

## **Sporozoite infection and Entomological Inoculation Rate as a Measure of Endemic Malaria Transmission**

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**Abstract:** *The study was conducted in Kontagora town of Niger State, North Central Nigeria. Sporozoite Infection Rate (SR) and Entomological Inoculation Rate (EIR) was assessed in spatial order. Indoor resting mosquitoes were collected using Pyrethrum Spray Catch (PSC). Morphological identification was carried out using a trinocular dissecting microscope with the aid of standard Taxonomic keys. Mosquito Salivary glands were carefully dissected for determination of Sporozoite Infection Rate. The proportion of individual mosquitoes infected with Plasmodium sporozoites were noted as the sporozoite rate (SR) in percentage. Entomological inoculation rate (EIR) was calculated as a product of Sporozoite Rate (SR) and Human Biting Rate (HBR) and expressed as infective bite per person (ib/p). Annual Sporozoite Infection Rate of 63.8 % was recorded. No significant variation was reported ( $p>0.05$ ) in Sporozoite Infection Rate between sampling locations. Annual Entomological Inoculation Rate (EIR) for Kontagora was calculated to be 1.84 infective bite per person (ib/p). A significant difference ( $P<0.05$ ) was however observed in EIR of Anopheline mosquitoes between sampling locations. The results established high Sporozoite Infection and Entomological Inoculation Rate which are key indices that sustains malaria transmission in Kontagora. Anopheline mosquitoes were reported to be infective throughout the study area. Vector control intervention based on local entomological data is strongly recommended.*

**Keywords:** sporozoite, infection entomological, inoculation

### **INTRODUCTION**

Malaria is a major cause of morbidity and mortality in developing countries, accounting for an estimated 300 to 500 million morbid episodes and 2 to 3 million deaths per year worldwide (Tiyong-Ifoue *et al.*, 2009). More than 90% of these deaths occur in Sub Saharan Africa, and most of them are due to *Plasmodium falciparum* (Tiyong-Ifoue *et al.*, 2009). In Nigeria,

malaria claims more lives than HIV/AIDS (World Health Organisation, WHO, 2013). The disease remained the leading cause of death in Nigeria with approximate 192,284 deaths in 2015 (Khanam, 2017). About 80 million Nigerians were infected with malaria in 2011, of which 1 million people died, most of them children under 5 years (WHO, 2011). A demographic surveillance has rated Niger State as the highest in percentage of death caused by malaria disease in the north central zone of Nigeria, with over 19,000 under-5 children dying every year (Eribake, 2016). Mosquito vector population density and the extent of their contact with human host are important factors in determining the transmission rates of malaria disease. Malaria transmission potential of *Anopheles* mosquitoes varies according to climatic and geographic conditions. Although malaria is endemic throughout Nigeria, but the actual vectors and transmission indices in each geographic location is not defined. Knowledge entomological indices of malaria transmission in any given location is essential to plan appropriate vector avoidance and control strategies. Therefore, precise evaluation of entomological indices of malaria transmission in each locality is worthwhile because control measures can only be effective if transmission indicators are known. Providing local data on such indices will improve the efficiency of control strategy thereby reducing cost.

## **MATERIALS AND METHODS**

### **Study Area**

Kontagora is a major town on the South bank of Kontagora River in the North -West of Niger State. The town is situated at 10.4<sup>0</sup> North latitude, 5.47<sup>0</sup> East longitude and 335 meters elevation above sea level. The area has a tropical climate with mean annual temperature, relative humidity and rainfall of 30.20 °C, 61.00 % and 1334.00 mm, respectively.

### **Sampling Locations**

Adult mosquitoes were collected from five sampling sites located in Kwangwara, Sabon Gari, Tudun Wada, Dadin Kowa and Usubu areas of Kontagora town. Two houses were randomly selected per area. Two rooms were selected from each house for indoor collection and the number of occupants in each room determined.

### **Adult Mosquito Collection and Preservation**

Indoor resting mosquitoes were collected using the Pyrethrum Spray Catch (PSC). Large white sheets of clothes were spread from wall to wall to cover the floors of the room while all doors and windows were shut. After about 20 minutes, the spread cloths were carefully folded starting from the corners. Knock down mosquitoes were collected with forceps into a damp petri dish.

### Identification of Mosquitoes

Morphological identification was carried out using a trinocular dissecting microscope with the aid of standard keys (Gillies and Coetzee, 1987; Gillies and De Meillon, 1968). The mosquitoes were identified using the gross morphology of the species, external morphology of the head, mouthparts, antennae, proboscis, patches of pale and black scales on the wings and legs and the terminal abdominal segments (Gillet and Smith, 1972).

### Dissection of salivary gland for determination of sporozoite infection rate

Dissection of the salivary gland was done according to the methods described by Looker and Taylor-Robinson (2004). The sporozoites were seen under a microscope (if present) and identified as minute needle like forms.

**Sporozoite rate (s)** = number of positive mosquitoes ÷ number of analysed mosquitoes

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

### Human Biting Rate (HBR)

Human biting rate (HBR) or Man Biting Rate (MBR) is the number of mosquitoes biting per person per night i.e. per human bait. The man-biting rate (ma) was expressed as the number of bites a person receives from a specific vector species per night (Aju-Ameh *et al.*, 2016).

Man biting rate (ma) = number of mosquitoes ÷ number of collectors ÷ number of collection hours

### Entomological Inoculation Rate (EIR)

Entomological inoculation rate was calculated as the product of sporozoite rate and human biting rate. Determination of the Entomological Inoculation Rates (EIR) was done according to (W H O, 2013) as shown below. Entomological Inoculation rate was expressed as infective bite per person (ib/p) for the study period.

$$\text{E I R} = \frac{\text{Human biting rate} \times \text{Sporozoite rate}(\%)}{100}$$

### Data Analysis

Data generated were analysed using SPSS software version 20.3 and excel package. Indoor Resting Density (IRD) of female *Anopheles* mosquitoes was computed using the method of Umar *et al.*, 2015) viz: Indoor Resting Density IRD= (total number of females collected divided by number of houses (rooms) used for the spray-sheet collection). The total number of females collected and the number of houses (rooms) were presented as means. Human biting rate (HBR) was calculated as the number of mosquito bites received per person per nights of collection. The proportion of individual mosquitoes infected with *Plasmodium* sporozoites were noted as the sporozoite rate (SR) in percentage. Entomological inoculation rate (EIR) was calculated as a product of sporozoite rate (SR) and human biting rate (HBR) and expressed as infective bite per person (ib/p). Chi-square test was used to compare the result

## RESULTS AND DISCUSSIONS

### Results

#### Sporozoite Infection Rate

A total of 2,911 were dissected and their salivary glands examined for *Plasmodium* parasite sporozoite. Of the 2,911 female salivary glands dissected, 1,845 were positive of *Plasmodium* sporozoites while 1,066 were negative as presented in Table 1. The sporozoite infection rate therefore stood at 63.4 % for the whole study area with no significant difference ( $p>0.05$ ) what so ever between sampling locations.

**Table 1: Sporozoite Infection Rate of Anopheline Mosquitoes**

Sampling Locations	Number Collected	Number Dissected	Number Positive	Number Negative	SR
Kwangwara	1,059	690	438	255	63.5
Tudun wada	887	587	371	216	63.2
Sabon gari	864	561	355	206	63.3
Dadin kowa	855	555	351	204	63.3
Usubu	827	518	330	188	63.7
<b>Total</b>	<b>4,492</b>	<b>2,911</b>	<b>1,845</b>	<b>1,069</b>	<b>63.4</b>

$X^2$  Cal = 0.43,  $X^2$  tab = 9.49, df = 4

#### Entomological inoculation rate (EIR)

The overall entomological inoculation rate (EIR) for Kontagora was calculated to be 1.48 infective bite per person as presented in Table 4. The EIR was highest in Kwangwara area at 1.75 ib/p. Tudun wada and Sabon Gari areas recorded an EIR of 1.46 ib/p and Dadin Kowa recorded an EIR of 1.4 4ib/p. Of the five sampling locations, Usubu area recorded the least EIR of 1.38 ib/p. Unlike EIR, human biting rate (HBR) for Kontagora stood at 2.34 bite per person per night. Kwangwara area still leads the other sampling locations with the highest HBR of 2.76 b/p/n and Dadin kowa area recorded 2.28 b/p/n as the lowest HBR. However, there was a significant variation ( $p<0.05$ ) in EIR and HBR of female Anopheline mosquitoes in Kontagora.

**Table 2: Entomological Inoculation Rate (EIR) of Female Anopheline Mosquitoes in Kontagora**

Sampling Locations	No. Collected	No. Dissected	Sporozoites Rate	HBR	EIR
Kgr	1,059	690	63.8	2.76	1.75
Tdw	887	587	63.5	2.31	1.46
Sgr	864	561	63.8	2.29	1.46
Ddk	855	555	63.5	2.28	1.44
Usb	827	518	64.7	2.65	1.38
Total	4,492	2,911	63.8	2.34	1.48

$X^2$  Cal = 1106.432,  $X^2$  tab = 15.51, df = 8

Key: Kgr=Kwangwara, Tdw= Tudun wada, Sgr=Sabon gari, Ddk=Dadin kowa, Usb= Usubu

## DISCUSSIONS

Sporozoite infection rate in this study stood at 63.4 % for Kontagora with no significant difference ( $p > 0.05$ ) between sampling locations. Meanwhile, annual sporozoite infection rate was 63.8 % with the month of March recording 58.8 % as the lowest while the month of May recorded 70.0 % as the highest. However, Chiphawanya (2003) reported sporozoite rate of 6.50, 5.20, and 4.50 % in *An. gambiae*, *An. funestus* and *An. arabiensis*, respectively, with an average sporozoite rate of 5.4 % in the three vectors. Okwa *et al.* (2009) while comparing sporozoite rate for different ecological zones of Nigeria reported 3.24 % for Guinea savannah, 9.9 % for rainforest, 4.3 % for savannah forest and 3.11 % for mangrove forest. The sporozoite rates between zones varied significantly ( $p < 0.05$ ). Mzilahowa *et al.* (2012) also reported the occurrence of infected mosquitoes all year round with highest prevalence occurring in April. In their findings, *An. gambiae* s.s had the highest year-round sporozoite rate of 10.6 % significantly greater ( $P < 0.05$ ) than both *An. funestus* 4.5 % and *An. arabiensis* 3.2 %. Aju-Ameh (2016) reported no significant difference in sporozoite rates of *An. gambiae* s.l. in relation to study sites in Otukpo Local Government Area of Benue state, Nigeria. Even though Ahmed *et al.* (2016) reported 37.65 % sporozoite rate for *An. gambiae* dissected from four localities in Makurdi, Benue state Nigeria, with significant variation ( $p < 0.05$ ) between study localities. Esperanca *et al.* (2018) reported 46 % sporozoite rate and Oyewole *et al.* (2005) reported 2.5 % in the coastal areas of Lagos state Nigeria. Anopheline mosquitoes in Kontagora were found to be significantly infected with *Plasmodium* sporozoites in all the sampling locations and all year round with a grave consequence of high malaria transmission potentials. The differences in the sporozoites rates recorded in this

study compared to others elsewhere can be explained by the fact that mosquito collection was done both in wet and dry seasons. Moawia and Osman (2010) reported that the seasons of mosquito collection greatly affects their sporozoite rates. An overall sporozoite rate of 63.4% (n = 1,845) recorded from the total number of mosquitoes dissected (n = 2,911) in this study is generally high compared to figures recorded from other malarious areas in Nigeria. However, this does not preclude that such high infection rate would be responsible for sufficiently high level of malaria in the population. The very low range between the lowest and highest infection rates between sampling location explained why there was no significant differences ( $p > 0.05$ ) in the sporozoites infection rates between locations. Further analysis of this result revealed that the highest proportion of infected mosquitoes were collected in May, which coincided with the onset of rainy season. This could therefore be attributed to favourable climatic environmental conditions that would influence mosquito survival and parasite development in the mosquito. High densities of sporozoite infected mosquitoes were also observed following the rains between May and October. The presence of infected mosquitoes between December and May, a period presumed to be free of malaria, clearly pointed to the need for sustained vector control operations all year round. However, variation in sporozoites infection rates between the twelve calendar months showed the variable nature of the risk of malaria infection in Kontagora. By this fact, the risk is therefore month or season specific and not site or location specific. This therefore, underlines the importance of generating intensive spatio-temporal information on risk status at a local scale. Entomological inoculation rate has a direct impact on malaria epidemiology. The intensity of malaria parasite transmission is normally expressed as the Entomological inoculation rate (EIR), and in Africa it is highly variable ranging from 1-1,000 infective bite per person per year (Aju-Ameh *et al.*, 2016). Entomological inoculation rates for Kontagora was calculated from five sampling locations and a significant variation ( $p < 0.05$ ) was reported. Over all EIR was 1.48 ib/p (infective bites per person). The study reported a very low EIR compared to previous estimates from other parts of Nigeria where annual EIR of 13.6 ib/p was recorded (Okwa *et al.*, 2009). However, the findings agreed with pervious literatures (Service, 2013). Aju-Ameh *et al.* (2016) also reported a low EIR of 0.4 ib/p in Otukpo, Benue state Nigeria. Elsewhere in Uganda, Kaliwal *et al.* (2010) reported annual EIR of 3.8 ib/p with two peaks from May-June and October – December. Entomological inoculation rates (EIR) are associated with prevalence rate of parasitaemia in human population (Chiphawanya, 2003). High EIR can have implication for malaria epidemiology, including the likely age to first malaria infection and the clinical disease pattern (Mzilahowa, 2012). 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. The biggest challenge in malaria control is to reduce the observed high EIR in order to achieve reductions in human infection rates sufficient to reduce the burden of the disease. It is necessary to note that the values of transmission indices in this study is only a reflection of captured species.

## CONCLUSION

Sporozoite infection and Entomological Inoculation rate have direct impact on malaria epidemiology. The study established that the study area is a malaria-exposed community as *Anopheles* mosquitoes were found to be significantly infected with *Plasmodium* parasite and the intensity of malaria transmission was equally high suggested in the entomological inoculation rate reported. The study clearly pointed to the need or sustained vector control operation.

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