ORIGINAL RESEARCH

Anopheles funestus sensu stricto Giles (Diptera:Culicidae) bites after sunrise at two rural villages in northern Malawi and its implications for malaria vector control

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Abstract

Introduction

Malawi has scaled up distribution and use of LLINs but their effectiveness depends on vector behaviour. This study reports information on where and when peak biting takes place by *Anopheles* vectors at two study sites in northern Malawi.

Methods

The study was carried out at a single village each in Nkhata Bay and Karonga districts, northern Malawi. Monthly, three teams of four people each sampled mosquitoes using Human Landing Collections (HLCs) from 6.00 pm to 6.00 am. Mosquitoes were counted and identified by PCR. *Plasmodium falciparum* sporozoites were detected by ELISA and an entomological inoculation rate was estimated.

Results

A total of 4,668 and 2,079 mosquitoes were sampled in Nkhata Bay and Karonga districts respectively. *An. funestus s.s* was common (91.3%; n = 2,611) in Nkhata Bay while *An. arabiensis* was common (96.9%; n = 706) in Karonga. *Pf sporozoite* rates varied from 0.8% (4/484) to 3.3% (51/1558). Individuals in Nkhata Bay received more bites (approx. 200 bites/ person/ night) compared to Karonga (approx. 50 bites/ person/ night). An. funestus was more likely to bite indoors (p=0.002) while An. arabiensis was (p=0.05) more likely to bite outdoors. Furthermore, An. funestus peak biting was in the early morning hours from 4:00 am (approx. 331 and 177 bites/ person/ night indoors and outdoors respectively) and remained high till 6:00 am. *An. arabiensis* peak biting (approx. 63 and 62 bites/ person/ night indoors and outdoors respectively) was around mid-night (12:00). An EIR of 108.4 infective bites/ person/ year was estimated for Nkhata Bay compared to 9.1 infective bites/ person/ year for Karonga.

Conclusion

An. funestus s.s. had a considerable *Pf sporozite* infection rate and EIR. The shift in biting behaviour shown by this species poses a challenge to malaria control. Further studies are required to understand the biting behaviour of Anopheles vectors in Malawi.

Keywords: *Anopheles funestus* sensu stricto; *Anopheles arabiensis*; biting behaviour; malaria control; Malawi

Introduction

Anopheles funestus s.s., *An. arabiensis* and *An. gambiae s.s* are important vectors of malaria throughout sub-Saharan Africa, including Malawi[1]. These three species naturally exhibit differences in their breeding habitat preferences but also in their feeding and resting behaviours which have implications on malaria transmission, disease epidemiology and control success. Both *An. funestus* and *An. gambiae s.s.* are said to be highly associated with human beings (anthrophilic) and have a tendency to rest indoors (endophilic) after taking a bloodmeal. On the other hand, *An. arabiensis* shows flexibility in its feeding and resting behaviour with a propensity to readily feed on alternative hosts where available[2–4].

Significant gain s to control malaria have recently been reported globally[5,6] and in Malawi[7], though the disease burden still remains unacceptably high in the country. The reported gains have been attributed to a number of factors including, possibly better reporting, improved diagnosis and treatment with artemesinin-based combination therapies (ACTs), high coverage of the general populace with insecticide-treated bednets (ITNs) and the introduction of indoor residual spraying (IRS) though localized and geographically skewed towards districts along the shores of Lake Malawi and the Lower Shire Valley where malaria transmission is intense and perennial (holoendemic)[1].

Large scale and prolonged use of malaria control interventions such as ITNs and IRS have been shown to illicit a wide range of behavioural responses in Anopheles vector populations. The resultant behavioural changes have implications on malaria transmission and control. For instance, historical data showed that use of DDT in malaria control programmes were associated with behavioural changes in Anopheles sp vector mosquitoes from being predominantly indoor (endophagic/endophilic) to outdoor biting and resting (exophagic/ exophilic) behavioural tendencies[8]. Similarly, consistent deployment of vector control interventions have resulted in predominantly outdoor seeking behaviours

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in Anopheles vectors on Bioko Island and in Tanzania[9,10]. Other data from Kenya, Tanzania and Senegal have recently shown shifts in Anopheles species composition from one predominated by *An. gambiae s.s.* to commonly *An. arabiensis* vectorial system as a result of wholesale use of ITNs[10–12].

The present study focused on temporal and spatial changes in the biting behaviour of Anopheles vector species. Results of two recent studies carried out in Benin and Senegal demonstrated a shift in the biting behaviour of An. funestus populations to early morning following a mass roll out of ITNs[13,14]. However, a separate study carried out in Kenya using retrospective data did not show any changes in the biting behaviour of two malaria vectors, *An. gambiae s.s* and An. arabiensis following wide scale use of ITNs[15].

The importance of understanding behavioural changes in Anopheles vector populations cannot be overemphasized. The effectiveness of malaria vector control strategies targeted at adult Anopheles mosquitoes such as ITNs and IRS rely on understanding biting and resting behaviour of the Anopheles vector species. This brief study set out to generate preliminary data on peak biting times and biting location of two principal malaria vectors, *An. funestus* and *An. arabiensis* in two geographically separate areas of Karonga and Nkhata Bay districts in northern Malawi in order to provide empirical evidence on which to base malaria control decisions.

Methods

Study sites

The study was carried out at Dambo village in Nkhata Bay (11° 58' 02"; 33° 33' 52" E) and Kanyuka village in Karonga district (10° 2' 47" S; 33° 35' 06" E), northern Malawi (Fig.1). Throughout the paper, the district names will be used instead of villages. Nkhata Bay has tropical weather conditions and receives heavy rains. A large proportion of people in the community engaged in fishing in Lake Malawi. On the other hand, Karonga is characterized by dry weather conditions that are typical of savanna climatic conditions. The main economic activity by communities in Karonga was rice cultivation under a formal rice irrigation scheme and cattle farming.

Village enumeration

At the beginning of the study both villages were enumerated in a complete census using a standard questionnaire. Demographic, house construction, agricultural practices (crops and animals) and malaria control interventions data were captured using electronic data capture devices (Tablets).

Selection of households

At each study site, 3 sentinel houses were randomly selected from the census master list for mosquito collection on each visit. On subsequent visits, a different set of 3 sentinel houses were selected. The decision to select different houses on each visit was to ensure wider coverage of the study area since mosquitoes tend to cluster in nature. If a selected house refused consent to participate in the study or it was not found because the owner was not home, that particular house was replaced from the excess random household list generated. In total 18 different sentinel houses were enrolled for mosquito sampling over the 24 collection nights.

Recruitment of mosquito collectors

Human volunteers were recruited from the local community to collect mosquitoes at night. Only men aged between 18 and 40 years of age were recruited. Women of all ages and males aged <18 years of age were excluded from the study.

A total of 12 volunteers were recruited from each study site. They were all screened for *Plasmodium falciparum (Pf)* malaria parasites at the beginning of the study using a malaria rapid diagnostic test (*mRDT*) kit (BIO LINE Malaria Antigen *P.f*; SD STANDARD DIAGNOSTICS, INC). Those found positive for Pf were treated according to national malaria treatment guidelines by a nurse from the district hospital or nearest health facility. Screening for Pf was repeated on subsequent visits. During the study period, study volunteers were provided with prophylaxis using Doxycycline (100mg daily dose).

Prior to commencement of field data collection, study volunteers were familiarized with the study protocol and trained in data collection tools. Training included, live mosquito sampling using mouth aspirators (pooter), human landing catches (HLCs) for mosquito sampling and work flow. The volunteers and supervisors carried out a dry run after the training to test the study tools. Further, volunteers were familiarized with ethical issues pertaining to HLCs and the need to adhere to routine malaria screening, treatment with Lumafantrine-Artemether (LA) and prophylaxis regimes.

Mosquito collections

Field mosquito collections were carried out on two consecutive nights in a month per village between January and August 2014. A total of 12 collection nights was observed in each of the study villages. The collections in Nkhata Bay preceded those in Karonga during each visit. Written informed consent was obtained from the household owner before mosquito sampling commenced.

Nightly collections were carried out by a team of four volunteers at each sentinel house plus one supervisor. One person was positioned inside the house at the sitting room and the second person sat outside on the veranda. The role of the supervisor was to ensure that volunteers arrived on time, adherence to hourly collections and that volunteers did not go to sleep on duty. Each volunteer was equipped with a head torch, a mouth aspirator and a complete set of twelve well labeled paper cups for storing hourly mosquito samples separately.

Volunteers rolled up their trousers up to the knees and using the torch they located and collected every mosquito that landed on their bare legs before it could bite. Collections commenced at 6 pm and stopped at 6 in the morning. At mid-night the first pair of volunteers went home to rest and their positions were taken up by a second pair who worked from midnight to 6 o'clock in the morning. At each house collections were carried out on two consecutive nights. On the second night mosquito collectors were rotated among the three houses and the night shifts within the team. Because the three houses were only visited on two nights there was a partial rotation among collectors per visit. Every hourly collection was placed in a separate paper cup labeled with a date, household unique identification number, collection time and location (inside or outside).

Mosquito processing

Every morning after the night's collection the study coordinator (technician) counted the mosquitoes and identified them to genus level under a dissecting microscope

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using morphological identification keys[16]. Because of the challenges to identify sibling species within the *An. funestus* group of mosquitoes and *An. gambiae* species complex, specimens of these mosquitoes were individually placed in separate appropriately labeled eppendorf tubes and preserved dry in a desiccant (silica gel) for later identification.

Anopheles sibling species identification

The polymerase chain reaction (PCR) technique was used to identify the various anopheline sibling species belonging to An. funestus group of mosquitoes and An. gambiae species complex[17,18]. Briefly, deoxyribonucleic nucleic acid (DNA) from individual leg and wing mosquito parts was extracted by LIVAK grinding buffer method. The primers used for identification of the An. funestus group were those of Koekemoer et al.[17,19] while the primers used for the identification of the An. gambiae complex were those of Scott et al.[18].

DNA amplification for *An. funestus* group of mosquitoes was performed at initial denaturation of 95°c for 2 minutes followed by 35 cycles of denaturation at 95°c for 30 seconds, annealing at 53°c 30 seconds and extension at 72°c for 40 seconds. For *An. gambiae*, initial denaturation was at 95°c 5 minutes followed 30 cycles of denaturation at 95°c for 30 seconds, annealing at 53°c for 30 seconds and extension at 72°c 40 seconds.

Plasmodium falciparum (Pf) sporozoite ELISAs

To determine *Pf* sporozoite infections within the anopheline vector specimens a standard protocol and procedures were used[20]. Assays were carried out only on head + thorax mosquito parts preserved dry in a desiccant (silica gel).

Data analysis

The data was analysed as proportions. The proportion of people living in thatched houses, houses with open eaves and open windows were determined and summarized. In addition, proportion of households owning >2 insecticide-treated bednets (ITNs) and the proportion of sprayed houses were summarized. Mosquito biting rates were expressed per person and per night. The mean biting rates were obtained by averaging the bites over the number of people who were bitten and over the nights that they were exposed to the mosquitoes. The biting rates were plotted against month of the survey as well as time of the night. The proportions of animals owned by households in each village were also determined and summarized. All proportions were expressed as percentages. The entomological inoculation rate (EIR) was calculated as the product of biting rate (BR) and sporozoite rate (SR). Fisher's exact test was used to test difference in proportions where appropriate. Statistical significance was declared if p-value was less than 0.05. Due to the small sample size, potential confounders were not controlled for, as including many covariates in the model with a small sample would make the model unstable.

Results

Study sites characteristics

Household size, demographic structure, house construction, bednet ownership and agricultural levels for the study sites are shown in Table 1. The two study villages were equal in size based on proportion of households and population size. Overall, the two study villages comprised 229 households with a total human population of 1,191 people and the majority of the people were aged >16 years old. Both villages were typically rural characterized by thatched houses (66.8%; n = 153) and a large number of houses had open eaves (76%; n = 174) and open windows (81.2%; n = 186).

The two villages had received the two major malaria control interventions implemented in Malawi with noticeable coverage differences. A higher proportion (84.7%; n = 94) of households in Karonga were reported to have been sprayed compared to Nkhata Bay (53.4%; n = 63). Similarly, a higher proportion (80.2%; n = 89) of households in Karonga reported owning at least 2 insecticide-treated bednets (ITNs) relative to Nkhata Bay (59.3%; n = 70).

Inhabitants of both sites kept a variable number of different types of animals including, pigs, cows, sheep, goats and dogs. However, more animals (n = 622) were registered in Karonga compared to Nkhata Bay (n = 194).

Anopheles distribution

A total of 4,668 and 2,079 female mosquitoes were collected over 24 collection nights between January to August 2014 in Nkhata Bay and Karonga districts, respectively (Table 2). The bulk of the mosquitoes collected in Nkhata Bay district were Anopheles spp (61.3%; n=2,860) while culicines were predominant (64.5%; n=1,340) in Karonga district. Although both *An. funestus* s.l and *An. gambiae* s.*l* were present at the two study sites, the former was predominant in Nkhata Bay (91.3%; n = 2,611) and the latter in Karonga (96.9%; n = 706). Molecular analysis of these two major species complexes revealed *An. funestus s.s* and *An. arabiensis* as the only sibling species present in the study areas.

Anopheles biting intensity

Pooled results of Anopheles biting at the two study sites over the study period are shown in Fig. 2. In general, the number of bites an individual person experienced in a night decreased over time at both study sites. There was more biting during the wet season (in January) and fewer at the end of the study in dry season. Individuals in Nkhata Bay received significantly more bites (approx. 200 bites/ person/ night) compared to those living in Karonga district (approx. 50 bites/ person/ night). Increased biting (46 bites/ person/ night) was detected in Karonga district at the end of the study in August.

Anopheles biting in space (where or location)

Data were analyzed to test whether there were any differences in preference for indoor or outdoor biting between the two vector species and the results are shown in Table 3. There was a significant difference in biting rates between inside and outside with a higher frequency of biting taking place indoors for *An. funestus* (p=0.002) than outside. By contrast, *An. arabiensis* showed an increased tendency to bite outdoors (p=0.05).

Anopheles biting in time (when)

Figs. 3a and 3b show hourly biting intensity from dusk (6:00 pm) to morning (6:00 am) for *An. funestus* and *An. arabiensis* in Karonga district where the latter species was predominant. For both species, biting took place throughout the night. But *An. arabiensis* peak biting was around mid-night for both indoors (62 bites/ person) and outdoors (63 bites/ person). Similar information is shown in Figs. 4a and 4b for Nkhata Bay district where *An. funestus* was the most common vector species.

Table 1: Baseline characteristics (demographic, house construction type, malaria control intervention coverage and domestic animal ownership) of the two study sites, Dambo Village in Nkhata Bay and Kanyuka Village in Karonga Districts in northern Malawi

		Nkhata Bay	Karonga
Variable	Overall	Dambo	Kanyuka
Number of households (hh)	229	118 (51.5)	111 (48.5)
Number of participants (N)	1,194 (1,191)	604 (50.7)	587 (49.3)
Gender Male n (%)	584 (49.0)	295 (48.8)	289 (49.2)
Age: under 5 n (%)	185 (15.5)	91 (15.1)	94 (16.0)
>5-15 years n (%)	447 (37.5)	229 (37.9)	218 (37.1)
>=16 n (%)	560 (47.0)	284 (47.0)	276 (46.9)
Eave type: open n (%)	174 (76.0)	89 (75.4)	85 (76.6)
Roof type: Thatched n (%)	153 (66.8)	83 (70.3)	70 (63.1)
Windows: Open n (%)	186 (81.2)	93 (78.8)	93 (83.8)
IRS: Yes n (%)	157 (68.6)	63 (53.4)	94 (84.7)
Nets owned: 0 n (%)	24 (10.5)	16 (13.6)	8 (7.2)
1 n (%)	46 (20.1)	32 (27.1)	14 (12.6)
>=2 n (%)	159 (69.4)	70 (59.3)	89 (80.2)
Animals: All n (%)	816(100.0)	194 (100.0)	622(100.0)
Pigs n (%)	125 (15.3)	17(8.8)	108 (17.3)
Cows n (%)	248 (30.4)	6 (3.1)	242 (38.9)
Sheep n (%)	2 (0.2)	0 (0.0)	2 (0.3)
Goats n (%)	220 (27.0)	57 (29.4)	163 (26.2)
Dogs n (%)	221 (27.0)	114 (58.8)	107 (17.2)
Birds All n (%)	1383(100.0)	668 (100.0)	715 (100.0)
Chicken n (%)	1318 (95.3)	639 (95.7)	679 (95.0)
Ducks n (%)	65 (4.7)	29 (4.3)	36 (5.0)
Crops Cassava #hh (%)	167 (72.9)	110 (93.2)	67 (60.4)
Maize #hh (%)	214 (93.4)	103 (87.3)	111 (100.0)
Rice hh (%)	165 (72.1)	78 (66.1)	87 (78.4)

Table 2: Species composition of female mosquitoes (An. funestus, An. arabiensis, Culex sp, Mansonia spp) sampled at the two study villages, Dambo and Kanyuka in Nkhata Bay and Karonga Districts, respectively

Mosquitoes	Dambo, Nkhata Bay	Kanyuka, Karonga
An. funestus s.l. n (%)	2,611 (91.3)	22 (3.1)
An. arabiensis n (%)	249 (8.7)	706 (96.9)
Total	2860	728
Anopheles sp n (%)	2860 (61.3)	728 (35.0)
Culicines n (%)	1,679 (35.9)	1,340 (64.5)
Others n (%)	129 (2.8)	11 (0.5)
Overall n (%)	4,668 (100.0)	2,079 (100.0)

Table 3: Comparison of Anopheles biting rates between inside and outside study houses using a paired t-test

Species	Location	Mean number of bites/ person/ night	95% CI	P-value
An. funestus	Inside	143	70-216	
	Outside	76	38-114	
	Difference	68	30-106	0.002
An. arabiensis	Inside	11	6-16	
	Outside	15	10-19	
	Difference	-4	-8-0	0.05

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Table 4: Plasmodium falciparum sporozoite rates detected by ELISA. Calculated as a proportion of infected mosquitoes but presented as percentages

		Sporozoite			
Site	Species	Negative	Positive	Total	SR
Karonga (Kanyuka)	Combined	480	4	484	0.8%
	An. arabiensis	463	4	467	0.9%
	An. funestus s.s	17	0	17	0.0%
Nkhata Bay (Dambo)	Combined	1507	51	1558	3.3%
	An. funestus s.s	1377	44	1421	3.1%
	An. arabiensis	127	7	134	5.2%

Table 5: Estimated number of infective bites received per person per year (EIR). A product of biting rate (number of total mosquitoes collected and divided by number of collectors) for the two study villages

	Total number of bites over 24 person nights	Mean number of bites/ person/ night	Total number of bites/person/ year		
Village				SR	EIR
Dambo	2,860	9.9	3,613.5	3.3%	108.4
Kanyuka	728	2.5	912.5	0.8%	9.1

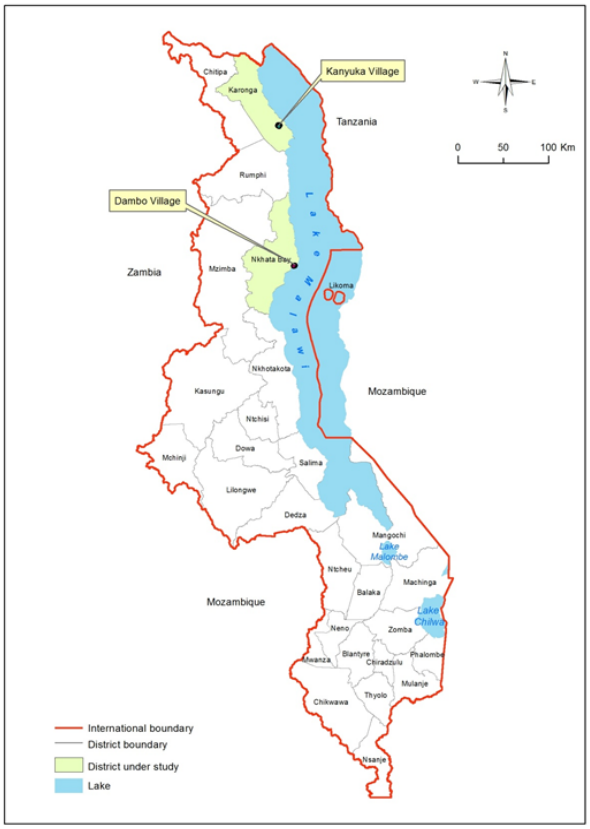

Figure 1: Map of Malawi showing the two study villages, Dambo in Nkhata Bay and Kanyuka in Karonga Districts in northern Malawi

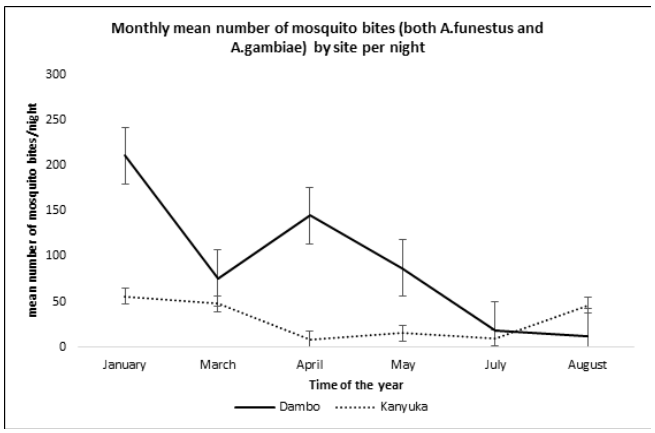

Figure 2: Monthly mean number of female *Anopheles* (combined for *An. funestus and An. gambiae*) per person and shown by study village

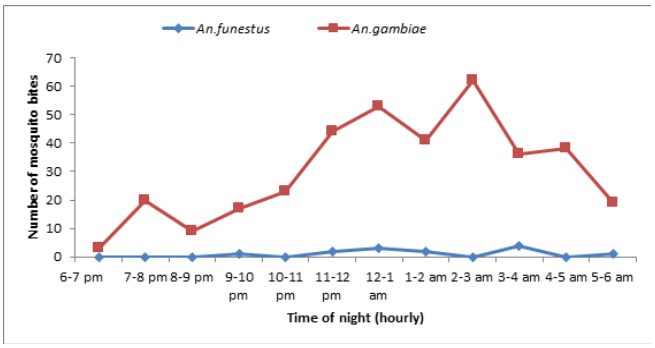

Figure 3a: Indoor hourly bites/ person/ night (from *An. funestus and An. arabiensis*) at Kanyuka Village in Karonga District

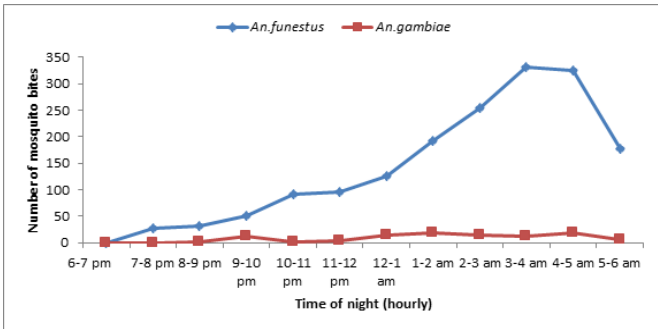

Figure 3b: Outdoor hourly number of bites/ person/ night (from *An. funestus* and *An. arabiensis*) at Kanyuka Village in Karonga District

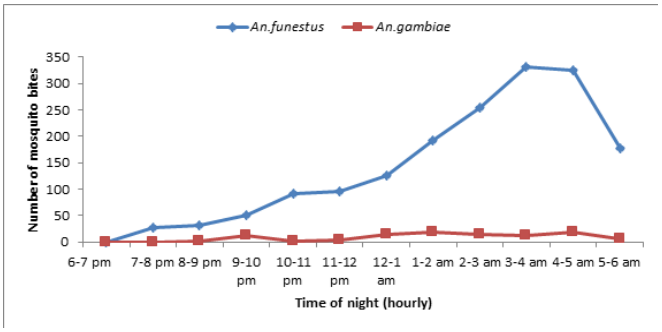

Figure 4a: Indoor hourly bites/ person/ night (from *An. funestus* and *An. arabiensis*) at Dambo village in Nkhata Bay district

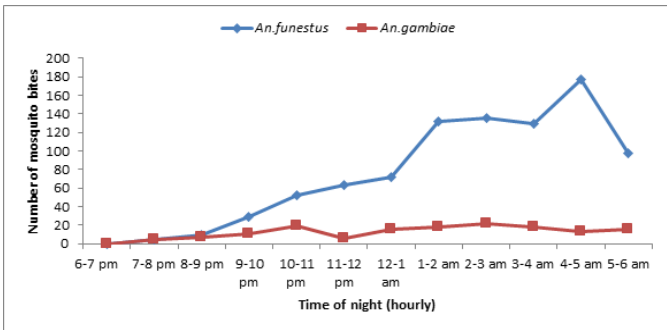

Figure 4b: Outdoor hourly number of bites/ person/ night (from *An. funestus* and *A. arabiensis*) at Dambo villag

An. funestus showed peak biting very late in the morning from around 4:00 am when approximately 331 bites per person were experienced by individuals and remained high till 6:00 am (177 bites/ person) when collections stopped.

Malaria transmission intensity

A total of 2,094 Anopheles mosquitoes were screened for *Pf* sporozoites in their salivary glands by ELISA (Table 4). There were marked differences in infection rates between study sites and Anopheles species. Infectivity rates were higher (3.3%; n = 1,558) in Nkhata Bay district where *An. funestus* was the predominant vector species than Karonga (0.8%; n = 484) where *An. arabiensis* was the most common vector species. However, *An. arabiensis* collected in Nkhata Bay showed very high infection rates (5.2%; n = 134) compared to *An. funestus* collected in the area.

Results of estimating entomological inoculation rate (EIR) are shown in Table 5. Residents of Nkhata Bay District experienced a high number (108.4 ib/ p/ yr) of infective bites per person per year compared to their counterparts (9.1 ib/ p/ yr) in Karonga district.

Discussion

This study investigated the biting behaviour of two important malaria vector species at two study sites in northern Malawi, *An. funestus* and *An. arabiensis*, to generate knowledge of their biology and its implications for malaria vector control. The study has demonstrated that *An. funestus* and *An. arabiensis* were markedly different in their geographical distribution and biting behaviour. The former was predominant in the wet, swampy and tropical environment in Nkhata Bay while the latter was commonly found in the much drier and arid area in Karonga District. Most importantly, this study showed that *An. funestus* was mostly an indoor biting species with its peak biting during later in the morning while *An. arabiensis* showed a propensity to bite outdoors and peak biting was experienced around mid-night. *An. funestus* was very infectious carrying high *Pf sporozoites* (3.3%) which translated into high number of infective bites per person per year in Nkhata Bay. By contrast, the sporozoite infection rate of *An. arabiensis* in Karonga District was 0.8%.

The geographic (rural location) and demographic (population size) parameters were very similar for the two study sites and therefore augured well with the original aim of the study. However, the study detected differences in uptake of malaria control interventions (ITN ownership and IRS coverage) which were both higher in Karonga compared to Nkhata Bay District. Such differences could be a reflection of geographical variation in coverage of interventions or simply a random error effect since only a single village was selected in an entire district. In general national intervention coverage data for ITNs showed high coverage in northern Malawi compared to the other two regions (central and south)[21,22]. Furthermore, there were differences in animal ownership (a wealth indicator) between the two study sites with people in Karonga owning more animals (mainly cattle) than those in Nkhata Bay. Such an observation is probably rooted in traditional practices of the two tribes that inhabit these districts. People in Karonga are predominantly Nkhondes whom traditionally keep cattle for various customary requirements including bride price (dowry). The Tongas of Nkhata Bay on the other hand are either fishermen or engage in cultivation of cassava which is their main source of carbohydrates (staple food).

The detection of two Anopheles vector species, *An. funestus* and *An. arabiensis* was expected and is in accordance to the findings of our President's Malaria Initiative (PMI) funded entomological monitoring efforts across the country. These two vectors currently enjoy a wide geographical distribution range across the country in varying proportions. Noteworthy was the distinct geographical separation in the distribution of the two vectors with *An. funestus* being predominantly high in Nkhata Bay and *An. arabiensis* being predominant in Karonga reflecting adaptation to local eco-environmental conditions. *An. arabiensis* is known to be adapted to arid dry weather environments which are typically characteristic of Karonga District (White 1974, Coetzee et al. 2000, Coetzee 2004) while *An. funestus* is adapted to areas with large and permanent water bodies[16] which were prevalent in Nkhata Bay District mainly during the wet season.

This study, however, did not detect *An. gambiae s.s.*, a known efficient malaria vector in sub-Saharan Africa. An earlier study[1] carried out in Chikwawa district in southern Malawi located approximately 800 km from the present study sites found this species to constitute about one quarter (25.0%)

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of the three sibling species (*An. arabiensis, An. gambiae s.s.* and *An. quadriannualtus*) within *An. gambiae* species complex that were found in the area. The present study findings agree with more recent studies carried out in Chikwawa by Mburu and others which showed that the two species, *An. funestus* and *An. arabiensis* are common[27–29]. Failure to find this species in the present study areas (especially in the wet tropical area in Nkhata Bay) can be attributed to several factors including wholesale use of LLINs. Recently some studies carried out in the region have reported a shift in *Anopheles* species composition due to large scale use of ITNs ([10–12,25,30,31]) or a response to indoor residual spraying (IRS)[32]. It is also possible to speculate that climate change (small increases in temperature and drier weather conditions) might have influenced the observed changes on species composition as studies carried out in Kenya seem to suggest[33]. Unfortunately, no similar studies have been carried out in Malawi to ascertain such claims.

An. funestus and *An. arabiensis* showed differential preference in their biting habits in space. The former was highly endophagic while the latter showed a propensity to bite outdoors (exophagic). Our study findings confirmed the conventional knowledge of the two anophelines. However, other studies have reported shifts in the biting behaviour of these two vector species. For instance, a study carried out in Benin before and after a wide scale bednet coverage showed an increased proportion of outdoor biting in *An. funestus* [14]. But no evidence of shift in location of biting was found in Asembo Bay in Kenya following many years of ITN use where this species continued to predominantly bite indoors[15]. The endophagic behaviour pattern exhibited by *An. funestus* in the present study would suggest that this species would be amenable to both ITNs and IRS using effective insecticides. However, the outdoor biting tendency displayed by *An. arabiensis* has wider implications for malaria control and indicates this species may be important in residual malaria transmission[34].

Similarly, there were differences in the biting times of the two vector species during the night with *An. funestus* showing peak biting late in the morning. This species actively continued biting as late as 6:00 in the morning when collections were deliberately stopped suggesting continued biting beyond the collection stoppage time. An extreme example of An. funestus biting quite later in the morning has been reported from Senegal where a substantial number of mosquitoes were collected biting indoors in broad day light[13]. On the other hand, the finding that An. arabiensis peak biting was around mid-night was not surprising. Results of this study are collaborated by a recent study carried out in Chikwawa by Mburu and others who also investigated the biting patterns of these two Anopheles malaria vector species[35]. In their study, there were no differences in the biting rate of An. arabiensis between outdoors and indoors. But this species fed largely between 21.00 hours and 23.45 hours. On the other hand, An. funestus predominantly fed indoors but its peak biting times varied with seasons. It showed high feeding activity early in the morning between 03.00 hours and 05.45 hours in the wet season.

This study detected high *P. falciparum* sporozoite infection rates in both *An. funestus* and *An. arabiensis* in Nkhata Bay District compared to the infection rate reported for Kanyuka Village in Karonga District. Such a finding would reflect heterogeneity in malaria infections and transmission driven by the Anopheles vector species involved or other epidemiological factors. The higher biting rates and low coverage of malaria interventions in Nkhata Bay could also partly explain the observed high infection rates. These high infective bites and the ability of the vectors to bite either in the morning hours or outdoors when people are not protected by vector control tools warrant the need for development of tools that target these vectors at such times/locations. Venezegho and others carried out a study in 2013 in Karonga District in which they reported zero *Pf infections* (n = 152) (36). In an earlier study carried out in Chikwawa District in southern Malawi reported a slightly higher sporozoite rate (4.85%) using polymerase chain reaction (PCR) (1).

Equally a high EIR was estimated for Nkhata Bay compared to Karonga district. Such an observation was most likely due to large number of *An. funestus s.s* caught biting in Nkhata Bay and its correspondingly high sporozoite rate (3.3%). Conversely, a low EIR reported for Karonga was likely due to the low number of *An. arabiensis* caught biting people in the study area at the time of the study. It is known that EIR or risk of infection can be quite heterogeneous (37). Furthermore, it is also possible that the observed low EIR estimated for Karonga reflects the effectiveness of malaria control interventions since this village had a high coverage of both ITNs and IRS at baseline.

The study was faced with a number of limitations ranging from design factors, sampling frame in terms of number of study sites and duration to effectively measure parameters such the entomological inoculation rate. The fact that three households were sampled on two consecutive nights did not allow for a complete rotation of human collectors to correct for differences in individual attractiveness to mosquitoes. Although the study was implemented during the wet season when *Anopheles* populations are naturally anticipated to be at peak abundance, there was no opportunity to estimate dry season transmission due to limited funds. Furthermore, the sampling effort was not intensive enough since data collection was carried out for two consecutive nights in a month.

Another important limitation was that only two villages were enrolled into the study. Malaria varies widely (heterogeneous) even within short distances due to a number of variables including ecological factors that support *Anopheles* vector species abundance and distribution. Since the two study sites were purposely selected for their inclusion into the study might also bias the overall findings of this study.

Having stated the above, one would argue that these study findings form very valuable information for malaria control in Malawi. These findings further provide most recent and empirical evidence of the behaviour of two important malaria vectors, *An. funestus* and *An. arabiensis* in the country and a platform for further studies. This study highlights aspects of *Anopheles* biting behaviour in Malawi.

Conclusions

This study has shown that there are behavioural differences between *An. funestus* and *An. arabiensis* at two study villages in Nkhata Bay and Karonga Districts in northern Malawi. Preliminary evidence has shown that the former bit people late in the morning while the latter largely bit around midnight. An. funestus was the most important malaria vector mosquito characterized by high *Pf* sporozoite rates and consequently contributed more to the estimated

entomological inoculation rate. Further studies with larger sample sizes and more replications are required to fully understand the importance of these behavioural attributes to malaria transmission in Malawi so that they can be exploited to effectively control the disease.

Declaration

Ethics approval and consent to participate

This study was approved by the College of Medicine Research Ethics Committee (COMREC) and its protocol number was P.10/12/1293. Furthermore, a written informed consent was obtained from individual mosquito collectors for their participation as project volunteers. Equally, household owners were consented both for the household survey and mosquito collections.

Consent for publication

All authors have read, commented and approved the manuscript for publication

Availability of data and material

The author agrees to make data freely available as per requirements of Biomedical Central.

Competing interests

The authors declare that there is no conflicting interest financial or otherwise

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Authors' contributions

The study was conceived by TM and JC. Coordination of the field work was done by TM and SG. Data analysis was done by MM, AB and TM. TM and MM contributed to the writing while the manuscript was critically reviewed by all authors. All authors have read and approved the final version of this manuscript.

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Authors' information

Not applicable

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