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Full Length Research Paper

Distribution, Abundance, and Vector Competence of Anopheles Species Across Six Regions in Lagos, Nigeria

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Malaria is a problem in Nigeria and the risk exists throughout the country. Anopheles mosquitoes have been incriminated as the major malaria vectors. Vectors competence, however, differs from one species to another and from place to place. The present study reports the species abundance, composition and vectoral competence of Anopheles species in six areas of Lagos in Nigeria. The human blood index (HBI) and sporozoite rates (SR) were the components of vectoral competence. Human landing catches was used in collection. Species identification morphologically and by polymerase chain reaction (PCR) was carried out on mosquitoes. Enzyme linked immuno- arsorbent assay (ELISA) was used to examine – sporozoite infected Anopheles and their blood meal origin. Anopheles *gambiae s.s.* was found to be most widespread and competent vector in all the six areas. The highest proportions of female Anopheles caught were from Alimosho area (49.8%), but they were mainly with no blood meal (47.9%). In Ajeromi, 85.7% of the female Anopheles had blood meals. Anopheles in Agege area, however, had the highest HBI. Sporozoite rate was highest in Mushin but lowest in Agege. The other Anopheles species were less relatively competent compared to Anopheles gambiae s.s, Anopheles *funestus s.s* was the second predominant and competent vector in all the areas. Anopheles moucheti nigeriensis was collected from Ajeromi and Amuwo Odofin areas alone and carried sporozoites in the two areas. Ojo area was identified as the most endemic because of the HBI 61.6% and SR of 62.9%. The present study has provided baseline data for formulating control programmes in Lagos State, Nigeria.

Key words: Anopheles Species, vectoral competence, mosquitoes, malaria, Lagos–Nigeria, Human blood index, sporozoite rate.

INTRODUCTION

It is an established fact that mosquitoes are the most im-portant insects affecting human health(Woodbridge and Edward, 2006) chiefly in spread of malaria the most com-mon lethal disease second only to HIV/AIDS (Rowton, 2005). More than 200 million die from it in Nigeria alone (Anthony et al., 2004) malaria accounts for 25% infant mortality and 30% of childhood mortality (Annon, 2003). Ninety percent of infection is caused by Plasmodium falciparium in Nigeria (Awolola et al., 2005).

On the basis of intensive studies carried out, during 2003- 2005, in Nigeria, Awolola et al. (2002, 2003, 2005) reported the existence of scanty information on the spo-rozoite rate of Anopheles mosquitoes in Southern Nige-ria.

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Malaria transmission is variable from one area to another and this has an impact on its epidemiology and control (CDC, 2004). In another study by Okwa et al. (2006), in Badagry a coastal area of southern Nigeria, several species of Anopheles occur in sympatry. These species all contributed to the transmission of malaria as potential vectors.

Vector capacity and vector competence has been used interchangeably to describe the ability of mosquitoes to serve as a disease vector. Vectoral capacity is defined qualitatively and is influenced by such variables as vector density, longetivity and vector competence (Rradraina-solo and Colluzzi, 1989). Vectoral capacity takes into account environment, behavioral, cellular and biochemi-cal factors that influence the association between vector, pathogen transmitted by the vector and the vertebrate host to which the pathogen is transmitted (Anthony et al., 2004; Rradrainasolo and Colluzzi, 1989).

Vector competence is a component of vectoral capacity

and is governed by intrinsic (generic) factors that influence the ability of a vector to transmit a pathogen. Host feeding preference or susceptibility to sporozoite stage of Plasmodium species are important components of vectoral competence (Osta et al., 2004)

The present study aimed to compare the Anopheles species, the sporozoite rate and human blood indexes of the Anopheles mosquitoes found in six areas of Lagos State, Nigeria. With objectives:

i. to compare the Anopheles species distribution in the six areas;
ii. to compare the sporozoite rates and human blood indexes, which are components of vectoral competence.

The results of this study will provide a baseline data for control of Anopheles mosquitoes in these areas, which are otherwise densely, populated areas of Lagos in Nige-ria.

MATERIAL AND METHODS

Study area

Nigeria is 923, 7685 km wide (Mabogunje, 1993), with a population of 140.3 million (2007 census). Lagos is the second most populous State in Nigeria-with a size of 3,577 square kilometers representing 0.4% of the country. It is the smallest state in the federation, 22% of 787 square kilometers is made up of lagoons and Creeks. The State is a socio-culture meeting point attracting both Nigerians and Non-Nigerians. Lagos was the former capital of Nigeria; still the commercial, industrial, socioeconomic and political nerve of the country.

Based on their dense population, six important sub-urban local government areas of the state were selected. These areas are Alimosho, Agege, Amuwo Odofin, Ajeromi, Mushin and Ojo. These areas are few kilometers apart from each other. Rainfall is the real climatic variable in Nigeria with June – September, the rainiest. These areas are usually characterized by stagnant pools in the rainy season where enormous numbers of mosquitoes breed.

Mosquito collection

Indoor collections were made monthly in the rainy season (July – September), simultaneously in the six areas. Five houses were selected randomly in each area. Human landing catches were carried out using World Health Organization (WHO) standard proce-dure (WHO, 1995) which adopts the stationed human bait collector method. Samples of mosquitoes caught were preserved dry on silica gel.

Morphological Identification of mosquitoes

Anopheline were distinguished from Culicine mosquitoes according to the morphological characteristics of their maxillary palps (Gilles and Coetzee, 1987). Anopheles *moucheti nigeriensis* was distinguished from other Anopheline by the presence of fringe spot opposite the 6th vein on the wings of the mosquitoes (Gilles and Coet-zee, 1987). The entire female Anopheles were dissected trans-versely at the thorax between the 1st and 3rd pairs of legs under a dissecting microscope (x20). The abdomens were preserved for blood meal analysis while the heads and thorax were preserved for circum - sporozoite – antigen (CSP) detection. The wings and legs were preserved for molecular identification.

Molecular Identification of Anopheles species

All other Anopheles, (except *An. moucheti*) that had been identified had Genomic DNA extracted from the wings and legs according to the standard procedures of Collins et al. (1987). The extracted DNA pellets were subjected to polymerase chain reaction (PCR) using species specific primers for Anopheles *gambiae* and Anopheles funestus complexes as described by Scott et al. (1993) and Koekemoer et al. (2002). Laboratory strains of the Anopheles complexes were used as controls. PCR products were electrophoresed on 1.4% ethidium bromide stained agarose gels at 80 volts for 40 min. The amplified fragments were then visualized by ultraviolet transilluminator and photographed with a syngene bio-imaging system.

Anopheline vectoral competence

Blood meals of engorged female Anopheles were subjected to direct ELISA using anti phosphatase conjugates Anti human 1 gG (Fab specific); Antibovine 1 gG (whole molecule); Anti goat 1 gG (whole molecule) (Sigma). They were used to identify Human, cattle and goat respectively based on the procedures of Beier et al. (1998). A total of 233 Anopheles with blood meal were assayed altogether. Para-nitrophenol phosphate (pnpp) was substrate used. Samples were considered positive on the ELISA plates if the optical densities (OD) were at least twice the mean of four negative wells on same plate. Positive controls were female Anopheles with kno-wn blood meals. Negative control contains male Anopheles tritu-rates.

The heads and thoraxes of the individual anophelines (439), in number, were tested for circumsporozoite antigen using pf 2A10 monoclonal antibody according to Wirtz et al. (1987) procedures. Peroxidase conjugated antibodies were also used in this Sandwich ELISA method according to the standard protocol of Beier et al. (1987) Five negative controls (male anophelines) and 3 positive controls (the appropriate synthetic peptide) were included in each plates. Samples were read like in the blood meal ELISA after addition of substrate ABTS (2, 2 azinodi -3-ethylbentiazoline) was used as peroxidase substrate.

Determination of human blood indices and sporozoite rates

The Human blood index (HBI) which is the proportion of female anopheles giving a positive reaction for human blood and multiple blood feeds with human blood was calculated (Shililu et al., 1998). Sporozoite rates were determined as the percentage of anophe-lines carrying *P. falciparium* (CSP) antigen i.e. those that tested positive on the ELISA plate.

RESULTS

The highest mosquito collection was made in Mushin area (dominated by Culicine mosquitoes that transmit yellow fever) while the lowest was in Agege which was dominated by Anopheles species. There was more Ano-pheles (586) (50.9%) than Culicine (572) (49.6%) though insignificantly different. There was also more female Ano-pheles (439) (37.9%) than female Culicine (316) (27.2%). The highest number of female Anopheles was caught in Alimosho area (148) (49.8%) . Table 1 show the morpho-logical identification into two genera and sexes of mos-quitoes in the six areas. There was more engorged fema-

Table 1. The morphological identification into two genera and sexes of mosquitoes collected in the six areas of Lagos, Nigeria.

Areas	Culicine Male (%)	Culicine Female (%)	Anopheles Male (%)	Anopheles Female (%)	Total (%)
Agege	10(10)	16(16)	17(17)	57 (57)	**100 (8.63)**
Alimosho	45 (15.6)	62(20.8)	42(14.1)	148 (49.8)	**297 (25.6)**
Ajeromi	40 (20.1)	36 (18.0)	39(19.5)	84 (42.2)	**199 (17.1)**
Amuwo-Odofin	16(14.8)	46 (42.5)	12 (11.1)	34 (31.4)	**108(9.3)**
Mushin	127 (38.8)	138(42.2	8 (2.4)	34 (10.3)	**327 (28.2)**
Ojo	18 (14.1)	18 (14.1)	29 (22.8)	62 (48.8)	**127 (10.9)**
Total	**256 (22.1)**	**316(27.2)**	**147 12.6)**	**439 (37.9)**	**1158**

Df = 5 P < 0.05

Table 2. The Anopheles with blood meal (engorged) and without in the six areas of Lagos, Nigeria.

Areas	Engorged (%)	Unengorged (%)	Total
Agege	12 (21)	45 (78.9)	57
Alimosho	77 (52)	71 (47.9)	148
Ajeromi	72 (85.7)	12 (14.2)	84
Amuwo-Odofin	22 (64.7)	11 (32.3)	34
Mushin	31 (57.4)	23 (42.5)	54
Ojo	19 (30.6)	43 (69.3)	62
Total	233 (53)	205 (46.6)	439

NB HBI calculated based on engorged Anopheles only. Df = 5 P < 00.5

le Anopheles in Ajeromi area 72 (85.7%) than others. Agege area had the lowest number of engorged Anopheles mosquitoes 12 (21%) . The largest proportion of unengorged Anopheles was caught in Agege area (78.9). Table 2 shows Anopheles with and without blood meals in the six areas.

The result of the molecular identification revealed that, *An. gambiae s.s.* constituted the bulk of the collection in all the areas with the highest prevalence of 87.7% in Agege while Mushin had the least 62.9%. *An. gambiae s.s.* abundance was therefore above average in all the areas.

An. funestus s.s was the next predominant species found in all the areas, but significantly less abundant than. Anopheles *gambiae s.s.* This species was most prevalent in Alimosho 40 (27%) and least in Mushin (2.3%). Anopheles *arabiensis* was collected in two areas; Alimosho 7 (4.7%) and Amuwo Odofin 4 (36.3%) *An. Moucheti nigeriensis* was collected only in Ajeromi 4 (4.7%) and Mushin areas 9 (16.6%).

Table 3 shows the species composition of female Anopheles. *An. gambiae s.s.* had the highest sporozoite rates and human blood indexes in all areas. All the Anopheles species were competent vectors of *Plasmodium falciparium* sporozoites. *An. funestus* was the second compe-tent vector after *An. gambiae s.s.* Alimosho area had the highest proportion of these two important vectors.

Overall, HBI was highest in Agege (100%) as the twelve mosquitoes sampled had bitten only humans. HBI was least in Amuwo Odofin (18.7%) sporozoite rate was highest in Mushin (75.9%) but lowest in Agege (14%). The least abundant and least competent vector was *An. arabiensis* which was found in Amuwo Odofin and Alimosho areas. It appeared as a competent vector only in Alimosho area. *An. moucheti nigeriensis* collected from Ajeromi and Mushin areas had sporozoites in both areas. Ojo area had HBI and SR above average (50%).

Table 4 shows human blood indices and sporozoite rates of individual species and combined (all) Anopheles species in the six areas. The overall HBI was 40.7% while SR was 35.5%.

DISCUSSIONS

Malaria has probably infected humans for over 50,000 years and may have been a human pathogen for the entire history of our species (Woodbridge and Edward, 2006). No thanks to the diverse Anopheles species that can transmit malaria. An estimated 60% of the African populations have malaria each year (Awolola et al., 2005) This is of course, attributed to the environmental condi-tions, a replica of which was found in all the six area cha-racterized by stagnant pools, open gutters and drainages. *An. gambiae s.s* had the highest HBI and sporozoite rate and this was followed by *An. funestus s.s.* According to Githeko (1994) species which feed on many host are less

Table 3. Species composition of Female Anopheles (identified molecularly) collected in the six areas of Lagos, Nigeria.

Areas	No caught	An. gambiae s.s. (%)	An. Arabiensis (%)	An. funestus s.s. (%)	An. Moucheti (%)
Agege	57	50 (87.7)	(0)	7 (12.2)	(0)
Alimosho	148	101(68.2)	7 (4.7)	40 (27)	(0)
Ajeromi	84	70 (83.3)	(0)	10 (11.9)	4 (4.7)
Amuwo-Odofin	34	22 (64.7)	4 (36.3)	8 (23.5)	(0)
Mushin	54	34 (62.9)	(0)	11 (2.3)	9 (16.6)
Ojo	62	54 (87)	(0)	8 (12.9)	(0)
Total	**439**	**331 (75.3)**	**11 (2.5)**	**84 (19.1)**	**13 (2.9)**

Df = 5 P < 0.05

Table 4. Combined species Human blood indexes (HBI) and sporozoite rate (SR) in the six areas of Lagos, Nigeria.

Areas	An. gambiae s.s		An. arabiensis		An. funestus s.s		An. moucheti		All Species	All species
	HBI	SR	HBI	SR	HBI	SR	HBI	SR	HBI (%)	SR (%)
Agege	12(100)	8 (14)	None		0 (0)	0 (0)	None		12 (100)	8 (14)
Alimosho	17(74)	20(91)	2(8.67)	1(4.5)	4(17.3)	1(4.5)	None		23 (29.8)	22 (14.8)
Ajeromi	14(45.1)	6(53.3)	None		16(51.6)	11(36.6)	1(3.2)	3 (10)	31 (43)	30 (35.7)
Amuwo Odofin	9(18.75)	8(50)	0 (0)	0 (0)	0 (0)	8 (50)	None		9 (18.75)	16 (43.24)
Mushin	9(29)	21(51.2)	None		0 (0)	11 (26.8)	0 (0)	9 (21.9)	9 (29)	41 (75.9)
Ojo	11(61.6)	31(79.4)	None		0 (0)	8 (20.5)	None		11 (61.6)	39 (62.9)
Total	**72(75.7)**	**04(66.6)**	**2(2.1)**	**1(0.64)**	**20 (21)**	**39 (25)**	**1(1.05)**	**12(7.69)**	$^{95}/_{233}$**(40.7)**	$^{156}/_{439}$**(35.5)**

The figures in parentheses () represent percentages.

likely to be such a good malaria vector as highly anthropophagic ones. The anthropophagy of these two important vectors has been documented by Awolola et al. (2002, 2003, 2005). The abundances of the vectors app-ear to correlate with their vector competencies. This stu-dy, however, confirms the sympatric distribution of Ano-pheles species. The omnipotency of *An. gambiae s.s.* in Nigeria has been settled (Annon, 2003; Gilles and Coet-zee, 1987).

Okwa et al. (2006) showed that *An. gambiae s.s* was the most prominent species in Badagry area of Lagos, Nigeria. A sporozoite rate of 4.2% and HBI of 67.3% was obtained. In a similar study by Oyewole et al. (2006), Anopheles melas were more abundant in Iworo area of Badagry Local Government of Lagos, Nigeria. In the above studies *An. arabiensis* was the least abundant and least competent. It had been described as a savannah and dry season zoophilic vector (Randrainsolo and Col-luzi, 1989). The study areas are located in the rain forest zone of Nigeria, so this is not surprising as the collections were also made in the rainy season. These results are in line with this present study.

Many projects have been directed at *An. gambiae s.s*, and this looks promising. More research should also be directed at the *An. funestus* group. Gilles and Coetzee (1987) stated that *An. funestus* is the next problematic specie in Africa, though more restricted in habitat choice. Three members of this group have been found to date in Nigeria (Awolola et al., 2005) only *An. funestus s.s* was identified within this specie complex, in this study.

An. moucheti nigeriensis is also a good relatively malaria vector though less abundant. It has been known as playing lesser roles compared to *An. funestus s.s* and *An. gambiae s.s.* It is also a member of complex species; (*An. moucheti* complex) (Antonio-Nkondijo et al., 2002). This species complex also deserves attention.

Most of the mosquitoes caught in this study, were caught attempting to bite the bait around the foot region. This is another interesting finding. Oduola and Awe (2006) learned that mosquitoes preferring the foot region were significantly higher when compared with other different parts of human host such as ankle, calf and thigh. Mala-ria resistant mosquitoes have been recently developed in the laboratory. This suggests that practically the compe-tent vectors could be manipulated by locating resistant genes (MSNBC, 2007). The malaria vectoral system in these six areas, which are just few kilometers apart, shows that an understanding of these local vectors biono-mics and transmission are vital for successful malarial control. Any strategy adopted by the Lagos government should take account this heterogeneities, so that relevant Anopheles species in each area can be targeted.

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