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

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. The implications of these results, in relation to the level of malaria risk are discussed.

Keywords: Malaria risk, Sporozoite rates, Gonotrophic stage, *Anopheles gambiae*, Teaching Hospital, Nigeria.

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. Species of the *Anopheles gambiae* complex are the most efficient vectors, because of their anthropophily endophagy and endophily. 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 wards of the teaching hospital and rooms in the contiguous village.

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.

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.

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].

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*).

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% gravid/half-gravid 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.

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**		

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. Fortunately, in 2000, WHO launched Mass Drug Administration (MDA) in Nigeria and other Africa 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 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. 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 retention of 93.0%. 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 19.00-22.00hrs 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.

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