

Sporozoite Infection Rate of Malaria Vectors in an Agrarian Community in Shongom Local Government Area of Gombe State, North-Eastern Nigeria

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Abstract

This study reports the mosquito species abundance and the sporozoite infection rate of malaria vectors in Latur Community Shongom Local Government Area of Gombe State, Northeastern Nigeria. The research was carried out in the dry season in March, 2016. A total of 412 adult female mosquitoes comprising of 373(90.5%) anophelines and 39 (9.5%) culicines were captured and morphologically identified using standard keys. Of these, 324(78.47%) were *Anopheles gambiae*, 49(11.89%) were *Anopheles funestus* and the remaining 39(9.47%) were *Culex* species. Chi –square statistic showed a significant difference in the mosquito species abundance (χ^2 test $P<0.05$). The *Anopheles* mosquitoes were dissected using standard procedures and an overall sporozoite infection rate of 12.6% was recorded. *Anopheles gambiae* had the highest sporozoite infection rate 8.6% while *Anopheles funestus* had the lowest sporozoite infection rate 4.0%. Chi-square statistic showed a significant different in the sporozoite infection rate in the two *Anopheles* species (χ^2 test $P<0.05$). The presence of stagnant water which serves as breeding sites of mosquitoes in the area explains this result. The study has made known Latur, as a malaria endemic area and that *Anopheles gambiae* and *Anopheles funestus* are incriminated as malaria vectors. Therefore, the findings of this research can serve as a baseline data for the implementation and evaluation of malaria control programs in Shongom Local Government Area.

Keywords: Mosquito species abundance, Malaria vector, Sporozoite infection rate, Malaria endemic area, Baseline data and Control programs.

INTRODUCTION

Malaria is considered as an endemic and important public health problem in Nigeria. According to the World Health Organization, an estimated 219 million cases of malaria occurs worldwide in 2017 (WHO, 2018). It also reported that, 200 million (92%) were in WHO African Region, noting that only fifteen countries in in Sub Saharan Africa and India accounted for 80% of the global malaria burden, with only five countries contributing half of all malaria cases worldwide: Nigeria (25%), Democratic Republic of Congo (11%), Mozambique (5%), India (4%), and Uganda (4%) (WHO, 2018). Half of Nigerians population is said to experience one or more malaria every year (Federal Ministry of Health, 2005). Oduola *et al.*, (2012) noted that a large percentage of the population affected with malaria in the country live below the poverty line in the villages with poor healthcare facilities.

There are four major species of human malaria parasites which are *Plasmodium falciparum*, *P. malariae*, *P. ovale* and *P. vivax*. However, *Plasmodium falciparum* is the most prevalent species of malaria parasite in Nigeria (Oduola *et al.*, 2012).

The female *Anopheles* mosquitoes serve as vectors that transmit *Plasmodium* parasites because they support the sporogonic development of human malaria parasites (Gimba, 2014). There are over 2,500 species of *Anopheles* mosquitoes, but less than 50 are capable of transmitting malaria (Ahmed, 2014). In some cases, different forms are found in varying ecological regions, thus the need to identify the prevalent malaria vectors in different ecological zones. Even though, major efforts were made in the past century to control the disease, vector resistance to insecticide and *Plasmodium* species resistance to a number of drugs remain a challenge to malaria control (Tarleton and Kissinger, 2001, Oyewole *et al.*, 2010). Vector control was recommended in 1993 by the World Health Organization as an important component of the global strategy to combat malaria and vector identification and entomological malaria

transmission indices forms an important aspect of the strategy (WHO, 1993). These information's on the vector profile and malaria transmission risk indices are lacking in most Nigerian communities and therefore evaluation of these malaria control measures remain a challenge (Oduola *et al.*, 2012). For example, Manyi *et al.*, 2014, reported an overall *Plasmodium* sporozoites infection rate of 54.9% in Makurdi the Benue State capital, but such information on the *Plasmodium* sporozoite infection rate of *Anopheles* is lacking in Gombe State though there are reports on species abundance, composition, indoor resting density, man biting rates of mosquitoes and susceptibility status of the vectors in some parts of the State (Yoriyo *et al.*, 2013). Hence, this study was carried out in an area where none of this information are available and attempts to report for the first time malaria vector species, sporozoite infection rate and parity rate in an agrarian community in Shongom Local Government Area of Gombe State, North-Eastern Nigeria.

Materials and Methods

Study area

This study was carried out in Latur an agrarian community in Shongom Local Government Area of Gombe State. The local government is located in the southern part of Gombe State and is approximately 77.2km from Gombe the state Capital. The community is located in the central part of the local government area on latitude 9°41'43.0"N and longitude 11°11'02.2"E. the settlement is located close to a river in the grass land savannah or the Sudan Savannah belt, having two distinct seasons, the dry season (November – March) and rainy season (April-October). Most of the inhabitants are farmers and farming is mainly during the rainy season. The houses are basically of three type (mud with thatch roof, mud with corrugated iron roof and cement block with corrugated iron roof).

Ethical Consideration

Ethical approval for this study was granted by the institutional ethical committee of Gombe State Ministry of Health, and the community gate keepers also gave their approval for the study.

Entomological Sampling

Fifteen houses were randomly selected in the village for mosquito collection to include five of each of the three housing type in the village. Indoor resting mosquitoes were collected in the month of March, 2016 in the rooms where people slept the previous night using pyrethrum spray catches (PSC) between the hours of 06:00am and 09:00am so as to prevent mosquitoes from escaping the rooms (Service, 2012). The materials used for collection were touch light, face mask, white sheet, petri dishes, forceps, insecticide, labels and a bag for keeping the collected samples. The aerosol sprayed to knockdown the mosquitoes was raid and the active ingredients are: D-allethrin (0.25%), tetramethrin (0.15%) and Deltamethrin (0.015%). The inert ingredient is (99.58%).

The rooms were prepared for PSC following the method of the WHO, (2013) by removing small furniture and food items. White sheets were then spread to completely cover the floor and all articles inside, doors and windows were closed and raid was then sprayed by the operator in a clock wise direction toward the ceiling with the operator standing in one position until the room was filled with the mist, the operator then exits from the room and immediately closed the door and waits for 10 minutes. At the end of the 10 minutes, the door was then opened, the white sheet were carefully retrieved, starting from the entrance, the corners of the sheet were carefully lifted thereby gathering the knocked down mosquitoes at the middle, then collecting them with forceps into labeled petri dishes. Mosquitoes collected in each room were stored in separate petri dishes and were labeled appropriately with the house type, and house serial number. A data form was also filled for each house with date, time, house type, name of head of household, house number, number of people who slept in the house the previous night and name of the collector.

Species Identification

The mosquitoes collected during Pyrethrum Spray Catch were examined under a dissecting microscope and were identified morphologically using identification keys by Gillies and Coetzee (1987) to determine the vector species. The abdominal states of the mosquitoes such as unfed, freshly fed, half gravid and gravid were recorded.

Detection of *Plasmodium* Sporozoite

The materials used for the dissection were dissection microscope, compound microscope, microscope slide, cover slip, distilled water, methanol, normal saline, giemsa stain, oil immersion, dissection needle, dissection blade and forceps.

Adult mosquitoes were examined for *Plasmodium* sporozoite by investigations of the salivary gland as described by Williams and Pinto (2012).

Just before dissection, the mosquito was held by one wing and the legs, proboscis and palps were detached one at a time after which the wings were then pulled off. The mosquito was soaked in distilled water with detergent for two minutes, rinsed with distilled water then placed on a clean slide with the head pointing to the right hand side. A drop of physiological saline was then added to keep the specimen fresh. The left dissecting needle was then placed gently on the thorax, just below the place where the salivary gland lies and the right needle placed at the same time but on the opposite side. This was then gently pulled towards the right direction to bring out the salivary glands. The glands are then placed on another slide with a drop of normal saline and covered with a cover slip after which a gentle pressure was exerted on the cover slip to rupture the salivary gland. A drop of methanol containing 90% absolute alcohol was added and allowed for one minute; a drop of Giemsa stain was added then left to air dry for 40 minutes as described by the World Health Organization, (1975) and adapted by Manyi *et al.*, 2014. This was then washed with distilled water; air dried and was then viewed under a microscope, using X40 objective. A drop of immersion oil was applied and viewed under X100 objective. *Plasmodium* sporozoite were seen as banana shaped cells (WHO, 2013) and the sporozoite Infection rates were calculated using the formula by Williams and Pinto, (2012) as follows:

Sporozoite rate = number of positive mosquitoes ÷ number of dissected mosquitoes × 100

Detection of Parity Rate of *Anopheles* Species

The abdomen of the female mosquitoes were dissected to determine their physiological age (parous and nulliparous) by examining their tracheolar skein on the surface of the stomach walls. The abdomens were dissected at the 7th and 6th sclerite under a dissection microscope (Williams and Pinto, 2012). The ovaries were pulled out, and a little pressure was applied to burst the abdomen to bring out the malpighian tubules and the stomach. This was then separated from other parts of the abdomen and transferred to a new slide; A drop of normal saline was added, then covered with a cover slip and examined under a compound microscope to determine if the tracheoles has terminal coiling (nulliparous) or is stretched (parous) (Williams and Pinto, 2012). The parity rate was calculated as:

Parity rate = number of parous females ÷ number of females dissected × 100

Data Analysis

The data obtained was analyzed using R console software version 3.2.2 to run chi-square (χ^2) statistic on the data collected. Significant levels were considered at 95% confidence level with significant differences taken at $P < 0.05$ chi-square statistic was considered as the appropriate statistic due to the homogeneity and frequency of the data.

Results

Table 1 shows that a total of four hundred and twelve (412) adult female mosquitoes, consisting of three species; *Anopheles funestus* (49), *An. gambiae* (324) and *Culex* species (39) were captured during the survey. The difference in abundance of the mosquito species was significant ($\chi^2 = 380.95$, $df = 2$, $P < 0.001$) in mosquito abundance in relation to species that were captured during the study. These were identified morphologically. The two anopheline species were dissected and investigated for sporozoites using standard procedures and identification keys. The number of *An. gambiae* collected were more than those of *An. funestus* and *Culex* species, though, the culicines were not incriminated as malaria vector. All the anophelines were found to be blood fed.

Table 2 shows that *Anopheles. gambiae* was the highest species 109(75.17%) collected in houses made with cement blocks and corrugated iron roof. this was followed by *An. funestus* 21(14.48%) and the least was *Culex species* 15(10.34%). Thus, there was a significant difference ($\chi^2 = 114.59$, $df = 2$, $P < 0.001$) in mosquito abundance between species resting indoors in houses made of cement block with corrugated iron roof. So also, *An. gambiae* 77(77.78%) was the highest species encountered in mud houses with thatch roof followed by *An. funestus* 13(13.13%), the lowest was *Culex species* 9(9.09%). Consequently, mosquito abundance between species resting indoors in mud houses with thatch roof showed a high significant difference ($\chi^2 = 88.242$, $df = 2$, $P < 0.001$). A similar results was obtained in mud houses with corrugated iron roof where *An. gambiae* was the highest 138 (82.14%), *An. funestus* and *Culex* species had the same abundance of 15 (8.93) each. Hence, there was a high significant difference ($\chi^2 = 180.11$, $df = 2$, $P < 0.001$) in mosquito abundance between species resting indoors in mud houses with corrugated iron roof.

Table 1. Pyrethrum spray collection of mosquitoes in Latur Village

Collection Date	House serial no.	House type	No of people who slept in the room	<i>A. gambiae</i>	<i>A. funestus</i>	<i>Culex</i> species
23/03/016	1	A	6	14	0	2
	2	C	7	35	5	4
	3	A	4	16	6	0
	4	B	4	17	5	3
	5	C	4	1	4	1
	6	A	5	6	4	4
	7	B	3	22	3	0
	8	A	2	45	5	5
	9	C	4	43	3	3
	10	A	8	28	6	4
	11	B	2	10	1	0
	12	C	7	31	0	4
	13	C	4	28	3	3
	14	B	5	13	1	2
	15	B	4	4	15	3
Total		15	69	324	49	39 GT 412
Percentage				78.64%	11.89%	9.47%

A (Cement Block with corrugated iron roof), B (mud with thatch roof) and C (mud with corrugated iron roof)

Table 2. Housing type and mosquito abundance in Latur community

House Type	<i>Anopheles gambiae</i>	<i>Anopheles Funestus</i>	<i>Culex</i> Species	Total for House type
A	109(75.17%)	21(14.48%)	15(10.34%)	145
B	77(77.78)	13(13.13%)	9(9.09%)	99
C	138(82.14%)	15(8.93%)	15(8.93%)	168

A (Cement Block with corrugated iron roof), B (mud with thatch roof) and C (mud with corrugated iron roof)

Table 3. Parity and sporozoite infection Rate

Species	N dissected	N nulliparous	N parous	N sporozoite	S rate
<i>A. gambiae</i>	324	201	123(37.9%)	28	8.6%
<i>A. funestus</i>	49	36	13(26.5%)	2	4.0%
Total	373	237	136	30	12.6%

N dissected = number of mosquitoes dissected, N nulliparous = number of nulliparous mosquitoes, N parous = number of parous mosquitoes, N sporozoite = number of mosquitoes infected with *Plasmodium* sporozoites and S rate = sporozoite rate

Table 3 shows that the ratio of nulliparous to parous *Anopheles* species was 237(63.54%):136(36.46%). This shows that there was a very high significant difference ($\chi^2 = 27.349$, df = 1, p-value = 1.699e-07) in relation to the number of nulliparous and parous *Anopheles* species. The overall sporozoite rates of infection of the *Anopheles* species was (12.6%), showing a significant difference in the number of the *Anopheles* species not infected with sporozoite to those infected with *Plasmodium* sporozoite ($\chi^2 = 262.65$, df = 1, P < 0.001).

Discussion

Records were made of the two most prevalent malaria vectors: *An. gambiae* and *An. funestus* which together accounted for 90.5% of mosquitoes collected. *Culex* species were the least mosquitoes observed accounting for only 9.5% of the

total mosquitoes collected though they were not incriminated as malaria vectors. The high proportion of *Anopheles* mosquitoes can be attributed to the fact that, the study community was less polluted and as such, provide favourable environment for the breeding of the anophelines compared to the Culicines which prefer polluted breeding grounds. This agrees with the findings of Okorie *et al.* (2014) in Ibadan South Nigeria, who observed that 1.9% (31) of the mosquitoes captured were *An. gambiae* and 98% (1756) were *Culex* species as a result of the polluted breeding grounds in residential areas of the town. Similarly, Yoriyo *et al.* (2013) also observed a low proportion of *Anopheles* species (17.3%) as against (82.6%) *Culex* collected in Gombe metropolis due to the polluted environment which favours the breeding of the culicines.

The higher number of *An. gambiae* observed in this work also agrees with the result of Kilama (2010) who pointed out that *An. gambiae* were more common than *An. funestus* as it is also observed in this research. This could be because *An. gambiae* were more anthropophilic, endophagic and endophilic as against *An. funestus* that could have been more zoophilic, exophagic and exophilic during the study period.

A contrary result was reported by Mzilahowa *et al.* (2012) in southern Malawi who observed that the proportion of *An. gambiae* was slightly higher than *An. funestus*. However, these works are not in agreement with that of Dadzie, Brenyah and Appawu (2013) in Ghana who revealed that *An. funestus* was a little higher than *An. gambiae* and *An. rufipes*. This could be as a result of variations in the ecology of the study area. The significant difference in the number of *Anopheles* species collected in houses in the study area is an indication of differences arising from the adaptations of the two species of *Anopheles*, in favour of *An. gambiae* than *An. funestus*, due to the dry season in which the work was done.

The overall sporozoite infection rate of 12.6% that was obtained in this work is lower when compared to the 54.9% overall sporozoite infection rate recorded in Manyi and his colleagues in Makurdi the Benue State capital. This could be attributed to the small sample size in the present work, and it could also be as a result of the difference in the environmental factors in that Makurdi provided more favourable environment for the breeding of mosquitoes than the area in consideration in this study. Nevertheless, sporozoite infection rate of *An. gambiae* in Manyi's study was higher than that of *An. funestus* and it is in agreement with the findings of this study which also shows a higher sporozoite infection rate of 8.6% in *An. gambiae* as against the 4.0% sporozoite infection rate obtained in *An. funestus*. This could also be that, *An. gambiae* is more endophilic while *An. funestus* is exophilic. Moawia and Osman (2010), observed an overall sporozoite infection rate of 14.01% in Central Sudan which is in agreement with the findings of the present study.

The overall parity rate observed in this work reveals that, there were more nulliparous *Anopheles* 237 (63.54%) than parous *Anopheles* 136(36.46%). This implies that there were more young anopheline population than the old ones, but for the fact that there were sporozoite positive and parous *Anopheles*, mean that malaria transmission was on going in the study community.

The sympatric occurrence of *An. gambiae* and *An. funestus* in Latur which are the major malaria vectors in Nigeria, can serve as a condition for the implementation of an integrated vector management campaign in Shongom Local Government Area such as the use of IRS and LLIN so as to have a successful malaria vector control in the area.

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