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Community Malaria Transmission Indices Implicates Sympatry Envelopment

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Abstract
Despite several measures put in place at controlling and eliminating malaria and its vectors, malaria still remains a public health problem that has continued to ravage humanity culminating into loss of man hours, hospital outpatients, school dropouts, loss of resources and several deaths.The aim of the study was todetermine the malaria transmission indices in Mazahfor effective control measures of the disease in the study area.The study was structured in aprospective cohort pattern to determine the possible outcome of interest and the exposure variables that necessitates the continuous stability of malaria in Mazah.Malaria vectors were sampled for a period of 5 months (from September 2014 to January 2015)using a Pyrethrum Spray Collection (PSC) from indoor houses of inhabitants. A total of 300 anopheline mosquitoes were collected, identified to species level (sensuslato), separated into males and females, and using standard procedures, were dissectedfor Sporozoite Rate (SR) and parity (egg laying status). Interestingly, 16.7% wereAnopheles funestuss.l. while 83.3% wereAnopheles gambiaes.l.Based on the Sporozoite rates (SR), the results obtained indicated that the Anopheles gambiaes.l. were 5.6% while Anopheles funestuss.l. were 4%.A further inquisition into the overall parity status indicates that 14.3% were parous while 85.7% of them were nulliparous. The parity rate for Anopheles funestuss.l. was 12 % while 14.8 % of the Anopheles gambiaes.l.The availability of the two species of mosquitoes in this study area, the sporozoites rate and parity of the female mosquitoes are indicators of the transmission indices of malaria predisposing the residents to an unstable form of malaria infection. This study therefore serves as a useful baseline data with a view to designing strategies for the control of mosquito borne diseases in Mazah and its environs.

Keywords: *Anopheles; Malariavectors; Transmission indices; Sporozoite; Parity; Nulliparity.*

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I. Introduction

In spite of advances in its treatment and prevention strategies over the past decades, malaria still threatens the lives of millions of people in tropical countries of the world[1]. Although over the years, increasing use of control measures such as insecticide treated nets, indoor residual spraying, and early treatment with Artemisinin-based Combination Therapies (ACT'S) has led to a reduction in morbidity and mortality caused by malariain some African countries[2]. The ability of the parasite to develop resistance to anti–malarial drugs and increasing insecticide resistance of the mosquito vector has served as militating factors against this effort.

Principally, the female mosquitoes of the genus *Anopheles* transmit malaria due to its support forthe sporogonic development of human malaria parasite[3]. In Africa, *Anopheles gambiae*species complex and *Anopheles funestus*species group dominate malaria transmission[4] and *Plasmodium falciparum* sporozoites have been detected in *Anopheles gambiaes.s.*, *Anopheles arabiensis*and *Anopheles funestus*[5,6]. In some cases, different forms are found in varying ecological regions hence, the need to identify the prevalent malaria vectors in the study area.A notable factor in the persistence of these vectors and its parasites are Environment conditions such as high humidity and warmth, which accelerates mosquito development, determine malaria transmission in these African countries. In addition, malaria transmission is influenced by poor quality housing as the populations are continually exposed to mosquito bites. Treated nets offer protection from mosquitoes, although bites can still occur outside the house[7].

The intensity of malaria transmission by mosquitoes is central to control eradication of the disease, and various methods to estimate it have been developed over the past 80 years[8].A complex interplay of epidemiological and entomological drivers of disease transmission has sustained the heavy burden of malaria transmission in the country for decades; the poor understanding of which has rendered well-meaning control interventions ineffective[9].

To provide basic data on malaria transmission indices in Mazah, Jos-North, Plateau State, Nigeria, we undertook a study to describe the relative importance of different mosquito species in transmission, their sporozoites infection rates and parous rate for malaria in the study area.The importance of the detailed knowledge of local determinants of malaria is of primary importance in the development of area-specific control interventions that will effectively lead to the control of the disease. Presently, there is little baseline information on these drivers of disease transmission in Mazah, Jos-North, and Plateau State, Nigeria, thus, the aim is to determine the entomological transmission indices of malaria for effective control measures in the study area.

II. Materials And Methods

Study Area

This study was carried out in Mazah Village, Gwong District of Jos-North Local Government Area (LGA). It is about 2km from Jos metropolis, the Headquarter of Jos, Plateau State, Nigeria. It lies within the North Eastern part between latitude 9.5^{o}N and longitude 8.5^{o}E and has an estimated population of about 2,500 people according to the 2006 population census. With the exception of Obudu in Cross River State, Jos is the coldest part of Nigeria by virtue of the Plateau[10]. The average minimum and maximum temperature are about 22^{o}C (72^{o}F) and 30^{o}C (86^{o}F) respectively[11]. The vegetation is grassland Savannah – Guinea Savannah belt[12].

There are two distinct seasons, the wet season which last from April to October and dry season from November to March[13]. The wet season is characterized by heavy rains and subsequent flooding of banks of rivers, streams, ponds, ditches and other hydrological sources, while the dry season is characterized by dry, cool and dusty winds December and January and high temperature in February and March which is completely devoid of rains. The study area is surrounded by two streams that flows from the Dilimi River through Mazah Valley, eastward towards ofBauchi State; dividing the village into Nahogom, Nabor and Andoho (Libah) clans. There are also few ponds and wells. These water bodies are few kilometers away from their habitats and that served as potential larval breeding pools for mosquito species and other insects. The surrounding of their houses sometimes remains permanently with thick and tall grasses and with poor drainage systems. Stagnant pools, rock pools, tree holes, pit latrines are also common around the houses. Mosquitoes move in easily from these breeding sites into the houses of inhabitants of this village. The principal occupation of the inhabitants of this area is farming and livestock rearing.

Advocacy and Pre – Survey Visit

A member of the community who was very familiar with the village served as a guide to get to the village members. Permission to work in the village was sought from the village chief and house heads, women group leaders, youth leaders and children group during community sensitization meetings. Later during each visit, a collection was carried out after the household owners have provided free and informed consent to do so.The houses were randomly selected. The right to refuse or withdraw at any time wasrespected.

Ethical Statement

This work was carried out in line with the guidelinesfor human experimentation in clinical research stated by Federal Ministry of Health of Nigeria[14]. The study was approved by the Department of Zoology of the University of Jos, Nigeria. Permission to work in the village was granted by the inhabitants after informed consent was sought.

Mosquito Collection, Preservation and Identification

Collection of mosquito was carried out using Pyrethrum Spray Catches[2] to estimate the members of mosquitoes resting inside the rooms where people slept the previous night. These collections were usually done during the morning between 7:00 – 12:00 hours. The population indoors was sampled by covering the floor with a white sheet of 5m x 5m each edge held to the wall by a masking tape. The room was sprayed with an insecticide, a pyrethroid, and then left for 10 minutes after the operator must have exits the room rapidly and closes the door. After a period of time, the mosquitoes found on the sheets were gathered and handpicked with forceps into petri dishes and were conveyed to the laboratory for identification.

Beginning from entrance, the corners of the sheets were lifted and the sheet was taken outside. All knockdown mosquitoes collected in the daylight with the forceps and placed in a labeled petri-dish, on top of a layer of damp cotton wool and / or filter paper.Mosquitoes collected from each house were stored in separated petri-dishes, appropriately labeled with date, time of collection, and household number/name of head of household.

Identification of the mosquitoes was morphologically carried out using the identification keys[15,16,17] prior to dissection. The mosquitoes were sexed and graded according to their abdominal conditions; namely; blood fed (BF), Unfed (UF), Gravid (G), and half gravid (HG) [18].

Specimen Processing and Dissection

Dissection of Adult Anopheline Mosquitoes for *Plasmodium* Sporozoite Rate

The adult anopheline mosquitoes were examined for *Plasmodium* sporozoites by investigating the salivary gland following the techniques of WHO[19], Inyama[20] and Williams and Pinto[17]. Prior to dissection, the wings and legs of the *Anopheles* mosquito were removed using a forceps and a dissecting needle. The mosquito was then placed on a slide with the head pointing to the right. A drop of normal/physiological saline was placed on the specimen to keep the specimen fresh. The left dissecting needle was placed gently on the thorax below the regions where the glands lie. The right needle was placed on the neck and a gentle pull towards the right was made. The lower part of the thorax was carefully teased and on a microscope slide. A little drop of normal/physiological saline was again added and a cover slip was placed on the specimen contained on the slide. A gentle pressure was applied on the cover slip to burst the salivary glands. A drop of methanol (90% absolute alcohol) was applied and left for a minute. Then a drop of Giemsa Stain was applied on the slide and left to air – dry. The slide was then washed with distilled water and viewed under a microscope with X40 objectives. A drop of immersion oil was applied and viewedunder X100 objectives. The *Plasmodium*sporozoites (if present) were seen as minute needle – like objects.

Dissection of Adult Anopheline for Parous Rate

The stomach of the female *Anopheles* mosquitoes were dissected to confirm the parity and nulliparity of the mosquitoes by examining the tracheolar skeins on the surface of the stomach walls[20]. The abdomens were dissected out at the 6^{th} and 7^{th}sclerite under a dissecting microscope[17]. After the extraction of the ovary, a gentle pressure was exerted at the abdomen to bring out the malphigian tubules and the stomach. When the stomach was partially extracted, the malphighian tubules were severed from around the stomach as close as possible without tearing the gut wall, while the rectum was cut off from the stomach just below the pyloric ampulla[20]. The stomach was transferred to another slide containing a drop of saline and covered with a cover slip. This was viewed under a compound microscope for the condition of tracheolar skeins on the surface of the stomach wall[17,20,21]. Tracheoles with terminal coiling signified parity.

Data Analysis

Records were made of the results in order to show the transmission indices with respect to the following parameters: Sporozoite Rate (SR) and Parous Rate (PR), Human Biting Rate (HBR) and Entomological Inoculation Rate (EIR). Chi – Square (χ^2) statistical analysis was used to evaluate these parameters. The Chi – square (χ^2) statistics was used because majority of sample data for this study were discrete, which can be referred to as frequency counts.

Determination of Transmission Indices

The entomological parameters/indices for each vector species were calculated as follows:

i.The HBR is number of vectors biting an individual over a fixed period of time. It was calculated as the total number of specimens collected from a room divided by the number of people that slept in the room the previous night[17,18].

i. Human Biting Rates = Number of mosquitoes collected
Number of collectors x Number of captures

ii. Indoor Resting Density (IRD) = Numbers of mosquitoes
Number of rooms sprayed

iii. The Main Biting Rate (MBR) was indirectly calculated as MBR = F/W

where: F = numbers of freshly fed mosquitoes of the particular species; and W = number of people who slept in the study house during the previous night (assuming that all fed mosquitoes collected in the houses took their blood meals from the occupants of the same houses and no fed mosquitoes left the houses after taking their blood meals until the time of collection[17,18] .

HBR (MBR) = Total number of mosquitoes
Total number of people that slept in the room the previous night

iii. *Plasmodium* Sporozoites Infection Rate (SR)

This is the number of sporozoites found in the salivary gland of dissected anopheline mosquitoes and it was calculated by dividing the number of sporozoites positive mosquitoes by the number of mosquitoes dissected.

S.R = Number of sporozoites positive mosquitoes
Number of dissected mosquitoes

v. Entomological Inoculation Rate (EIR): The EIR is the number of infectious bites per unit time and it is obtained as the product of the Human Biting Rate (HBR) and the Sporozoite Infection Rate (SR).
EIR = HBR x SR x Time unit (Expressed per year).

vi. Parity/Parous Rate (PR)

This was determined by the dissection of the ovary of the collected specimen and was calculated by dividing the number of parous females by the number of dissected mosquitoes. This will show the cycle of ovipositor.

PR = Number of parous female mosquitoes / Number of dissected mosquitoes

Chi – square (χ^2) test was used to compare the parous rates between species. Proportions were compared using Chi – square (χ^2) test at 0.05level of significance.

Scope and Limitation

The study was carried out in Mazah, Jos North Local Government Area, Plateau State, Nigeria.

III. Results

A total of 300 Anophelines were collected during the study. Of the 300 Anophelines collected from September 2014 to January 2015 (table 1), a breakdown indicates that the month of September recorded the highest catch with the least month being January. A further breakdown indicates that more of *Anopheles gambiaes.l.*were collected as compared to *Anopheles funestuss.l.* To our chargrin, the laboratory investigations/identifications of the collected Anophelines showed that in the months of December and January, no single*Anopheles funestuss.l.* were recorded.

Table 1: Monthly Distribution of Anopheline Mosquitoes Captured from Mazah

Sampling date	*Anopheles funestuss.l.*(%)	*Anopheles gambiaes.l.*(%)	Total (%)
September 2014	22(44)	163(65.2)	185(61.7)
October 2014	27(54)	51(20.4)	78(26.0)
November 2014	1(2)	22(8.8)	23(7.7)
December 2014	0(0)	6(2.4)	6(2.0)
January 2015	0(0)	8(3.2)	8(2.7)
Total	**50(16.7)**	**250 (83.3)**	**300(100)**

Sporozoite Rates of Anophelines of Mazah

The findings of this work (table 2) estimated an overall Sporozoite Rate (SR) of 0.053 (5.3%). The parous/Parity Rate was 0.143 (14.3%). The sporozoite and parous rates of the anophelines reflected on Table 2 shows that *Anopheles gambiaes.l.* had a higher sporozoite rate of 0.056 (5.6%) when compared to *Anopheles funestus.l.* of 0.04 (4%).

Chi – square (χ^2) analysis shows that the differences in the sporozoite rates were found not to be significant during the study ($P<0.05$). Table 2 also revealed that the parous rate for *Anopheles gambiaes.l.* was 0.148 (14.8%) while 0.12 (12%) was recorded for *Anopheles funestuss.l.* It was noted that there were more nulliparous anopheline mosquitoes during the survey. Although statistical analysis on the difference between the parous and nulliparous mosquitoes indicates no significant difference ($P>0.05$). It was also shown that there was no significant difference between the sporozoite positive and parousanophelines($P>0.05$).

Table 2: Sporozoite and parous rates of Anopheline mosquitoes of Mazah

Entomological Parameter	Value
Overall SporozoiteInfection Rate (%)	0.053 (5.3%)
Anopheles gambiaes.l(SR%)	0.056 (5.6%)
Anopheles funetuss.l(SR%)	0.04 (4%)
Overall Parous/Parity rate (%)	0.143 (14.3%)
Anopheles gambiaes.l(SR%)	0.148 (14.8%)
Anopheles funetuss.l(PR%)	0.12 (12%)

IV. Discussion

The species composition of mosquitoes, their relative abundance and the role of these vectors in transmission of malaria in Mazah, Jos North Local Government Area of Plateau State, Nigeria as revealed in this study could be attributed to the ecological settings of the area. Gillies and de Meillon[15];Okorieet.al.[18] had both advocated that the knowledge of vector species is important to understanding the epidemiology of the disease.In our findings of the ecological settings as reported in the studied, the breeding sites for the anopheline mosquitoes in the area constitutes of many rock pools, tyre prints, animal hoof prints, tree holes and a stream. In addition, the study area had limited basic infrastructure and is characterized by a few wells; sparsely populated and less waste generated which accounted for the existence of the vectors.Our findings were consistent with the

findings and the assertion of Gillies and de Meillon[15] and Okorieet al.[18] where anopheline mosquitoes breed in transient habitats, hoof prints and tyre tract prints, hence the presence of the malaria vectors in the study area.

The species composition of Anopheline mosquitoes in the studied area indicates more*Anopheles gambiaes.l.* -250 (83.3%) were recorded as compared to 50 (16.7%) *Anopheles funestuss.l.*Coincidentally, these species were incriminated as malaria vectors in the study area.Our findings are in conformity with the work of Coluzziet al.[22];Kilama et. al.[8] in Uganda; Tchouassiet al.[23]in Ghana; Mzilahowaet al.[4] in southern Malawiwhoall established that *Anopheles gambiaes.l.* is the most important vector of malaria in Africa and in particular sub – Saharan Africa. However our findings are in contrary to that of Dadzieet al.[24] in Ghana who reported that *Anopheles funestuss.l.* was slightly higher than *Anopheles gambiaes.l.* and *Anopheles rufipes.* It is worthwhile to note that the variation in ecological zones of the study area, which was Guinea Savannah and Ghana Sahel Savannah, offers a possible explanation for this disagreement in our findings. It is apparent that certain climatic factors like annual precipitation and temperature appear to exert some effects on mosquitoes' relative abundance.

Ourfindings from the relative abundance of Anophelines in the studied area offered exciting results.We noted that *Anopheles gambiaes.l.* was the most dominant species as compared to *Anopheles funestuss.l.* whose population declined from September to January. The result of this work is in agreement with the reviewed report of[2]in which they noted that often there is one anopheline species that occurred regularly throughout the year while others were highly seasonal.Previously, similar results were have been reported by[25] whose work in defunct Zaria Province in northern Nigeria showed that *Anopheles funestus* was the dominant species for all but two months of the year in the northern part of the province whereas in the south*Anopheles gambiae*was dominant in the rainy season. He further noted that both *Anopheles gambiae*and *Anopheles funestus*occurred in equally low numbers in the dry season; but to his chagrin an additional species, *Anopheles nili*, was sampled only during the rains. Excited with the report of[25,26]in their study on anopheline mosquitoes from defunct southern Zaria Province obtained almost an entirely*Anopheles gambiae*in the wet season and *Anopheles arabiensis*in the dry season. Our findings are also in line with the reports of [27] from the coastalareas of Lagos, southern Nigeria who identified *Anopheles gambiae*and *Anopheles arabiensis*to be dominant in the wet and dry season, respectively, whereas*Anopheles melas*and *Anopheles moucheti* were observed throughout the year. We are therefore of the opinion that the quality and preponderance of mosquitoes vary with location and season.

In the same vein, other studies have also confirmed to the significant variations in monthly densities of adult anopheline species across Africa. For instance, in a survey conducted by[28] on mosquitoes in Sierra Leon, *Anopheles gambiaes.l.* was recorded in very large numbers throughout the year whereas *Anopheles funestuss.l.* were observed in considerably lower densities and coincidentally they were dry season species.Krafsur[29], observed that anopheline mosquitoes in Ethiopia became numerous during the months of August to November when flooding occurred. Similar observation were made in Sudan of the marked seasonality in the abundance of *Anopheles arabiensis;* following the end of the rainy season in October, the number of species surveyed dropped gradually until February when it totally disappeared, only to reappear in June, as humidity rose with the onset of the rains[30]. A contradiction to the above results were however reported by[31]who noted that *Anopheles arabiensis*occur in large numbers throughout the year. Similarly, Olayemiet al.[9] have also observed in Nigeria significant an increase in anopheline densities starting from April to peak in August, before declining steadily till January, with densities in the months of April and November significantly higher and lower than the dry season and wet season averages, respectively.

It is evident that, the occurrence of sporozoites in the salivary glands of the mosquitoes portrays another transmission index in the study area. The surveyreveals that an overall sporozoiterate for the vectors was 0.053(5.3%).Although this result is relatively low compared to the findings of[9] whose work in Ilorin revealed 1.7% for *Anopheles funestus*; Konateet al.[32]who reported 1.3% in Senegal; Appawuet al.[33] 7.1% in Ghana; andShililuet al.[34]who reported 9.5% in Kenya.

It was also established that a sporozoite rate of 0.056 (5.6%) was recorded for *Anopheles gambiaes.l.* and 0.04 (4%) for *Anopheles funestuss.l.*The variation insporozoite rate between the two vectors sampled could be due to in addition to feeding on humans, the Zoophilic nature of*Anopheles funestuss.l.*.The slightly higher sporozoite rate of *Anopheles gambiaes.l.* (5.6%) when compared to*Anopheles funestuss.l.* (4%)had been confirmed in other reports like[23,35,36]. Their individual studies have all implicated*Anopheles funestuss.l.* as the second most important vector after *Anopheles gambiaes.l.* In addition, the low sporozoite rate, hence the infection, in *Anopheles funestuss.l.*couldalso be attributed to the low numbers caught during biting. It is imperative that the extension of the study over a longer period is required to ascertain the significance of this vector in the spread of the disease at Mazah.In clear terms, malaria transmission was maintained by *Anopheles gambiaes.l.* and *Anopheles funestuss.l.* a confirmation of their vectorial capacity in the study area. Our findings are in agreement with[15,24,37] who all noted that the vectorial and behavioural variations found within the species group are the major reasons why accurate identification is needed to understand malaria transmission patterns.

Although the study established that there is sympatry of*Anopheles gambiaes.l.* and *Anopheles funestuss.l.* as members of the anophelines in the study area, their level of parity differs.The overall parous rate of 0.143(14.3% n=43) of the malaria vectors were analyzedas compared to the nulliparous (85.7% n=25.7). This seems to be at variancewith the works of Tchouassiet al.[23] in Ghana;Okorieet al.[18] in Ibadan-Nigeria; who both recorded more parousanophelinemosquitoes than the nulliparous ones. An indication that the older populations of mosquitoes tend to accumulate with time[23,38,39].Okorie et al.[18] position corroborates those of[40] who noted that the high number of parous mosquitoes compared to the nulliparous ones indicates that not only is the population an older population with potentials of continuous supply of the area with young mosquitoesbut also signifies the high survival rate of the mosquitoes. The low parous rate in our findings as noted by Tchouassiet al.[23]could be attributed to the fact that most members of the community have long lasting insecticides treated nets and this could have resulted in the low mosquitoes' abundance and risk of infective bites in the area during the period of investigation.

V. Conclusion

Our findings have established malaria transmission indices as well as explain theendemicity of the infection in the studied area. In addition, the investigation provides a baseline for evidence based planning and implementation of malaria control strategies targeting vectors.

VI. Recommendations

Efforts should be intensified by government towards the monthly environmental sanitation, health education through community sensitization and mobilization exercises. Moreover,the use of insecticide treated bed nets, repellents, protective clothing, screening houses and zooprophylaxis should be encouraged, as these will reduce the abundance of mosquitoes and consequently the burden of the disease.

Conflict of Interest: The authors declare that there is no conflict of interest

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