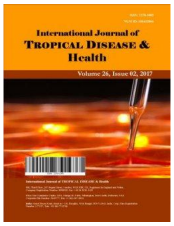

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Plasmodium falciparum Sporozoite Rates in *Anopheles gambiae s.l.* at a University Teaching Hospital and Contiguous Village, Rivers State, Nigeria

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Authors' contributions

This was a collaborative work among authors. Author MAEN designed the study, supervised the project, managed the literature searches and wrote the first draft of the manuscript. Author MOE conducted field and laboratory experiments, obtained consents and educated the participants in the study. Author SNO managed analyses, fine-tuned and revised the final manuscript that all authors read and approved.

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ABSTRACT

Malaria is a major burden to human health in tropical and sub-tropical areas. In Nigeria, the entire population is at risk. Over the past decade, there had been persistent reports of mosquito nuisance and an increase in malaria prevalence at the University of Port Harcourt Teaching Hospital in lowland rainforest, Rivers State, Nigeria. It was therefore decided to obtain provisional malaria risk data, by determining the sporozoite rates of anthropophilic, endophlic and endophagic *Anopheles gambiae s.l.* in the hospital wards and rooms in the contiguous village. Standard keys and guides were used for mosquito identification and dissection to observe sporozoites in their salivary glands. More than half of all female *Anopheles gambiae* caught at the hospital had fed; similarly, 40.9% of *Anopheles gambiae* collected from the contiguous village had fed. The sporozoite rates were 75.0% and 73.29% at the hospital and the village respectively. These high sporozoite rates and the

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preponderance of endophilic, endophagic, anthropophilic *An. gambiae s.l.* among anophelines indicate high malaria risk at both sites. Since indoor residual spraying is not advisable in hospitals, physical (bed/outlet netting) and chemical (ITNs/LLINs) barriers and larval source management are the recommended alternatives.

Keywords: Malaria risk; sporozoite; gonotrophic; Anopheles gambiae; hospital; Nigeria.

1. INTRODUCTION

Malaria is a major burden to human health in tropical and sub-tropical areas [1]. Of the estimated 300 million cases of malaria worldwide and annual deaths of 1 million, 90% occur in Africa [2]. It is estimated to cost US$1.8 billion annually in Africa [2]. In Nigeria, the entire population (154,728,895) is at risk of malaria infection and in 2009, there were 4,295,686 confirmed cases, 658,732 in-patient malaria cases and 7,522 malaria-attributed deaths [3]. It is estimated that ₦132 billion are lost to malaria as treatment cost and loss of man-hours in Nigeria [2].

The disease is caused by a protozoan parasite in the genus *Plasmodium*, *Plasmodium falciparum* is the most virulent. The life cycle of *Plasmodium* is complex in humans and *Anopheles*. Sporozoites (the infective stage) are transmitted from the salivary glands of an infected female *Anopheles* during a bite [3]. Species of the *Anopheles gambiae* complex are the most efficient vectors, because of their anthropophily endophagy and endophily [4]. Sporozoite rates are provisional indicators of the malaria risk level in an area. There is limited information on sporozoite rates of *Anopheles* in southern Nigeria [4].

Nigeria has a 3-tier health system: Primary health care at the Local Government Area (LGA) level, Secondary Healthcare at the State level and Tertiary healthcare funded by the Federal Government. The Tertiary facilities consist of teaching, specialist, national hospitals, etc. Over the past decade, there had been persistent reports of mosquito nuisance and concomitant increase in malaria cases among patients at the University of Port Harcourt Teaching Hospital, located at the northern outskirts of the village, Alakahia, in lowland rainforest, Rivers State, Nigeria. It was therefore decided to obtain provisional estimates of malaria risk at the hospital and contiguous village by dissecting endophilic *Anopheles gambiae s.l.* caught in ward of the teaching hospital and rooms in the contiguous village.

2. MATERIALS AND METHODS

The coordinates for the hospital and village are 4°8867′N, 6°9285′E and 4°8867′N, 6°9242′E respectively (Fig.1). They are separated by a busy interstate expressway. The village had poor road network and drainage system. Consequently, there were several breeding sites. The hospital is a massive edifice, surrounded by vegetation. It was constructed on a marshy area with a below-ground recyclable water/sewage system that has been badly managed because it was poorly understood. As a reference hospital, the University of Port Harcourt Teaching Hospital houses staff and patients from various geographical locations and ecological zones, with many other heamatophilic pathogens. Also, Alakahia shelters university students from various States of Nigeria and international students from other African countries (Cameroon, Ghana, Kenya, Rwanda, etc) with potentially different vectors and pathogen strains.

2.1 Sampling Procedure

At the hospital, six wards were selected: Gynaecology, Antenatal, Postnatal, Male, Female, Paediatric. Permission was obtained from the respective Heads of Departments and supervising nurses. At the village, permission was obtained from the village Head and occupants of the 12 houses, selected from the southern, northern, eastern and western sections of the village. Resting mosquitoes were caught with the aid of a mouth aspirator from the underside of beds, on nets, hosts' skin, curtains, under chairs, desks and in dark corners (which yielded the highest numbers). Collected mosquitoes were placed in paper cups, covered with netting and taken to the laboratory for sorting and identification. Standard keys of Gilles [5], Gilles and Coetzee [6] and Service [7] were used for identification.

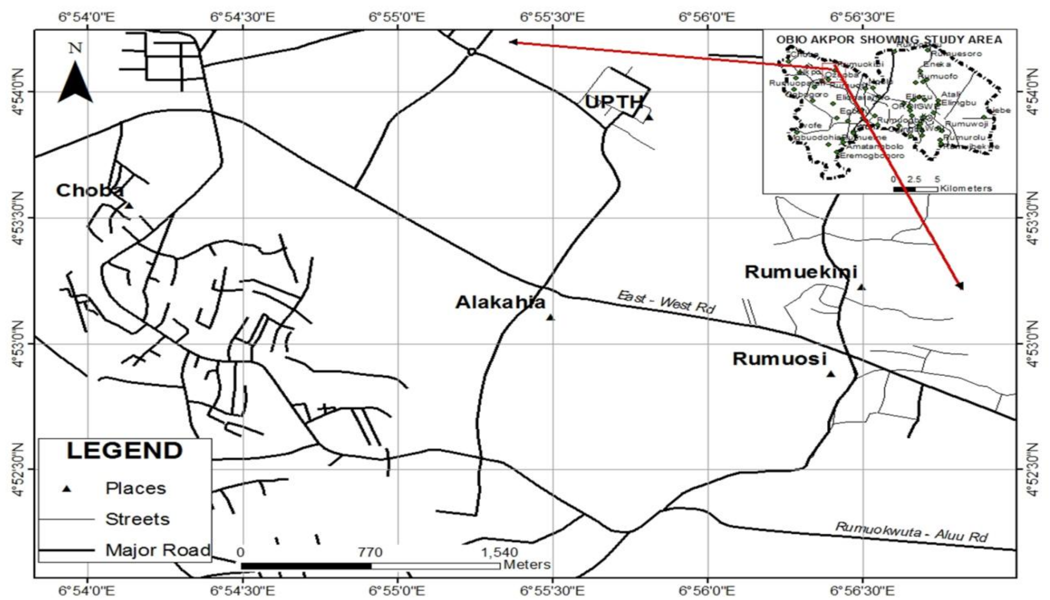

Fig. 1. Study sites- Alakahia and at the university of Port Harcourt teaching hospital (UPTH)

2.2 Dissection

Anopheles were grouped, based on their sex and gonotrophic stage (Fed, Gravid, Half-gravid, Unfed). Unfed and gravid mosquitoes were dissected immediately, while fed and half gravid were dissected a few days later. The exposed salivary glands were placed on a slide to air-dry, fixed with methanol for 1 minute and stained with Giemsa for 40 minutes, covered with a clean coverslip and viewed under the microscope for sporozoites [8]. The midgut was also stained with iodine and examined for oocysts and sporozoites detection using a modified technique [9].

3. RESULTS

A total of 407 mosquitoes were collected at the hospital (57 *Anopheles gambiae s.l.*, 312 *Culex quinquefasciatus*, 38 *Aedes aegypti*); at Alakahia there were 266 (151 *Anopheles gambiae s.l.*, 100 *Culex quinquefasciatus,* 15 *Aedes aegypti*).

Table 1. Numbers of males and females anophelines in different gonotrophic stages at wards in UPTH (*sex ratio and gonotrophic stages at the UPTH*)

Wards	Males	Gravid	Half-gravid	Unfed	Fed	Total
Gynaecology	7	6	11	11	26	61
Antenatal	6	4	9	10	35	64
Postnatal	3	6	5	7	48	69
Female	4	11	13	8	34	70
Male	0	13	17	15	37	82
Paediatric	2	7	14	10	28	61
Total	22	47	69	61	208	407
Percent (%)	5.4	11.5	17.0	15.0	51.1	

Table 2. Numbers of male and female *anopheles* in different gonotrophic stages at Alakahia *(sex ratio and gonotrophic stages at Alakahia)*

Stages	Males	Gravid	Half gravid	Unfed	Fed	Total
Numbers	16	50	53	46	109	266
Percent	6.0	18.8	19.9	17.3	40.9	

Table 3. Sporozoite rates of *Anopheles gambiae s.l.* at UPTH and Alakahia

Location	No dissected	No infected	%infection
UPTH	48	36	75%
Alakahia	122	95	73.29%

Table 4. Distribution of individuals at collection sites in the teaching hospital

Wards	Males	Females	Children	Infants	Neonates	Total	Percent/ward
Gynaecology	12	42	00	00	00	54	14.52
Antenatal	09	25	00	03	00	37	9.95
Postnatal	15	39	11	00	09	74	19.89
Female	17	48	07	00	00	72	19.35
Male	34	13	02	00	00	49	13.17
Paediatric	26	28	14	10	08	86	23.12
Total Patients	113	195	34	13	17	372	
Percent at risk	30.38	52.42	9.14	3.49	4.57		

At the hospital, 51.1% of the female *Anopheles* were fed at the time of capture, 28.5% gravid/half-gravid and 15.0% unfed (Table 1). In the village, 40.9% were fed at the time of capture, 38.7% graved/half-graved and 17.3% unfed (Table 2). Sporozoite rates at both locations were in the range, 73.29-75.0% (Table 3). Numbers of individuals in the different wards at collection were in the range, 34-65; the highest was in the female ward (Table 4). At Alakahia, collections from 130 rooms recorded 195 residents; 99 males and 96 females.

4. DISCUSSION

The dominance (74.22%) of *Culex quinquefasciatus* at the hospital was similar to results obtained from some rural areas in lowland rainforests of Rivers [10], Akwa Ibom [11] and Bayelsa [12] States. This was in contrast to the ratio in the village, where *Cx. quinquefasciatus* constituted only 37.28% of all mosquitoes. Reasons for these differences are still being investigated. The high numbers of *Cx. quinquefasciatus* in rural areas are alarming. The species had been associated with urban filariasis, but may complement *Anopheles gambiae s.l.* in the epidemiology of rural bancroftian filariasis. The dominance of *Cx. quinquefasciatus* increases the probability of coinfections (malaria, bancroftian filariasis) among patients. Fortunately, in 2000, WHO launched Mass Drug Administration (MDA) in Nigeria and other African countries [3].

Okorie et al. [13] analyzed the Nigerian *Anopheles* vector database, 1900-2010. Sporozoite rates varied significantly across eco-vegetational zones: 0.0% in Borno (Bama) [14], 0.4% in Sokoto [15], 0.4% in Rivers (Bonny) [16], 91.0% in Alimosho, Lagos [17], 21.9% in Mushin, Lagos [16], 6.3% in Badagry, Lagos [18]. The highest rates were in the coastal, highly populated areas and lowest were in the Sudan savanna and Sahel zones. More recent studies have followed the same pattern of variations in sporozoite rates: Msugh-Ter et al. [19] recorded 31.5% in *Anopheles gambiae s. l.* at Makurdi, Benue State; Obembe and Awopetu [20] obtained sporozoite rates in *Anopheles gambiae s.l.* of 82.6% at Ado-Ekiti and 79.55% at Ibadan; Aju-Ameh et al. [21] obtained sporozoite rates of 0.0-0.4% in rural and urban areas of Oturkpo and Gboko LGAs, Benue State. The high rates of 75.0% and 73.2% obtained at the hospital and village respectively, were in accord with the findings of Okorie et al. [13] who established high sporozoite rates in coastal areas.

The Presidents' Malaria Initiative (PMI) Nigeria, final Entomology Report of November, 2014 - December, 2015, on Africa Indoor residual Spray (AIRS) Project (2016) that undertook entomological surveillance in six sentinel States: Enugu, Lagos, Nasarawa, Plateau, Rivers and Sokoto established a mean sporozoite rate of 5.0% in Rivers State [22]. They used Pyrethrum Spray Catch (PSC) and Human-baited Center for Disease Control (CDC) Light traps for collections, but did not indicate locations of the collections.

Moffett et al. [23] calculated three different types of malaria risk: (a) multiplied the probability of the occurrence of vector by both human population and the human blood Index (HBI) of the vector. The relative risk of malaria infection was the sum of these values (b) the maximum probability of vector occurrence was multiplied by its HBI and human population density; the relative risk of malaria infection was the product of these 3 values (c) the probability of occurrence of vectors was multiplied by the human population density and the HBI of the vector. The relative risk of malaria infection was calculated as the maximum of these values. Although HBI values were not obtained in these studies, the high proportions (40-50%) of fed *Anopheles gambiae* from the hospital and village and high sporozoite rates were indicators of high HBI. Moffett et al. [23] found high HBI values in *Anopheles gambiae s.l. An. funestus* and *An. moucheti*. The dominant malaria vector in both hospital and village was the anthropophilc, endophilic and endophagic *Anopheles gambiae s.l.*

Human population densities were high both in the hospital and village. Irrespective of the type of malaria risk calculated, the conditions in the hospital and village were near optimal for high malaria risk. It is therefore not surprising that there had been a continuous demand for action on vector control. Indoor Residual Spray is not advisable in hospital wards. The AIRS 2016 study revealed that only 2,784,319 LLINs had been distributed in Rivers State and 93.0% retained. One of the major limitations of LLINs is that in Rivers State, there is a steep rise in biting trends of *Anopheles gambiae s.l.* outdoors and indoors 19.00-22.00 hrs when many people are not yet in bed [22]. Global burden of disease study in 2016 among 195 countries showed that Nigeria has a Healthcare Access Quality (HAQ) index of 41.9 compared to Cape Verde, 54.8; Botswana 51.5 in Sub-saharan Africa [24]. Against the background of low HAQ index, prevention of vector-borne diseases is advisable. Larval source management is recommended at the hospital. If all the potential breeding sites were eliminated (man-made) or treated (natural), it could reduce the Entomologic Inoculation Rate - EIR (number of infective bites per person per year), thereby reducing malaria transmission in well-defined setting such as the hospital, where it is feasible. The elimination of larval habitats can be a cost effective and long-term solution to the malaria burden.

The high sporozoite rates also allude a potential high risk of multiple infections even through a single bite, the spread of chemoresistant and insecticide resistant genes, the emergence of new or previously eliminated diseases are exponentially enhanced by the constant migration of hosts as previously reported by Noutcha [14]. This migration sustains the presence of patients, staff and students with diverse genetic and immunological backgrounds from various geographical locations and ecovegetational zones (possibly with different malaria vectors and *Plasmodium* strains in addition to other heamatophilic pathogens) at the teaching hospital and Alakahia (a university village). Service [7] explained that it is because the existing vectors need just a pathogen source or reservoir in the environment to spread/ establish an outbreak of the corresponding disease.

5. CONCLUSION

The high sporozoite rates and preponderance of *An. gambiae s.l.* among anophelines indicate high malaria risk. Since indoor residual spraying is not advisable in a hospital setting, physical barriers (bednets; screens on outlets: doors/windows), chemical barriers (ITNs, LLINs), adequate waste management programmes, proper environmental sanitation sessions and larval source management are the only promising alternatives for now. Considering the high relative humidity and the heat at these study locations especially at night, the provision of constant power in the hospital will minimize host-vector interactions hence reduce potential infections. Schools, hospitals and camps for internally displaced persons or refugees, pull together people from various ecological/genetic backgrounds and thus must be monitored for outbreaks of vector-borne diseases because they provide favorable conditions for host substitutions, vector colonization and cross infections.

CONSENT

Permission was obtained from the respective Heads of Departments and supervising nurses. At the village, permission was obtained from the village Head and occupants of the 12 houses, selected from the southern, northern, eastern and western sections of the village.

ETHICAL APPROVAL

As per international, community and university standards, written ethical approval was collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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