



ISSN: 2348-5906  
CODEN: IJMRK2  
IJMR 2016; 3(5): 31-35  
© 2016 IJMR  
Received: 06-07-2016  
Accepted: 07-08-2016

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## Malaria transmission indices of two dominant anopheles species in selected rural and urban communities in Benue state North Central, Nigeria

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### Abstract

An entomological survey was piloted to generate baseline entomological data in some communities in Benue State where such information was not previously available. Indoor resting mosquitoes were captured using Pyrethrum Spray Catch (PSC) in 2015. Of the 1,734 mosquitoes captured 276 (16%), were *Anopheles* species. Molecular assays revealed the presence of *Anopheles gambiae* sensu. stricto and *Anopheles arabiensis*. Human blood meal source was within the range of 97% to 100% clearly expressing a high degree of human-vector contact. Circumsporozoite (CS) proteins infection status of engorged female species were determined by Enzyme Linked Immunosorbent Assay (ELISA). The calculated Sporozoite Infection Rate (SR) for rural was 1.9% and 0% for urban communities respectively. The Entomological Inoculation Rates (EIR) recorded was 0.4% per person per night culminating in an annual 146 infective bites per person per year. Our observed data highlights some malaria risk indices in the study communities.

**Keywords:** Malaria, transmission indices, vectors, rural, urban, Nigeria

### 1. Introduction

Malaria is a preventable and curable but life-threatening disease caused by parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes. These *Anopheles* species are widely distributed in Nigeria across all the ecological zones <sup>[1, 3, 4]</sup> *Anopheles gambiae* and *Anopheles arabiensis* are the two dominant vectors of human malaria in sub-Saharan Africa. They occur in sympatry and are of the greatest medical importance as very efficient malaria vector species <sup>[2, 1, 5]</sup> Four species of the protozoan parasite exclusively transmitted to man by competent anophelines are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium Ovale*, of which *Plasmodium falciparum* is the most pathogenic <sup>[6]</sup>. The main activity of these *anopheline* vectors of malaria that is the basis for this entomological survey is their blood feeding behaviour. Blood sucking arthropods were established as agents of human and animal disease in the last quarter of the 19<sup>th</sup> century <sup>[7]</sup>. Most female *Anopheles* species feed on warm-blooded animals predominantly mammals; anthropophagic ones having a preference for man and zoophilic ones preferring other animals. The blood meal intake and the gonotrophic cycle are intricately related and blood feeding is the channel for parasite acquisition and transfer of malaria infection, <sup>[8]</sup>. This study presents the results of a semi-longitudinal entomological survey highlighting some of the malaria risk indices in the study communities.

According to the latest WHO estimates, released in December 2015, <sup>[9]</sup> there were 214 million cases of malaria in 2015 and 438 000 deaths. Sub-Saharan Africa continues to carry a disproportionately high share of the global malaria burden with 88% of malaria cases and 90% of malaria deaths. It is estimated that 15 countries accounted for 80% of malaria cases and 78% deaths globally. The overall decrease in malaria incidence (32%) between 2000 and 2015 in the 15 countries that accounted for 80% of cases lags behind that in the other countries (53%). <sup>[9]</sup> The Carter Center in 2012 <sup>[10]</sup> reported that approximately 20-30% of all African malaria cases occur in Nigeria and Ethiopia while the WHO reports that both countries now account for more than 35% of the global total of estimated malaria deaths <sup>[9]</sup>.

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Inadmissibly Nigeria now bears the heaviest malaria burden in Africa. Malaria is endemic in Nigeria, with seasonal peaks during the rainy season [10]. This survey was piloted to generate baseline entomological data in some communities in Benue State where such information was not previously available.

## 2. Materials and Methods

### 2.1 Study Area

Fourteen communities in both Gboko (7° 19' 30" N, 9° 0' 18" E) and Otukpo (7° 19' 30" N, 9° 0' 18" E) local government areas of Benue State, north central Nigeria were selected for the study. Seven of the study communities were rural and the inhabitant subsistence farmers while six were classified urban and semi-urban where the inhabitants engage more in secondary economic activities. Based on Koppen climate classification, Benue State lies within the AW (tropical wet & dry) climate and experiences two distinct seasons, the Wet season and the Dry season. The wet-rainy season lasts from April to October with annual rainfall in the range of 100-200mm. The dry season begins in November and ends in March. Benue State experiences temperature fluctuations between 21 – 37 °C in the year. The rural dwellings are traditional ancient mud huts, some partially plastered with cement and roofed with thatched palm fronds or zinc. The urban dwellings were made from local burnt bricks or cement blocks roofed with zinc, or long span aluminium sheets. In all the study communities several domestic animals such as goats, chicken, sheep, dogs, and pigs were sheltered in nearby buildings at nights but roamed about freely during the day. Some communities had even monkeys as not too far from their bedrooms.

### 2.2 Specimen Collection and Preservation

Indoor resting mosquitoes were collected using the Pyrethrum Spray Catch (PSC) between the hours 06:00 and 09:00 am in the study communities. Individual samples were each preserved dry over silica gel in well labelled Eppendorf tubes (1.5ml) prior to identification. This was to ensure preservation of delicate significant features that would be needed for morphological identification in the laboratory. They were then carried to the molecular laboratory at the Nigerian Institute of Medical research, Yaba, Lagos, Nigeria.

2.3 Laboratory analysis of Field Collected Mosquitoes: Morphological identification was carried out using a trinocular dissecting microscope (Amscope SZMT2/MU1000 10APTINA COLOR CMOS) with the aid of standard keys [11-13]. Polymerase Chain Reaction (PCR) assay [14] employed to determine the vector specie compositions. Polymerase Chain-Restriction Fragment Length Polymorphism (PCR-RFLP) [15] was used to detect the molecular forms.

### 2.4 Detection of Sporozoite Infection in the Mosquito Using Elisa

Using a sterile blade and forceps the head and thorax was separated from the rest of the body and homogenised in phosphate buffer (PBS-pH7.8). Then tested for plasmodium circumsporozoite antigen [16] Results were read visually by colour change (19) or at 405-414 nm using an ELISA plate reader 30 and/or 60 minutes after the substrate has been added.

2.5 Entomological Inoculation Rates (EIR): Determination of the Entomological Inoculation Rates (EIR) necessitated two

other measurements, the sporozoite rate and the human biting rate [17] as shown below:

$$\text{Sporozoite Rate} = \frac{\text{Number of mosquitoes with sporozoites}}{\text{Number of females examined}} \times 100$$

$$M = \frac{F}{W}$$

F=total number of freshly fed mosquitoes of a particular species  
w=total number of human occupants in houses used for collection  
M is human biting rate

$$\text{EIR} = \frac{\text{Human biting rate} \times \text{sporozoite rate (\%)}}{100}$$

**EIR** is Entomological Inoculation Rate

### 2.6 Determination of Blood Meal Source Using ELISA

Blood meal source was determined using the direct Enzyme-Linked Immunosorbent Assays (ELISA) method. The remaining mosquito abdomen from the specimen preserved over silica gel was homogenized in 1.5ml Eppendorf tube containing 50 µl Phosphate Buffer Solution (PBS) (0.01M, pH=7.4) A dilution solution of the mosquito titrate (1:50) was used to coat the microliter wells, the plates were covered and incubated at room temperature for 1:30minutes. The wells were washed twice with PBS after incubation and strained over neat batches of tissue folds. Fifty (50 µl) of prepared enzyme conjugate was added. The primary antibody was Affinity purified Antibody to human IgG (H+L) and the secondary antibodies was Peroxidase labelled Affinity Purified Antibody to human IgG (H+L) both in the ratio of 1:5 each. This was incubated for 30 minutes and snapped.

During this study the Molecular Entomology Laboratory, Public Health Division of the Nigerian Institute of Medical Research, Yaba, Lagos provided standard DNA obtained from known specimens that we used as our positive controls and the Standard 1-Kb plus ladder used confirmed the sizes of the amplified products.

### 2.7 Ethical consideration

Advocacy visits were conducted and verbal informed consent sort and gotten from community and family heads at the onset of indoor collections. Written local government consents were gotten and used to drive confidence levels and authenticate research work in the selected communities. Mosquito nets were bought and distributed to households without nets or inadequate number of nets for free.

### 2.8 Data Analysis

Data obtained was analysed using R Console software version 3.2.2. Welch two sample t-test was used to compare the indoor resting density of *Anopheles gambiae* s. l. in relation to sites. Also, two sample t-test was used to compare human biting rate in relation to sites. Pearson's Chi-square test was used to compare the proportion of the sporozoite rate as well as entomological inoculation rate in relation sites. The P-value < 0.05 was considered statistically significant.

## 3. Results

A total of two hundred and forty (240) *Anopheles* mosquitoes were analysed using sandwich ELISA. Out of these only one (OOL -AU 222 -research identification code) tested positive to the *Plasmodium falciparum* circumsporozoite antigen. The sporozoite rate calculated for *Anopheles gambiae* s.s captured

in August 2015 at Olahimu a rural community in Otukpo local government area was 1.9% for the compromised female *Anopheles*. The sporozoite rate of *An. gambiae* s. l. in relation to study sites showed no significant difference ( $\chi^2 = 1.7322 \times 10^{-27}$ , df = 1, P = 1, Tables 1, & 2). The human biting rate of *An. gambiae* s. l. which ranged from 1.0 to 2.1 bites per night per person showed a significant difference (t = 2.6488, df = 12, P = 0.02122 (Figure 1) in relation to study sites. The Entomological Inoculation Rate (EIR) was calculated per person per night and had (0.4) culminating in 146 bites per

year per person for the study community that had one infective mosquito. The Entomological Inoculation rate of *An. gambiae* s. l. in relation to study sites showed no significant difference ( $\chi^2 = 0.4$ , df = 1, P = 0.5271, Table 3) The blood meal sources of both the *Anopheles gambiae* s.s. and *Anopheles arabiensis* were analyzed to determine their host preference. About 97.9 to 100% had human blood meal including the four *Anopheles arabiensis* (Figure 2) that were captured with engorged abdomen.

**Table 1:** Sporozoite Infection Rates of female *Anopheles* Species in Selected Communities of Gboko and Otukpo Local Government Areas of Benue State in 2015.

Name of Community	LGA	Location Index	Number tested	Number positive	Sporozoite % rate
Chember-Ipav	Gboko	Rural	47	0	0
Vende	Gboko	Rural	45	0	0
Yiase	Gboko	Rural	12	0	0
Mbayande	Gboko	Rural	16	0	0
Otukpo-Icho	Otukpo	Rural	2	0	0
Otobi	Otukpo	Rural	0	0	0
Otada	Otukpo	Rural	28	0	0
Olahimu	Otukpo	Rural	54	1	1.9
Total			204	1	1.9
GRA-Gboko	Gboko	Urban	2	0	0
Genyi	Gboko	Urban	2	0	0
Mkar	Gboko	Urban	0	0	0
GRA-Otukpo	Otukpo	Urban	2	0	0
Sabon-gari	Otukpo	Urban	0	0	0
Efa-Epu (Babylon)	Otukpo	Urban	4	0	0
Total			10	0	0

**Table 2:** Sporozoite rate of *Anopheles gambiae* s. l. between urban and rural sites in Gboko and Otukpo Local Government Areas of Benue State in 2015

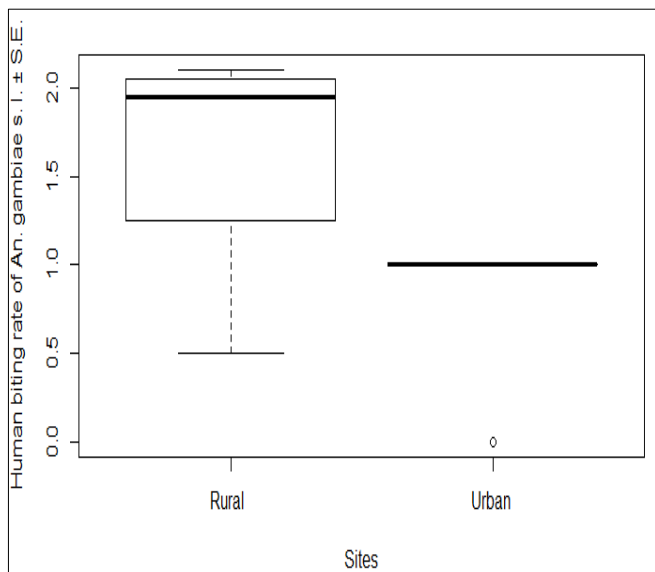
Site	No. analyzed	No. infected	No. uninfected
Rural	204	1	203
Urban	10	0	10
Total	214	1	214

$\chi^2 = 1.7322 \times 10^{-27}$ , df = 1, P = 1

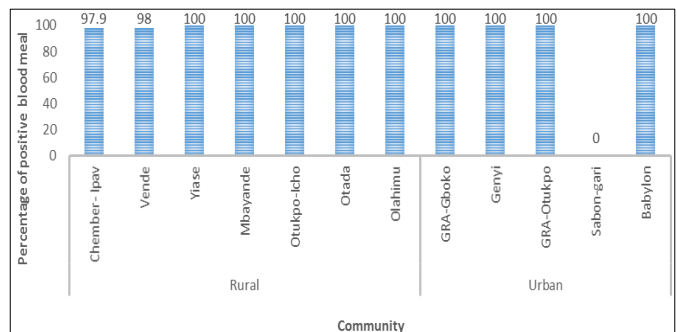
**Table 3:** Entomological Inoculation rate of *Anopheles gambiae* s. l. between urban and rural sites in Otukpo Local Government Area of Benue State in 2015

Site	Entomological Inoculation Rate
Rural	0.4
Urban	0
Total	0.4

$\chi^2 = 0.4$ , df = 1, P = 0.5271



**Fig 1:** Human biting rate of *Anopheles gambiae* s. l. in relation to sites in Gboko and Otukpo Local Government Areas of Benue State in 2015.



**Fig 2:** Human Blood-Meal Source for *An.gambiae* s.l. from Gboko and Otukpo Local Government Areas of Benue State in 2015.

#### 4. Discussion

Polymerase Chain Reaction (PCR) assays in a previous study revealed *An. gambiae* sensu stricto and *An. arabiensis* as the major vector species present in the study communities with *Anopheles gambiae* s.s having a much higher density (P= 0.015) than *Anopheles arabiensis* [18]. In sub-saharan African (SSA) *Anopheles gambiae* s.s and *Anopheles arabiensis* are listed among the most effective and efficient dominant vector

species (DVS) of human malaria [5, 13] The anthropophagy of these two competent malaria vectors is well documented by Awolola and co-authors [19, 20] This research went further to highlight some transmission indices of these very important malaria vector species. In all the study communities these vectors had human blood meal within the range of 97% to 100% clearly expressing a high degree of human-vector contact. These mosquitoes preferred human host to other vertebrate (goats, pigs, dogs, chicken, sheep) host present similar to other reports [21, 22] This extreme preference for human blood meal is a specialization that allows them sustain high levels of transmission in Africa than elsewhere [23]. In effect they showed a remarkable degree of responsiveness to human host cues such as carbon dioxide, odour and warmth [24, 25]. Further insight into the *Anopheles* anthropophilic specialization is the fact that the mean duration of exploratory period recorded for *Anopheles* species is about 16 s which is the highest so far compared to that of three species of *Aedes* which is between 3 to 11 s [7].

Beier, Killeen and Githure [26] have stated that the intensity of malaria parasite transmission is normally expressed as the Entomologic Inoculation Rate (EIR) and that in Africa it is highly variable ranging from <1 to > 1,000 infective bites per person per year. The sporozoite rate of 1.9% and the entomological inoculation rates of 0.4% per person per night recorded in this study are within range and in agreement with available literature [27-30]. This one infective female that is compromised with the *plasmodium* parasite can put the entire human population in the study community at risk of malaria disease. It is necessary to note that the values of our transmission indices here is only a reflection of the captured species. It is known that control measures especially long term use of insecticides in malaria vector control has been shown to alter the behaviour of vectors. Such behavioural shifts have the potential of undermining the effectiveness of insecticide-based control interventions [31]

An attempt has been made by this study to provide baseline information on the anthropophagic mosquito which indicates the need to develop and implement effective vector control strategies in the study areas.

## 5. Recommendations

Malaria is a focal disease with peculiar challenges that may be localized. Our study has revealed the presence of very competent malaria vector species and their interaction with man. Our recommendations at this point will be as follows:

1. Vector control should be targeted at the vector species that have been accurately identified for effective control and judicious use of scarce or limited resources.
2. Zooprophyllaxis for *Anopheles arabiensis* will not be effective for these communities at this point since they were all anthropophagic.
3. The higher vector species density in the rural communities suggest the need for more deliberate malaria control efforts in these areas. Farmers should be educated on personal hygiene to reduce intense dour which is an attractant to the mosquitoes.
4. The housing types in which the rural dwellers live in should be researched further in order to help build malaria out, as presently their houses confer no real protection against these competent vector species.

## 6. Competing interests

We declare that there is no conflict of interest with respect to this study

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