

# Sporozoite Infection Rate and Identification of the Infective and Refractory Species of *Anopheles gambiae* (Giles) Complex

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## Abstract

The ability of *Anopheles gambiae* complex mosquitoes to transmit *Plasmodium* infection is known to be variable within sibling species of the complex with strains that cannot transmit the parasite. High sporozoite infection rate recorded showed that *A. gambiae* mosquitoes are potent malaria vectors in southwestern Nigeria. The aim of this study was to identify the infective and refractory strains of *A. gambiae* mosquitoes and to determine the sporozoite infection rate in this area. The infective strains were *A. gambiae* (*sensu stricto*) and *A. arabiensis*, while the refractory strains were *A. gambiae* (*sensu stricto*). However, ovarian polytene chromosome banding patterns could not be used to distinguish between the infective and refractory strains of *A. gambiae* (*sensu stricto*). This study showed that the refractory strains of *Anopheles gambiae* complex are present, but in low frequencies, in southwestern Nigeria, and that the sibling species of *Anopheles gambiae* (*A. gambiae* s.s. and *A. arabiensis*) are potent malaria vectors.

**Keywords:** arabiensis, chromosome, falciparum, malaria, mosquito, parasite, plasmodium, polytene

## Introduction

Malaria is one of the most deadly diseases affecting many people worldwide, particularly in the tropical and sub-tropical areas. It remains a major burden to human health in these regions (WHO, 1993, 2009). Malaria that affects humans is caused by *Plasmodium* parasites that are adapted to propagating alternately in two different hosts, i.e. the primary hosts, *Anopheles* mosquitoes (vectors) and the secondary hosts, human beings. The four species of *Plasmodium* that infect humans and cause human malaria are *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*, among which *P. falciparum* is the most infectious (Hoffman *et al.*, 1996). Awolola *et al.* (2005) reported that ninety percent of infections are caused by *P. falciparum* in Nigeria. It has been reported that, in Nigeria alone, more than 200 million people have died from malaria (Kiszewski *et al.*, 2004).

The most important vectors of *P. falciparum* are the members of the *Anopheles gambiae* (complex), which are the most widespread and potent vectors of malaria in sub-Saharan Africa (Gregory and Yoosook, 2013). *A. gambiae* is a complex of eight sibling species, which presently includes *A. gambiae* (*sensu stricto*) Giles; *A. arabiensis* Patton; *A. bwambae* White; *A. melas* Theobald; *A. merus* Dönitz; *A. quadriannulatus* Theobald; *A. amharicus* Hunt, Coetzee and Fettene and *A. comorensis* Brunhes, le Goff and Geoffroy (Coetzee *et al.*, 2013; Gillies and DeMeillon, 1968; Harbach, 2003; Hunt *et al.*, 1998; White, 1974; White, 1985). These sibling species are morphologically identical, but genetically distinct (Coluzzi *et al.*, 1979). Ten fixed inversions can be

used to identify individual species (Coluzzi *et al.*, 1979, 2002). Drawings depicting the banding patterns of *A. gambiae* s.l. polytene chromosomes are available to identify the divisions and subdivisions and locations of paracentric inversions (Coluzzi *et al.*, 2002; Holt *et al.*, 2002).

It has been reported that infective capacity varies among the sibling species of these complex, with strains of *A. gambiae* being poor vectors of *P. falciparum*, as the parasites do not develop well (or not at all) within them (Cirimotich *et al.*, 2011; Collins *et al.*, 1986). The strains that lack the capacity to transmit the parasites are said to be refractory to infection by the parasite. The ability of a mosquito to transmit malaria parasites depends on the ability of the parasite to complete its life cycle inside the mosquitoes' organism, i.e. reaching the infective (sporozoite) stage in the salivary glands of the host (Sanofi, 2013). The life cycle of *P. falciparum* may be incomplete in the refractory strains of *A. gambiae* due to two factors, namely (i) the vector's innate immune system, through lysis, melanization and hemocyte-mediated phagocytosis (Collins *et al.*, 1986; Hurd *et al.*, 2005; Vernick *et al.*, 1995) and (ii) bacterial competition with the parasite in the midgut of the mosquitoes (Cirimotich *et al.*, 2011).

Macdonald (1952) defined sporozoite rate as the proportion of mosquitoes with sporozoites in their salivary glands. The parameter has been used as an epidemiological index to determine the infectivity of the species of *Anopheles* (Beier *et al.*, 1990). Sporozoite rate can be determined by dissection and ELISA (Adungo *et al.*, 1991; Beier *et al.*, 1990).

Determination of sporozoite rate by dissection has been found to be more accurate and sensitive than the ELISA method. ELISA is limited by overestimation (Adungo *et al.*, 1991; Beier *et al.*, 1990; Sanofi, 2013) and it cannot distinguish between infected and infectious mosquitoes.

There is paucity of information on the infective capacity and refractoriness of *Anopheles gambiae* complex in southwestern Nigeria; in addition, the refractory strains and the infective strains are yet to be identified. However, there is need for identifying these strains in order to know the effective malaria vectors among the sibling species of *A. gambiae* complex as it would contribute to the discovery of effective ways of controlling the malaria parasite and vector, because the effective vector will be targeted.

## Materials and methods

### *Collection and processing of the samples*

Samples of mosquitoes larvae and pupae were collected from five towns of south-western Nigeria, Ado-Ekiti (7°37'16"N 5°13'17"E), Ekiti State; Ilesa (4°44'0"E 7°37'0"N) and Ile-Ife (7°28' N 4°34' E), Osun State; Ibadan (7°23' 47" N 3°55' 0" E), Oyo State and Akure (7°15' 0" N 5°11' 42" E), Ondo State. The mosquitoes larvae and pupae were collected by a dipper, made of a big plastic spoon, from temporary breeding places of rain pools in residential areas of the towns mentioned above. They were transported in bottles with mosquito net covers containing water from their natural habitats, to the laboratory in the Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria. The *Anopheles* larvae were selected within the samples using the resting position of the larvae in water and the diagnostic feature, lack of a respiratory siphon but spiracles on the 8<sup>th</sup> abdominal segment.

### *Rearing of the Anopheles larvae*

The *Anopheles* larvae that were selected from the samples were transferred into new trays with water from their natural habitats and reared in wooden cages (32 x 32 x 32 cm) in the laboratory, under fluctuating temperature between 26 °C and 32 °C; they were fed with finely ground dry biscuit powder lightly sprinkled on the water surface daily. The biscuit was chosen because its powder floats and forms suspension in water and in the same time it does not form colloids that could entangle the larvae and kill them. The larvae were maintained on this diet until they all emerged as adults. The trays that contained the larvae were transferred into new cages two days after the first emergent adults were observed. The transfer of the trays into new cages continued every two days, until all the larvae emerged as adults. This was vital for obtaining adults of the same age groups. The cages were labelled according to the last days of emergence and the locations of collection. The emerging adult mosquitoes were fed with 10% sugar solution. Cotton wool was inserted into a small bottle of sugar solution and the mosquitoes were feeding from the soaked part of the cotton wool that protruded out of the bottle.

### *Breeding F<sub>1</sub> generations of Anopheles mosquitoes from the emerged adults*

Breeding *Anopheles* mosquito involved giving the adult mosquitoes blood meal so that they might lay eggs and

afterwards rearing the laid eggs in order to obtain F<sub>1</sub> adult mosquitoes.

According to Clements (1992) male mosquitoes become sexually mature within 24 hours (one day), while females become sexually mature between 48 hours (two days) and 72 hours (three days) after emergence. Female mosquitoes become receptive to males, usually prior to blood feeding, between two and three days after emergence (Marks *et al.*, 2007). Shute (1936) reported that maximum mating occurs between three and five days after emergence. Therefore, the emergent female *Anopheles* mosquitoes from each location were blood-fed for 15 minutes on the fifth day after emergence in order to lay eggs there are to be collected (Marks *et al.*, 2007). This was done by allowing the mosquitoes to feed on an anaesthetized and immobilized mouse and this continued at intervals of three days. The mouse was anaesthetized following to the ethical guidelines of Canadian Council of Animal Care (CCAC, 2005) and International Society for Applied Ethology (ISAE, 2012). The mouse was restrained by tying its legs. The belly of the mouse was shaved prior to blood-feeding the mosquitoes in order to make the skin easily accessible to the mosquitoes.

A cup of water was then placed in the cage on the third day of blood-feeding the mosquitoes (8<sup>th</sup> day after emergence) for egg collection, since the duration of the gonotrophic cycle (i.e the period between the blood meal, the egg maturation and the subsequent oviposition, is within three days) (Bruce-Chwatt, 1985). The sides of the cups were lined with filter paper and placed in the cages that contained the blood-fed mosquitoes for three days. The mosquitoes laid eggs on the moist filter paper at the sides of the cup.

The filter paper, on which the *Anopheles* mosquitoes laid eggs, was removed from the cups and the eggs on the filter paper were carefully washed with boiled and cooled water into hatching trays that also contained boiled and cooled water. The trays were then placed in the wooden rearing cages 32 x 32 x 32 cm, where they hatched to larvae under the fluctuating laboratory temperature between 26 °C and 32 °C; they were daily fed with finely ground dry biscuit powder lightly sprinkled on the water surface.

In order to ensure the true breed of the emerged F<sub>1</sub> *Anopheles* larvae, species authentication was carried out by using their resting position in water, absence of breathing siphon and presence of palmate hairs on the eighth and fourth abdominal segments respectively (WHO, 1997). These diagnostic features were observed under a light microscope according to the method of Mark *et al.* (2007). The authenticated F<sub>1</sub> *Anopheles* larvae were selected from the stock larvae and then transferred into new rearing trays.

The F<sub>1</sub> *Anopheles* larvae were maintained on the biscuit powder diet until they pupated. The pupae were separated from the larvae by picking them with a picker and transferred into new trays that contained boiled and cooled water. The trays that contained the pupae were then transferred into new cages. They were kept in the cages until they emerged as adults. The emerged F<sub>1</sub> adult mosquitoes were fed with 10% sugar solution.

### *Gender grouping of the F<sub>1</sub> adult mosquitoes*

On the third day of emergence, test tubes were used to collect the emergent F<sub>1</sub> adult *Anopheles* mosquitoes from the cages and they grouped upon the gender, based on the

structures of their antennae and palps (Service, 1980; WHO, 1997). Female mosquitoes were transferred into new cages, while the males were killed by starvation.

#### *Selection for female Anopheles gambiae from the stock*

On the fourth day after emergence, test tubes were used to collect the female *Anopheles* mosquitoes from their cages and the open ends of the test tubes were clogged with cotton balls. The mosquito in the test tube was then placed in a killing jar containing chloroform and anaesthetized for 45 to 60 seconds. The anaesthetized mosquitoes were removed from the killing jar and their morphological characteristics were observed under a stereomicroscope in order to identify and select *A. gambiae* females from the stock. The identification key developed by Mark *et al.* (2007) was first used for this purpose. The species of the female *A. gambiae* mosquitoes were then authenticated by using the Identification Key compiled from those published by Evans (1938), De Meillon (1947) and Hamon and Adam (1963). The identified female *A. gambiae* mosquitoes were then transferred into separate rearing cages and used for the subsequent experiments.

#### *Infection of the F<sub>1</sub> A. gambiae females with Plasmodium falciparum*

For the infection experiment, the female mosquitoes were starved for 18 to 20 hours prior to feeding them with the infectious blood meal (Gnémé *et al.*, 2013). On the 5<sup>th</sup> day of emergence, the identified *A. gambiae* females were infected by allowing them to feed on an individual who had been diagnosed positive for infection by *P. falciparum*, following the regulations of Department of Health and Human Services (HHS, 2009). The infected individual dipped his hand through the sleeve into the cage that contained the female *A. gambiae* for 15 minutes, so that the mosquitoes obtained the infected blood meal from the individual and became engorged. The mosquitoes became infected by the parasite *P. falciparum* from the blood meal they took from the infected individual.

Mosquitoes that were engorged were identified by the colour and nature of their abdomen; they were removed from the cages and transferred into new cages. Female *A. gambiae* mosquitoes that took the blood meal and were engorged were identified by their dark red and swollen abdomen. These infected mosquitoes were then maintained on 10% sugar solution in new cages in the laboratory with fluctuating temperature between 26 °C and 32 °C.

#### *Dissection of the infected mosquito ovaries and salivary glands*

In order to determine the infective capacity of the mosquitoes and to identify the mosquito sibling species, the salivary glands and the ovaries of the mosquitoes were dissected under a dissecting microscope 16 and 18 days after infection, i.e. the time required by *P. falciparum* to reach salivary glands of the mosquitoes (WHO, 1975). The infected mosquitoes were blood fed a day before the dissection, after being starved for 18 to 20 hours. The dissection of ovaries and the salivary glands of the mosquitoes were carried out at the same.

Half gravid females (with ovaries at Christopher III stage) were selected from the mosquitoes 20-24 hours after blood

feeding. Half gravid females are identified when the ovaries take up to 3/5 of the abdomen (Clements, 1992). The half gravid female mosquitoes were anaesthetized for 30 to 50 seconds with ethyl ether in an anesthetizing chamber. The anaesthetized mosquito was then placed on a clean microscope slide and dissected under a dissecting microscope. The wings and legs of the mosquito were first removed. The mosquito was then held down with a dissecting pin at the base (8<sup>th</sup>/9<sup>th</sup> segment) of the abdomen and simultaneously, the last two abdominal segments (genitalia) of the mosquito were gently pulled out with a forceps in order to extract the ovaries. The ovaries were immediately placed in a vial containing modified Carnoy's solution (Cornel, 2007). The ovaries were fixed and preserved in the modified Carnoy's solution in a refrigerator for 24-30 hours at 4 °C.

The mosquito, after extraction of the ovaries, was then placed on its side on a clean microscope slide and gentle pressure was applied on the thorax towards the mesonotum end in order to squeeze the salivary glands into the neck. While gently pressing the thorax, a dissecting needle was used to gently pull the head of the mosquito in such a way that the salivary glands were pulled out of the thorax with it. The salivary glands were gently detached from the head. The remains of the head and the thorax/abdomen were then removed from the slide and a drop of physiological solution (Hayes, 1953) was placed on the salivary gland. The physiological solution was vital for keeping the salivary glands from drying out immediately and to maintain the tissues in a reasonably normal state for observation under a microscope. A cover slip was then placed on the glands and gentle pressure was applied on the glands which helped to rupture the tissues and freed the sporozoites from the salivary glands of infected mosquitoes into the physiological solution.

The salivary glands were stained with Giemsa using the method of Marks *et al.* (2007). The stained salivary glands were inspected for infection by *P. falciparum* under x40 objective of the microscope. The pictures of the salivary glands were taken by an Accuscope LCD Digital Microscope DMS012 (Accuscope Inc., 2011). The number of the infective mosquitoes that had the parasite in their salivary glands and the refractory mosquitoes that did not have the parasite in their salivary glands were recorded.

#### *Estimation of the sporozoite infection rate*

The sporozoite infection rate (SIR) of each study area was determined (Macdonald, 1952; Kakkilaya, 2006).

The sporozoite infection rate was used as an indicator to determine the potential infectivity of the *A. gambiae* mosquitoes, and hence the infective capacity, if they successfully gained access into the natural environment.

#### *Mosquito sibling species identification*

The sibling species of the mosquitoes were identified by developing the cytogenetic maps of the polytene chromosomes prepared from the ovaries of the mosquitoes.

The polytene chromosome spread preparation and staining was done from the fixed ovary (Cornel, 2007). The slide that contained the chromosome spreads was then scanned at 100X magnification and the spreads that showed suitable levels of polytenization were then imaged by the digital imaging system of the Accuscope 3000 LED phase-contrast microscope (Accuscope Inc., 2011) under oil

immersion at 1,000X magnification. The images of the chromosomes were processed using the Macromedia Fireworks image editing software version 8.0 (Macromedia Inc., 2005) on a personal computer. The software was used to increase the quality and the resolution of the polytene chromosome images. The cytogenetic maps of the chromosome images that showed well spread arms and suitable levels of polytenization were then prepared. The arms of the polytene chromosomes were recognized using the arm recognition landmarks of George *et al.* (2010). The cytogenetic map of the 2R arm of the mosquito polytene chromosomes was developed and the localization of each inversion breakpoint or landmark was determined with reference to the *A. gambiae* polytene chromosome maps published by Coluzzi *et al.* (2002) and George *et al.* (2010).

With reference to the data and information from Coluzzi *et al.* (2002) and George *et al.* (2010), a simple identification key was developed. The identification key, consisting of five inversion polymorphisms (2Rj, 2Rb, 2Rc, 2Ru and 2Rd) of 2R arm of the mosquito ovarian polytene chromosomes (Tab. 1), was employed in identifying the sibling species of the mosquitoes.

Tab. 1. Simple identification key for identifying the sibling species of *A. gambiae* complex using common polymorphic inversions. (Tab. derived from Coluzzi *et al.*, 2002 and George *et al.*, 2010)

Inversion	<i>A. gambiae</i>	<i>A. arabiensis</i>	<i>A. buxambae</i>	<i>A. melas</i>	<i>A. merus</i>	<i>A. quadrimaculatus</i>
2Rj	+	-	-	-	-	-
2Rb	+	+	-	-	-	-
2Rc	+	+	-	-	-	-
2Ru	+	-	-	-	-	-
2Rd	+	-	-	-	-	-

+ = present, - = absent

**Statistical methods**

The data collected were subjected to statistical analyses. The proportions of the refractory and the infective mosquitoes and the sporozoite infection rate were estimated by using the Microsoft Office Excel 2007 software. Statistical tests were also carried out by using one-way analysis of variance (ANOVA) using the computerized database SPSS (Version 16.0) for Windows to determine the differences between proportions of the refractory and infective mosquitoes within each location and between the locations. Treatments were considered significant at  $P < 0.05$ .

**Results**

Sporozoite stages of *P. falciparum* were observed in the dissected salivary glands of *A. gambiae* mosquitoes that were

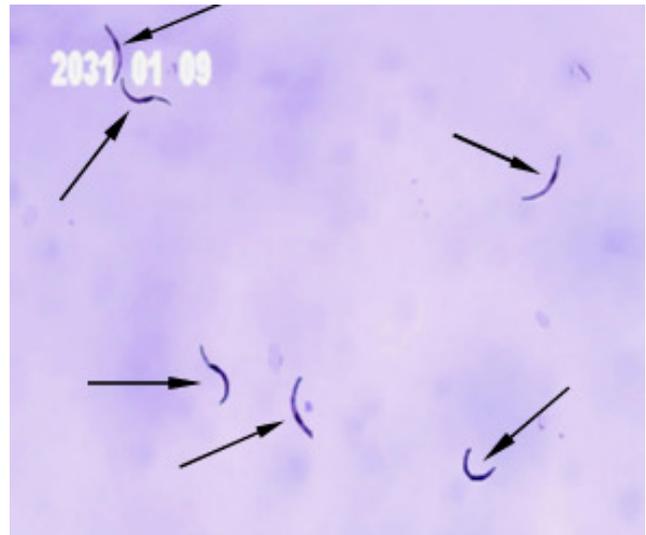


Fig. 1. Sporozoite stage of *P. falciparum* (spindle shaped and darkly stained) in the stained dissected salivary glands of an infective mosquito (→ shows *P. falciparum* sporozoite)

susceptible to infection by the parasite (Fig. 1). Sporozoites were not observed in the salivary glands of the unsusceptible (refractory) mosquitoes. The infective strains, as well as refractory strains, of *A. gambiae* complex mosquitoes were recorded in all the locations.

*Proportions of the infective and the refractory strains*

The proportions of the infective (I) and the refractory (R) strains of female *A. gambiae* mosquitoes from each study area are shown in Tab. 2. There was a significant difference between the proportion of infective and the proportion of refractory strains in each study area ( $P < 0.05$ ), but the proportion of refractory strains as well as the proportion of infective strains between the study areas were not significantly different for  $P > 0.05$ . However, the highest proportion of refractory strains of *A. gambiae* (0.205) was recorded in Ibadan, while the highest proportion of infective strains (0.826) was recorded in Ado-Ekiti.

*Sporozoite infection rate*

Tab. 3 shows the sporozoite infection rates of the mosquitoes from the study areas. The sporozoite rates did not significantly differ among the study areas ( $P > 0.05$ ). A highest sporozoite index of 82.61% was obtained from Ado-Ekiti, while the least sporozoite rate of 79.55% was recorded in Ibadan.

Tab. 2. Proportions of Refractory (R) and Infective (I) *A. gambiae* mosquitoes from the study areas

Study Area	A	b	c	d	e
	Total number of mosquitoes dissected	Number of R mosquitoes	Number of I mosquitoes	Proportion of R Mosquitoes (b/a)	Proportion of I mosquitoes (c/a)
Ilesa	122	24	98	0.197	0.803
Ado-Ekiti	92	16	76	0.174	0.826
Akure	99	18	81	0.182	0.818
Ile-Ife	113	21	92	0.186	0.814
Ibadan	88	18	70	0.205	0.795
Overall	514	97	417	0.189	0.811

R mosquitoes = Refractory mosquitoes; I mosquitoes = Infective mosquitoes

Tab. 3. Sporozoite infection rates of *A. gambiae* mosquitoes from the study areas

Study Area	a	b	c
	Total number of mosquitoes dissected	Number of infective mosquitoes	Sporozoite infection rate (%) (b/a x 100)
Ilesa	122	98	80.33
Ado-Ekiti	92	76	82.61
Akure	99	81	81.82
Ile-Ife	113	92	81.42
Ibadan	88	70	79.55
Overall	514	417	81.12

#### Sibling Species Identification

Two sibling species of the *A. gambiae* complex, viz. *A. gambiae* (*sensu stricto*) and *A. arabiensis* were identified. The infective mosquitoes were *A. gambiae* (*sensu stricto*) and *A. arabiensis*, while the refractory mosquitoes were *A. gambiae* (*sensu stricto*).

The polytene chromosomes of the infective and refractory strains of *A. gambiae* (*sensu stricto*) mosquitoes had the same banding patterns.

#### Discussion

There are several reports on the species abundance and composition, sporozoite and infectivity rates, as well as vector competence of *Anopheles gambiae* in Lagos State, Nigeria (Awolola *et al.*, 2002; Okwa *et al.*, 2006; Okwa *et al.*, 2007) and also in Igbo-Ora, Oyo State, Nigeria (Noutcha and Anumdu, 2009), but the available information on the sporozoite rate of *Anopheles* mosquitoes in southwestern Nigeria is scanty (Awolola *et al.*, 2002; Awolola *et al.*, 2005) and no direct evidence regarding the refractoriness of *A. gambiae* to infection by *P. falciparum* in southwestern Nigeria has been published. In addition, no reports of the identification of the refractory and infective strains of *A. gambiae* complex have been published. Hence, this study was carried out in areas where less or none of these pieces of information are available.

The significantly high proportions of infective strains of *A. gambiae* recorded showed that the species of *A. gambiae* complex are highly susceptible to *P. falciparum* infection. Similar reports have also been recorded showing that *A. gambiae* mosquitoes are highly susceptible to *P. falciparum*, (Molina-Cruz *et al.*, 2012; Collins *et al.*, 1986). It also corroborates the idea that despite the immune response of mosquitoes against *Plasmodium* infection, the parasites are capable of using some molecules produced by the mosquito to evade the mosquito innate immune response (Osta *et al.*, 2004), which makes the mosquito susceptible.

The lack of significant differences among the sporozoite infection rates between the study areas might probably be due to the fact that the parasite and the vector originated from the same geographical origin (United States, 2004). This suggests a geographic scale of adaptation and sympatric association between the parasite and the vector. Similar reports of geographic scale of adaptation (Haris *et al.*, 2012) and sympatric association (Gn  m   *et al.*, 2013) have been posited. This is also in agreement with the reports of Collins *et al.* (1986), Molina-Cruz *et al.*, (2012) that showed the survival of *P. falciparum* in *A. gambiae* mosquitoes seemed to correlate with their geographical origin. It also shows the

importance of sympatric association in vector-parasite association, and hence the ability of the parasite to evade the mosquito immune response and survive in the vector, which in turn determines the infective capacity and vector competence to transmit the parasite. Collins *et al.* (1986) and Molina-Cruz *et al.* (2012) also reported that the evasion of the *A. gambiae* immune system by *P. falciparum* may be the result of the parasite adaptation to the sympatric mosquito. However, the fact that some of the mosquitoes were refractory corroborates the reports of Cirimotich *et al.* (2011), Collins *et al.* (1986), Dimopoulos *et al.* (2001), Juli  n (2010) that state the fact that the mosquito's innate defence surveillance system is also capable of triggering defence reactions, which in some cases can terminate the development of all the parasites, leading to total refractoriness.

The high sporozoite infection rates in all the study areas, which ranged between 79.55% in Ibadan and 82.61% in Ado-Ekiti, showed that the species of *A. gambiae* complex are effective vectors of *P. falciparum* in southwestern Nigeria. The sporozoite infection rates were significantly higher than the *P. falciparum* sporozoite infection rates for *A. gambiae* reported in some other studies. For instance, the *P. falciparum* sporozoite infection rates for *A. gambiae* that were recorded at Igbo-Ora in 2001 and 2002, a rural community of Oyo State in south-west Nigeria, were 6.70% and 6.30% respectively (Noutcha and Anumdu, 2009). Along Badagry Axis of Lagos Lagoon, Lagos, Nigeria, *P. falciparum* sporozoite infection rates of 4.8% and 6.5% for *A. gambiae* s.s and *A. melas* respectively were reported (Oyewole *et al.*, 2010). Oduola *et al.* (2012) reported that *P. falciparum* sporozoite infection rates of *A. gambiae* s.s. in six rural communities of Oyo State, Southwestern Nigeria, varied between 1.9% and 3.1%. Also in Western Kenya and in the Kiruhura District (formerly part of Mbarara District) in southwest of Uganda, low *P. falciparum* sporozoite infection rates of 6.3% and 0.84 - 5.26% for *A. gambiae* were recorded respectively (Echodu *et al.*, 2010). However, high *P. falciparum* sporozoite rates for *A. gambiae* were similarly reported in some other studies. Okwa *et al.* (2006) reported *P. falciparum* sporozoite rates of 62.9% for *A. gambiae* in Badagry area of Lagos, Nigeria. In another study, Okwa *et al.* (2007) reported *P. falciparum* sporozoite rates of 50%, 51.2%, 53.3%, 79.4% and 95.5% for *A. gambiae* in Amuro Odofin, Mushin, Ajeromi, Ojo and Alimosho areas of Lagos State, Nigeria respectively. In a similar study in which *A. gambiae* mosquitoes were infected with *P. falciparum*, using direct membrane feeding, in laboratory, 83.52% sporozoite infection rate was recorded (Ndiath *et al.*, 2011); this is

virtually equal to the sporozoite infection rates obtained in this study. Hence, the high *P. falciparum* sporozoite infection rates for *A. gambiae* recorded in all the study areas in this study justify the reports from other studies that *A. gambiae* is highly infective in southwestern Nigeria (Okwa *et al.*, 2007) and it is a prominent malaria vector in Nigeria (Annon, 2003; Gilles and Coetzee, 1987). It has also been reported that *A. gambiae* is the most prominent malaria vector of *P. falciparum*, the mosquito species with the highest sporozoite rate and the most infected mosquitoes in the rain forest zone, south-west, of Nigeria (Okwa *et al.*, 2008).

The fact that the infective and refractory strains of *A. gambiae* (*sensu stricto*) had the same polytene chromosome banding patterns showed that the infective and refractory strains cannot be distinguished using their polytene chromosome banding patterns/photomaps.

### Conclusions

This study showed that the refractory strains of *Anopheles gambiae* complex are present, but in low frequencies, in southwestern Nigeria, and that the sibling species of *Anopheles gambiae* (*A. gambiae* s.s. and *A. arabiensis*) are potent malaria vectors in the rain forest region of Nigeria. Even so, no conclusion could be drawn that refractoriness is species-specific, i.e. it might not be specific to *A. gambiae* (*sensu stricto*); hence, further studies are recommended.

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