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Malaria Transmission Risk Indices of Three *Anopheles* Species in Selected Rural Communities in Oyo State South-Western Nigeria

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Abstract: An entomological survey was carried out in selected rural communities in Oyo State Nigeria where entomological baseline data required for implementation and evaluation of vector control interventions is lacking. A total of 6806 Anopheles mosquitoes were collected in six rural communities out of which 10 *Anopheles* species were identified. *An. gambiae*, *An. funestus* and *An. coustani* were the only vector species that occurred in all the communities. Only two species: *An. gambiae* 2596 (38.1%) and *An. funestus* 3785 (55.6%) were found to be predominant. Polymerase Chain Reaction (PCR) analysis showed that *An. gambiae* s.s and *An. arabiensis* occurred in sympatry in all the communities while *An. funestus* s.s and *An. leesoni* occurred together in Oko Agric and Idi Ose. Cicumsporozoite (CSP) infection status of each Anopheles mosquitoes determined by Enzyme Linked Immunosorbent Assay (ELISA) showed that the *Plasmodium falciparum* sporozoite infection rates of *An. gambiae* s.s varied between 1.9 and 3.1% in the study communities as compared to 1.5 and 4.5% in *An. funestus*. Only one sample of *An. arabiensis* from Akufo community tested positive to the *P. falciparun* CSP antigen, all the other samples including *An. leesoni* tested negative to either *P. falciparum* or *P. malariae*. The annual EIR in Akufo, Ikere and Idi Ose were 139, 153 and 110 infective bites/person/year, respectively with *An. gambiae* s.s contributing a higher overall transmission potentials when compared with *An. funestus* and *An. arabiensis*. This result shows the abundance of malaria vector species and their potential role in malaria transmission in rural communities.

Key words: *Anopheles* species, malaria, vectors, transmission risk indices, rural communities, Nigeria

INTRODUCTION

The reported case of malaria around the world is put at 243 million with an estimated death of 863,000. About 89% of these deaths occurred in Africa (WHO, 2009). Out of the 109 countries where malaria is reported to be endemic, 45 are within the WHO Africa region. Nigeria accounted for one fourth of all estimated malaria cases in the WHO African region in 2006 (WHO, 2009). A large percentage of the population affected with this disease live in extreme poverty in rural communities with few having access to good healthcare facilities (Otubanjo and Mafe, 2002; Amexo *et al*., 2004; Obrist *et al*., 2007).

The human malaria parasite in Nigeria includes: *Plasmodium falciparum*, *P. ovale* and *P. malariae*. However, *Plasmodium falciparum* is responsible for >95% of all malaria cases transmitted. Malaria parasites are usually transmitted through the bites of infected female mosquitoes of the genus *Anopheles*. These *Anopheles* species are widely distributed across the different ecological zones in Nigeria where suitable sub-Saharan climatic conditions exist (Molineaux and Gramiccia, 1980; Kiszewski *et al*., 2004). Despite several efforts being made in the implementation of vector control interventions, baseline data on malaria vector profile and malaria transmission risk indices in most Nigerian communities are lacking.

Hence, evaluations of these malaria control programmes remain a challenge (Smith *et al*., 2004; Mabaso *et al*., 2007).

This study seeks to bridge this gap by providing information on malaria vector profiles and malaria transmission indices such as: vector species identification, Biting Rates (BR) and Entomological Inoculation Rate (EIR) in selected rural communities in Oyo State, Nigeria.

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MATERIALS AND METHODS

Study area: The study was carried out in rural communities in Ibadan (N07°29.059' E003°49.050'), Iseyin (N08°09.785' E003°40.323') and Eruwa (N07°32.585' E003°27.219') between June 2005 and May 2007. Two communities were randomly chosen from each of the selected localities; Akufo and Ido in Ibadan, Ikere and Olobo in Iseyin, Oko Agric and Idi Ose in Eruwa. The chosen settlements were all rural communities located in the forest-savannah transition ecosystem. The houses chosen for mosquito collection had surrounding vegetation and either a river or streams within a walking distance. The people in the area are mostly farmers, growing cassava yam and maize while some of them are involved in lumbering and charcoal production as their main source of income earning.

Entomological sampling: Ten households were randomly selected for the collection of indoor resting anopheline mosquitoes in each of the rural communities (WHO, 2003). Altogether, 60 households were marked out for mosquito collection throughout the period. Night human landing catches were conducted by volunteers in one representative community (Akufo, Ikere and Idi Ose) from each of the localities in order to estimate the man biting rates (Service, 1977). Mosquito samples were collected with aspirators into labeled paper cups and knocked down with chloroform. Specimens were preserved in eppendorf tubes filled with desiccated silica gel overlaid with tissue paper. These specimens were transferred to the laboratory for further analysis. Preserved samples were identified using morphological keys of Gillies and De Meillon (1968) and Gillies and Coetzee (1987).

Laboratory analysis of mosquitoes: Mosquitoes identified as belonging to the major vector groups; *An. gambiae* s.l and *An. funestus* s.l were separated during the identification procedure for separate individual PCR. Members of the *An. gambiae* s.l and *An. funestus* s.l. were molecularly identified to species level following the procedures of Scott *et al*. (1993) and Koekemoer *et al*. (1999). In order to determine the infection rates, the head and thorax of each identified mosquito sample was separated from the body and homogenised in blocking buffer. The homogenates were aliquoted into microtiter plates coated with monoclonal antibodies of *Plasmodium falciparum* and *P. malariae* in a double antibody enzyme-linked immunosorbent assay (Burkot *et al*., 1984; Wirtz *et al*., 1987). The positive controls (supplied by Kikergaard and Perry Laboratories, USA) and negative controls from uninfected laboratory reared colony were also assayed.

Entomological Inoculation Rates (EIR): The man biting rate was estimated from night human landing catches conducted in 4 houses from each of the 3 selected communities (Akufo, Idi Ose and Ikere). The Entomological Inoculation Rate (EIR) was determined by multiplying the man biting rate estimated for each community with the proportion of mosquito specimens that tested positive for the circumsporozoite protein (sporozoite rate).

Ethical consideration: Informed consent of volunteers willing to participate in mosquito collections were obtained through series of individual discussions and meetings organized with the family heads and community leaders before the project started. For volunteers that participated in the mosquito collection, malaria prophylaxis treatment was given during the course of the study.

Data analyses: Paired t-test was conducted to determine significant differences in species composition, infection rates, man biting rates of each vector species in the rural communities using SPSS Version 15.0 (SPSS Inc. Chicago Illinois).

RESULTS

Composition of Anopheles mosquitoes in the rural communities: A total of 6,806 Anopheles mosquitoes were collected indoors in 6 rural communities between June 2005 and May 2007 (Table 1). Ten *Anopheles* species namely; *An. gambiae*, *An. funestus*, *An. rhodesiensis*, *An. rhodesiensis rupicolus*, *An. maculipalpis*, *An. coustani*, *An. nili*, *An. longipalis*, *An. ziemani* and *An. moucheti* were morphologically identified from these collections. *An. moucheti* and *An. nili* were not identified in all the communities when compared with *An. gambiae*, *An. funestus* and *An. coustani* identified in all the communities

Table 1: Numbers and species of *Anopheles* mosquitoes collected within six rural communities

	Communities sampled							
Anopheles species	Akufo	Ido	Ikere	Olobo	Oko Agic	Idi Ose	Total	Anopheles caught (%)
An. gambiae	474	394	548	610	324	246	2596	38.1
An. funestus	895	453	1157	551	456	273	3785	55.6
An. rodesiensis	21	43	25	20	20	7	136	2.0
An. rhodesiensis rupicolus	12	12	20	12	0	5	61	0.9
A. maculipalpis	8	36	6	2	0	2	54	0.8
An. coustani	17	7	5	1	3	4	37	0.5
An. nili	9	5	7	3	5	0	29	0.4
An. longipalis	16	15	16	8	16	7	78	1.1
An. ziemani	5	3	4	7	2	0	21	0.3
An. moucheti	2	3	0	4	0	0	9	0.1
Total	1459	971	1788	1218	826	544	6806	-

Table 2: Species composition of *Anopheles gambiae* s.l and *An. funestus* s.l in rural communities

Location	Species composition (%)			
	An. gambiae	*An. arabiensis*	*An. funestus*	*An. leesoni*
Akufo	449 (94.7)	25 (5.3)	325 (100.0)	0.0
Ido	369 (93.6)	25 (6.4)	266 (100.0)	0.0
Ikere	480 (87.6)	68 (13.4)	332 (100.0)	0.0
Olobo	560 (91.8)	50 (8.2)	323 (100.0)	0.0
Oko Agric	297 (92.8)	23 (7.2)	312 (68.6)	143 (31.4)
Idi Ose	229 (95.0)	12 (5.0)	199 (72.8)	74 (27.1)
Total	2384.0	203.0	1757.0	217.0

(Table 1). Two major vector groups; *An. gambiae* 2596 (38.1%) and *An. funestus* 3785 (55.6%) were found to be predominant in the mosquito sample collection (Table 1).

Species composition of the major vector groups: The Polymerase Chain Reaction (PCR) assay identified the *An. gambiae* s.s and *An. arabiensis* as the only two members of the *Anopheles gambiae* complex. Species identified as members of the *Anopheles funestus* group were *An. funestus* s.s and *An. leesoni* (Table 2). The composition of these species occurred in varying proportion across the rural communities surveyed. Out of the 2,587 mosquitoes positively identified by the PCR, 2384 (92.2%) were characterized as *An. gambiae* s.s and 203 (7.8%) as *An. arabiensis* (Table 2). The difference in the proportion of *An. gambiae* s.s over *An. arabiensis* was found to be significant (p = 0.000). Similarly, out of a total of 1974 *An. funestus* s.l mosquito positively identified by the PCR Assay, 1757 (89%) were characterized as *An. funestus* s.s while 217 (11%) were characterized as *An. leesoni* (Table 2).

An. funestus s.s was found widely distributed in all the rural communities surveyed compared to *An. leesoni* identified in two rural communities (Oko Agric and Idi Ose) (Table 2). The proportion of *An. funestus* s.s was not significantly different from *An. leesoni* in these two communities (p = 0.687).

Sporozoite infection rates in the vector species: A total of 4561 mosquitoes identified as *An. gambiae* and *An. funestus* were assayed for *P. falciparum* and *P. malariae* Circumsporozoite Protein (CSP) antigen. Out of the 2384 mosquitoes identified by the PCR as *An. gambiae* s.s, 54 (2.3%) samples tested positive for *P. falciparum* CSP. Only 48 (2.7%) *An. funestus* mosquitoes tested positive for *P. falciparum* out of a total of 1732 assayed. The *P. falciparum* sporozoite infection rates of *An. gambiae* s.s varied between 1.9 and 3.1% in the study communities as compared to 1.5 and 4.5% in *An. funestus* (Table 3). While only one sample of *An. arabiensis* from Akufo community tested positive to the *P. falciparun* CSP, all the other samples identified including *An. leesoni* tested negative to either *P. falciparum* or *P. malariae*. Though,

Table 3: Malaria transmission indices of major vectors identified in 6 rural communities

Locality	EIR	Transmission indices	*Anopheles gambiae*	*Anopheles funestus*	*Anopheles arabiensis*
Akufo		Number tested	449	325	25
		Mbr	5.65	7.15	1.23
		Tested +ve for CSP	12	8	1
		SPR	2.7	2.5	4
		ib/p/night	0.153	0.179	0.049
	139	Annual EIR	56	65	18
Ido		Number tested	369	266	25
		Mbr	ND	ND	ND
		Tested +ve for CSP	7	4	0
		SPR	1.9	1.5	0
		ib/p/night	-	-	-
		Annual EIR	-	-	-
Ikere		Number tested	480	332	68
		Mbr	10.05	3.575	2.7
		Tested +ve for CSP	15	10	0
		SPR	3.1	3	0
		ib/p/night	0.312	0.107	0
	153	Annual EIR	114	39	0
Olobo		Number tested	560	323	50
		Mbr	ND	ND	ND
		Tested +ve for CSP	8	7	0
		SPR	1.4	2	0
		ib/p/night	-	-	-
		Annual EIR	-	-	-
Idi Ose		Number tested	229	199	12
		Mbr	6.5	3. 975	0.875
		Tested +ve for CSP	7	5	0
		SPR	3.1	2.5	0
		ib/p/night	0.202	0.099	0
	110	Annual EIR	74	36	0
Oko Agric		Number tested	297	312	23
		Mbr	ND	ND	ND
		Tested +ve for CSP	5	14	0
		SPR	1.7	4.5	0
		ib/p/night	-	-	-
		Annual EIR	-	-	-

the average sporozoites rate of *An. funestus* in the study communities 2.67 was higher when compared with sporozoite infection rates of 2.32 in *An. gambiae*. There was however no significant difference in the sporozoites rate of both *An. gambiae* s.s and *An. funestus* s.s in all the rural communities (p = 0.861).

The human Biting Rates (BR), Entomological Inoculation Rates (EIR) of the major malaria vector species: The human biting rate per night recorded in *An. gambiae* in Ikere (10.05) was the highest when compared to HBR contributions by the 3 major vectors from the 3 study communities (Table 3). The total HBR from all the species in each of the communities; Akufo, Ikere and Idi Ose were 14.03, 16.35 and 11.35, respectively. Though the total biting rates in Ikere was higher when compared to the 2 other study communities the difference was however not significant (p = 0.06). The numbers of infective bites per person per year by vector species were estimated for the localities using the 3 representative study sites (Table 3).

The EIR for *An. gambiae* s.s in Akufo, Idi Ose and Ikere were 56, 74 and 114 infective bites/person/year while the EIR for *An. funestus* was 65, 36 and 39 infective bites/person/year.

An. arabiensis was another major vector found to be involved in malaria transmission in Akufo contributing a total of 18 infective bites/person/year (Table 3). The annual EIR in Akufo, Ikere and Idi Ose were 139, 153 and 110 infective bites/person/year (Table 3).

DISCUSSION

The main species of Anopheles identified in the transition forest of Oyo state are *An. gambiae*, *An. funestus*, *An. rodesiensis*, *An. rhodesiensis rupicolus*, *An. maculipalpis*, *An. coustani*, *An. nili*, *An. longipalis*, *An. ziemani* and *An. moucheti*. Out of 10 *Anopheles* species morphologically identified only five; *An. gambiae*, *An. funestus*, *An. nili*, *An. moucheti* and *An. coustani* have been reported to be malaria vector species. However, only *An. gambiae* s.l and *An. funestus* s.l were found to be the major vector group involved in malaria transmission as they were found to be infected in this study area. The non-infection status of *An. nili*, *An. moucheti* and *An. coustani* could be attributed to the fact that only a small proportion (about 1%) of these population were present in the total mosquitoes collected. In addition, these samples were mainly from human landing catches suggesting their low indoor resting bahavioural tendencies when compared to the other 2 predominant species. Similar results of only 2 major vector species (*An. gambiae* and *An. funestus*) confirmed to be infected with *P. falciparum* out of ten *Anopheles* species identified have also been reported in Western Cameroon (Tchuinkam *et al*., 2010).

Using PCR analysis, both vector and non vector members of the *An. gambiae* complex and *An. funestus* group were revealed. While both *An. gambiae* s.s and *An. arabiensis* had a wide distribution in all the rural communities, *An. funestus* s.s was found to co-exist with *An. leesoni* in only 2 out of the 6 communities surveyed. Till date very few data exist on the distribution and role of members of the *An. funestus* group in malaria transmission in Nigeria. Earlier entomological research carried out in Garki area located in Sudan savannah Nigeria before the advent of PCR assays for sibling species identification had reported an uneven distribution in the density of *An. funestus* showing varying behaviour and karyotypes in villages that are just few kilometers apart (Molineaux and Gramiccia, 1980). Hence, the application of the Polymerase Chain Reaction technique in identifying *Anopheles leesoni* (a non vector species) occurring in sympatry with *An. funestus* (a major vector species) in two of the study localities is of great importance in malaria control programs (Hargreaves *et al*., 2000; Awolola *et al*., 2005; Temu *et al*., 2007). Despite the co-existence of *An. gambiae* s.s and *An. arabiensis* in all the communities, the proportion of *An. gambiae* s.s was significantly higher than *An. arabiensis* in the communities. The low occurrence of *An. arabiensis* in the communities may exclusively be due to the preference of these species for drier ecological conditions tending towards the arid zones as against the forest-savannah ecotype where this study was conducted (Taylor *et al*., 1993; Robert *et al*., 1998; Onyabe and Conn, 2001). Noutcha and Anumudu (2009) also reported that *An. arabiensis* constituted about 8% of the total *An. gambiae* s.l identified from neighboring community located in the same ecological zone as the study site. However, the findings in this study still conform to earlier reports of Coluzzi *et al*. (1979) on the abundance of *An. gambiae* s.s in indoor resting and rural populations of *A. gambiae sensu lacto*.

In this study, *An. gambiae* s.s remains a major vector well distributed and involved in malaria transmission in all the 6 communities surveyed. The total sporozoites rates in the rural communities ranged between 1.4-3.1%. The highest sporozoites rate (3.1%) was observed in Idi Ose and Ikere and this compared favourably with 3.6 and 4.3% reported in coastal Lagos and Aba Onilu in Nigeria (Awolola *et al*., 2002a). Another study conducted in the tropical rain forest also reported a sporozoites rate of 2.5% for *An. gambiae* s.s (Oyewole *et al*., 2005). Sporozoites rates in this study were lower when compared with sporozoites rates of 5.9% (Bruce-Chwatt, 1951) and 5.3% (Hanney, 1960) reported in Nigeria. Sporozoite rates of 7.6 and 1.4% were reported in *An. gambiae* s.s and *An. arabiensis*, respectively during Garki Malaria Control Programme (Molineaux and Gramiccia, 1980).

Despite the efficiency of *An. arabiensis* as a malaria vector and its high association with arid habitats (Coetzee *et al*., 2000). it was found to be involved in transmission in Akufo community. This result therefore confirmed *An. arabiensis* as a major vector in Oyo State. Several studies have confirmed *An. arabiensis* to be the major vector involved in malaria transmission in the urban environment, forest and sahel ecological zones (Awolola *et al*., 2002a, b; Shililu *et al*., 2003). As with other studies, the lower infection rates in *An. arabiensis* was mainly an issue of low abundance when compared to *An. gambiae* s.s. Earlier reports have confirmed *An. arabiensis* as a major vector in Sahel Nigeria where its population density was significantly higher than that of *An. gambiae* s.s (Samdi *et al*., 2006). Anopheles funestus was another major vector that contributed mainly to

malaria transmission in all the rural communities. The result of this study has shown the transmission potentials of *An. funestus*. This agrees with earlier reports indicating *An. funestus* as a major vector in different parts of sub Saharan Africa (Hargreaves *et al*., 2000; Awolola *et al*., 2002a; Temu *et al*., 2007). The total sporozoite infection rates (4.5%) reported for *An. funestus* in this study was found to be comparable to 4.3% previously reported in Ibadan, Nigeria (Awolola *et al*., 2002b) and 4.5% in Mbalayo, Cameroon (Antonio-Nkondjio *et al*., 2005). Lower sporozoites rate of 2.3 and 1.0% had been reported in the forest and savannah ecological zones in Nigeria (Awolola *et al*., 2005). In the tropical forest, infection rate in *An. funestus* was put at 2.9% (Oyewole *et al*., 2005).

The higher circumsporozoite infection rate shown by *An. funestus* over *An. gambiae* s.s may be density related based on the dynamics of temporal and permanent breeding sites created by seasonal fluctuations which favour the abundance of *An. funestus* in dry season when temporary breeding sites of *An. gambiae* have been taken over by permanent water bodies favourable to *An. funestus*. However, similar findings by Wanji *et al*. (2003) have reported a higher sporozoite infection rate in *An. funestus* than *Anopheles gambiae* during the dry season. Similarly, a study carried out in coastal southwestern Cameroon, showed that *An. funestus* had higher circumsporozoite infection rates (17.8%) compared to *An. gambiae* (8.3%) when they both coexisted (Bigoga *et al*., 2007).

The overall EIR over the period of the study for each of the study sites showed that annual infective bites/ person/year in Akufo, Idi Ose and Ikere were 139,110 and 153. It was observed that the total EIR was higher in Ikere community where 2 major vectors were identified in contrast to Akufo where 3 major vectors were actively involved in transmission. Similar findings by Wanji *et al*. (2003) reported that 2 major vectors to have constituted a higher infective biting rates/man/night (0.56) during the dry season when compared to the wet season whereby 3 major vectors contributed a total of 0.18 infective bites/ person/night. This assertion however did not hold in another study conducted in Cameroon by Bigoga *et al*. (2007) where 3 major vectors (*An. gambiae*, *An. funestus* and *An. nili*) identified in a locality constituted a higher EIR than 2 other locations where only 2 major vectors were found.

The role of each vector was established by estimating the transmission indices of each vector species across the localities. *Anopheles gambiae* s.s was the main vector species and was responsible for 243 infective bites/person/year. This suggests that the EIR of *An. gambiae* s.s, *An. funestus* s.s and *An. arabiensis* were one infective bite in every 2, 3 and 20 days, respectively. This agrees with other findings from Nigeria and Cameroon with reported cases of *An. gambiae* constituting a higher infective biting rate than all other major vectors (Awolola *et al*., 2002a, b; Wanji *et al*., 2003; Bigoga *et al*., 2007). An EIR value of 0.049, though low, still suggested that *An. arabiensis* was involved in transmission.

CONCLUSION

This study shows that the total EIR at Akufo, Idi Ose and Ikere is one infective bite per person every 2.6, 3.3 and 2.4 days, respectively. Therefore, the chance of being infected from a mosquito bite is higher in Ikere community when compared with the other communities. This study has provided information on the occurrence of malaria vectors in rural communities in Oyo State. Data on the malaria transmission potentials of the major vectors and malaria transmission risk indices in the study localities are important indicators of monitoring the impact of future malaria control interventions in these localities.

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