Malarial Entomological Indices of Ijebu-North District of Ogun State, Nigeria

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Abstract

Malaria is a life-threatening parasitic disease transmitted from person to person through the bite of the female Anopheles mosquitoes. Geographically, the dominant species in the Southern part of Nigeria are the Anopheles gambiae sensu stricto and An. funestus s.s. especially in the Southwest. There is paucity of information on the vectorial capacity of Anopheles mosquitoes that transmit malaria in Ijebu-North Local Government Area (INLGA) which necessitates this study on entomological indices of malaria vectors in the study INLGA. Ten apartments were selected each from the six communities; Ojowo, Oke-Agbo, Oru- Ijebu, Awa-Ijebu, Oke-Igan and Ibipe used. Mosquito samples were collected using Pyrethrum Spray Catch (PSC) method with modified exit trap on a monthly basis for a period of one year. A total of 1,316 collected mosquito specimens were morphologically identified using reference guides from which 266 were selected and used for the molecularly analyses using polymerase chain reaction- randomly amplified polymorphic DNA (PCR-RAPD) method. The indoor resting behaviour, abundance of the mosquitoes and sporozoite (infection) rate were

estimated. Behaviourally, the collected mosquitoes significantly showed endophylic habit (P=0.0023). For the presence ***of An. gambiae had 100%*** *sporozoite infection rate (SIR) for the presence of Plasmodium falciparum while* ***for the Entomological Incubation Rates (EIR) or risk of getting infected by mosquito per person per year, An. gambiae (0.42) had highest followed in decreasing order by An. funestus (0.26) then An. arabiensis (0.16) and lastly by An. leesoni (0.12).*** *Consequently, periodic surveillance is suggested to be carried out in order to subsequently update the malaria vector database, while Government at all levels should improve funding for researches on malaria vectors.*

Keywords: *Anopheles, Entomological indices, Ijebu North Local Government, Plasmodium falciparum, Pyrethrum Spray Catch.*

1. Introduction

Malaria is a life-threatening parasitic disease transmitted from person to person through the bite of the female *Anopheles* mosquito. In recent years, malaria has caused increased human mortality and morbidity (Shanks *et al.*, 2000; Chen *et al.*, 2004; Talapko *et al*., 2019). Most cases and deaths are estimated to have occurred in the WHO African Region and followed by the WHO South-East Asia Region (Gunathilaka *et al.*, 2015; WHO, 2016). One of the most successful methods of malaria prevention and eradication has been through control of the mosquito vector (CDC, 2013).

Malaria entomological indices are parameters used to establish man-vector contact and risks of malaria parasite transmission with a focus to understand the relationship between the vector, the parasite and the host in order to develop and implement effective vector control strategies (WHO, 2013). Some entomological indices include; Indoor Resting Density (IRD), Human Blood Index (HBI), Man Biting Rate (MBR), Sporozoite Infection Rates (SIR), Entomological Infection Rate (EIR), Vector Capacity (VC), Parity Rate (PR), Age Grading (AG) and Longevity and Infectivity (LI).

Oduola *et al.* (2012) reported that the Sporozoite Infection Rates (SIR) of *An. gambiae s.s.* varied between 1.9 and 3.1%, while

those of *An. funestus* were from 1.5 to 4.5%. They also reported that the annual Entomological Innoculation Rates/ Entomological Infection Rate (EIR) in Akufo, Ikere and Idi-Ose communities in Oyo State, Nigeria were 139, 153 and 110 infective bites per person per year respectively. In another survey carried out by Obembe *et al*. (2018) in Kwara State, pyrethrum spay catch (PSC) was used. The mean annual mosquito Man-Biting Rates (MBR) increased significantly in both intervention (0.88 ± 0.18 vs 1.06 ± 0.38; $F_{(1, 10)} = 9.50$, $P = 0.012$) and control (1.45 ± 0.31 vs 1.61 ± 0.34; $F(1, 10) = 10.18$, $P = 0.010$) sites along with increase (≥ 1.6 times) in sporozoite rates within intervention (0 - 2.13%) and control (2.56 - 4.04%) communities. Aju-Ameh *et al*. (2016) also reported a work carried out in Benue State using PSC for malaria vector collection. The SIR for the rural was 1.9% and for the urban communities was 0%. EIR recorded was 0.4% per person per night culminating in an annual 146 infective bites per person per year.

Presently, there is paucity of records and information from research to unveil the status of malaria vectors in Ijebu-North Local Government and some other areas in Nigeria This therefore necessitated this study using four major entomological indices; Indoor Resting Density, Man Biting Rate, Sporozoite Infection Rate and Entomological Incubation Rate.

2. Materials and Methods

2.1 Study Area

This study was conducted in six (6) communities Health Centres-HCs in Ijebu-North Local Government Area (INLGA) of Ogun State, Nigeria on coordinates 6°57`N 4°00`E. Six Primary Health Centres (HCs) based communities were randomly selected for the study. Residents are mainly farmers, while some also engage in secondary activities like trading (Olufunso, 2018). The locations of the survey sites and coordinates are presented in Table 2.1.

Table 2.1: Coordinates of the survey Communities

S/N	Localities/ Health Facility (HC)	Coordinate
1	OJOWO HC, IJEBU-IGBO	N6^{0}58'41.946", E3^{0}59'43.506"
2	OKE-AGBO HC, IJEBU-IGBO	N6^{0}57'31.854", E4^{0}0'13.572"
3	ORU HC, ORU-IJEBU	N6^{0}57'0.828", E3^{0}56'37.044"
4	AWA HC, AWA-IJEBU	N6^{0}57'54.624", E3^{0}56'7.644"
5	IBIPE HC, AGO-IWOYE	N6^{0}56'25.482", E3^{0}55'14.736"
6	OKE-IGAN, AGO_IWOYE	N6^{0}56'55.584", E3^{0}54'37.188"

2.2 Protocols and Ethical approval

Series of individual and group discussions and meetings were also organized before and during the study and permissions given by apartment owners. Malaria drugs were given to households with direction on usage at the end of the study as incentives. Ethical approvals were given by the Federal Ministry of Health, Abuja (NME/IVM/VS/S.1/01) and Ministry of Health, Abeokuta, Ogun State (HRS/381/230).

2.3 Entomological Sampling

Collection of mosquitoes was done in sixty apartments by Pyrethrum Spray Catch (PSC) (Oduola *et al.*, 2012) in six (6) communities (Ojowo, Oke-Agbo, Oru-Ijebu, Awa, Ibipe and Oke-Igan), in INLGA from May, 2017 to April, 2018. Prior to the collection day (mostly 4-6pm), a modified exit trap **(**Plate 2.1) was carefully affixed to the window of each study apartment in order to catch the exophilic (outdoor resting) mosquitoes (Govella *et al*., 2011).

Plate 2.1: Modified Exit Trap

The trapped and PSC collected female Anopheline mosquitoes were separated and counted which were used for further analysis due to their role in the transmission of malaria as a result of their inherent blood sucking habit (Okorie *et al*., 2011; Helmesson, 2013; Aju-Ameh *et al*., 2016; Ondiba *et al*., 2017; Obembe *et al*., 2018). Collected and sorted mosquito samples were kept in separate 1.5ml Eppendorf tube with desiccated gel (Gonzalez *et al.*, 2017) overlaid with filter paper (Oduola *et al.*, 2012) to preserve them. Each tube was labeled appropriately with place, time and date of collection, serial number, and name of collector.

2.4 Morphological Identification

Each preserved mosquito was identified using the morphological keys of Gillet (1972) and Gillies and Coetzee (1987). This was carried out in the Laboratory of Department of Zoology and Environmental Biology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria

2.5 Molecular Analysis of Mosquito Samples

A total of two hundred and sixty-six (266) anopheline samples were collected from the bulk (1,316) to reduce the samples after the morphological identification process for separate individual PCR targeting the cytochrome oxidase I gene for the presence of *Plasmodium falciparum* (Scott *et al.*, 1993). The DNA extraction, PCR and Gel Electrophoresis were carried out at the Molecular Laboratory of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria.

2.6 Entomological Indices

i. Indoor resting density (IRD): This was calculated for each anopheline species collected per house per month on Health facility basis as the total number of females of a particular species divided by the total number of houses inspected. It is the average number of bites per person per night by a vector species.

ii. Man-biting rates (MBR) was calculated using the **indirect approach** by dividing the total number of freshly-fed mosquitoes of a particular species by the total number of human occupants in houses used for collection (Oyewole *et al*., 2005).

iii. Sporozoite Rate (SPR) was also estimated as the number of *Anopheles* mosquitoes found with *Plasmodium* sporozoite divided by number of blood fed Anopheles mosquitoes assayed multiplied by 100.

iv. Entomological Inoculation Rates (EIR) which is an expression of the risks of getting infective mosquito bites per person per month was calculated thus; EIR (infective bites/person/night) = MBR × [SPR (%)/100] (Oyewole *et al*., 2005).

2.7 Statistical Analyses

The abundance of each mosquito species was expressed as the total number of the species collected in a descriptive form. A paired sample t-test was carried out to determine the difference in abundance between the indoor and exit trap collected mosquitoes.

3 Results

3.1 Abundance of *Anopheles* collected Indoor and using Exit Traps

The number of *Anopheles* collected indoor (1,219) was more than the exit trap (97) counterpart recorded during this study (Figure 3.1). There was a significant difference (P = 0.023) between the group of *Anopheles* collected indoor and those collected using exit traps (Table 3.1).

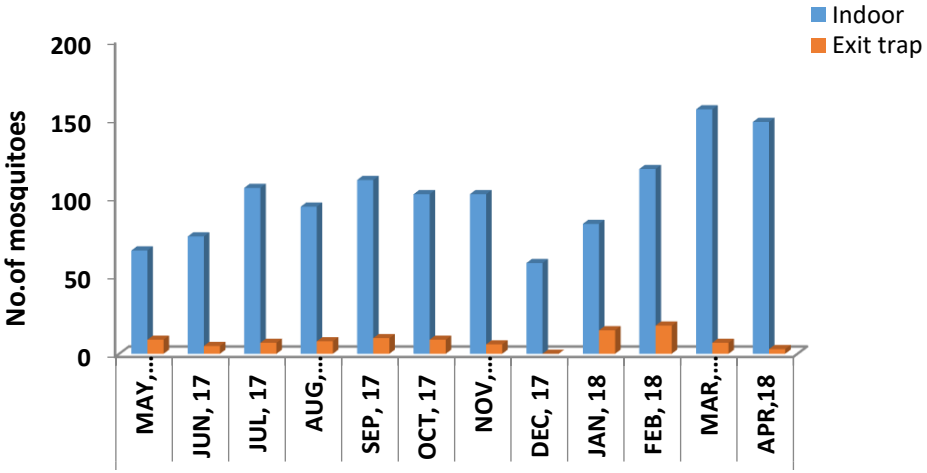


Figure 3.1: Abundance of *Anopheles* collected Indoor and using Exit Traps in selected Ijebu-North Communities

Table 3.1: T-test for resting behaviour comparison

Parameter	Paired Difference					
	Mean	N	Std. Deviation	Std. Error Mean	Correlation	Sig.
Indoor	101.58	12	29.801	8.608	0.097	0.023
Outdoor	8.08	12	4.652	1.401		

At the 0.05 level P value =0.023 t = 10.897

There is significant different when the p- value is < 0.05

3.2 Molecular Diversity

After the molecular procedures, four (4) anopheline species, *An. gambiae s.s.*, *An. funestus s.s.*, *An. arabiensis*, and *An. leesoni* were identified which cut across all the locations (Fig. 3.2) where the survey was carried out. Most of the *Anopheles* species habour *P. falciparum* DNA in them as shown following specific PCR amplification using *P. falciparum* DNA primer (Plate 3.1).

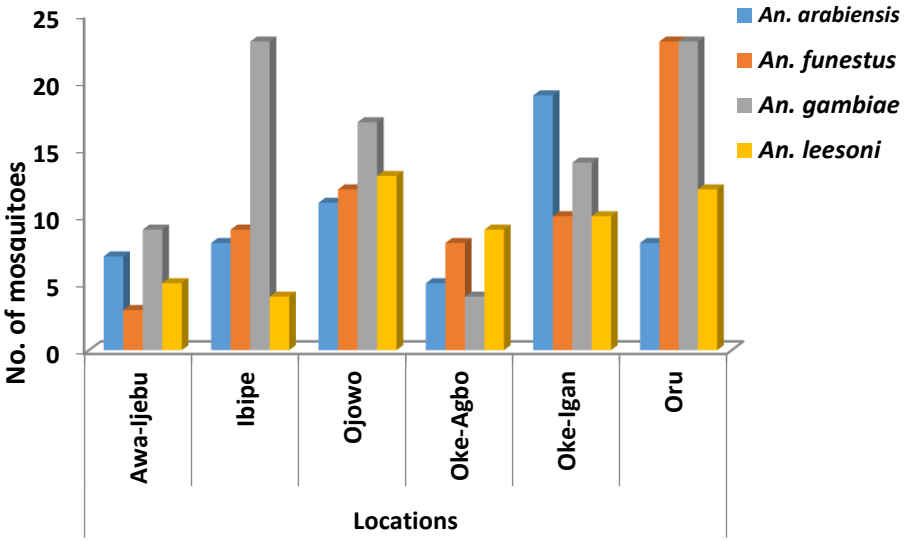
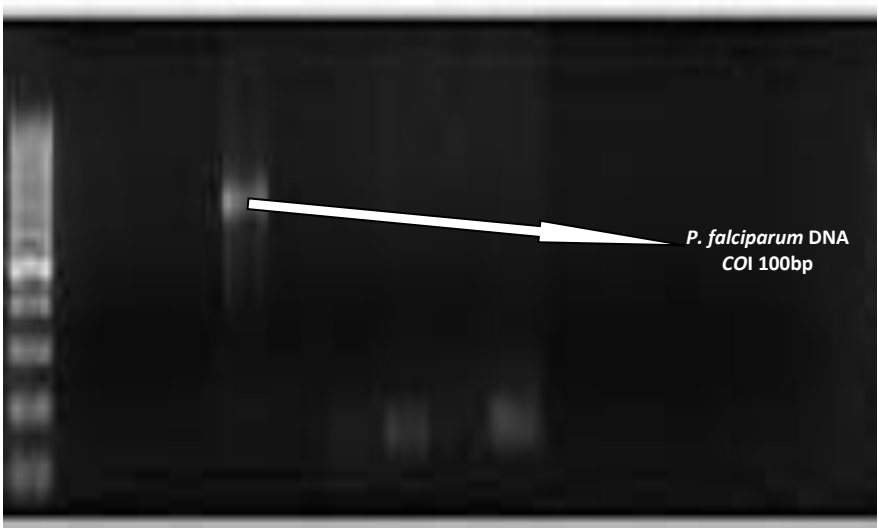


Figure 3.2: Mosquito-based distribution in INLGA with molecular diversity

M AF AF AF AG AL AL AF AG AG AG AF AA AG AG AG



M = DNA Ladder, **AG** = *Anopheles gambiae*, **AF** = *Anopheles funestus*, **AA**= *Anopheles arabiensis*, **AL** = *Anopheles leesoni*

Plate 3.1: Amplification of *Plasmodium falciparum* DNA in the sampled *Anopheles* species

3.3 Assessment of *Plasmodium falciparum* DNA in the sampled mosquitoes

Table 3.2 summarises the occurrence of *Plasmodium falciparum* DNA in the samples of mosquito species collected during the survey. *Anopheles gambiae* had 90 (100%) as positive and none of them was negative, while other species in decreasing order had; *Anopheles funestus*- 60 (92.3%), *Anopheles arabiensis*- 41 (70.7%) and *Anopheles leesoni*- 36 (67.9%) respectively. In total only 85.3% of the sampled Anophelines were positive to the presence of *P. falciparum* out of the 266 samples analysed.

Table 3.2: *Plasmodium falciparum* DNA in sampled mosquito species from Ijebu-North, Southwest Nigeria

Mosquitoes	**Presence of *Plasmodium falciparum* DNA**	**Absence of *Plasmodium falciparum* DNA**
Anopheles gambiae	90 (100.0%)	0 (0%)
Anopheles funestus	60 (92.3%)	5 (7.7%)
Anopheles arabiensis	41 (70.7%)	17 (29.3%)
Anopheles leesoni	36 (67.9%)	17 (32.1%)
Total	**227 (85.3%)**	**39 (14.7%)**

3.4 Assessment of Entomological Indices

Table 3.3 reveals that the indoor resting density of snopheline mosquitoes in INLGA was highest in *An. gambiae* followed by *An. funestus* while their siblings (*An. arabiensis* and *An. leesoni*) also follow the trend, where the sequence was reported for MBR and Sporozoite infection rate (SPR). Only *An. gambiae* had 100% (i.e. all individual samples were infected with *P. falciparum*). For the Entomological Incubation Rates (EIR) which is the risk of getting infected by mosquito per person per year, in decreasing order, *An. gambiae* (0.42) will transmit more malaria infections followed sequentially by *An. funestus* (0.26) *An. arabiensis* (0.16) and *An. leesoni* (0.12).

Table 4.10: Some Entomological Indices of mosquito species from Ijebu-North, Southwest Nigeria

Anopheles spp.	No. blood fed	No. of Females	No. with *Plasmodium*	IRD	MBR	SPR (%)	EIR
An. gambiae	90	90	90	1.50	0.42	100.0	0.42
An. funestus	65	65	60	1.08	0.28	92.3	0.26
An. arabiensis	58	58	41	0.97	0.23	70.7	0.16
An. leesoni	53	53	36	0.85	0.17	67.9	0.12
Total	266	266	227	4.40	1.10	330.9	0.96

IRD – Indoor Resting Density (per house per year),
MBR – Man Biting Rate (per person per year),
SPR – Sporozoite Infection Rate,
EIR – Entomological Incubation Rate (per year)

4. Discussion

4.1 Abundance of *Anopheles* collected Indoor and using Exit Traps

Generally, indoor collections are always more in numbers than exit trap collections. This also occurred in the present study in all the months of collection. This could be due to the arrangement of most of the apartments where the collections were made; they were rather disorganized with clothing hanged on the wall and with tattered sit and sometimes no cementing of the walls internally, hence, making adequate provision for resting places for blood-fed mosquitoes to rest which resembles the surveillance sites in the work of Ajayi *et al*. (2010) also conducted in Ogun State.

4.2 Morphological Assessment

Basically, two species of malaria vectors (*Anopheles gambiae* and *Anopheles funestus*) were identified using the morphological method (Aina *et al*., 2023). The two malaria vectors were also reported as the major malaria vector in this part of Nigeria by CDC (2012) and Kiszewski *et al.* (2004) in their malaria vector maps. But due to its higher occurrence, *An. gambiae* could be implicated for the huge and persistent transmission of malaria in INLGA of Ogun State. This finding is in line with what was observed in all locations where entomological survey was carried out in Oyo State by Oduola *et al*. (2012), *An. gambiae* was reported to be most abundant and this could be because the study area falls in the same geo-political and climatic belt as INLGA.

4.3 Assessment of *Plasmodium falciparum* DNA in the sampled mosquitoes

The presence of *P. falciparum* DNA in the Anophelines samples analysed revealed the potential of *An. gambiae* in transmission of the protist. This shows that there is a high risk of malaria being transmitted in the localities surveyed. It also implies that virtually all the female mosquitoes collected have had blood meal and was

even freshly-fed. Proliferation of the rarely noticed *Anopheles* in all the study communities may have been encouraged by the inhabitants' ways of disposing refuses, their jobs, daily activities, as well as retention of used containers in and around their houses.

The presence of *P. falciparum* sporozoites was found in the *An. coluzzii* (a sibling of *An. gambiae*) and said to increase in numbers from the first collection year to the second in the control communities while in that of intervention communities, infected mosquitoes were identified as *An. gambiae* (Aju-Ameh *et al*., 2016). The similarity observed between Aju-Ameh *et al*. (2016) and the present study may be due to the environmental conditions and anthropogenic activities which could rather facilitate mosquito breeding in the study locations.

However, in the work of Oduola *et al*. (2012), only 54 (2.3%) of *An. gambiae* and 48 (2.7%) of *An. funestus* mosquitoes tested positive for *P. falciparum* out of the total of 1,732 assayed. The reason for this low plasmodia infestation may be due to the collection method (Night human landing catching) which does not give free chance for the mosquitoes to take the blood meals and also because this makes only one human available for the mosquitoes. In PSC on the other hand, there is probability that the entire inhabitant's blood could be fed upon and there is no hindrance to the mosquitoes while taking the blood meals.

4.4 Assessment of Entomological Indices

These results show that malaria transmission in INLGA is done by all the four species reported in this study. The recorded high density of each species could be due to availability of ample human source of blood as most of the apartments visited for the survey are inhabited by 3 people on the average. In the work of Obembe *et al*. (2018), the MBR increased in the control surveillance from 0.88 to 1.06. The difference so noticed therein may have been due to some environmental and human factors.

In contrary, Oduola *et al*. (2012) reported the SPR for *An. gambiae* as 3.1% and *An. funestus* as 4.5% in the communities

surveyed in Oyo State, Nigeria. The differences observed could be due to the collection method they used which involves only one human used as bait unlike the present study where mostly all inhabitants in the apartments visited are fed upon by the mosquitoes. Aju-Ameh *et al*. (2016) in a similar report stated that the EIR in the Rural Communities surveyed in Otukpo Local Government Area of Benue State was 0.4. This is because they also used PSC method in the specimen collection giving rise to a high number of blood-fed and infected mosquitoes.

5 Conclusion

This study was able to provide a long needed resting behaviour as entomological indices of *Anopheles* species that transmit malaria in INLGA which is intended to serve as background information for subsequent studies. The need for periodic surveillance in order to monitor the molecular diversity and behavioral change in *Anopheles*, unstoppable enlightenment on waste disposal, creation of drainages and removal of water storage in and around their houses, whether knowingly or unknowingly as this will help to reduce these and other vectors drastically. It also informed Governments at all levels that malaria endemic status as reported is a reality as malarial mosquitoes in INLGA are almost all infected with the malaria parasite in the study communities.

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Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

SAA, OAL OMA, AO and TFS designed the experiment and preparation of manuscript. The surveillance and morphological identification was done by SAA, RYO and ATA. TFS, IBO, and SAA were involved in the molecular analysis. GAR, OAO, TFS and SAA were saddled with the statistical analysis. IBO, ONA and SAA handled the logistics part of the project while OMA, OAL, TFS, RYO and SAA participated in the writing of the final versions of the manuscript.

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