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Assessing the Entomological Parameters of Malaria Vectors in Anambra State, Southeast Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Adequate knowledge of entomological parameters within a defined geographic endemic area is basic for effective planning of malaria vector control for malaria elimination. The study investigated sibling species of malaria vectors, indoor resting density (IRD), human biting rate (HBR), blood meal source (BMS), human blood index (HBI), sporozoite rate (SR) and entomological inoculation rate (EIR) of malaria vectors in Awka North, Awka South and Njikoka Local Government Areas in Anambra State, southeast Nigeria. Pyrethrum spray collection (PSC), Centre for Disease Control (CDC) light trap, and human landing catch (HLC) techniques were the methods used for collection of indoor and outdoor malaria vectors. Mosquitoes collected were sorted according to species and sex; and identified using standard morphological and molecular techniques. Chi-square test was used for data analysis. A total of 2,870 *Anopheles* mosquitoes were collected, male 1949 (67.9%) and female 921 (32.1%). All female species identified morphologically belong to the *Anopheles gambiae* s. l. complex. From the molecular and siblings species separation, *Anopheles gambiae* recorded the highest abundance of 54.2% and *Anopheles coluzzii* the least abundance of 45.8%.

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The IRD was found to be 1.40 per man per night with an average HBR of 5.05. The blood meal source showed that human blood source was the highest number with 46.2%, followed by goat blood source with 32.7%, and combination of human and goat blood was the least with 21.2%. The result also showed a HBI of 0.51 whereas the test for the presence of circumsporozoite protein (CSP) of *Plasmodium falciparum* shows that none of the malaria vectors was positive for sporozoite; thus, the EIR could not be determined and compared among the study population. The values of the entomological parameters and the allopatric breeding of the two sibling species of *Anopheles gambiae* and *Anopheles coluzzii* reported in this study which is probably the second of such report in Nigeria has a huge implications for malaria vector control and malaria control in Anambra State, Southeast Nigeria.

Keywords: Blood meal source; entomological parameters; malaria vectors; Anambra State.

1. INTRODUCTION

Anopheles gambiae remains the dominant and most efficient vector of human malaria in the tropics including Nigeria [1] based on its high abundance, longevity, high propensity for humans feeding and high vectorial capacity [2]. The correct analysis of the distribution of specific malaria vectors and its subsequent entomological parameters remains the prerequisites for meaningful epidemiological studies and for planning and monitoring of successful malaria control or eradication program. In the past, large areas of Nigeria had no reliable data on presence and absence of vectors, yet it has been established that there are diversities of malaria vectors in Nigeria and they have different bionomics and vector competences. Several studies conducted in the past observed that *Anopheles arabiensis* has a low Human Blood Index (HBI) and shows a marked preference for cattle and other warm-blooded animals [3]. For instance, *Anopheles* species has a high degree of zoophily in some countries as demonstrated by HBI reported from various environmental settings [4,5]. Lower proportion of human blood meals (26%) were also recorded in *An. arabiensis* collected from sites where cattle were kept closer to human housing than in those collected from sites where cattle were kept some distance from humans [6-8]. From the data available, it is on record that the entomological parameters allows a better understanding of the transmission dynamics and the design of more effective malaria vector control strategies. The information collected in this study will both complement and support other studies that describe the entomological parameters of malaria vectors which include but not limited to the indoor resting density (IRD), human biting rate (HBR), blood meal source, human blood index (HBI), sporozoite rate (SR) and entomological inoculation rate (EIR) of the study area. The collection and integration of these information will characterize malaria in the LGAs and establish a robust framework for developing future interventions against malaria and in planning effective malaria vector control strategies.

2. MATERIALS AND METHODS

2.1 Study Area

The research was carried out in Awka South, Awka North and Njikoka LGAs of Anambra State Nigeria from September 2021 to August, 2022 covering the two major seasons of the year. The State has twenty one Local Government Areas with a total land kilometer of 4,844km² (1,870sqm) [9]. The 2020 projected population of Anambra State is 6,182,924 [10]. The official language of the people of Anambra State is Igbo although English language is widely spoken throughout the state as a secondary language [9]. The State is located on Latitude 6˚ 12'45.68''North of Equator and Longitude 7° 04'19.16''East of Greenwich and its average daily temperature is 26.8°C / 80.2 °F [9]. Anambra State has seasonal climatic conditions, the rainy season (which falls between April and October) and the dry season (which falls within November and March) with a short spell of harmattan between November and January which is a period of cold weather when the atmosphere is generally misty [11]. The total annual rainfall of the State is above 11,450mm for the six to seven months of the rainy season whereas the mean annual temperature range is 30.0–36.0°C [12]. Awka South Local Government Area (LGA) is made up of nine (9) communities namely; Awka, Amawbia, Ezinato, Isiagu, Mbaukwu, Nibo, Nise, Okpuno and Umuawulu [9]. Awka South LGA has one ethnic group and the LGA is located on Latitude 6˚10' North of equator and Longitude 7° 04' East of

Greenwich. Awka North is also a Local Government Area in Anambra State with coordinates of 6° 15' North of equator and 7°10' East of Greenwich. Communities that make up the local government are ten (10) in number which includes: Awba Ofemili, Amansea, Ugbene, Ebenebe, Achalla, Urum, Amanuke, Isu Aniocha, Mgbakwu and Ugbenu with its local government headquarters at Achalla [9]. Njikoka is a Local Government Area in Anambra State, South-Central Nigeria lying between latitude 06° 20' 58 '' N to 06° 21' 00'' N and longitude 06° 52' 55''E. The area is characterized by a temperature range of 27˚ - 30˚ C and has a double maxima rainfall peaks in July and September. Six (6) towns make up the local government which include; Abagana, Enugwu-Ukwu, Enugwu-Agidi, Nawfia, Nimo and Abba. The area has a land mass of 84.40 km² area with an annual population change of 2.8% and 2020 projected population of 219,571 [13].

2.1.1 Selection of communities and households

One community was randomly selected from each LGA to represent all the communities in the local government area. A village was randomly selected from a pool of villages with equal representation from the different sites with each village having an equal chance of being selected. According to the method described by WHO [14], households were randomly selected from a pool of village with equal representation from the different sites with each household having an equal chance of being selected. Rooms were randomly selected from the different collection sites at the three study areas from a pool of houses with equal representation with each houses having an equal chance of being selected. Mosquitoes were captured between September 2021 and August 2022, covering the rainy and dry seasons of the year.

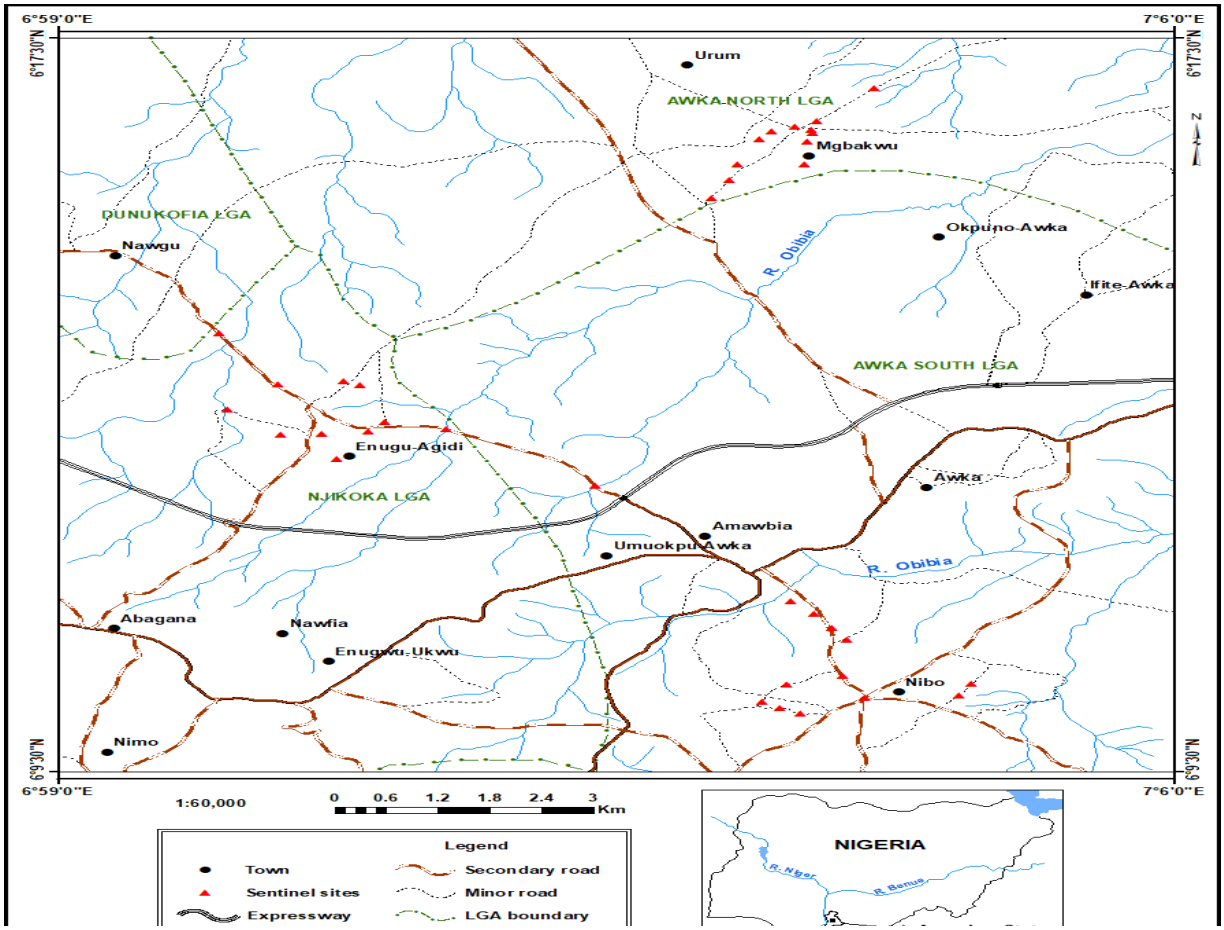

Fig. 1. GIS map of the study areas showing Nibo in Awka South, Mgbakwu in Awka North and Enugwu Agidi in Njikoka local government areas

Source: Modified from Google map on 20th December, 2022

2.2 Study Design

Descriptive and comparative study design was used in the study and simple random sampling as described by WHO [14] was used to select households for the study.

2.2.1 Community sensitization and mobilization

Sensitization was done through churches, markets and kindred enclaves while community mobilization which involved youths, women, and men in the study communities was done by a sensitization rally during which the objective of the study, explanation of the project intent and its methodology was made. Study participation was voluntary and participants had the liberty to withdraw from the study without giving any reason for doing so.

2.2.2 Sampling procedures for entomological investigations

The following sample procedures were engaged: pyrethrum spray trap collections for indoor mosquito vectors, CDC light trap collections for outdoor and indoor mosquito vectors, human landing catches for outdoor and indoor mosquito vectors.

2.2.3 Collection of adult mosquito vector

Outdoor and Indoor resting mosquitoes were collected from the study locations using pyrethrium spray collection techniques, CDC light trap collection and human landing catches methods. The collections were performed monthly during three consecutive nights/days, and each collection period lasted for twelve hours, from 6:00pm to 6:00am for the Human Landing Catch and CDC Light Trap, from 6:00am to 9:00am for the Pyrethrum Spray Collections. Under these conditions, the mosquitoes were captured concomitantly in the intra (indoor) and peridomestic (outdoor) environments. In all the collections, mosquitoes collected were transferred into moist filter papers in Petri dishes as described by WHO [15] properly labeled with location, date, hour, collection method.

2.2.4 Pyrethrum spray collections for indoor mosquito vectors

Indoor mosquito collection was done monthly using pyrethrum spray catches (PSC) method as described by Abdoon and Alshahrani [16]. The rationale behind selection of this method is that it is considered most successful in capturing anthropophagic (human biting) and endophilic (indoor resting) mosquitoes. According to Curtis [17], it is also considered far less ethically objectionable in areas of multidrug resistance. In each site, households were randomly selected for the spray collections and sampled monthly. Pyrethrum spray collections took place between 06.00am and 09.00am using BMC branded pyrethroid mosquito spray which constitutes the following active ingredients (0.26% Esbiothrin, 0.28% Permethrin, 0.1% Beta-Cypermethrin and 0.31% Lemon). Food and water were removed from the house and white sheets spread out on the floor and over the furniture in the house. Two field workers were engaged, one inside the house and the other outside who sprayed around the eaves with this non-residual pyrethroid. The field worker inside the house then sprayed the roof and walls. The house was closed for 20 minutes after which the white sheets were brought outside (where there is sufficient light to see the dead and dying mosquitoes).

2.2.5 CDC light trap collections for indoor and outdoor malaria vectors

Indoor and Outdoor mosquito collection was also conducted monthly using modified miniature light traps as described by the Centers for Disease Control (CDC) (John W. Hock Company, model 512). The (CDC) was used to collect mosquitoes during night time for three consecutive nights. The CDC light traps were placed approximately 1.2m above the floor in and outside the room. Traps were switched on from 6:00 pm and removed by 6:00 am the next day. Each night, 6 traps (one trap indoor and one trap outdoor at three different locations) were set for three nights for each site each month and they were rotated in the same order each month.

2.2.6 Human landing catches for outdoor and indoor malaria vectors

The human landing catch (HLC) method which is the standard reference method for measuring human exposure to mosquito bite was used to assess the human biting rate (HBR) of mosquito in the study areas as described by WHO [15]. HLC was performed for 50 minutes each hour with 10 minutes break for the collectors. Human-landing catches was conducted indoors and outdoors in and around the randomly selected

houses at each site each month. At each site, different houses were selected each night, at least 300 m apart, for three consecutive nights. Thus, all households were sampled in the same week each month. Catches were designed to replicate normal human subject behaviour, assuming many residents were outdoors in the early evening, and that most will retire to bed before 22.00. At each house two adults were stationed outdoors 10 m from the house, and two were stationed indoors. Outdoor collections were conducted from 6.00pm to 9.50pm, after which time few people were outdoors, and indoor collections from 6.00pm to 05.50am. Collectors sat on chairs with their legs exposed. Using flashlights, collectors caught landing mosquitoes with a hand – held mouth aspirator and improvised test tubes and each hour's collection were kept separately and the collectors were rotated between sites on different nights.

2.2.7 Preservation of adult malaria vector

All adult malaria vectors collected were temporary preserved on moist filter papers in petri dishes as described by WHO [15] and transferred to the Vector Research Department at the National Arbovirus and Vectors Research Centre (NAVRC), Enugu, Nigeria for identification and further analysis.

2.2.8 Morphological and molecular identification of malaria vectors

All the mosquito vectors collected were identified and sorted out under a Stereomicroscope Leica model NSW series IMNS 210 entomological microscope. The mosquitoes were identified to species level using morphological keys of Gillies and De Meillion [18], Gillies and Coetzee [3]. Adult female mosquitoes were stored dry on silica gel for species identification and molecular assays (PCR and ELISA) subsequently of the *Anopheles gambiae* complex. Each mosquito sample was preserved individually in an eppendorf tube and were transferred to the Molecular Entomology and Vector Control Research Laboratory of the Department of Public Health and Epidemiology at the Nigerian Institute of Medical Research (NIMR) Yaba Lagos, Lagos State Nigeria for further molecular identification and analyses. Molecular assays used were the species specific PCR techniques for confirmatory identification of the species within *An. gambiae* complex. The PCR were performed with universal and species specific primers for the *An. gambiae* complex. Molecular identification of *An. gambiae* species complex were based on the species specific nucleotide sequences in the ribosomal DNA intergenic spacers (IGS) following the procedure as described by Wilkins et al., [19]. The amplified DNA were separated on a 2.0% agarose gel stained with ethidium bromide and viewed on a UV transilluminator

2.3 Analysis of Data

Descriptive statistics (mean and standard deviation) was used for sample characterization. One-way analysis of variance (ANOVA). Data were subjected to Chi-square using the SPSS Software package (Version 20) to assess possible correlation whereas *P-values* < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

A total number of 2,870 *Anopheles* mosquitoes were collected in the study. Of this number, 67.9% (1949/2870) were males and 32.1% (921/2870) were females. All the female species identified morphologically belonged to the *Anopheles gambiae s. l.* complex. The highest number of female malaria vectors was 35.7% (329/921) which was collected from Awka South LGA, followed by 33.8% (311/921) that was collected in Awka North LGA and then 30.5% (281/921) that was collected in Njikoka LGA in Anambra. The total number of mosquitoes caught in the current study has a significant implication for vector control although the 32.1% of female mosquitoes caught was relatively low compared to that caught by Kevan et al., [20] where a total of 7146 female *Anopheles* mosquitoes of seven different species (*An. bancroftii*, *An. farauti s.s*, *An. farauti* no. 4, *An. hinesorum*, *An. koliensis*, *An. longirostris* and *An. punctulatus s.s*) were collected, including (36.5% blood-fed and 63.5% unfed) to investigate species composition, abundance, and nocturnal activity of *Anopheles* populations in the study villages. The number of mosquitoes caught in that study was very high because mosquitoes were sampled from 6 pm to 6 am in five villages from 2012 to 2016 (a four years survey) whereas the present study was conducted under one year.

The IRD in Awka South LGA was found to be 1.36 per man per night while Awka North and Njikoka LGAs recorded an IRD of 1.44 per man

per night and 1.39 per man per night respectively. The average IRD in the overall study was found to be 1.40 per man per night. The IRD recorded in the study was very high when compared with the overall indoor resting density (IRD) of 0.02 bites per man per night required for maintaining transmission. This high IRD might also account for high malaria prevalence in the area. For instance, earlier in 2020, WHO observed that *A. gambiae* has high indoor resting density and typically long lived mosquitoes connected with stable malaria in equatorial Africa. The high indoor resting density of the anopheline mosquitoes in the present study was perhaps an outcome of favorable ecological conditions, good breeding environment (presence of conducive habitats) and may probably be attributed to high malaria incidence in this part of the state. Nevertheless, the result was lower than the total average indoor resting density recorded by Dash et al. [21] which was (46.32/man hr) in the region. Separately, a very high average density for *Anopheles* and *Culex* which was 30.80 and 12.95, respectively was reported by Kulkarni [22] in the study area. Furthermore, Indoor resting density of anopheline species was 66.79 as reported by Sharma [23] who observed high density (11.3–125.1) of *A. culicifacies* in their study areas where the average density was high in August–September and February–March 2003, declined gradually and was lowest in summer season. This report concurred with the previous reports of Kulkarni, [24] and Mahesh et al., [25] where several of the climatic factors viz. rainfall, humidity and temperature and biological activities like housing style, pattern of villages, larval breeding grounds, socioeconomic conditions and adopted control measures are found to be the regulatory factors of indoor mosquito density in the study areas.

Awka North LGA recorded the highest HBR of 6.35 followed by Njikoka LGA which had the HBR of 5.21 while Awka South recorded the least HBR of 3.58. The average HBR in the 3 LGAs was found to be 5.05. The result is lower than the result study done by Oljira et al., (2016) who reported an overall (indoor and outdoor combined) mean human-biting rate (HBR) of *Anopheles* mosquitoes of 85.2 mosquitoes/ person/night (m/p/n). The total (indoor and outdoor combined) mean HBRs for *An. zeimanni* was 56.7, *An. arabiensis* was 21.1. The mean hourly human-biting density of *An. arabiensis* was greater outdoors than indoors and peaked between the months of January and March. However, *An. arabiensis* parous population showed high indoor man biting activities during February and also during bedtimes (22:00 to 05:00 h) when the local people were indoor and potentially protected by IRS and LLINs. The finding in the present study was consistent with previous reports by (Abose et al*.,* 1998) [26] where *Anopheles gambiae* was more exophagic than *Anopheles coluzzi* which appeared to have less chance to come in contact with LLINs and IRS. This might have favored *Anopheles gambiae*, while *Anopheles coluzzi* population was affected by IRS and LLINs interventions. Results from this study showed that mosquito human-biting activities occurred both outdoors and indoors and during early parts of the night, implying higher outdoor malaria transmission potential in the area. However, high bedtime (22:00 to 05:00 h) indoor biting activities of parous *Anopheles gambiae* and *Anopheles coluzzi* suggest high potential intervention impact of IRS and LLINs on indoor malaria transmission. The scale up of IRS and LLINs during the last decade has substantially reduced malaria incidence in many parts of the study areas as reported by D'Acremont et al., [27] and Mharakurwa et al., [28]. These interventions reduce the density, feeding frequency and longevity of malaria vectors by killing the vectors with insecticides or blocking their contact with humans [29,30] and primarily target malaria vectors that feed indoors and at night on sleeping humans [31].

Table 1. Vector composition and abundance of *Anopheles* species collected from Awka South, Awka North and Njikoka local government areas in Anambra state

Sex of *Anopheles* species	Awka South (%)	Awka North (%)	Njikoka (%)	Total (%)
Male	412 (21.1)	540 (27.7)	997 (51.2)	1949 (67.9)
Female	329 (35.7)	311 (33.8)	281 (30.5)	921 (32.1)
Grand total	741 (25.8)	851 (29.7)	1278 (44.5)	2870 (100)

χ^2=119.125, df=2, Pv=0.000

Table 2. Determination of Indoor Resting Density (IRD) of malaria vectors in Awka South, Awka North and Njikoka LGA, Anambra state

		Number of Indoor Anopheline Mosquitoes Collected														
LGA/	**T No. of Rooms sprayed per month**	**SEP**	**OCT**	**NOV**	**DEC**	**JAN**	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**T. number of mosquitoes collected**	**T. number of rooms sprayed**	**IRD**
AWKA SOUTH																
	36	72 (12.2)	123 (20.9)	15 (2.5)	13 (2.2)	52 (8.8)	41 (7.0)	76 (12.9)	31 (5.3)	20 (3.4)	24 (4.1)	37 (6.3)	85 (14.4)	589 (100)	432	1.36
AWKA NORTH	36	17 (2.7)	30 (4.8)	11 (1.8)	27 (4.3)	100 (16.1)	35 (5.6)	43 (6.9)	51 (8.2)	21 (3.4)	120 (19.3)	85 (13.7)	81 (13.0)	621 (100)	432	1.44
NJIKOKA	36	55 (10.7)	46 (9.0)	14 (2.7)	12 (2.3)	18 (3.5)	58 (11.3)	51 (9.9)	19 (3.7)	25 (4.9)	49 (9.6)	85 (16.6)	81 (15.8)	513 (100)	432	1.39
TOTAL		144 (8.4)	199 (11.5)	40 (2.3)	52 (3.0)	170 (9.9)	134 (7.8)	170 (9.9)	101 (5.9)	66 (3.8)	193 (11.2)	207 (12.0)	247 (14.3)	1723 (100)	1296	1.40

χ^2=290.370, df=22, Pv=0.000

Table 3. Determination of Human Biting Rate (HBR) of malaria vectors in Awka South, Awka North and Njikoka local government areas, Anambra state

Months	Awka South LGA				Awka North LGA				Njikoka LGA				
	Average No. of Occupants	Total No. of Mosquitoes Sampled	Average No. of freshly fed *Anopheles spp*	HBR	Average No. of Occupants	Total No. of Mosquitoes Sampled	Average No. of freshly fed *Anopheles spp*	HBR	Average No. of Occupants	Total No. of Mosquitoes Sampled	Average No. of freshly fed *Anopheles spp*	HBR	Average HBR
Sept	3	72	24	8	3	17	0	0	3	55	19	6.33	
Oct	2	123	0	0	4	30	0	0	4	46	45	11.25	
Nov	3	15	3	1	4	11	0	0	3	14	0	0	
Dec	4	13	2	0.5	4	27	1	0.25	4	12	4	1	
Jan	4	52	31	7.75	2	100	87	43.5	3	18	16	5.33	
Feb	3	41	9	3	4	35	12	3	2	58	12	6	
Mar	3	76	33	11	3	43	29	9.67	2	51	39	19.5	
Apr	3	31	5	1.67	3	51	7	2.33	4	19	3	0.75	
May	3	20	3	1	3	21	2	0.67	3	25	3	1	
Jun	2	24	4	2	3	120	15	5	3	49	9	3	
Jul	3	37	6	3	4	85	9	2.25	4	85	12	3	
Aug	3	85	12	4	2	81	19	9.5	3	81	16	5.33	
Total	36	589	132	3.58	39	621	181	6.35	38	513	178	5.21	5.05

R-value = -99.23 (negative correlated at 0.7). Between 0.5 and 1 = strong positive correlation between IRD and HBR recorded

Table 4. Identification of blood meal source in *Anopheles gambiae* and *Anopheles coluzzi* collected indoors in Awka South, Awka North and Njikoka LGA, Anambra state

LGA	T. Collected	Blood Meal Source in *An. gambiae s. l.*				
		N. Human (%)	N. Goat (%)	N. Bovine (%)	N. Human (%) + N. Goat (%)	T. Blood fed (%)
AWKA SOUTH	60	15 (36.6)	14 (34.2)	0 (0)	12 (29.3)	41 (39.4)
AWKA NORTH	60	24 (63.2)	9 (23.7)	0 (0)	5 (13.2)	38 (36.5)
NJIKOKA	60	9 (36.0)	11 (44.0)	0 (0)	5 (20.0)	25 (24.0)
TOTAL	180	48 (46.2)	34 (32.7)	0 (0)	22 (21.2)	104 (57.8)

χ^2=18.408, df=6, Pv=0.005

T= total of mosquitoes; N = number of mosquitoes having taken their blood meal on: Human (H), Goat (G), and Bovine (B)

Table 5. Determination of Human Blood Index (HBI) of malaria vectors in Awka South, Awka North and Njikoka local government areas, Anambra state

LGA	T. Collected	T. Blood fed (%)	N. Human (%)	Human Blood Index (HBI)
Awka South	60	41 (68.3)	15 (36.6)	0.37
Awka North	60	38 (63.3)	24 (63.2)	0.63
Njikoka	60	25 (41.7)	9 (36.0)	0.36
Total	180	104 (57.8)	53 (51.0)	0.51

χ^2=69.35768, df=1, Pv=0.0992

Table 6. Determination of malaria sporozoite / infection rate in Awka South, Awka North and Njikoka local government areas, Anambra state

LGA	T. Collected	*Anopheles gambiae* (%)	*Anopheles coluzzii* (%)	T. Blood fed / Processed (%)	Positive for Sporozoite (%)	Sporozoite Rate (%)
Awka South	80	45 (56.3)	35 (43.8)	41 (51.25)	0 (0)	0 (0)
Awka North	80	61 (76.3)	19 (23.8)	38 (47.50)	0 (0)	0 (0)
Njikoka	80	24 ()30.0	56 (70)	25 (31.25)	0 (0)	0 (0)
Total	240	130 (54.2)	110 (45.8)	104 (43.33)	0 (0)	0 (0)

Sporozoite rate = number positive/ number processed (WHO, 2013)

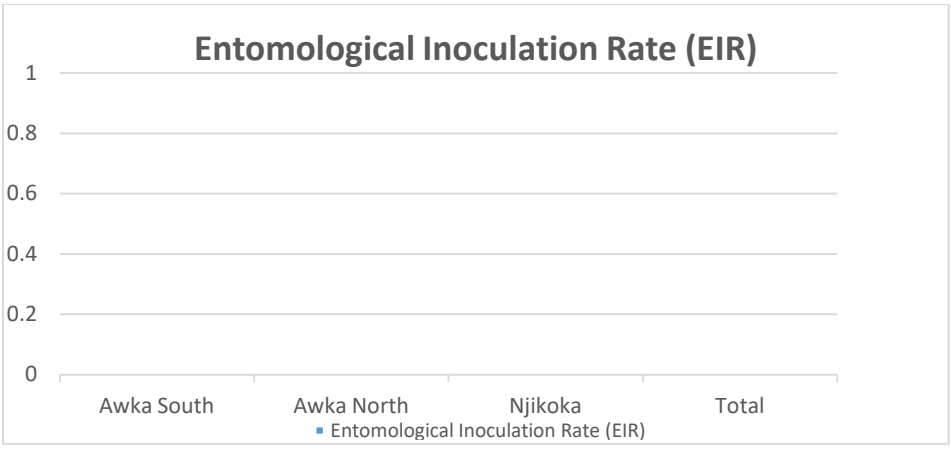

Fig. 2. Determination of EIR of malaria vectors in Awka South, Awka North and Njikoka local government areas, Anambra State

Since female mosquitoes require blood meals for their egg maturations, it is very crucial that the blood meal source of *Anopheles gambiae* and *Anopheles coluzzi* in the current study be identified. The blood meal source of malaria vectors in the study were found to be goat, human, combination of human and goat, but no malaria vector had bovine blood meal source. Human blood source recorded the highest number with 46.2% (48/104) followed by goat blood source 32.7% (34/104), whereas (human and goat) recorded the least number of 21.2% (22/104). Awka South LGA had the highest number of blood fed malaria vectors 39.4% (41/104) followed by Awka North 36.5% (38/104) and Njikoka respectively 24.0% (25/104). The result was in congruent with the result of another study done by Mwanga et al*.,* [32] who used the logistic regression (LR) model to achieve the highest accuracy, correctly predicting true vertebrate bloodmeal sources with overall accuracy of 98.4%. It was reported that the model correctly identified 96% goat blood meals, 97% of bovine blood meals, 100% of chicken blood meals and 100% of human blood in *Anopheles arabiensis*. The study was also in line with that of Kent and Norris, [33] who reported a total of 194 blood-fed *An. arabiensis* (identified by PCR) that were collected by indoor resting collections and tested for vertebrate host blood source (human, bovine, goat, dog), the overall proportion of *An. arabiensis* that fed on humans (including mixed blood meals on both human and animal) was 59.3% (115/194), with cattle blood being the most common non-human source. An estimated 32.5% (63/194) of *An. arabiensis* fed on both human and animals, demonstrating opportunistic feeding behaviour, while only 26.8% fed only on humans [34]. The figure for blood meal source in this study was very high and this probably accounts for high malaria prevalence in the area. Earlier, [35] observed that *Anopheles gambiae* has high human blood index, and are typically long-lived mosquitoes connected with stable malaria in equatorial Africa.

The Human Blood Index (HBI) represents the proportion of blood meals derived from humans by mosquito vectors and may be used to estimate the human biting habit, an important component of vectorial capacity, as a proxy measure of malaria transmission. From the result of current study, it was observed that the HBI of malaria vectors in Awka South LGA was 0.37 (15/41), 0.63 (24/38) in Awka North and 0.36 (9/25) in Njikoka LGAs. Moreover, the overall HBI in the study was 0.51 was almost the same when compared with other results from previous studies. Kent and Norris, [33] reported that the overall human blood index in their study was 0.62 whereas [36] recorded the HBI of 0.55 in their study. Marco Pombi et al., 2018 in their study noted that low values of human blood index (0.12) observed in the major malaria vectors in the area (*Anopheles coluzzii*: N = 263, 20.1%; *An. arabiensis*: 5.8%, N = 103) are consistent with the hypothesis that LLINs reduced the availability of human hosts to mosquitoes. The result was also in line with the report of the study done by Vasiliki et al., [37], who reported that the majority of blood meals were derived from humans. Four mosquitoes took mixed blood meals (human and dog), and one took a blood meal derived from dog and the crude Human Blood Index (HBIs) among the different mosquito species ranged from 0.56 to 1, whereas all adjusted HBIs were equal to 1. This shows that *Anopheles gambiae* is widely regarded as a highly anthropophilic and domestic mosquito vector [38]. However, some studies reported that *An. gambiae* preferentially feeds on dogs or cattle over human hosts [39,40]. The zoophily of these vectors could be explained as a response to indoor residual spraying (IRS) interventions combined with the use of long-lasting insecticide-treated nets (LLINs) [41]. The insecticides used in such interventions have excito-repellent effects that drive host-seeking mosquitoes outdoors, where they may come into contact with non-human hosts. It may be that, as a consequence of indoor-based antivector interventions, host-seeking mosquitoes are diverted to seek blood meals in outdoor venues, where a greater diversity of non-human hosts may be encountered and fed on. The high degree of anthropophily observed in the present study was consistent with the findings reported in other studies [42-46]. Although some studies report zoophily in Afro-tropical vector populations [47], although such behaviors were not detected. In these situations, ongoing antimalarial interventions may not be enough to divert malaria vectors to non-human hosts. Different results may also be attributed to behavioral differences in local vector population or differences in collection methods. A high degree of anthropophily in the vector population was observed in this study and this suggests that LLINs and IRS activities may not be sufficient to reduce the HBI because of a variety of factors, including diminished residual insecticidal activity and household coverage and lack of available alternative vertebrate hosts. And this call into

question the appropriateness of current methods of assessing host preferences among disease vectors and have important implications for strategizing vector control.

The epidemiological significance of *Anopheles* as malaria vectors is defined by a range of factors, the most important among them including: the rate of *Anopheles* species susceptibility to infection by human plasmodia, the duration of sporozoites' preservation in salivary glands of the mosquitoes, the species numbers, and the degree of relationship with man and the individual life span of the mosquito. In the present study, a total of 130 *Anopheles gambiae* and 110 *Anopheles coluzzii* from Awka South, Awka North and Njikoka LGAs were tested for the presence of CSP of *P. falciparum*. However, none was found positive for sporozoite, which has a significant implication for malaria control and intervention. The result was in line with another report of the study done by Ledayane et al*.,* [48] who also recorded that using the PCR technique, no *Anopheline* was found infected with the malaria parasite**.** But in contrast with the report of the study done by Burkot et al*.,* [36] who reported that between 2016 and 2017, the overall *P. falciparum* sporozoite rate across all sites for all *Anopheles* (*An. funestus*, *An. arabiensis*, *An. gambiae*, and *An. parensis*) combined was estimated as 1.72% (286/16,670). Marco Pombi et al., [49] in their study noted that a regression meta-analysis of data from a systematic review of published studies reporting HBI and sporozoite rates (SR) for *An. gambiae* complex revealed that the observed SR values (*An. coluzzii*: 7.6%, N = 503; *An. arabiensis*: 5.3%, N = 225) are out of the ranges expected based on the low HBI observed. Sporozoite rates of *An. coluzzii* females were higher for the human-fed sub-sample (22.9%; N = 35) than for animal-fed (8.4%; N = 143) or unfed-gravid subsamples (5.5%; N = 200), as well as for the sub-sample of fed-females with unidentified blood-meals (5.6%; N = 125). This suggests that females that have already taken a blood-meal on an (infected) human host and/or that are ready to transmit the malaria parasite, have a higher tendency to bite human again [50-52]. It is believed that *Plasmodium* can potentially affect the complex mosquito host-seeking behaviour due to neurophysiological mechanism, based on changes in the responsiveness of mosquito odorant receptors [53]. Thus, the higher SR observed in human-fed *A. coluzzii* could be due to a significant increase in infected mosquito attraction to human odours triggered by the parasite itself in order to enhance its chances of transmission to the appropriate host [50,51]. Entomological inoculation rate (EIR) is the product of man biting rate and sporozoite rate. In the present study, none of the malaria vectors were found to be positive for CSP of *P. falciparum* (sporozoite). For this reason, the EIR could not be determined and compared among the study populations [54-56].

4. CONCLUSION

The findings of entomological data of malaria vectors is necessary to check for vigilance of local populace to the disease and equally generate more information regarding the management and complete elimination of malaria vectors in the study area. So assessing the entomological parameters of malaria vectors has significant gaps in interpretation to malaria eradication. To this effect, the allopatric breeding of the two sibling species of *Anopheles gambiae* and *Anopheles coluzzii* reported in this study was probably the second of such report in Nigeria and therefore has huge implications for malaria control and vector interventions in Anambra State, Nigeria. As was observed in the present study, the IRD from the 3 LGAs was very high when compared to the WHO standard IRD of 0.02 bites per man per night required for maintaining transmission in an area. Also, the BMS in *Anopheles gambiae* and *Anopheles coluzzi* collected indoors implicated human, goat, and a combination of human and goat blood meal source, whereas there was no record for bovine blood meal source. Furthermore, the HBI in this study presented a high degree of anthropophily in the vector population which suggests that IRS activities alone may not be sufficient to reduce the HBI because of a variety of factors, including but not limited to diminished residual insecticidal activity and household coverage alongside lack of available alternative vertebrate hosts. This call into question the appropriateness of current methods of assessing host preferences among disease vectors and have important implications for strategizing vector control. The HBR of malaria vectors across the three LGAs was high in the present study and this might have favored *Anopheles gambiae* and *Anopheles coluzzi* population. In this study, no *Anopheles gambiae* and *Anopheles coluzzii* tested for the presence of CSP of *P. falciparum* was found positive and the EIR could not be decided because none of the *P. falciparum* mosquitoes were found to be positive for sporozoite. This shows that preventive

measures and eradication of malaria vectors by various methods is not only imperative or for academic knowledge but also urgent so to attain malaria free status like some countries have already done.

5. RECOMMENDATIONS FOR FUTURE RESEARCH

The study therefore recommends that regular studies on the ecology, bionomics, development, emergence and evolution of mosquito vectors and the impact of human component of mosquito vector ecology which is a crucial components for sustainable long term disease management, control, eradication or elimination should not only be supported but promoted. Furthermore, it is not clear whether changes in mosquitoes' avoidance behaviour act as an ecological obstacle is geared towards limiting the effectiveness of vector control. To this regard, a regular study to understand the behavioural ecology of malaria vectors will help to develop tailor-made vector control strategies to generate sustainable control effects.

DATA AVAILABILITY

The data used to support the findings of this study is available upon reasonable request.

CONSENT AND ETHICAL APPROVAL

Advocacy visits were carried out first to the heads in charge of state ministry of health department through the local government chairman of the selected LGAs, then to the community leaders/president general of the selected communities and finally to the households/individuals that will participate in the study. The advocacy visit was followed up with a letter of introduction obtained from the Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka that assisted to obtain cooperation from the study participants. Approval and informed consent of the participants for the study was obtained before the commencement of the study and the identity of the participants were anonymous during and after the survey.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Center for disease Control. *Anopheles* mosquitoes. Global Health Division of parasitic diseases and malaria; 2015.
2. Autino IJ, Andersen FS. The negative binomial distribution and the sampling of insect populations. Proceedings of the 12th International Congress of Entomology. 2012;395:395-399.
3. Gillies MT, Coetzee M. A supplement to the *Anophelinea* of Africa south of the Sahara (Afrotropical region). South African Institute for medical Research, Johannesburg. 1987;55:143.
4. Scott EB, Chareonviriyaphap T, Coetzee M, Mbogo CM. A global map of dominant malaria vectors. Parasitology Vectors. 1993;(5):69.
5. Kirby MJ, Green C, Milligan PM, Sismanidis C, Jasseh M, Conway DJ, Lindsay SW. Risk factors for house-entry by malaria vectors in a rural town and satellite villages in The Gambia. Malaria Journal 2018;7:2-10.
   DOI: 10.1186/1475-2875-7-2
6. Smith D, Battle K, Hay S, Barker C, Scott T, McKenzie F. Ross, Macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens. Plos Pathogen. 2012;8:e1002588.
   DOI: 10.1371/journal.ppat.1002588.
7. Lindsay EB, Marbiah NT, Maude G, Curtis CF, Bradley DJ, Greenwood BM, Petersen E, Lines JD. Effects of community-wide use of lambdacyhalothrin-impregnated bednets on malaria vectors in rural Sierra Leone. Medical and Veterinary Entomology. 2018;11:79–86.
8. Onori OD, Conn JE. The Distribution of Two Major Malaria Vectors, *Anopheles gambiae* and *Anopheles arabiensis*, in Nigeria. Mem Inst Oswaldo Cruz, Rio de Janeiro. 2001;96(8):1081±1084.
9. Microsoft Encarta College Dictionary and Encyclopedia. New York: St. Martin's Press. 2009:276.
   Access on 23 March, 2020
10. National Bureau of Statistics. National Population Commission 2006 Nigeria Census; 2016.

11. Enete IC. Managing aviation operations during harmattan in Nigeria for maximum operations. Inpritx Lagos State, Nigeria. 2008:12-23.
12. Enete IC. A study of Enugu rainfall patterns from the viewpoint of precipitation dynamics. “Nigeria Journal of Research and Production”, Nigerian Researchers Forum Enugu. 2004;5(4):98-108.
13. National Population Commission. Nigerian Malaria Indicator Survey; Key Indicators survey in 2017. Descriptive Support System. 2016:1–37.
14. World Health Organization. Entomological field techniques for malaria control part I learners guide. World Health Organization, Geneva; 1992.
15. World Health Organization. Antimalarial drug resistance; 2015. Available:http://www.who.int/malaria/areas/drug_resistance/overview/en/ Access on March 11, 2021).
16. Abdoon JK, Alshahrani S. Age-dependent acquired protection against *Plasmodium falciparum* in people having two years exposure to hyperendemic malaria. American Journal of Tropical Medicine and Hygiene. 2003;45:65-76.
17. Curtis WG. A distribution map of the member species of *Anopheles gambiae* complex. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2003;61:454.
18. Gillies MT, Meillon DB. The Anophelinae of Africa South of the Sahara. Publications of the South African Institute for Medical Research. 1968;15:1-343.
19. Wilkins RU, Nicholas T, Fontenille D. Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome B. American Journal of Tropical Medicine and Hygiene. 2006;3:36–42
20. Keven JB, Michelle K, Rebecca V, Gussy K, Naomi V, Edward K, Thomsen SK, Lisa J, Reimer DW. Species abundance, composition, and nocturnal activity of female *Anopheles* (diptera: Culicidae) in malaria‐endemic villages of Papua New Guinea: Assessment with barrier screen sampling. Malaria Journal. 2019;18:96.
21. Dash AP, Hazra RK, Mahapatra N, Tripathy HK. Disappearance of malaria vector *Anopheles sundaicus* from Chilka lake area of Orissa state in India. Medical Veterinary and Ecology. 2000;14:445–449.
22. Kulkarni SM. Density pattern of anophelines and their relation to malaria in Bastar district, Madhya Pradesh. Journal of Malariology. 1990;27:187–194.
23. Sharma VP. Fighting malaria in India. Current Science. 1998;75:1127–1140.
24. Kulkarni SM. Density pattern of anophelines and their relation to malaria in Bastar district, Madhya Pradesh. Journal of Malariology. 2019;27:187–194.
25. Mahesh RK, Jauhari RK. Seasonal abundance of vector anophelines in Doiwala area of Doon Valley, Uttaranchal. Journal of Parasitology and Applied Animal Biology. 2004;13:65–70.
26. Krafsur CS. The bionomics and relative prevalence of Anopheles species with respect to the transmission of *Plasmodium* to man in western Ethiopia. Journal of Medical Entomology. 2017;14:180–94.
27. D’Acremont V, Lengeler C, Genton B. Reduction in the proportion of fevers associated with *Plasmodium falciparum* parasitaemia in Africa: A systematic review. Malaria Journal. 2010;9:240.
28. Mharakurwa S, Mutambu SI, Mberikunashe J, Thuma PE, Moss WJ, Mason PR. Changes in the burden of malaria following scale up of malaria control interventions in Mutasa District, Zimbabwe. Malaria Journal. 2013;12:223.
29. Mutuku FD, King CH, Mungai P, Mbogo C, Mwangangi J, Muchiri EM. Impact of insecticide-treated bed nets on malaria transmission indices on the south coast of Kenya. Malaria Journal. 2011;10:356.
30. Gimnig JE, Vulule JM, Lo TQ, Kamau L, Kolczak MS, Phillips-Howard PA. Impact of permethrin-treated bed nets on the entomologic indicies in an area of intense year-round malaria transmission. American Journal of Tropical Medicine and Hygiene. 2013;68:115–20.
31. World Health Organization. Control of residual malaria parasite transmission: Guidance note. WHO Geneva. 2014;12:222-267.
32. Mwangan EP, Salum A, Mapua DJ, Siria HS, Ngowo FN, Joseph M, Francesco B, Mario GJ, Heather M, Ferguson KW, Prashanth S, Simon AB, Fredros OO. Using mid‐infrared spectroscopy and supervised machine‐learning to identify vertebrate blood meals in the malaria vector, *Anopheles arabiensis.* Malaria Journal. 2019;18:187.

33. Kent WH, Norris SM. Density pattern of anophelines and their relation to malaria in Bastar district, Madhya Pradesh. Journal of Malariology. 2005;27:187–194.
34. Chow E, Wirtz RA, Scott TW. Identification of blood meals in *Aedes aegypti* by antibody sandwich enzyme-linked immunosorbent assay. Journal of American Mosquitoes Control Association. 1993;9:196–205.
35. World Health Organisation. Vector control for malaria and other mosquito-borne diseases. Technical Report. 2020:23-33.
36. Burkot TR, Dye C, Graves PM. An analysis of some factors determining the sporozoite rates, human blood indexes, and biting rates of members of the *Anopheles punctulatus* complex in Papua New Guinea. American Journal of Tropical Medicine and Hygiene. 1989;40:229–34.
37. Vasiliki P, Michael R, Hans J, Overgaard SA, Adalgisa C. Estimation of the human blood index in malaria mosquito vectors in equatorial Guinea after indoor antivector interventions. American Journal of Tropical Medicine and Hygiene. 2011;84(2):298–301.
DOI: 10.4269/ajtmh.2011.10-0463
38. Muirhead-Thomson RC. Mosquito behaviour in relation to malaria transmission and control in the tropics. London: Edward Arnold & Co. 1957:56–70.
39. Sousa CA, Pinto J, Almeida AP, Ferreira C, do Rosario VE, Charlwood JD. Dogs as a favored host choice of *Anopheles gambiae* sensu stricto (diptera: Culicidae) of São Tomé West Africa. Journal of Medical Entomology. 2001;38:122–125.
40. Duchemin JB, Leongpocktsy JM, Rabarison P, Roux J, Coluzzi M, Costantini C. Zoophily of *Anopheles arabiensis* and *An. gambiae* in Madagascar demonstrated by odour-baited entry traps. Medical and Veterinary Entomology. 2001;15:50–57.
41. Teklehaimanot HD, Teklehaimanot A, Kiszewski A, Sacramento Rampao H, Sachs JD. Malaria in São Tomé and Principe: on the brink of elimination after 3 years of effective antimalarial measures. American Journal of Tropical Medicine and Hygiene. 2009;80:133–140.
42. Githeko AK, Service MW, Mbogo CM, Atieli FK, Juma FO. Origin of blood meals in indoor and outdoor resting malaria vectors in Western Kenya. Acta Tropica. 1994;58:307–316.
43. Okwa OO, Akinmolayan FI, Carter V, Hurd H. Transmission dynamics of malaria in four selected ecological zones of Nigeria in the rainy season. Annals of African Medicine. 2009;8:1–9.
44. Wanji S, Tanke T, Atanga SN, Ajonina C, Nicholas T, Fontenille D. *Anopheles* species of the mount Cameroon region: Biting habits, feeding behaviour and entomological inoculation rates. Tropical Medicine and International Health. 2003;8:643–649.
45. Tanga, MC, Ngundu WI, Judith N, Mbuh J, Tendongfor N, Simard F, Wanji S. Climate change and altitudinal structuring of malaria vectors in South-Western Cameroon: Their relation to malaria transmission. Transactions of Royal Society of Tropical Medicine and Hygiene. 2010;104:453–460.
46. Magbity EB, Marbiah NT, Maude G, Curtis CF, Bradley DJ, Greenwood BM, Petersen E, Lines JD. Effects of community-wide use of lambdacyhalothrin-impregnated bednets on malaria vectors in rural Sierra Leone. Medical and Veterinary Entomology. 1997;11:79–86.
47. Silver JB. Mosquito ecology: Field sampling methods. New York, Springer. 2018:372–492.
48. Ledayane MC, Barbosa RN, Picanc S, Ricardo S, Anjos F, Margarete S. Behavioral patterns, parity rate and natural infection analysis inanopheline species involved in the transmission of malaria in the northeastern Brazilian Amazon region. Acta Tropica. 2016;164:216–225.
49. Marco P, Maria C, Wamdaogo M, Guelbeogo MM, Eleonora P, Verena P, Emiliano M, N'Fale S, Hilary R, Alessandra della T. Unexpectedly high *Plasmodium* sporozoite rate associated with low human blood index in *Anopheles coluzzii* from a LLIN-protected village in Burkina Faso. Scientific Reports. 2018;8(2018):12806.
50. Koella Koekemoer LL, Spillings BL, Christian RN, Lo T-CM, Kaiser ML, Norton RAI. Multiple insecticide resistance in *Anopheles gambiae* (diptera: Culicidae) from Pointe Noire, Republic of the Congo. Vector Borne Zoonotic Diseases. 1998;11:1193–2000.
51. Nyasembe VO. *Plasmodium falciparum* infection increases *Anopheles gambiae* attraction to nectar sources and sugar uptake. Current Biology. 2014;24:217–21.

52. Smallegange RC. Malaria infected mosquitoes express enhanced attraction to human odor. Plos One. 2013;8:8–10.
53. Cator LJ. 'Manipulation' without the parasite: altered. Proceedings of Biological Sciences. 2013;65:73–78.
54. Autino B, Noris A, Russo R, Castelli F. Epidemiology of malaria in endemic areas. Meditter Journal Hematology Infectious Disease. 2012;4(1):60.
55. Onori E, Grab B. Indicators for the forecasting of malaria epidemics. Bull World Health Organization. 1980;58: 91-98.
56. World Health Organization. World malaria report, Geneva. 2016:280.

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