean hourly adjusted biting exposure in unprotected individuals by dominant mosquito vector species in Massavasse village. Biting exposure were adjusted by multiplying the crude biting rate by the proportion of people indoor and outdoor at night.

smaller peak around 02:00in the morning, was similar to that described by Haddow[61] and Chandler[64], except that in their study the largest peak was the latter one. Indoors, *An. pharoensis* bit at a steady low rate throughout the night. This pattern differed from the unimodal rhythm reported in Burkina Faso[62], and Kenya[75], where peak activity occurred from 21:00 - 23:00. However, in the Kano plain, also in Kenya, Chandler and colleagues[64] reported a peak around 05:00-06:00 in the morning. These data show that differences in nocturnal host-seeking peaks of activity also exist in *An. pharoensis*, underscoring the need for a thorough investigation of this vector species for implementation of site appropriate malaria control interventions. *An. pharoensis* is an important vector of malaria and lymphatic filariasis across its range of occurrence[43,76].

However, information on the bionomics of this vector is poor for most of Africa, as is knowledge of its role as a malaria vector. The high level of biting recorded when people were active outdoor suggests that, while it is normally only a secondary vector *An. pharoensis* could play an far greater vectorial role in maintaining outdoor residual malaria transmission in Massavasse village following scale-up of indoor-targeted control.

Anopheles tenebrosus and *An. ziemanni,* were the two most abundant sibling species of the *An. coustani* group, and both showed different biting patterns. Host-searching activity was concentrated outdoor for both species, but while activity of *An. tenebrosus* extended from 21:00–03:00, *An. ziemann*i activity increased gradually peaking around early morning. An irregular biting rhythm of a population of *An. ziemanni* was reported by Haddow[52] in Uganda. Published studies on the biting behaviour of *An. tenebrosus* and *An. ziemanni* and, as well as their contribution to residual malaria transmission are few with most reports describing the biting behaviour of the *An. coustani* group, which is unlikely to be of much use, in the light of our study data. With recent evidence showing that the two species might be vectors of secondary importance in Tanzania[77] and Cameroon[78,79] there is renewed interest. The presence of these vector species in Massavasse village is a concern, as they could contribute to the persistence of malaria in the region. Moreover, they should not be overlooked as part of the ongoing cross boarder malaria elimination initiative involving Mozambique, South Africa, and Swaziland (MOSASWA).

To our knowledge, there are no recent published reports describing the patterns of human exposure to bites by culicine mosquitoes in Africa, though many are known to be vectors of arboviruses and of global or regional medical importance. The recent global expansion of *Aedes aegypti,* together with the relentless urbanisation trends of human populations have led to increased contact between this vector and humans resulting in more frequent and larger outbreaks of dengue, chikungunya and Zika, threatening vast human populations worldwide. While this vector is of major concern, it must not distract from the very real threat to rural or peri-urban human populations, associated with the presence of so many nocturnally active vector mosquitoes.

The present and earlier surveys conducted at the Massavasse site[31] confirm the presence of a diverse and range of abundant culicine mosquitoes, notably *Cx. tritaeniorhynchus*, *Cx. antennatus*, *Cx. poicilipes*, and *Cx. pipiens s.l*, *Ma. africana* and *Ma. uniformis*. Some are known to be vectors of important arboviral diseases. *Cx. tritaeniorhynchus* and *Cx. antennatus* are two important vectors of Japanese Encephalitis virus and Rift Valley fever virus in Asia[80] and Africa[47,81], respectively. *Cx. poicilipes* and *Cx. pipiens s.l* are important vectors of West Nile fever virus[82–84], while *Ma. africana* and *Ma. uniforms* have been incriminated as vectors of lymphatic filariasis in Ghana[85]. The transmission risk of both Rift valley fever and lymphatic filariasis is high in the central and northern regions of Mozambique[12,86,87]. Given the high risk of miscarriage in pregnant women infected with Rift valley fever virus[88], a thorough study to identify the actual vector species among the potential vectors present is an essential preliminary step before planning measures to mitigate RVF outbreaks. In this study, we found that *Cx. tritaeniorhynchus* were essentially exophagic, biting outdoors at a relatively high intensity from early evening through to the following morning before sunrise. In contrast, indoor activity was consistently low. The pattern of biting rhythm observed here is inconsistent with the bimodal biting rhythm commonly reported in Asia[89–92]. Yajima *et al.*,[93] have argued that the bimodal rhythm in *Cx. tritaeniorhynchus* population is related to variations in physiological age in the host-seeking populations, with a higher proportion of younger females biting earlier at the evening than the older females, which would usually bite later in the night[93]. Other authors also suggested that variations of environmental temperature and humidity may also have an important role[90]. So far, we have not found a published report on the biting behaviour of an African population of *Cx. tritaeniorhynchus*. The knowledge gap may probably be driven by the fact that the species does not appear to have yet been incriminated as a vector of any human or mammalian pathogen in the African continent. However, the large numbers of host-seeking *Cx. tritaeniorhynchus* collected in Massavasse villag is a matter of concern and should stimulate re-evaluation of health system preparedness for possible outbreaks of *Cx. tritaeniorhynchus*-transmnitted pathogens in the region. Moreover, the rapid widespread demographic transformation, characterized by increasing mobility and connectivity between Japanese encephalitis virus (JEV) endemic regions in Asia and African may exacerbate the risk of JEV outbreaks in the African continent, where the vector species is also endemic.

As with *Cx. tritaeniorhynchus*, *Cx. antennatus* was also highly aggressive outdoors, with a unimodal pattern of activity and a notable peak in the morning. A not dissimilar unimodal pattern, but with the peak in activity occurring around 21:00-23:00, was reported in Kenya[94]. The discrepancies are likely to be driven by the variability between hosts and vector habitats between the two geographic regions, ecological adaptations or in the type of sampling methods employed, in this study (HLC) and in the Kenyan study (baited net trap).

The remaining vector species, *Cx. poicilipes*, *Cx. pipiens s.l*, *Ma. africana*, and *Ma. uniformis* displayed rather similar unimodal intense patterns of activity both indoor and outdoor, usually peaking from dawn to late morning (01:00 - 04:00). The biting patterns of *Cx. poicilipes*, *Ma. africana* and *Ma. uniforms* were similar to those reported previously in studies from other geographic locations[52,94–96]. The biting activity of *Cx. pipiens s.l* was similar to that reported in west and east Africa for one of the sibling species of the *Cx. pipiens* group, *Cx. quinquefasciatus*[95,97]. The estimates of mean biting exposures adjusted for local people nocturnal habits corroborate the crude biting exposures estimates indicating that the main "hotspot" of high biting exposure by both malaria and arboviral disease mosquito vectors in Massavasse village is outdoor before bedtime. For some members of the *An. gambiae* complex found in the village, possibly *An. arabiensis*, and non-Anophelines such as *Cx. pipiens s.l. Cx. poicilipes*, *Ma. africana* and *Ma. uniformis*, a great exposure to bites may also occur indoor throughout bedtime, and outdoor before sunrises. The answers to queries on sleeping habits indicated that residents would retire indoor early in the evening to sleep but emerge early in the morning before sunrise. This pattern of sleeping behaviour is common in agricultural and livestock producing areas where people tend to rise earlier to take care of livestock and work on the land before it becomes too hot. Indoor residual spraying (IRS) and long-lasting insecticide treated (LLINs) spearhead vector control interventions worldwide[23]. However, the efficacy of both methods depends on the propensity of the vectors to feed and rest inside human shelters or houses[98]. Our findings indicate that malaria and arbovirus vectors in Massavasse village may be beyond the reach of IRS and ITN interventions, given the high tendency to outdoor feeding shown by the local mosquitoes. The findings emphasize the importance of baseline study of the target vector population before selecting control methods, and ensuring that all methods integrate fully when implemented, with each other and with other public health interventions. The study also highlights the urgent need for novel effective control tools that aer effective against outdoor biting mosquitoes whose vectorial role includes malaria and numerous arboviruses.

Abbreviation

HBR: Human biting rate

HLC: Human-landing Catch

ITN: Insecticide Treated Net

MBD: Mosquito-borne disease

PCR: Polymerase Chain reaction

Data availability

Underlying data

Open Science Framework: Characterisation of human exposure to nocturnal biting by malaria and arbovirus vectors in a rural community in Chókwè district, southern Mozambique: https://doi.org/10.17605/OSF.IO/AYFM6[99]

This project contains the following underlying data:

- Dataset_1: Raw dataset of mosquito species collected using human-landing catch approach in Massavasse village from February to April 2016
- Dataset_2: Raw dataset of sleeping and waking time of people in Massavasse village from February to April 2016

Extended data

Open Science Framework: Characterisation of human exposure to nocturnal biting by malaria and arbovirus vectors in a rural community in Chókwè district, southern Mozambique: https://doi.org/10.17605/OSF.IO/AYFM6[99]

This project contains the following extended data:

- Questionnaire_1: A copy of the questionnaire used to investigate the sleeping habits of Massavasse village residents

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Open Peer Review

Current Peer Review Status: ✔ ? ? ? ?

Version 1

Reviewer Report 07 September 2023

https://doi.org/10.21956/wellcomeopenres.21361.r62978

?

John B Keven
Department of Population Health and Disease Prevention, University of California Irvine, Irvine, California, USA

This work is generally sound, but I have a few comments and suggestions, all minor issues that can be easily addressed by the authors.

Methods
Paragraph 1, line 6: "a well characterized mosquito population".

Comment: "Community" is a more appropriate word to use as it encompasses all mosquito species in the area. "Population" is used to refer to one species only.

Paragraph 4, line 10: "participate".

Comment: Did you mean "participant"?.

Paragraph 8 (Estimation of Human Biting Rates), line 3: "greater than 1% of all mosquitoes".

Comment: Why 1% and not another value? Please explain the reason for setting this criteria at 1%.

Paragraph 13, line 7: "level of 80% (P = 0.8) for ITNs".

Comment: The P = 0.8 can be confused for a P value associated with a statistical significance test.

Results
Paragraph 1, subtitle: "Relative abundance of nocturnally active anthropophagic mosquitoes".

Comment: I think that "anthropophilic" is a more appropriate term to use in this context than "anthropoophagic". Anthropohagic specifically means that the mosquitoes had fed on human blood, which is not the case here because these mosquitoes were unfed ones.

Paragraph 1, line 3-4: "paired human landing collections indoor (n=19,752) and outdoor (n= 50,006)"

Comment: By "paired", do you mean that mosquitoes were collected outside and inside a house simultaneously (i.e., at the same time)? If so, then your results might be biased by superficially showing a higher outdoor biting rate relative to indoor biting rate. This is because as host-seeking mosquitoes commute from peridomestic habitats towards houses to seek human hosts, most of the mosquitoes that would have otherwise entered into the house (indoor) opportunistically encounter humans outdoor, preventing them from going indoor, causing a superficially high number of outdoor compared to indoor biting rate. An unbiased sampling approach would be to conduct one sampling method (indoor or outdoor but not both) per house but not simultaneously. Please address this potential caveat.

Discussion

Paragraph 2, line 6-11: "However, the discrepancy
may be explained by the fact that the vector population
in the present study was not homogeneous. Molecular analyses confirmed the presence of An. arabiensis, An. merus and An. quadriannulatus and An. gambiae s.s, in descending order of abundance".

Comment: Why not produce biting activity profile for each species separately, or at least the dominant species, e.g. An. arabiensis? This will help solve the discrepancy.

Paragraph 2, line 12-14: "An. arabiensis, An. merus and An. quadriannulatus are well known exophagic malaria vectors which can blood-feed both indoors and outdoors."

Comment: Contradictory statement. An "exophagic" mosquito species feeds predominantly outdoors not both indoors and outdoors as stated here. Did you mean to say exophilic? If so, then this may solve the contradiction because exophilic means a different thing from exophagic.

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

Yes

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?

Yes

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

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical entomology, vector biology, molecular biology

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.

Reviewer Report 20 July 2023

https://doi.org/10.21956/wellcomeopenres.21361.r57228

? **Gerry F Killeen**
School of Biological, Earth & Environmental Sciences and Environmental Research Institute, University College Cork, Cork, County Cork, Ireland

Generally speaking, this is a nice, well thought-through and well-implemented study, which has been nicely written up into a smooth-flowing, well-crafted manuscript. Having said that, the are several substantive opportunities to improve the technical rigour of the estimations and statistical contrasts, as well as the transparency and presentation formats for the results, all of which I detail as follows.

1. While it's nice to see our old paper from 2006 being used and cited, we have recently published two more suitable manuscripts summarizing the most up-to-date and carefully annotated versions of these analytical models. I recommend the authors use these latest papers, which capture all the various iterative stages of evolution of these approaches to calculating and graphing exposure patterns, and include lots of useful templates in the supplementary materials:

   Monroe et al 2020 Malaria Journal 19: 207;
   https://malariajournal.biomedcentral.com/articles/10.1186/s12936-020-03271-z [1]
   Killeen et al 2021 Parasites & Vectors 13: 384;
   https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-021-04884-2 [2]

2. Figure 3 also needs the human sleeping pattern data included, so that readers can see how, where and when it coincides with mosquito activity, to yield figure 4.

3. I would strongly prefer a stacked graph format for figure 4, which is otherwise quite good, as per the examples provided above and below.

4. A Williams' mean is inappropriate as it underrepresents the small proportion of nights and locations during which the majority of actual exposure occurs: while log transformations

are great for making valid statistical comparisons for over dispersed data, they inaccurately dampen down the important contributions of the highest density and articially smoothen off activity profiles that are naturally every bit as dynamic as the raw data and simple arithmetic means indicate. Please use simple arithmetic means instead, which may well yield somewhat higher but more accurate estimates for the proportions of exposure occurring while asleep and preventable by LLINs for both *An. gambiae* and *Cx. quinquefasciatus*.

5. For simple ways to analyze mosquito preferences for feeding indoors versus outdoors, during sleeping versus waking hours, or the interaction between the two, logistic regression with simple binomial distributions for binary format expression of where and when mosquitoes are captured is far more appropriate and powerful than Poisson or Negative Binomial regression. Please see the second paper above, plus the following example of a practical application, for details: Huho et al Int J Epidemiol 42: 235; https://academic.oup.com/ije/article/42/1/235/698545 [3]

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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?
Yes

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

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

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Malaria vector biology and control

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.

Reviewer Report 19 July 2023

https://doi.org/10.21956/wellcomeopenres.21361.r59350

?

Dickson Wilson Lwetoijera
Environmental Health and Ecological Science Department, Ifakara Health Institute, Ifakara, France

This study summarizes important findings on how biting risk of different malaria and non-malaria disease mosquitoes resonate with human sleeping patterns. Although with notable limitations, the article highlights the importance of analyzing mosquitoes biting patterns by including secondary malaria vectors and arboviruses transmitting mosquitoes even in locations that are well covered with bednets, and its contribution to outdoor and residual malaria transmission.

1. This sampling period is also characterized by high densities of mosquitoes in the area, and one would expect the actual risk to be estimated from proportion of infectious bites out of all received bites either by protected or unprotected individual, which isn't the case. This represents a major limitation of this study.

2. Another limitation is that the collection were done in a simulated house environment (experimental hut) that by far differs from an actual house which would have had concentrated indoor host's air plume differently to host seeking mosquitoes and probably caused variability in the recorded numbers.

3. At a sentence "*Paired indoor and outdoor human landing catches were conducted in neighbourhoods two to six of Massavasse village*" it is a little confusing, did the author meant to say that collections were conducted in six neighbourhood of Massavasse village?

4. In the methodology, it reads as collections were done for only six hours, given that collectors had to break for every following our, but in the biting patterns graph, it looks like there is 11 collection points. Can authors clarify on this.

5. Given that this was done in the field, with history of high malaria prevalence, authors didn't clarify if the mosquito collectors were protected with malaria prophylaxis or not, even if they were fully trained and experienced mosquito collectors.

6. Authors can also fix minor typos, such as "aer" to be replace with "are"; villag with village, bit

with bite; *Cx. Antennatus* with *Cx. antennatus*

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

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

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

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

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

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical entomologist, specialized in malaria vectors behaviors and its interaction to control interventions.

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.

Reviewer Report 19 July 2023

https://doi.org/10.21956/wellcomeopenres.21361.r59342

?

Patric Stephane Epopa
Direction Régionale de l'Ouest, Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso, South Africa

In the study entitled: “**Characterisation of human exposure to nocturnal biting by malaria and arbovirus vectors in a rural community in Chókwè district, southern Mozambique**” the authors describe the night biting patterns of all mosquitoes present in the village of Massavasse in southern Mozambique during a 3 months period of rainy season 2016 (February to April 2016). Results indicates significant nocturnal biting activities from almost all the species found. Outdoor

biting activities was in general very high, not only for malaria mosquitoes but also for other Culicidae of public health importance, which can act as vectors for some important arboviral disease (such as dengue, West Nile fever, Japanese Encephalitis virus or Rift Valley fever). The authors use these results confirm the threat of malaria mosquito vectors in the village but also to highlight the increasing importance of the other mosquito vectors and the potential risk that these could have in generating unexpected outbreaks if nothing is done.
In general, the manuscript is well written and most of the time easy to understand. The language is simple with a quite good English. Nevertheless, a number of edits are still needed in my opinion to improve the understanding and bring some details necessary to ensure study reproduction.

The abstract is generally well presented, with nevertheless some minor edits needed to improve understanding of the study (see detailed comments below).

The introduction section is globally well written. The context and aims of the study are quite well described. Nevertheless, some minor edits will be needed to improve understanding by the readers (see detailed comments below). I will also recommend adding one or two sentences to explain a little bit more the rationale behind the study for this is a bit confusing in the present manuscript. Is the study tries to describe the dynamic of all mosquitoes of health importance in the village to highlight the need of more structure surveys at country level ? or rather aim to give a background data to support future control interventions ? etc… This really needs to be clarified.

The Methods section quality is just average. Though generally well written with a simple language and a quite good level of details in general, some important details are lacking and will needed completion to ensure study reproducibility. In addition, some aspects of the study design are confusing. Also, some information given in the figures illustrating the Methods section do not match with the text in the manuscript. Nevertheless, almost all of these lacks will just require minor edits in general. I strongly recommend the authors to address those issues in the revised version of the manuscript to ease understanding of their study and its reproducibility.

The results section is very well structured, and results presented in a simple way easy to understand and generally well-illustrated. The tables and figures are in general of good quality. Only minor edits are needed here to improve readership.

The Discussion section is also of good quality. The references and comparisons given are generally appropriated. The only thing that is lacking is a description of limitations of the study.

The conclusion section is also well written. Conclusions given are in general appropriate even though some of them will need to be moderated.

<u>Abstract (pdf version)</u>
Background:
- Line 2 : "*is fundamental <u>to reduce</u>…*" I will suggest changing to "is fundamental <u>to control</u>…"

Methods:
- The period of survey (period of the year) and the collection duration is lacking in the abstract, this is very important for the understanding of your results, I recommend adding them.

Results:
- Just after given the general number of host-seeking female mosquitoes, it could be

interesting to add even in brackets the general overall mean exposure per night)

Conclusion

- "*identify a wide range of arbovirus vectors nocturnally active in rural areas, many with outbreak potential*" In my opinion, this conclusion is a little bit exaggerated. Unless you add evidence that the corresponding pathogen are already circulating the studied area, there is no real potential of outbreak. So, I will suggest that you moderate your conclusions here. For example, you may say something like: "identify a wide range of arbovirus vectors nocturnally active in rural areas, many of these could present interest in term of public health"

Introduction (pdf version)

1. Please update your WHO reference and corresponding statistics whenever possible to the newest malaria report 2022.

2. Paragraph 3: I will recommend to moderate a little bit your conclusions about health authorities disease control policies. I do understand the frustration pf the authors about decision from Mozambican health authorities to allocate resources mainly to malaria control. In fact, I am not really sure that their willing is to consciously neglect the other vector borne disease. Resources for disease control in a low-income country like Mozambique is usually very limited. So the way they are allocated is a result of complex considerations sometimes out of authorities control. This usually aim to optimize the cost/benefits ratio and gives priority to the most urgent needs.

3. Paragraph 5: Line 4-7: "*Effective vaccines are few, and vector control interventions remain the most reliable and cost-effective control measures against virtually all known* ***mosquito-borne arboviruses***"

   I think "**arboviral diseases"** rather than **"mosquito-borne arboviruses"** is more appropriated.

   Line 7: please delete "**Therefore**."

4. Line 13: "*HBR gives the most realistic estimation of the magnitude of human exposure* ***to a mosquito vector*** *over time and space*" I will suggest to say: "HBR gives the most realistic estimation of the magnitude of human exposure **to given host seeking mosquito vector** over time and space"

Methods

Mosquito collection

This is no very ease to follow and there are lots of details that are not clear at all or even confusing:

- The rotation design: Does it follow a particular scheme (like latin square)? or just random distribution of collector each time?

- What was the minimum distance between the sampling areas?

- How many collectors per day of sampling?

- What do the sampling locations refer to? is it a compound? a house? and what is the correspondence with the “sampling sites” indicated in Figure1?

- Are the terms “sampling sites”, sampling locations and sampling areas referred to the same thing? If yes, you better choose only one expression, if not you should define each in the manuscript to avoid confusion
- In figure 1, 6 sampling sites are indicated. This number do not correspond to nothing (nor the number of neighbourhoods studied, nor the sampling location of the manuscript, nor the number of huts. This will need additional edits from the authors.
- Line 6: “*a well characterized mosquito population*” do you mean “a well characterized malaria mosquito population”? If so, please correct accordingly.
- Line 12: I will suggest to keep the term ‘cold/dry” as previously stated rather than “winter/dry”
- Line 13: if available, add the confidence interval to the mean rainfall

Sleeps surveys
Additional information needs to be provided here

- Statement on how you managed individual data privacy/protection
- Inclusion/exclusion critaria
- Compound selection mode (how compounds were selected (systematic? Randomly?)
- Why this sample size? (was this related to a particular expected ratio of compounds? or may be just to the number of acceptance received)
- To ease reader understanding, I will suggest that you start by given a general overview of you study design before going into detailed description. You can also add a small diagram to illustrate your sampling design.

Data analysis

- Estimation of human-biting rates: “*Estimation of human exposure to mosquito bites was only carried out for species where the total catch was equal to or greater than 1% of all mosquitoes caught*” is there a particular reason for that ? please add a rationale to explain a little bit more for the context may be different for other similar study.
- Please be consistent with the mosquito collecting period: in the mosquito collection section it is mentioned 19h00 to 05h00 and in the Data analysis section it is mentioned 19h00 to 06h00.

Consent

- I will suggest that you add 2 or 3 sentences to explain a little more about how the information was given and the consent form were taken: by who (field team, specialized stakeholder engagement team); what type of information given (study objectives, risks, etc.), form of signature (formal signature, finger print)

<u>Results</u>

1. I suggest you to split the total number of Anopheles (806) into 2 indicating the respective number of mosquitoes selected indoor and outdoor and for each the number of successful

amplification. The same comment applies to Anopheles funestus PCR results.

2. Page 7, Paragraph 2: "*whereas biting by all other vector species were far more likely to occur outdoors*" I will suggest rephrasing for this gives the impression that indoor collections are negligible. You can say something like: "whereas biting by all other vector species were far more likely to occur outdoors despite significant indoor biting."

3. Table 3: I suggest adding a column next to the mosquito species to indicate for each the main transmitted diseases (malaria, arboviral diseases). This will highlight the importance of your work in public health perspectives.

Discussion

The author used the density index of vector to indicate the potential risk of disease occurrence in the studied area. The results are used to highlight the potential risk of arboviral diseases outbreak in the area due to significant presence of related biting mosquito vectors.

Nevertheless, unless the presence of such pathogen has been observed in this area, it is unlikely in my opinion to see such event (vector borne disease being the result of contact between a host, a pathogen and his vector0 for vector and host only are not enough.

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

Yes

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

Yes

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

Partly

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

I cannot comment. A qualified statistician is required.

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

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: medical entomology, malaria vector control, malaria mosquito ecology and biology

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.

Reviewer Report 29 June 2023

https://doi.org/10.21956/wellcomeopenres.21361.r59347

Yeromin P Mlacha
Environmental Health and Ecological Science, Ifakara Health Institute, Ifakara, Morogoro Region, Tanzania

The study's findings have significant implications for understanding the entomological drivers of persistent malaria transmission and the potential for arbovirus outbreaks in Mozambique.

The paper is well-written, and its findings are succinct and straightforward. The methodology is sound and makes use of tried-and-true methods for collecting and analyzing mosquitoes. Although HLC raises ethical concerns because it exposes the person performing the operation, its usage at hourly intervals enables a thorough assessment of the host-seeking mosquito composition throughout the night.

The discovery of several nocturnally active arbovirus vectors highlights the necessity of setting up a surveillance system to keep track of these vectors across the country.

However, It is important to consider the limitations of the study. It would be beneficial to include information on the generalizability of the findings beyond Massavasse village. The discussion and conclusions section may have benefited from a brief paragraph examining limitation and potential solutions.

Furthermore, the study could benefit from additional discussion on the implications of the findings for vector control strategies. How can the information on biting patterns and the effectiveness of bed nets be used to improve vector control interventions and reduce disease transmission in Mozambique?

Overall, this study significantly contributes to the understanding of human exposure to mosquito biting and the identification of key vector species in a rural area of Mozambique.

With some minor additions, this manuscript would be a valuable addition to the journal, providing cohesive insights into mosquito behavior and offering important considerations for vector control efforts.

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

Yes

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

Yes

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

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

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

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Vector-borne diseases

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.