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Research Article

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Sporozoite Infection Rate of Malaria Vectors along Altitudinal Gradients of Plateau State, Nigeria

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ABSTRACT

African Plateau areas are known of malaria hypoendemicity due to low temperatures and relative humidity, as these factors have significant impacts on the anopheline reproductive suitability and abundance. However, changes in abiotic factors especially temperature in African highlands could result in temporary favourable conditions for unstable transmission within populations that have acquired little functional immunity. To this end, a descriptive longitudinal study to determine the sporozoite infection rate of malaria vectors along altitudinal gradients of 1330 m, 633 m, 211 m above sea level of Plateau State was conducted between 2018 and 2019 using pyrethrum spray collection (PSC) and CDC light trap. Of the one thousand one hundred and ninety-two (1,192) adult female mosquitoes collected, the predominant was *Cx. quinquefasciatus* 948 (79.5%) followed by *An. gambiae* 241 (20.2%) and the least was *An. funestus* 3 (0.2%), and differences was significant ($P < 0.05$). Sporozoite infection rates 0.9% from ELISA result among female mosquitoes where Vwang had 0%, Jing 0.74%, while Pangwasa had the highest sporozoite rate of 1.28%. *Anopheles* species composition was higher in Pangwasa. There was a significant difference between infected and non-infected *Anopheles* in the three study areas ($P < 0.05$). This study shows that Vwang, Jing and Pangwasa, are endemic to malaria with *An. gambiae* and *An. funestus* being the primary malaria vectors. These findings shows variation in the sporozoite rates across the three study sites which will also serve as a baseline data for malaria control program implementation and evaluation along altitudinal gradients of Vwang, Jing, and Pangwasa sites.

Keywords: Sporozoites, altitudes, malaria vectors, house types, seasonality, Jos, Plateau State

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INTRODUCTION

Malaria is considered an endemic and important public health problem in Nigeria (WHO, 2020). It is transmitted by the bites of an infected female *Anopheles* mosquito of the genus *Plasmodium* (WMR, 2020). The global malaria cases from the 87 malaria endemic countries which was 238 in 2019 declined to 229 the year 2000 (WHO, 2020). Of these, only 29 countries including Nigeria accounted for 51% of global malaria deaths and 95% of cases worldwide. Sub-Saharan African nations bear the majority of the global malaria burden, with Nigeria and the

Democratic Republic of the Congo combined accounting for more than 44% of all malaria deaths worldwide (WHO, 2020). In the WHO African region, Nigeria accounted for 27% of all anticipated cases of malaria in 2019 (WHO, 2020). Oduola *et al.* (2013) noted that a large percentage of the population affected by malaria in the country lives below the poverty line in the villages with poor healthcare facilities. The four major human malaria parasites are *Plasmodium falciparum*, *P. malariae*, *P. ovale* and *P. vivax*. However, *Plasmodium falciparum* is the most

dominant and lethal species in Nigeria (Oduola *et al* 2012, Oyewole *et al.*, 2010). The World Health Organization (1993) recommended vector surveillance and control as an important component of the global strategy to combat malaria. The vector profile and malaria transmission risk indices are deficient in a good number of Nigerian communities and therefore evaluation of these malaria control measures remain a challenge (Oduola *et al.*, 2012). There were reports on species abundance, composition, indoor resting density, man biting rates of mosquitoes, susceptibility status of the malaria vectors in some parts of Plateau, Nasarawa and Gombe States (Yoriyo *et al.*, 2013; Mwansat, 2014), but, information on the sporozoite infection rates of *Anopheles* species in relation to altitudes is deficient in Plateau State. Therefore, this study was carried out in an attempt to report for the malaria vector species and some entomological malaria transmission indices such as sporozoite infection rate, endophagic index and exophagic index along altitudinal gradients of three selected study sites located in three Local Government Areas of Plateau State, North-Central Nigeria.

MATERIALS AND METHODS

Study area

This study was carried out in three different altitudinal gradients of Vwang, Jing, and Pangwasa, of Plateau state, Central Nigeria, all positioned on altitude 1,330 m above sea level (asl), N 9°43', 44.76 E 8°47', 28.97, 663 m asl, N 09° 13.057' E 009° 18.364' and 211 m asl N 08° 53.292' E 009° 32.398', respectively. Plateau state is characterized by temperature fluctuations of as low as 15°C in December and January about 30°C in February and March (the hottest months in a year). Temperature falls during raining season to about 20°C. Vwang, Jing, Pangwasa of Plateau State has a tropical climate of wet and dry seasons driven by the movement of the two dominant winds—the rain-bearing southwesterly winds and the cold, dry, and dusty Northeasterly winds, usually referred to as the Harmattan. The dry season occurs from November to March, with a spell of coolness accompanied by the dry, dusty Harmattan wind, felt mostly in the North in December and January. The wet season occurs from April to October. The houses are basically of three types (A=cement block with corrugated iron roof, B=mud with corrugated iron roof, and C=mud with thatch roof), (Tusting *et al.*, 2016).

Ethical consideration

Jos University Teaching Hospital (JUTH) institutional ethics committee with reference number JUTH/DCS/ADM/127/XXVIII/1280 and Plateau State Health Ministry of Nigeria Ethics Committee (PHMEC) with reference number MOH/MIS/202/VOL.T/X approved

the research protocol and provided ethical consideration and approval for the implementation of the study. Similarly, the house owners and mosquito gatherers gave their informed consent to partake in the study after due explanation of the objectives and collections methods through individual discussions and group meetings that took place.

Entomological Sampling

Study design

Data collections were done four times in a year corresponding to different transmission seasons, early wet (April-June), late wet (July-September), early dry (October-December), and late dry (January-March). Ten houses were randomly selected in each site of Vwang, Jing, and Pangwasa communities for mosquito collection to include five of each of the three-housing types in the village (mud with thatch roof, mud with corrugated iron roof and cement block with corrugated iron roof).

Collection of indoor resting mosquitoes

Indoor resting mosquitoes were collected in the rooms where people slept the previous night using pyrethrum spray catches (PSC) between hours of 06.00 am and 09.00am so as to prevent mosquitoes from escaping the rooms (Service, 2012). Before spraying, the rooms were prepared for PSC by removing small furniture and food items. White sheets were then spread to completely cover the floor and all articles inside leaving the doors and windows shut. Raid aerosol was then sprayed by the operator in a clockwise direction toward the ceiling until the room was filled with the mist. The operator then exits from the room without delay. At the end of the 10 minutes, the door was opened and the white sheet retrieved carefully, starting from the entrance by lifting the corners of the sheet carefully to gather the knocked down mosquitoes in the middle before picking them with a forceps into labelled petri dishes. Knocked down Mosquitoes collected in each room were kept in separate petri-dishes with appropriate labeling such as the house type, and 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 collector. All mosquitoes were kept separately in a well-labelled Eppendorf tubes containing silica gel and frozen at -20°C for laboratory molecular examination. Upon collection, Anopheline were sorted out from other mosquitoes, and taken to the laboratory and identified individually under a stereoscopic dissecting microscope (Celestron Model #44204). The pyrethroid 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%.

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 and, half gravid were recorded.

Dissection of mosquitoes for detection of *Plasmodium* sporozoite

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 and 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 was placed at the same time but on the opposite side. This was then gently pulled in the right direction to bring out the salivary glands. The glands were 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 and air dried before viewing under a microscope using an x40 objective. A drop of immersion oil was applied and viewed under x100 objective. Minute needle like objects are indication of *Plasmodium* sporozoite which was not seen (WHO, 2013).

Preparation of mosquitoes and detection of circumsporozoite protein (CSP) Detection

Enzyme-Linked Immunosorbent Assay (ELISA) was then used in determining infection rates in mosquitoes according to Wirtz *et al.* (1987). The heads and thoraxes of individual mosquitoes were tested for *Plasmodium falciparum* circumsporozoite protein (CSP) using Pf2A10 monoclonal antibody, as recommended and modified by Wirtz *et al.* (1987). The monoclonal and peroxidase-conjugated antibodies were obtained commercially. A sandwich ELISA was carried out on dried mosquitoes, the heads and thoraxes were grounded individually in 50 µl grinding buffer containing bovine serum albumin (2.5 g), boiled casein (1.25 g) in phosphate buffered saline

(250 ml), phenol red (100 µl) and Nonidet P-40 (1,250 µl) and the final volumes of each triturate were brought to 250 µl.

Data analysis

Data obtained was analyzed using the Chi-square test and significant levels were considered at 95% confidence level with significant differences taken at $P < 0.05$. The Chi-square test was considered as the appropriate test due to the homogeneity and frequency of the data. 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%

RESULTS AND DISCUSSION

A total of one thousand one hundred and seventy-two (1,192) adult female mosquitoes were collected from the three sites, 93 were collected from the CDC Light Trap, while 131 were from Pyrethrum Spray Catch (PSC). Similar data was obtained from Mwangangi *et al.*, (2012) and Amaechi *et al.*, (2018) as greater number of mosquitoes (78.5% and 75.4%) were collected with Pyrethrum spray sheet than with Human Landing Catch method (18.1% and 24.6%), respectively. In contrast, Kweka *et al.*, (2018) and Chanyalew *et al.*, (2022) captured higher number of mosquitoes (3,861 and 231), respectively using CDC light trap and 3,022 using the pyrethrum spray catch method. Differentiating them, the study areas composed of 3 *Anopheles funestus*, 221 *Anopheles gambiae* and 948 *Culex quinquefasciatus* and differences in abundance significantly varied between species ($\chi^2 = 380.95$, $df = 2$, $P < 0.001$). This is similar to the study by Chanyalew *et al.*, (2022) who recorded *Culex* mosquitoes as the most dominant (80.4%) followed by *Anopheles* mosquitoes. Reports from Amaechi *et al.*, (2018); Kweka *et al.*, (2018) and Kwi *et al.*, (2022) also showed that *Anopheles gambiae*, *Anopheles funestus* and *Culex quinquefasciatus* as the three most common species which aligns with our study. *An. gambiae* were more than those of *An. funestus* though *Cx. quinquefasciatus* was more in number (Esayas *et al.*, 2024; Ntabi *et al.*, 2024) but was not incriminated as a malaria vector. All the anophelines were found to be blood fed. Mwangangi *et al.* (2012) also reported that *Anopheles gambiae*, *Anopheles funestus* and *Anopheles coustani* were predominant in the rural stratum while *C. quinquefasciatus* was mostly found in urban and peri-urban strata.

Figure 1 shows housing types and mosquito abundance in the three altitudes in this research. The proportion of *Anopheles gambiae s. l.* collected in houses made with cement block and corrugated roof was the highest 80 (31.77%) followed by houses made with mud blocks and corrugated roof 66(29.46%) while the least

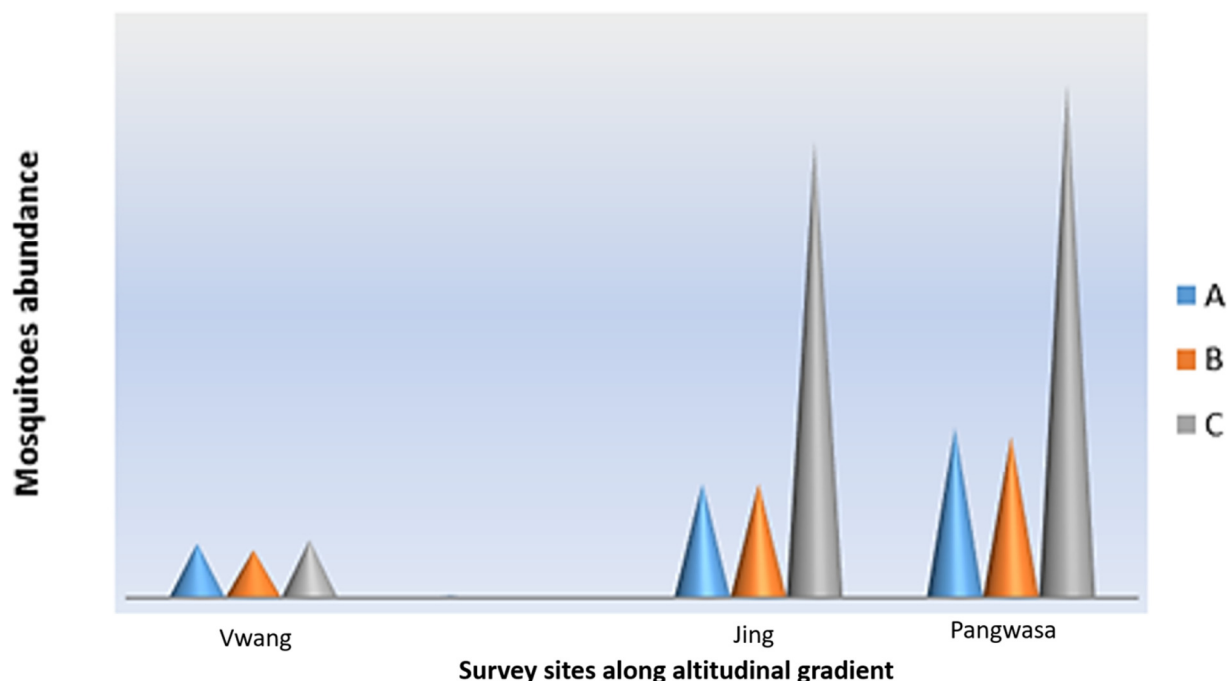


Figure 1: Mosquitoes abundance in relation to housing types along altitudinal gradients sites
Key: A- Cement block with zinc roof; B- Mud with zinc roof; C- Mud with thatch roof

was observed 80 (11.23%) in houses made with mud and thatch roof. *An. funestus* 3 (1.27%) was the least of species collected from houses made with cement block with corrugated roof. Thus, there was a significant difference ($\chi^2 = 114.59$, $df = 2$, $P < 0.001$) in mosquitoes abundance between species resting indoors in houses made of cement block with zinc roof. This agrees with the findings of Ebenezer and Woyinzuosindor (2019), who noted that 38.03% of mosquitoes were collected from batcher houses with zinc roofs. This was followed by mud wall houses with thatched roofs, which accounted for 29.58%. Houses made of block walls with corrugated roofs but lacking ceilings and screens had a collection rate of 29.11%, while modern block houses with ceilings and protected windows recorded the lowest number of mosquitoes at 3.29%. Their study also highlighted significant differences in mosquito species distribution across various building types ($F = 11.2247$, $df = 3$, $p < 0.05$). The structure of a house and its environmental characteristics have consistently been shown to impact indoor mosquito densities, thereby influencing malaria transmission risk (Mburu *et al.*, 2018; Ngadjeu *et al.*, 2020; Ombugadu *et al.*, 2022). Supporting this, Ye *et al.* (2006) indicated that using iron-sheet roofs instead of thatched roofs could significantly reduce the risk of malaria infections.

So also, *An. gambiae* 80 (11.23%) was the highest species encountered in mud houses with thatch roof

while *Cx. quinquefasciatus* 632 (8.93%) was collected, and mosquitoes' abundance between species in mud houses with thatch roof showed a high significant difference ($\chi^2 = 88.242$, $df = 1$, $P < 0.001$). Similar results were obtained in mud houses with zinc roof where *An. gambiae* was the highest 66 (29.46%), but there was 0 (0.00) *An. funestus* and *Cx. quinquefasciatus* had abundance of 158 (9.09%). Hence, there was a significant difference ($\chi^2 = 180.11$, $df = 2$, $P < 0.001$) in mosquito abundance between species resting indoors in mud houses with zinc roof. Thatch-grass roofs and mud walls provide benign resting sites for blood fed mosquitoes. Conversely, houses with brick walls are usually installed with screened windows and iron sheet roofs. These types of houses protect residents from being bitten by malaria carrying mosquitoes. However, the higher construction costs of brick houses with corrugated iron-sheet roofs and screened windows limit the wide use of these construction materials by local residents (Zhou *et al.*, 2007).

The indoor abundance of *Anopheles* mosquitoes was highest in Pangwasa area 56.3% followed by Jing 35.7% while the least abundant site was Vwang 7.9%. Therefore, there was a significant difference ($\chi^2 = 7.6594$, $df = 2$, $P = 0.02172$), in the abundance of *Anopheles* mosquitoes in relation to the three altitudinal gradients. Pairwise multiple comparisons of the mosquitoes' averages recorded across the altitudinal gradients

showed that there was significant variation in mosquito abundance between Jing versus Vwang as well as Pangwasa versus Vwang whereas there was no significant difference between Jing versus Pangwasa gradients. This finding is similar with the work of Dagyna *et al.* (2017), who observed significant variations in the density of *Anopheles* mosquitoes across different altitudinal transects in their study area ($F = 53.7$, $df = 2$, $P < 0.001$). Additionally, Kwi *et al.* (2022) reported that the overall infectivity rate of these vectors was notably higher at lower altitudes (19.7%, 37 out of 188) compared to intermediate altitudes (12.0%, 15 out of 125). Moreover, Garba *et al.* (2023) highlighted a decrease in both abundance and species diversity of mosquitoes with increasing altitude, noting that *An. gambiae* ss. was the predominant sibling species of the *Anopheles gambiae* complex found in highland areas (93.92%).

Anopheles mosquitoes' abundance was found the most during the early wet season period in the least gradients site (Pangwasa). This observation aligns with findings by Amaechi *et al.* (2018), who documented a lower number of mosquitoes during the dry season compared to the wet season, with a minimum count of 114 in February and a peak of 445 in July. Similarly, Kwi *et al.* (2022) noted that the abundance and species diversity of mosquitoes fluctuated significantly across seasons. Their research showed a clear variation in mosquito distribution linked to seasonal changes, with diverse larval habitats influencing mosquito numbers and diversity. These seasonal effects were further supported by Tsegaye *et al.* (2023), who found significant differences in habitat contributions to mosquito populations between seasons. However, the abundance of mosquitoes in relation to altitudinal gradients and the four seasons showed no significant difference ($F_{91} = 2.252$, Adjusted $R^2 = 0.0612$, $P = 0.05578$). The effect of altitudinal gradients on *Anopheles* species composition and abundance using Pyrethrum Spray Catch (PSC), shows the presence of *Anopheles* species across the three study sites. Travelling from the highlands to the lowlands for activities increases the risk of malaria vectors in highland residents (Lindsay & Martens, 1998). Female anopheline examined for *Plasmodium falciparum* circumsporozoite protein in the Pangwasa, Jing and Vwang, shows a low rate of the sporozoites. This could be attributed to the interventional activities that might have taken place over the years (WHO, 2018). Although, lower sporozoite rates of 0.4% and 0.4% was recorded in Pangwasa and Jing, respectively, which could be as a result of unfavorable environmental conditions for the vector to thrive. Although, the two sites recorded high mosquitoes' abundance but recorded low *Plasmodium falciparum* circumsporozoite, this clearly shows that infection was influenced by altitude.

The overall sporozoite rates infection of the *Anopheles* species was 2(0.08%) showing a significant difference in

the number of the *Anopheles* species not infected with sporozoite to those infected with sporozoites ($\chi^2 = 262.65$, $df = 1$, $P < 0.001$). This is similar to the study of Aschale *et al.*, (2023) who recorded an overall *Plasmodium* sporozoite infection proportion which ranged from 0.0 to 3.88% and only four *Anopheles* species (*Anopheles arabiensis*, *An. pharoensis*, *An. coustani*, and *An. funestus*) were found positive for sporozoites with infection proportion of 1.09%, 0.79%, 0.13%, and 2.71%, respectively; whereas 4.8% sporozoite rate in Mambilla Plateau Taraba, North-East Nigeria (Garba *et al.*, 2023) and Gbêkê region, central Côte d'Ivoire, respectively (Koffi *et al.*, 2023). The *Plasmodium falciparum* sporozoite rate from the ELISA test across the altitudinal gradients revealed that Vwang with a high altitude value of 1330m asl had 0% sporozoite rate, followed by Jing with an altitude of 663m asl had 1.28% sporozoite rate, while Pangwasa with a low altitude of 211m asl had 0.74% sporozoite rate.

Conclusion

The significant variation in the number of *Anopheles* species collected in houses in the study areas 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 seasons in which the work was done. This also implies that there was a stable malaria transmission in the areas. Other malaria transmission indices such as the overall sporozoite rate of 0.8% that was obtained in the survey show the potential roles of the two *Anopheles* species as malaria vectors in the area and could also indicate the stability/intensity of malaria transmission.

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