Original article

Estimation of Infection Reservoir of *Plasmodium* Malaria by Measurement of Oocyst Rate in a Natural Vector Population

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ABSTRACT

The study was conducted to estimate the infection reservoir of *plasmodium* malaria by measurement of oocyst rate in a natural vector population in Kontagora, Niger State Nigeria. Seasonal and Spatio-temporal variation in prevalence and intensity of *Plasmodium* parasite oocyst was determined. 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. The stomach of female *Anopheles* mosquitoes was dissected and closely examined to estimate the oocyst rate. The presence of oocyst was detected with a low power magnification and young oocysts were detected because of their refractiveness and characteristic parasite pigment. Oocyst prevalence was determined as the proportion of mosquitoes infected with *Plasmodium* parasite oocyst, and intensity was calculated as the geometric mean number of oocysts per mosquito. This study revealed an oocyst rate of 73.8 %, and a geometric mean number of oocyst per mosquito of 38.7 o/m. Monthly prevalence and intensity of oocyst varied significantly s($p<0.05$), with no significant variation however in the seasonal prevalence and intensity of *Plasmodium* oocyst of Anopheline mosquitoes in the study area. The study established a high infection reservoir of Anopheline mosquitoes in Kontagora with a grave consequence on high malaria transmission potentials. This necessitated the need to regularly obtain reliable information about the risk factors associated with malaria transmission on a local scale.

Keywords: Estimation, Infection, Reservoir, Oocyst, Measurement, Vector

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INTRODUCTION

Human malaria is caused by infection with a mosquito transmitted parasite. Various vector control methods are now available to decrease transmission and cure infection. However, the efficiency of the interventions cannot provide universal coverage because of variability in physiological and environmental factors surrounding vector development. There has been marked reduction in malaria transmission intensity in Sub Saharan Africa due to intensification of malaria control interventions [1]. Access to malaria diagnosis also increased, supported by increased supply of rapid diagnostic tests [2]. Despite these successes, malaria remains an important public health burden in many African countries including Nigeria. The global strategy is to reduce malaria burden by 90% by 2030 and eliminate malaria in at least 35 countries actively pursuing malaria elimination [3]. As has been reported elsewhere, rapid gain in malaria control does not necessarily lead to malaria elimination, as large reservoir of parasites may persist in human population [4]. Asymptomatic infections may be important sources of onward transmission to mosquitoes. Understanding malaria transmission requires measuring the probability of a mosquito becoming infected after feeding on a human. Parasite prevalence in mosquitoes is age-dependent and the unknown age structure of fluctuating mosquito populations impedes estimation [5]. Malaria transmission can be quantified by estimating oocyst rate in female mosquitoes. This can be very informative in understanding the endemic nature of malaria in a given location. Measuring of oocyst rate in natural population of mosquitoes provides simple way of obtaining estimate of infection reservoir. Therefore, the prevalence and intensity of *Plasmodium* parasite oocyst can be important indicators of human infectivity in mosquitoes.

MATERIALS AND METHODS

Adult Mosquito Collection and Preservation

Indoor resting mosquitoes were collected using the Pyrethrum Spray Catch (PSC) between the hours 06:00 am and 09:00 am in the study areas. Food items were covered properly, and moveable furniture were taken care of before spraying. 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. All cracks and openings in walls were stocked with rag papers to prevent mosquitoes from escaping. After about 20 minutes, the spread clothes were carefully folded starting from the corners. Knock down mosquitoes were collected with forceps into a damp petri dish. Outdoor mosquitoes were collected using the Centre for Disease Control (CDC) light traps (J.W. Hock Ltd, Gainesville, Florida, USA). These traps were set from 6.00 pm and retrieved at 7.00 am). Individual samples were preserved in dry silica gel in well labelled Eppendorf tubes (1.5 ml) prior to identification. This is to ensure preservation of delicate significant features that will be needed for morphological identification in the laboratory.

Identification of Mosquitoes

Morphological identification was carried out using a trinocular dissecting microscope (Amscope SZMT2/MU100010APTINA COLOR CMOS) with the aid of standard keys [6, 7]. 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 [8].

Preparation of Mosquitoes for Dissection

Live mosquitoes were immobilized, and each mosquito was placed on a slide and held by one wing while the legs were being removed one at a time and afterwards, the other wing was pulled off. The mosquito was then placed on a fresh dry slide and arranged in a more suitable position for dissection of the stomach and salivary glands as described by [9].

Oocyst examination and estimation

The stomach of the female *Anopheles* mosquitoes was closely examined to estimate the oocyst rate after mercurochrome staining [10]. The midguts were dissected in 0.4 % mercurochrome solution, and the infection intensity of each individual female was determined by counting oocysts under a light microscope. Examination of the stomach for oocysts began from the posterior end, proceeding towards the anterior end. This is because the oocysts are usually located in the posterior half of the stomach in low and moderate infections. The presence of oocysts was detected with a low power magnification and young oocysts were detected because of their refractiveness and characteristic parasite pigment.

DATA ANALYSIS

Data generated were analysed using the Statistical Package for Social Scientists (SPSS) software version 20.3 and excel package. Oocyst prevalence was determined as the proportion of mosquitoes infected with *Plasmodium* parasite oocyst, and intensity was calculated as the geometric mean number of oocysts per mosquito

RESULTS

The spatial variation in prevalence of *Plasmodium* parasite oocyst in female Anopheline mosquitoes in Kontagora is presented in Table 1. A total of 2,911 female Anopheline mosquitoes were dissected and checked for oval oocysts, of which 2,148 (73.8 %) had *Plasmodium* oocysts and the geometric mean number of oocysts per mosquito was 38.7 oocysts per mosquito. Prevalence and intensity of *Plasmodium* oocyst in Anopheline vectors in Kontagora varied significantly according to the sampling locations. While the overall oocysts rates were 73.8 %, Usubu area recorded 76.8 % as the sampling locations with the highest oocysts rate. Tudun Wada, however, recorded the lowest oocyst rate of 71.6 %. An average mean value of 42.02 o/m oocysts per mosquito was reported in Kwangwara area as the A highest, while 35.2 o/m oocysts per mosquito was reported in Sabon gari as the lowest. The number of oocysts per mosquito also varied between sampling locations. While Anopheline mosquitoes in Kwangwara area harboured an average of 42.02 o/m oocysts per mosquito. Anopheline vectors in Sabon gari area were reported to carry an average of 35.2 o/m oocysts per mosquito. Kwangwara and Sabon gari produced vectors with the highest and lowest oocysts intensity respectively. The monthly prevalence and intensity of *Plasmodium* oocysts in *Anopheline* vectors in the study area is presented in Table 2. A total of 2,911 Anopheline mosquitoes were dissected for the study period (April 2017 – March 2018), out of

which 2,069 had *Plasmodium* oocyst in their guts which represented 71.1 % annual prevalence rate. However, the highest prevalence rate of 84.5 % was recorded in April while the lowest oocysts rate 6.0 % was recorded in September. The overall average number of oocysts per mosquito was 39.5 o/m for the period of the study. The highest number of oocysts per mosquito was reported in December at 45.6 o/m and the lowest was fond in April 31.5 o/m. Monthly prevalence and intensity of *Plasmodium* parasite oocyst varied significantly ($p<0.05$). However, the results revealed no significant variation ($p>0.05$) in prevalence and intensity of Anopheline oocysts between wet and dry season Table 2. Oocyst rate in wet season was 70.4 % and 71.4% in dry season.

Table 1: Spatial Variation in Oocyst of Anopheline Mosquitoes in Kontagora

Sampling Locations	Number Collected	Number Dissected	Number positive (Oocysts Rate)	Average no. of oocysts per mosquito
Kwangwara	1,059	690	510(73.9) *	42.02
Tudun wada	887	587	420(71.6)	41.4
Sabon gari	864	561	411(73.3)	35.2
Dadin kowa	855	555	409(73.6)	36.1
Usubu	827	518	398(76.8)	40.5
Total	4,492	2,911	2,148(73.8)	38.7

X^2 Cal= 509.901, X^2tab=9.49, df =4

Table 2. Monthly Variation Oocysts of Anopheline Mosquitoes in Kontagora

Months	Number Collected	Number Dissected	Number positive (Oocysts Rate	Average no. of oocysts per mosquito
April	267	168	142(84.5) *	31.5
May	379	244	189(77.5)	32.3
June	443	289	211(73.0)	39.2
July	500	323	243(75.2)	42.9
August	561	360	260(72.2)	41.02
Sept	513	332	206(62.0)	44.2
Oct	437	285	190(66.7)	37.9
Nov	442	287	196(68.3)	39.9
Dec	325	211	143(67.8)	45.6
Jan	227	148	112(75.7)	40.3
Feb	211	141	91(64.5)	39.8
March	187	123	86(69.9)	33.9
Total	4,492	2,911	2069(71.12)	39.5

X^2 Cal=45.68, X^2 tab=19.68, df=11

The average number of oocysts per mosquito for wet and dry seasons was 40.0 o/m and 39.0 o/m respectively. Seasonal variation in prevalence and intensity of *Plasmodium* parasite oocysts of Anopheline mosquitoes was presented Table 3. The results revealed no significant variation ($p>0.05$) in oocysts rate and average number of oocysts per mosquitoes between wet and dry season. Oocyst rate in wet season was 70.4 % and 71.4% in dry season. The average number of oocysts per mosquito for wet and dry seasons was 40.0 o/m and 39.0 o/m respectively.

Table 3. Seasonal Variation in Oocysts of Anopheline Mosquitoes in Kontagora

Seasons	Number Collected	Number Dissected	Number positive (Oocyst Rate)	Average no. of oocysts per mosquito
Wet season (May-Oct)	2,833	1,833	1,299(70.9) *	40.0
Dry season (Nov-April)	1,659	1,078	770(71.4)	39.0

X^2 Cal=0.10, X^2 tab=3.84, df=1

DISCUSSION

The oocyst is the non-transmissible stage of *Plasmodium*. It must develop for several days in the mosquito mid-gut wall before it can produce sporozoites. Measurement of oocyst rate in wild-caught mosquitoes after blood meal digestion provides a simple way of obtaining estimate of the infections reservoir. The detection of oocyst regardless of their density provides an acceptable estimate of mosquito infectivity. This study revealed an oocyst rate of 73.8 %, and a geometric mean number of oocysts per mosquito of 38.7. Monthly prevalence and intensity of oocyst varied ($p<0.05$), with no significant variation however in the seasonal prevalence and intensity of *Plasmodium* oocyst of Anopheline mosquitoes in the study area. This result was supported by previous estimates of oocysts by [11] who reported 43 oocysts per mosquito and [12] reported 44 oocysts per mosquito. Furthermore, [13] reported 63% oocyst rate and infection prevalence for separate experiments (n=30-104) ranged between 33 and 86.5 % with mean oocyst intensities from 0.57 and 4.7 oocyst per mosquito. [14] Reported an oocyst intensity range of 1 to 5 in a naturally infected population. On the contrary, lower oocyst rate were however reported elsewhere; 8.0 % Kenya [14] and 5.3 % and 1.30 % [16]. In line with previous studies (Stone, 2013[11]), oocyst prevalence and intensity are highly related in the resent study. The detection of any number of oocysts should reasonably predict their likelihood of causing

mosquito infectivity [17]. This study established a high infection reservoir of Anopheline mosquitoes in Kontagora compared to Makurdi where 22.08 % oocyst rate was reported [18]. Oocyst prevalence is reliable but not a perfect indicator of mosquito infectivity. Not all oocysts contribute effectively by releasing sporozoite into the haemocoel [11]. But since most oocyst positive mosquitoes had at least one ruptured oocyst from which sporozoite reached the salivary gland [19] and as the number of sporozoite egested during probing (<100) is considerably less than the number produced by single oocyst (1,359 – 14,000) [20]. It is possible that infectivity can be predicted with seasonality from oocyst prevalence. However, the impact of oocyst arrest in low intensity infections might decrease the reliability of oocyst prevalence as an indicator of infectivity because it could lead to the total failure of sporozoite release [21]. The proportion of mosquitoes that become infective is the most relevant outcome measure for assessment of the human infectious reservoir for malaria and for evaluating the impact of transmission reducing interventions on human infectivity. If sporozoites invasion of salivary gland is effective, then the detection of oocyst regardless of their density provides an acceptable estimate of mosquito infectivity.

CONCLUSION

Understanding malaria transmission requires measuring the probability of a mosquito becoming infected after feeding on a human. Measuring of oocyst rate in natural population of mosquitoes provides simple way of obtaining estimate of infection reservoir. The study revealed high infection reservoir and established the importance of oocyst prevalence and intensity as a standalone indicator of mosquito vector infectivity in the study area.

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