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A Survey of Man-Biting Mosquito Species in a Tropical Rainforest Community in the Southeastern Nigeria

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ABSTRACT

Mosquitoes remain a public health nuisance and principal vectors of human and animal diseases. A survey on man-biting adult mosquito vector species was conducted in Awka South Local Government Area, Anambra State, Nigeria from October, 2017 to September, 2018. The objectives were to determine the distribution, the biting and resting behavior and the physiological state of the adult mosquitoes. The outdoor and indoor biting mosquitoes were collected by human bait collection and pyrethrum-based insecticide knockdown methods respectively. Four human volunteers were involved in the outdoor collection and Baygon® a pyrethroid-based insecticide was used in the indoor collection. The indoor resting density and man-biting rates were studied using calculations, while the physiological states of mosquitoes where studied by observation of their abdomen. A total of 2,663 mosquitoes were collected from the study. Of this number, 1,393 (40.05%) were outdoor biting adults while 1,270 (36.51%) were indoor biting adults. There was no significant difference in the number of mosquitoes collected using these two methods ($p > 0.05$, $p = 0.122$). Seven mosquito species namely ; *An. gambiae* 80 (5.79%), *An. funestus* 62 (4.45%), *Ae. aegypti* 149 (10.69%), *Ae. albopictus* 77 (5.53%), *C. quinquefasciatus* 1023 (73.44%), *C. annulioris* 1 (0.07%) and *Eretmapodites chrysogaster* 1(0.07%) were collected outdoor. *An gambiae* 131 (10.31%), *An. funestus* 47 (3.70%), *Ae. aegypti* 84 (6.61%), *Ae. albopictus* 57 (4.4%) and *C. quinquefasciatus* 951 (74.88%) were collected indoor. There was a significant difference in the numbers of indoor and outdoor mosquito species collected ($p < 0.05$, $p=0.000$). *Culex quinquefasciatus* had highest room density of 2.20 mosquitoes / room / night and the highest man-biting rate of 1.0 bites / man / night. A total of 570 (44.88%) mosquitoes collected indoors were freshly fed. The high preponderance of man-biting mosquitoes and their wide distribution in the area calls for mosquito control intervention strategies to save the people from imminent endemic mosquito-borne infections.

Keywords Man - biting, Mosquitoes, Adults, Vectors, Distribution.

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INTRODUCTION

Mosquito-borne diseases have emerged as a major public health concern. Mosquitoes are the most common blood sucking arthropods vectors of human and animal diseases (Jerome et al. 2019). The diseases are common in most developing countries and even in developed economies of the world (Nnamonu et al. 2019). Some of the diseases include dengue fever transmitted by *Aedes* mosquitoes, yellow fever transmitted by *Ae. aegypti* and *Ae. albopcitus* (Jerome et al. 2019), lymphatic filariasis transmitted by *Culex* mosquitoes (Onyido et al. 2016) and the parasites of malaria ; *Plasmodium falciparum*, *P. ovale*, *P. vivax* and *P. malariae* transmitted by the *Anopheles* mosquitoes (Irikannu and Chukwuekezie 2015).

Mosquitoes also cause serious biting annoyance, noise nuisance, sleeplessness and allergic reactions through their bites (Onyido et al. 2009a). The main route of transmission of mosquitoborne diseases is through their bites (Aribodor 2012). Recently, the incidence of mosquito-borne diseases has increased at an alarming rate, with approximately 700 million infected cases and more than one million deaths recorded annually (Caraballo and King 2014).

Ecological studies aimed at investigating the abundance, distribution, resting and biting habits of mosquito vectors of human diseases are important for formulating and implementing their control strategies. This is necessary to ensure focused and well directed control intervention and to avoid waste of resources in combating harmless species. Therefore, the objectives of the study were to determine the distribution, the biting and resting behavior and the physiological state of the man-biting adult mosquitoes in Awka South Local Government Area, Anambra State, Nigeria.

MATERIALS AND METHODS

The study was conducted in Awka-South Local Government Area (LGA) of Anambra State, South-Eastern Nigeria. The geographical coordinates of Awka South LGA is 7°04´E and latitude 6°10´N (Fig. 1). Awka is in the tropical rainforest zone of Nigeria and experiences two distinct seasons in a year ; eight months of heavy rain (March – October) and is followed by four months of dryness (November–February). The temperature range of the area is between 27-30°C from June to December and rises to 32–34°C between January and April. The

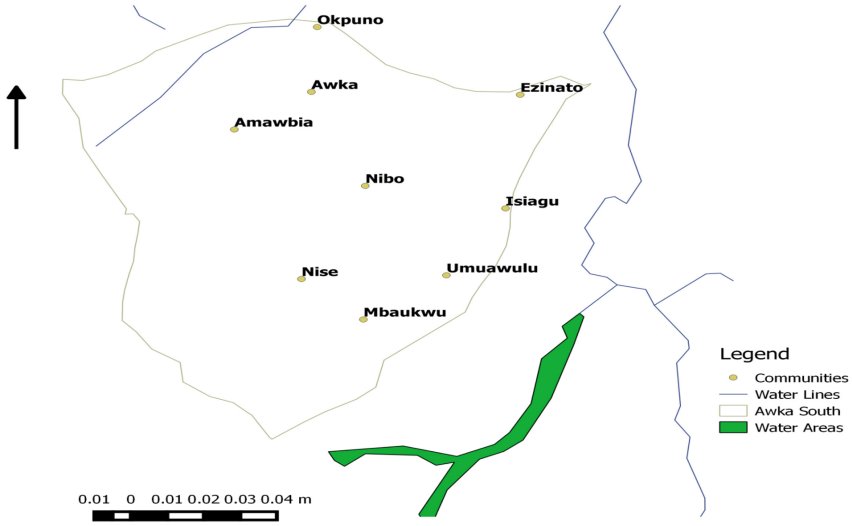

Fig. 1. Map of Awka South Local Government Area, showing the study communities (using geographical coordinates of the communities with QGIS version 2.10.1).

relative humidity of the area is about 70% in the dry season but may rise to 80% during the wet season. The annual rainfall is between 2000–3000 mm. The community is about 150 m above sea level. Awka South LGA has a population of 189,049 inhabitants (NPC 2006). The inhabitants of the communities are mainly farmers and civil servants. A few others are traders and blacksmiths.

Study design

The study was conducted for a period of twelve (12) months starting from October, 2017 to September, 2018. The study was a longitudinal survey of adult mosquitoes. It included a monthly field survey of the biting and resting habits of the adult mosquitoes.

Selection of sampling area

Six of the nine communities that made up Awka-South Local Government Area namely ; Amawbia, Awka, Okpuno, Nibo, Nise and Mbaukwu were judgementally selected (Onuoha et al. 2011).

Advocacy visits and community sensitization

Several advocacy visits were made to the opinion leaders of the communities and their permission to carry out the project was obtained. The communities were sensitized and mobilized through meetings organized with the aid of their community leaders. The study intent and their significance were explained to them and their consent was also obtained for the use of their environment for the study. In addition, the 4 volunteer collectors were vaccinated with yellow fever vaccines for at least 10 days before the commencement of the study. Each volunteer was also properly instructed on the techniques of the study.

Collection of indoor-biting and resting adult mosquitoes

Indoor biting and resting adult mosquitoes were collected from the communities using pyrethroid-based insecticide knock down (PKD) as described by Onyido et al. (2016). Collections were made in 18 houses (3 houses per community) in the study area. The adult mosquitoes were collected from living rooms where people slept the previous night. In the rooms, the doors and windows were shut and white spread sheets laid from wall to wall, covering furniture and other non-movable items in the rooms. Edible items, cooking utensils and movable household furniture were carried outside to avoid contamination with insecticide. A pyrethroid-based insecticide aerosol (Baygon®) were sprayed in the room and allowed to remain for 20 minutes before collection. At the end of the 20 minutes interval after spraying, the white spread sheets were folded starting from the edges to ensure that no knocked down mosquito escaped.They were taken outside the room and spread out again to collect the knocked down mosquitoes using a pair of entomological forceps into damp-petri dishes.

Collection of outdoor-biting mosquitoes

Outdoor-biting mosquitoes were collected using human-bait collection (HBC) as described by Onyido et al. (2016). The collections were done all-night from 6.00 pm–6.00 am (local time). Four volunteer collectors exposed their legs and hands for mosquito bites by rolling up their trousers and shirts sleeves to knee and elbow level respectively. Mosquitoes alighting on the volunteers were collected with the aid of test tube vials and torchlight. Each vial was quickly covered with a ball of cotton wool to avoid escape of the mosquito. The time of collection of each mosquito was properly recorded and collations were made hourly.

Indoor resting density of mosquitoes

The indoor resting density of mosquitoes collected indoors in the study area was calculated from the result of PKD using the methods described by Ezihe et al. (2017). It was calculated by the number of mosquitoes collected divided by the total number of house sampled and the total number of night collections made. It is expressed as :

Indoor Resting Density (D)= (Number of females ÷Number of houses) ÷ Number of nights

Man biting rate of mosquitoes

Man-biting rate (Mbr) is expressed as the number of bites a person receives from a specific vector species

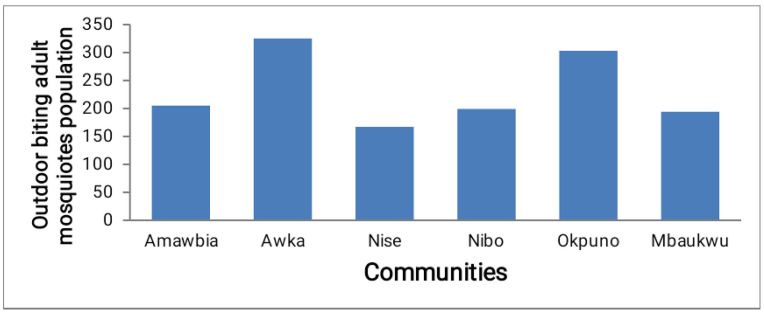

Fig. 2. Outdoor biting adult mosquito populations from different communities ($p < 0.05$, $p = 0.386$).

per night. This was calculated from PKD collections as the total number of freshly fed females of a species divided by the total number of occupants who spent the night in the rooms and by the total number of nights that were used for the collection according to Ezihe et al. (2017). It is expressed as :

Man-biting rate (Mbr) = (Number of freshly fed females ÷ Total number of occupants) ÷Total number of nights

Physiological state of adult female mosquitoes collected indoors

The physiological states of female mosquitoes collected indoors was determined in order to observe mosquitoes that had blood meal and those that had not fed. The mosquitoes were grouped into four categories ; unfed, freshly fed, half gravid and gravid (Service 1985).

Morphological identification of the mosquitoes

At the end of each collection period, all the mosquitoes collected were properly labeled and sent to the Entomology Laboratory of the Department of Parasitology and Entomology, Nnamdi Azikiwe University for identification. The morphological identifications were later confirmed at the Laboratory of National Arbovirus and Vectors Research Center, Enugu. The mosquitoes were identified using the gross morphology of the species especially the body color, patches of scales on the palps, antennae, proboscis, patches of pale and black scales on the wings and legs and the terminal abdominal segments using standard keys (Gillies and De Meillon 1968, Gillet 1972, Gordon and Lavoipierre 1976, Russell et al. 2005).

Data analysis

Data collected from the study were analyzed using the statistical package for social sciences (SPSS) version 2.10. Analysis of variance (ANOVA) at 5% significant level was used to compared the indoor and outdoor-biting adults.

RESULTS AND DISCUSSION

A total of 2,663 adult mosquitoes comprised of 1,393 (52.31%) outdoor biting adults and 1,270 (47.69%) indoor biting and resting adults were collected from the study. There was no significant difference between the numbers of mosquitoes collected outdoors and indoors ($p > 0.05$, $p=0.122$). Of the 1,393 outdoor biting adult mosquitoes collected, the highest number 325 (23.33%) was from Awka and the least 167 (11.98%) was from Nise community (Fig. 2). Outdoor mosquito collection from other communities were 205 (14.72%) from Amawbia, 199 (14.28%) from Nibo, 303 (21.75%) from Okpuno and 194 (13.92%) from Mbaukwu. There was no significant difference in the numbers of the outdoor biting mosquitoes from the different communities ($p > 0.05$, $p = 0.386$). Seven mosquito species, including two *Anopheles* species and five Culicine mosquito species were collected outdoors (Fig. 3). These were *An. gambiae* 80 (5.79%), *An. funestus* 62 (4.45%), *Ae. aegypti* 149 (10.69%), *Ae. albopictus* 77 (5.53%), *C. quinquefasciatus* 1023 (73.44%), *C. annulioris* 1

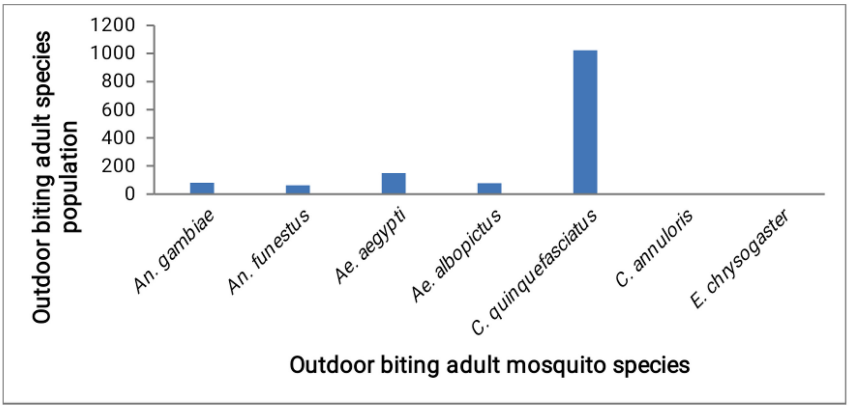

Fig. 3. Outdoor biting adult mosquito species from different communities ($p < 0.05$, $p = 0.000$).

(0.07%) and *Eretmapodites chrysogaster* 1 (0.07%). Among the Culicine mosquitoes, *C. quinquefasciatus* was the most abundant outdoor biting mosquitoes in the community followed by *Ae. aegypti* and *Ae. albopictus*. There was a significant difference in the numbers of the different outdoor mosquito species collected from the different communities ($p < 0.05$, $p = 0.000$).

Five dominant mosquito species namely ; *An. gambiae*, *An. funestus*, *Ae. aegypti*, *Ae. albopictus* and *C. quinquefasciatus* were collected outdoors during all night collection (Fig. 4). *Ae. aegypti* and *Ae. albopictus* had their peaks between 7.00–8.00 pm and virtually stopped biting by 9.00 pm. *C. quinquefasciatus* continued biting until dawn (6.00 am) the next day, with a major peak between 9.00 pm and 11.00 pm. *An. gambiae* and *An. funestus* had their biting peaks by 1.00 am followed by a gradual decline in population until dawn. Of the 1,270 indoor biting adult mosquitoes collected (Fig. 5), the highest number 279 (21.96%) were from Okpuno and the least 160 (12.59%), from Nise. The collection of indoor biting adult mosquitoes from other communities were 169 (13.30%) from Amawbia, 257 (20.23%) from Awka, 197 (15.51%) from Nibo and 208 (16.37%) from Mbaukwu. There was no significant difference

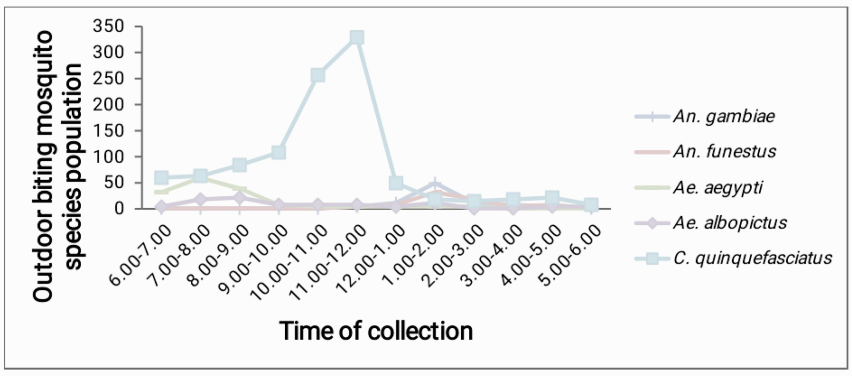

Fig. 4. All night-hourly collections of outdoor biting adult mosquitoes in Awka South LGA Anambra State, Nigeria.

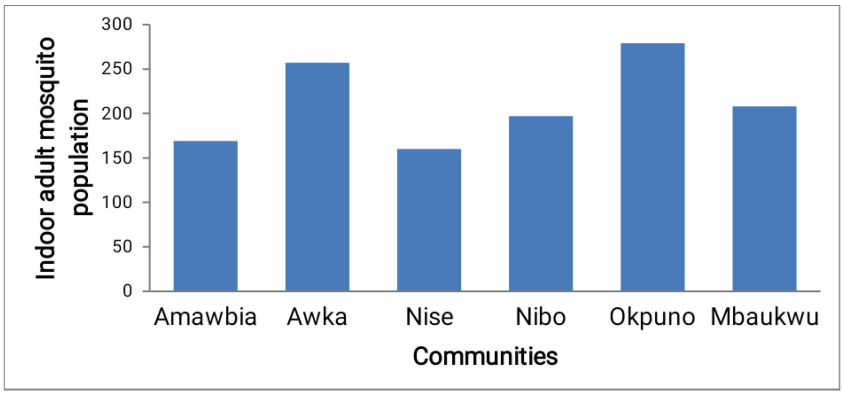

Fig. 5. Indoor biting adult mosquito populations collected from different communities ($p > 0.05$, $p = 0.796$).

in the populations of indoor biting mosquito in the different communities ($p > 0.05$, $p = 0.796$).

Five mosquitoes species namely *An. gambiae*, *An funestus*, *Ae. aegypti*, *Ae. albopictus* and *C. quinquefasciatus* were collected indoors. *C. quinquefasciatus* 951 (74.88%) was the most prevalent indoor-biting mosquitoes and *An. funestus* 47 (3.70%) the least (Fig. 6). *An. gambiae* 131 (10.31%) and *An. funestus* 47 (3.70%) which are important malaria vectors were collected indoors. *Ae. aegypti* 84 (6.61%) and *Ae. albopictus* 57 (4.4%) which are important vectors of arboviral infections were collected indoors. *C. quinquefasciatus* 951 (74.88%) and *An. gambiae* which are important vectors of lymphatic filariasis were collected indoors. There was a significant difference mosquito species population indoors in the different communities ($p < 0.05$, $p = 0.000$). *Culex quinquefasciatus* had the highest room density of 2.20 mosquitoes / room / night, followed by *An. gambiae* 0.30 mosquitoes / room / night, *An. funestus* 0.10 mosquitoes / room / night, *Ae. aegypti* 0.20 mosquitoes / room / night and *Ae. albopictus* 0.13 mosquitoes / room/ night (Table 1). *Culex quinquefasciatus* had the highest indoor biting rate of 1.0 bites / man / night followed by *An. gambiae* 0.017 bite / man / night, *An. funestus* 0.010 bites / man / nights, *Ae. aegypti*

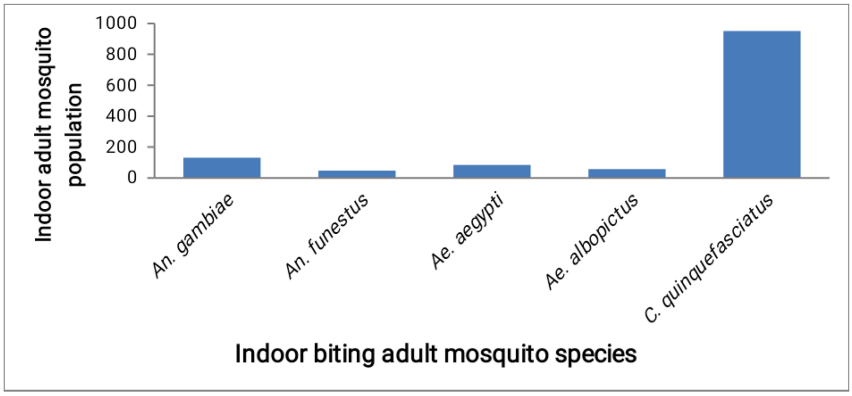

Fig. 6. Indoor biting adult mosquito species collected from different communities ($p < 0.05$, $p=0.00$).

Table 1. Indoor resting density and man-biting rate of different mosquito species in Awka South Local Government Area, Anambra State.

Mosquitoes species collected indoors	No. of females collected	No. freshly fed females	Indoor resting density (IRD) No. of mosquitoes/ room/ night	Man biting rate (MBR) No. of bites/ man/ night
An. gambiae	131	11 (1.9%)	0.30	0.017
An. funestus	47	6 (1.0%)	0.10	0.010
Ae. aegypti	84	10 (1.8%)	0.20	0.020
Ae albopictus	57	9 (1.6%)	0.13	0.014
C. quinque fasciatus	951	534 (93.7%)	2.20	1.0
Total	1270	570 (44.9%)	3.00	1.10

0.020 bites / man / nights and *Ae. albopictus* 0.014 bite / man / night. Of the 1,270 (100%) adult mosquitoes collected indoors, 125 (9.84%) were unfed, 570 (44.88%) were freshly fed, 409 (32.20%) were half gravid and 166 (13.07%) were gravid (Table 2).

A relatively high population of mosquitoes which included seven species was collected in the study. *An. gambiae, An. funestus, Ae. aegypti, Ae. albopictus, C. quinquefasciatus, C. annulioris* and *E. chrysogaster* collected both indoors and outdoors reveal that diverse species are biting in the area. All the mosquitoes collected in this study have also been reported in different places in Nigeria. Okonkwo et al. (2014) collected *An. gambiae, An. funestus, Ae. aegypti* and *Ae. albopictus* in Oba. Mbanugo and Okpalaononuju (2003) collected *Ae. aegypti* and *C. quinquefasciatus* in Awka metropolis. Umeanaeto et al. (2017) reported *An. gambiae, An. funestus, C. quinquefasciatus* and *C. annulioris* in Nnamdi Azikiwe University female hostels. Onyido et al. (2016) reported *An. gambiae*, *C. quinquefasciatus, Ae. aegypti* and *Ae. albopictus* in Nibo community. Also, Okogun et al. (2005), Umaru et al. (2006), Oguoma and Ikpeze (2008), Adeleke et al. (2008), Onyido et al. (2009a and b) also made similar collections in different parts of Nigeria.

Table 2. Abdominal grading of indoor-biting adult mosquitoes collected using pyrethroid knock down (PKD) to determine their physiological state.

Mosquitoes species	Unfed	Freshly fed	Half gravid	Gravid	Total
An. gambiae	4	11	87	29	131
An. funestus	4	6	26	11	47
Ae. aegypti	7	10	44	23	84
Ae. albopictus	6	9	35	7	57
C. quenquefasciatus	104	534	217	96	951
Total	125	570	409	166	1270

Five of the mosquito species namely *An. gambiae* and *An. funestus* which are efficient vectors of malaria parasites (Aribodor 2012) and *Ae. aegypti, Ae. albopictus* and *C. quinquefasciatus* which are efficient vectors of well-known human diseases (Jerome et al. 2019, Onyido et al. 2016) were collected biting both indoors and outdoors. These observations suggest that inhabitants of the study area are exposed to the bites of these vectors and may contract infections if the mosquitoes are infected. Outdoor biting and resting mosquitoes were highest in Awka, but least in Nise community. Also, the numbers of indoor biting adult mosquitoes were highest in Okpuno while the population was least in Nise community. The uneven distribution of mosquitoes in the communities may be dependent on the availability of human hosts, since more mosquitoes were collected from highly populated communities where human host were readily available. Thus ; least number of adult mosquitoes was collected in sparsely populated area like Nise community.

Culex quinquefasciatus mosquitoes were the most abundant Culicine mosquitoes biting both indoors and outdoors in the study area. *Culex quinquefasciatus* has also been reported to be the most abundant in other studies in the different parts of the same study area and in nearby communities (Mbanugo and Okpalaononuju 2003, Irikannu and Chukwuekezie 2015, Onyido et al. 2016, Umeanaeto et al. 2017) and also in Midwestern Nigeria (Okogun et al. 2005). This could be most likely explained by the presence of large blocked drainages with very dirty

stagnant water and septic tanks among others which serve as their breeding sites found in the study area. However it was in contrast with the findings from Katstina State Nigeria (Bunza et al. 2010) and North Central Nigeria (Oguoma and Ikpeze 2008) where *Anopheles* species were the most abundant mosquito species. The variation in the abundance of different species in the different regions may be because of the environmental factors such as availability of peculiar breeding sites of species which contributes to their distribution.

With the exception of *C. quinquefasciatus*, most of the culicines had their peaks between 7.00–8.00 pm and virtually stopped biting by 9.00 pm. This observation shows that these species are twilight biters. *C. quinquefasciatus* continued biting until dawn (6.00 am) with a major peak between 9.00 pm and 1.00 am. This also shows that *C. quinquefasciatus* are highly nocturnal, having increased biting activities at midnight. The observations are in tandem with Onyido et al. (2016) who made similar observation on Culicine species in Nibo community. Also, *C. quinquefasciatus* had highest indoor density and man-biting rate compared to other mosquitoes species. The epidemiological implication of the high indices is that *C. quinquefasciatus* can be efficient vectors of lymphatic filariasis in the study area during any outbreak of the disease. *Anopheles gambiae* and *An. funestus* had their biting peak between 1 am – 2 am when human are fast asleep. The findings on *Anopheles* species which are important malaria vectors is in agreement with Aribodor (2012) who reported that *Anopheles* species are midnight biters, as such, the mosquitoes could transmit malaria parasites to humans while they are fast asleep.

CONCLUSION

The study has shown that there were high abundance of the different genera of man-biting mosquitoes namely *Culex*, *Aedes* and *Anopheles* in the study area. These mosquitoes pose serious biting nuisance and risk of disease transmission to the inhabitants. Interventions with mosquito control programs together with massive health education programs to the inhabitants will aid in curbing the vectors.

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