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Molecular detection of the infectivity status of *Anopheles gambiae* stricto lacto in Osun State, Nigeria

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Abstract
The present study reports the infectivity of An. gambiae s.l with sporozoite of Plasmodium. falciparum. Adult mosquitoes were caught quarterly in three communities across the state between 1800hr – 0600hr using the Centre for Disease Control light trap and 0600hr – 0700hr for pyrethrum spray catch (PSC) using protocol by the WHO and identified using morphological keys. Molecular analysis for sibling species identification was conducted using Polymerase Chain Reaction. The CDC light trap had a total of ninety (90) catches while the PSC had a relatively low number (1) of catch. A total of 4 samples were positive for P. falciparum. Ido-Osun 2 (50%) recorded the highest infectivity status as compared to Inisa 1(50%) and Ife 1 (50%). Infectivity was found in the first, second and third quarters respectively. The outdoor catch had the highest number of sporozoites detected across the study areas as compared to the indoor catch ($p = 0.59$; $P > 0.05$). Similarly, 1 (33.3%) sporozoite was detected in the outdoor catches across all the study areas. The only sporozoite detected indoor was found at Ido-Osun. No sample was positive for P. vivax sporozoite across the study areas. The present study therefore suggests the possibility of an ongoing transmission of falciparum malaria in the state. Therefore, the need to devise proactive control and preventive measures targeted at containing the transmission.

Keywords: *Anopheles*, Infectivity, Molecular, Osun State, Nigeria

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INTRODUCTION

The public health importance of vectors in disease transmission cannot be overemphasized. Mosquitoes are vectors that constitute a serious biting nuisance and transmit most deadly and life-threatening diseases such as malaria, dengue fever, yellow fever and bancroftian filariasis (Adeleke *et al.*, 2013). Furthermore, they are regarded as the most dangerous animals on earth (WHO, 2020). However, female mosquitoes of the *Anopheles gambiae* complex which include *An. gambiae sensu stricto, An. arabiensis, An. funestus, An. rufipes, An. pharoensis, An. wellcomei, An. squamosus, An. coustani, An. maculipalpis, An. nilli,* and *An. pretoriensis* of which two species; *An. gambiae* and *An. funestus* are regarded as the main vectors (Oyewole and Awolola, 2006; Oduola *et al.*, 2010; 2012) involved in the transmission of the malaria parasites (*Plasmodium spp*) due to their blood-sucking habit. They are also involved in the transmission of arboviral diseases (WHO, 2020).

In 2022, 249 million estimated malaria cases were reported in 85 malaria-endemic countries and an increase of 5 million cases compared with 2021(WHO, 2023). The increase in the case numbers over the past 5 years occurred in countries in the WHO African Region with 93.6% of cases and 95.4% of deaths globally; 78.1% of all deaths in this region were among children aged under 5 years in 2022, compared with 90.7% in 2000 (WHO, 2023). *Plasmodium falciparum* is the main species of malaria parasite that is found in Nigeria and is responsible for over 80% of the total malaria burden while *Wuchereria bancrofti* is responsible for lymphatic filariasis (FMoH, 2013).

Nigeria (26.8%) accounted for almost half of the global cases of malaria in 2022 (WHO, 2023). It (31.1%) also accounted for half of all malaria deaths globally (WHO, 2023).
In Osun state, the burden of malaria in its mortality and morbidity is worrisome. This has necessitated proactive strategies by both the federal and state government in its control and elimination through vector control and environmental management programs. Despite this, the incidence of malaria persists making futile all efforts adopted. However, previous studies by Oduola *et al*. (2013) had reported the absence of *Plasmodium* sporozoite in a study conducted in the state. Therefore, the need to investigate the current infectivity status of *Anopheles* mosquitoes in the state. This will assist in understanding the cause of the infection and the development of stringent control and preventive measures.

MATERIALS AND METHODS

Study Area

The study was conducted across the three senatorial districts of the state which are Osun West, Osun East and Osun Central. Three communities were selected in each of the senatorial districts through gridding. They are Ido-Osun (N7.779272 and E4.480356), Ife (N7.485694 and E4.556917) and Inisa in Osun West, Osun East and Osun Central senatorial districts respectively.

The state is known majorly for tourism due to the presence of ancient cultural edifices such as the Osun Groove and the Erin-Ijesha waterfall. The major occupation of the inhabitants is agriculture and trading.

Adult *Anopheles* mosquito collection

Collection of adult *Anopheles* mosquitoes using Centre for Disease Control (CDC) light trap

Adult *Anopheles* mosquitoes were collected using WHO protocol between January and December, 2023. Collection was carried out quarterly in the three study locations using the

WHO CDC light trap. The adult *Anopheles* mosquitoes were collected between 1800-0600hr for two days in each of the study locations. Two CDC light traps were set up in each study area with one indoor and the other outdoor for both indoor and outdoor catches respectively. The light traps were placed close to the leg of the occupant of the room in each location while sleeping under an untreated mosquito net. Hourly mosquito catch was recorded. The collected mosquitoes were demobilized in a chloroform container and afterward kept in collecting cups where they are carefully covered using foil to prevent losing them and preserving them until they are transported to the laboratory of the Animal and Environmental Biology Department of the Osun State University, Osogbo, Osun State, Nigeria. The collecting cups were well labelled with details such as the hour of collection and whether the catch was indoor or outdoor. The cups are properly covered with foil paper and fastened with a rubber band to prevent mosquito loss. The mosquitoes were then transported to the Department of Animal and Environmental Biology Labouratory of Osun State University, Osogbo, Osun State, Nigeria, where they were morphologically identified using both the digital and conventional dissecting microscopes using keys by Gillies (1972) and Coetzee (2020). The flies after identification were preserved in 1.5ml Eppendorf tubes containing silica gel for further molecular analysis.

Collection of adult *Anopheles* mosquitoes using Pyrethrum Spray Catch (PSC)

Ten rooms were selected for PSC in each of the three study areas used for adult mosquito collection across the state.

Each room was sprayed using a pyrethrum based insecticides at 0600hr in the morning at the end of the CDC catch due to the anthropophilic nature of the mosquitoes before they fly out. Prior, to spraying the rooms, a white cloth was spread cutting across the four walls of the room to ensure the easy identification and collection of knocked-down mosquitoes. After about 5mins, knocked-down mosquitoes were picked into well labelled petri dishes using forceps. The petri dishes were properly wrapped with paper tapes to prevent losing the mosquitoes and transported to the same laboratory for analysis.

Detection of Sporozoite in *An. gambiae* s.l

DNA Extraction

The genomic deoxyribonucleic acid (DNA) was extracted from the head of the individual female *Anopheles* mosquitoes using genomic DNA purification kit manufactured by NIMR BIOTECH. The genomic DNAs from 51 randomly selected mosquitoes was extracted by crushing the head and thorax of each mosquito placed in 2ml Eppendorf tube with pestle, then homogenized in 500µl lysis buffer. The mixture was vortex and incubated at 56℃ for 10min then centrifuged at 10,000 rpm for 1 minute, after spinning, 200µl of absolute ethanol was added to the tube. The mixture was transferred into spin column and centrifuged at 10,000rpm for 30 sec. Then the flow-through was discarded, followed by blotting of the collection tube on tissue paper. 500µl of wash buffer 1 was added to the spin column and then centrifuged at 10,000 rpm for 30 sec. Then the discarding of the flow-through and blotting of the collection tube on tissue paper. The spin column was centrifuged again at 12,000rpm for 3 minutes to remove all the traces of ethanol hereafter and then placed in another microcentrifuge tube. 50µl of elution buffer was added and then incubated at room temperature for 1 to 2 mins and centrifuged at 10,000rpm for 1 minute to elute the DNA. The DNA obtained was stored at -20℃ for PCR.

PCR Amplification

Protocol provided by Snounou *et al*. (1993) was used for the amplification of extracted DNA using nested PCR. All PCR reactions were carried out in a total volume of 20µl. In all cases,

amplification was performed in 2 mM $MgCI_2$/50 mM KC1/10 mM Tris pH 8.3 (HC1)/0.1 mg ml^{-1} gelatin/125 µM of each of the four deoxyribonucleotide triphosphates/ 250 nM of each oligonucleotide primer/ 0.4 unit of AmpliTaq Polymerase (Perkin Elmer Cetus, USA). 1µ1 of the purified template DNA was used for the first PCR reaction, in which the fragment spanned by rPLU5 (CCTGTTGTTGCCTTAAACTTC) and rPLU6 (TTAAAATTGTTGCAGTTAAAACG) is amplified. The rPLU5 and rPLU6 are genus-specific primers used for the first cycle of amplification. A 1 µ1 aliquot from the product of the first PCR reaction was then used as a template in each of the four separate reactions in which the species-specific primer pairs rFAL (TTAAACTGGTTTGGGAAAACCAAATATATT and ACACAATGAACTCAATCATGACTACCCGTC) and rVIV (CGCTTCTAGCTTAATCCACATAACTGATAC and ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA) for *P. falciparium* and *P. vivax* respectively are employed. The PCR assays were performed using a heating block (PTC-100, MJ Research Inc., USA). The cycling parameters for the first amplification reaction were as follows. step 1, 95°C for 5 min; step 2, annealing at 58°C for 2 min; step 3, extension at 72°C for 2 min; step 4, denaturation at 94°C for 1 rain; repeat steps 2-4 24 times, then step 2, and finally step 3 for 5 min. On termination of the amplification cycle, the temperature was reduced to 20°C. For the subsequent four species-specific amplification reactions, 30 cycles were performed.

Gel electrophoresis

A 1.5g of agarose gel with 100 ml of Tris-acetate- Ethylene-diamine tetra acetic acid (EDTA) was used in the electrophoresis of the PCR product. The agarose was melted in a microwave for about 2 minutes and allowed to cool satisfactorily. The gel was stained with 5µl Ethidium Bromide for the visualization and detection of amplified DNA fragments, after cooling, the gel was poured into a clean well-casting chamber and an electrophoresis comb was inserted to create wells into which amplicons were loaded. The cast was placed in the electrophoresis tank containing 1X to cover the gel and wells followed by the removal of the comb from the well. The molecular ladder (5µl) was dispensed into the first well followed by 7µl of each (amplicons) were appropriately loaded and run at 80v with 150mA for an hour. The gel was viewed and taken under a UV transilluminator for documentation.

Data Analysis

The data obtained from the study were analyzed using ANOVA to determine the significant difference in the infectivity along the study area and the quarters respectively. A p-value of less than 0.05 ($p < 0.05$) at a 95% confidence interval (CL) would indicate significance in correlation.

RESULTS

Molecular detection of sporozoite in the female *An. gambiae* s.l collected in the study areas

The adult female *Anopheles* mosquito samples collected by the CDC across the study areas were analyzed for the detection of sporozoite. A total of 4 samples out of the 58 adult female *Anopheles* mosquitoes were positive for *P. falciparum* with a base pair of 205bp while none of the samples was positive for *P. vivax* (Figure 1). The positive samples include two samples 2 (50%) from Ido-Osun and one each from Inisa 1(25%) and Ife 1 (25%) respectively (Table 1). Although not statistically significant, the outdoor catch had the highest number of sporozoite detected across the study areas as compared to the indoor catch ($p = 0.59$; $P > 0.05$). Similarly, 1 (33.3%) sporozoite was detected in the outdoor catches across all the study areas. The only sporozoite detected indoor was found at Ido-Osun 1 (100) (Table 2).

Furthermore, the first quarter had the highest number of sporozoites detected with two sporozoites, one each from Inisa 1 (50%) and Ido-Osun 1 (50%). The only sporozoite detected from the second and third quarters was at Ido-Osun 1 (100%) and Ife 1 (100%) respectively. There was no sporozoite detected from the fourth quarter (Table 3).

Table 1: Infectivity of adult female *Anopheles* with *falciparum* and *P. vivax* across the study areas

Location	Number tested	Number positive for *P. falciparum* (%)	Number positive for other *P. vivax* (%)
Ife	40	1 (25)	0 (0)
Inisa	5	1 (25)	0 (0)
Ido-Osun	13	2 (50)	0 (0)
Total	58	4	0

NB: Percentages were calculated relative to each column

Table 2: Infectivity of adult female *Anopheles* across the outdoor and indoor catches

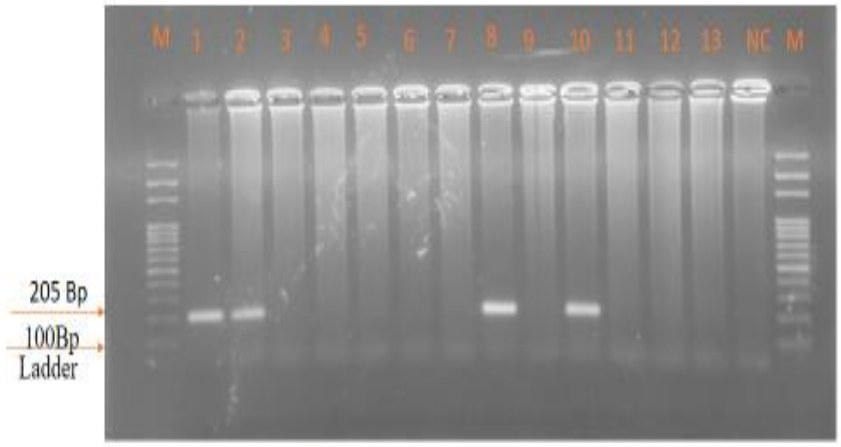
Study locations	Outdoor		Indoor	
	Number collected (%)	Number positive (%)	Number collected (%)	Number positive (%)
Ife	7 (15.2)	1 (33.3)	6 (50)	0 (0)
Inisa	5 (10.9)	1 (33.3)	0 (0)	0 (0)
Ido-Osun	34 (73.9)	1 (33.3)	6 (50)	1 (100)
Total	46	3	12	1

NB: Percentages were calculated relative to each column

Table 3: Infectivity of adult female *Anopheles* across the quarterly catches

Locations	1st Quarter		2nd Quarter		3rd Quarter		4th Quarter	
	Number screened (%)	Number positive (%)	Number screened (%)	Number positive (%)	Number screened (%)	Number positive (%)	Number screened (%)	Number positive (%)
Ife	3 (9.1)	0 (0)	0 (0)	0 (0)	8 (80)	1 (100)	2 (100)	0 (0)
Inisa	3 (9.1)	1 (50)	2 (15.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ido-Osun	27 (81.8)	1 (50)	11 (84.6)	1 (100)	2 (20)	0 (0)	0 (0)	0 (0)
Total	33	2	13	1	10	1	2	0

NB: Percentages were calculated relative to each column



Lane 1-14=DNA molecular ladder; Lane 1,2,8 and 10 = *An. coluzzi* positive for *Plasmodium falciparum*; Lane 3,4,5,6,7 = *An. coluzzi* negative for *P. falciparum*; Lane 9, 11,12, 13= *An. coluzzi* negative for *P. vivax*; Lane 14= Negative control.

Figure 1: Gel electrophoresis plate showing sporozoite of *P. falciparum*

Discussion

The public health menace of malaria continues to remain a global concern with one of the risk factors attributed to the infectivity status of female *Anopheles* mosquitoes.

The infectivity of mosquito vectors is mainly determined through the detection of sporozoite in adult female mosquitoes. The present study detected sporozoite of *P. falciparum* (the major malaria parasite in Nigeria) in the *An. gambiae s.l* vector caught during the study period. Although there were variations in the number of sporozoites detected across the study areas. In Ido-Osun 50% sporozoite was detected in the two mosquitoes with 25% each from one mosquito each in Ife and Inisa. The sporozoite was specifically detected in the *An. coluzzi* sibling species as reported by a previous study by Busari *et al*. (2024) that revealed *An. coluzzi* as the predominant sibling species of *An. gambiae* complex in Osun State. This indicates the infectivity of the mosquitoes viz-a-viz their vectorial competence, thus their potential for the transmission of malaria in the aforementioned locations or the possibility of an ongoing infection. This undoubtedly is a risk factor that places residents of the aforementioned study areas at risk of being infected with malaria and other mosquito-borne diseases (MBDs). This is consistent with the report by Oduola *et al*. (2021) in a study in Kwara State who reported the detection of *P. falciparum* sporozoite in *An. coluzii*. Similarly, Ojurongbe *et al*. (2018) had reported in a study in Osun State the presence of sporozoite of *P. falciparum* in pregnant women. Although the study was not entomological but rather parasitological, it nevertheless, agrees with the presence study which showed its presence across the state. However, this contradicts previous reports by Oduola *et al*. (2013) in Osun State, who reported zero infectivity with *P. falciparum* of the *Anopheles* mosquitoes which was recorded in communities such as Egebda, Iwikun and Odeyinka. Although, it was attributed to the cross-sectional nature of the study and the low number of samples. Moreover, compared to the study by Oduola *et al*. (2013), the report from the present study on vector infectivity and competence could be attributed to migration issues which possibly could be as a result of the entry of malaria-infected individuals into the study area from other places. This could with time result in an increase in the vector parasitic load thus making them infective.

In addition, the detection of a higher sporozoite from the outdoor mosquito catch than the indoor catch indicates the possibility of infection outdoor in contrast to reports by previous studies by Oduola *et al*. (2021) which reported more indoor biting preference. Ido-Osun had sporozoite detected for both indoor and outdoor catches. Thus portending the possibility of a higher prevalence of malaria cases in Ido-Osun as compared to Inisa and Ife. This could be attributed to the poor environmental management arising from improper drainage systems leading to the indiscriminate littering of the environment with waste water and stagnation of rain water after rainfall. The result of the quarterly collection shows that infection occurs in the first, second and third quarters in ascending order of prevalence. The reason could be due to the rainfall attributed to the wet season in the first and second ~~and~~ quarters respectively. However, there was no sporozoite detected from the fourth quarter. This could invariably be due to the lack of rainfall which is characteristic of that quarter referred to as the dry season. Furthermore, the absence of *P. vivax* sporozoite in the vector across the study areas is an indication of the absence of *P. vivax* transmitted malaria in the study areas as it is not majorly involved in malaria transmission in Africa but rather it has its highest prevalence in Latin America and Southeast Asia (WHO, 2020).

CONCLUSION

The present study reveals the infectivity of the *An. gambiae* s.l in the state with *P. falciparum* sporozoite. This invariably could portend an ongoing transmission of *P. falciparum* malaria in the state. Thus, the need for stringent action on mosquito vector surveillance, chemotherapy through MDA and public health education and enlightenment to break the transmission chain. However, there could be a need for further parasitological studies to augment MDA.

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REFERENCES

Adeleke, M.A., Adebimpe, W.O., Hassan, A.O., Oladejo, S.O., Olaoye, I, Olatunde, G.O., *et al*. (2013). Larval habitats of mosquito fauna in Osogbo metropolis, Southwestern Nigeria, *Asian Pac J Trop Biomed* 3(9): 673-677. doi: 10.1016/S2221-1691(13)60137-9.

Busari, L.O., Iwalewa, Z.O., Adeogun, A.O., Surakat, O.A., Rufai, A.M., *et al*. (2024).Dynamics of *Anopheles gambiae* s.l (Diptera: Culicidae) 2 Sibling Species Composition and Biting Preference in 3 Osun State, Southwestern, Nigeria. *DUJOPAS*, Vol. **10** No. 2b. https://dx.doi.org/10.4314/dujopas.v10i2b.27

Coetzee M (2020). Keys to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). *Malaria Journal* **19**(70): 1–20. https://doi.org/10.1186/s1236-020-3144-9.

Federal Ministry of Health, Department of Public Health, Guidelines for Malaria-Lymphatic Filariasis Co-Implementation in Nigeria, September, 2013.

Gillet, J.D. (1972). Common African mosquitoes and their medical importance (with colourillustrations). London: *W Heffer & Sons*.

Oduola, A.O., Idowu, E.T., Oyebola, M.K., Adeogun, A.O., Olojede, J.B., *et al*. (2012). Evidence of carbamate resistance in urban populations of *Anopheles gambiae s.s.* mosquitoes resistant to DDT and Deltamethrin insecticides in Lagos, South-Western Nigeria. *Parasites and Vectors* **5**: 116.

Oduola, A.O., Obembe, A, Adeogun, A.O and Awoloa T.S (2013). Entomological and transmission risk indices of malaria vectors in selected communities in Osun State, Nigeria. *Animal Research International*, **10**(3): 1805 – 1808.

Oduola, A.O., Obembe, A, Lateef, S.A., Abdulbaki, M.K., Kehinde, E.A., *et al*. (2021). Species Composition and *Plasmodium falciparum* Infection Rates of *Anopheles gambiae* s.l. Mosquitoes in Six Localities of Kwara State, North Central, Nigeria, *J. Appl. Sci. Environ. Manage*. Vol. 25 (10) 1801-1806.

Oduola, A.O., Olojede, J.B., Ashiegbu, C.O., Adeogun, A.O., Otubanjo, O.A. *et al*., (2010). High level of DDT resistance in the malaria mosquito: *Anopheles gambiae s.l.* from rural, semi urban and urban communities in Nigeria. *Journal of Rural and Tropical Public Health* **9**: 114-120.

Ojurongbe O., Nkem-Okorie P., Opatokun, R.L., Ojurongbe, T.A., Mabayoje, V.O., *et al*. (2018). Prevalence and associated factors of *Plasmodium falciparum* and soil helminths infections among pregnant women in Osun State, Nigeria. *Afr Health Sci* 18(3): 542-551.

Oyewole, I.O. and Awolola, T.S. (2006). Impact of urbanization on bionomics and distribution of malaria vectors in Lagos, Southwestern Nigeria. *J. Vect. Borne Dis* 43: 173-178.

Snounou, G., Viriyakosol, S., Zhu, X.P., Jarra, W., Pinheiro, L., *et al.* (1993). High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. *Mol Biochem Parasitol* 61(2):315-320.

WHO (2023). Guidelines for malaria. www.who.int/teams/global-malaria-programme.

World Health Organization (2020) World malaria report. 300pp. https:// www.who.int/publications/i/item/9789240015791. Accessed 21 Nov 2021.