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acta SATECH **2**(1): 6-10 (2005)

Research

Anthropophilic mosquitoes and malaria transmission in a tropical rain forest area of Nigeria

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Received: 8 June, 2005 *Revision accepted: 23 Aug., 2005*

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Abstract

Adult female Anopheles mosquitoes attracted to man were collected twice a month (December 2003-January 2005) from communities located in tropical rain forest region of Ikenne and Remo North Local Government Areas of Ogun State, Nigeria. The collection was made on Human volunteers from indoor and outdoor between 1900-0600h following WHO (1975) standard procedure. A total of 1500 Anopheline mosquitoes were collected and identified using both morphological and molecular techniques. This constitutes 790 An. gambiae (52.7%), 555 An.arabiensis (37%), and 155 An. funestus group (10.3%). The indoor catch 807(53.8%) predominated over the outdoors 693(46.2%) which constitute mainly of An. rivulorum and An. arabiensis. The biting activity observed indoor was significantly higher than outdoor (P<0.05) with a ratio of 10.1: 9.60, indoor to outdoor. Although, there was no significant difference in the seasonal biting activity (P>0.01), the mosquito species that fed exclusively on man were significantly higher than those with mixed blood (P< 0.05). The implicated malaria vectors were An. arabiensis, An.gambiae s. s. and An. funestus s. s. with overall infection rates of 2.3%, 2.5% and 2.9% respectively. This study emphasizes the need for accurate identification of Anopheles mosquitoes attracted to man in order to determine vector-host contact necessary for malaria transmission and to provide information required to formulate vector control programme.

Keywords: Anopheles mosquitoes, PCR identification, ELISA test, malaria, Nigeria

Introduction

Anopheline vector of malaria consists of various species with unique behaviour associated with their biting activities and transmission dynamics.

In Nigeria, the Anopheles mosquitoes involved in malaria transmission belong to *An. gambiae* and *An. funestus* group (Boreham et al., 1979; Molineaux & Gramiccia, 1980; Rishikesh *et al.*, 1985). Others regarded as minor/secondary vectors include *An. moucheti* and *An. nili* (Gillies & De Meillon, 1968; Gillies and Coetzee, 1987).

The *An. gambiae sensu lato* consist of seven species, five of which are vectors of human malaria. Amongst these are *An. gambiae* and *An. arabiensis (*the two major malaria vectors in Africa) (Gillies & De Meillon 1968), *An. merus*, *An. melas*, and *An. bwambae* (the minor vectors). The other species *An. quadriannulatus* A and B are the non-vector species from southern Africa, and Ethiopia respectively (Gillies & Coetzee, 1987; Hunt *et al.*, 1998). The *An. funestus* group also consists of nine morphologically indistinguishable species. These include, *An. funestus sensu stricto, An. vaneedeni, An. parensis* Gillies, *An. aruni* Sobti, *An. fuscivenosus* Leesoni, *An. confusus* Evans & Leesoni, *An. leesoni* Evans, *An. rivulorum* Leesoni and *An.brucei* (Gillies & De Meillon, 1968; Gillies & Coetzee, 1987).

Studies on the biology of Anopheles species

in Nigeria had described *An. gambiae* s. s, *An. arabiensis*, *An. funestus*, *An. rivulorum* and *An. Leesoni* as vectors of malaria (Boreham *et al*., 1979; Coluzzi *et al*., 1979; Molineaux & Gramiccia, 1980; Rishikesh *et al*., 1985; Onyabe & Conn, 2001; Awolola *et al*., 2002). The role of anthropophilic mosquitoes in malaria transmission in southwestern Nigeria has also been documented (Awolola 2003, 2005).

The present study embarked on a longitudinal collection of man-biting mosquitoes with a view to investigating activities and habits related to malaria transmission, epidemiology and control.

Materials and Methods

Study Area

This study was carried out in Akaka, Ilara and Ijesa-Isu communities located in a forest area characterized with moderately hot and humid tropical climate in southwestern Nigeria. The two distinct seasons in this area are rainy season, which lasts from March/April to October/November, and the dry season, which lasts for the rest of the year, October/Novemb-er till March/April. The mean temperature is 30^{o}C during the dry season and 24^{o}C in the rainy season. The mean annual rainfall is about 2000mm (Anon, 2005).

The study communities were selected based on altitude, size, house types, presence of water bodies especially slow running ones and prevailing vegetation type.

The inhabitants usually engaged in subsistence farming and petty trading for a living. The houses were constructed with either block and cement or mud with corrugated iron sheet, bamboo or palm fronds.

The study areas lack social amenities such as pipe borne water, modern health facilities and waste disposal system. The residents often keep domestic animals within their houses or nearby sheds.

Mosquito Collection and Identification

Adult female Anopheles was collected twice a month from December 2003 to January 2005. The collection was made on human volunteers stationed indoor and outdoor from 19:00-06:00 hr following the standard of WHO (1975) procedure. The samples were then preserved dry on silica gel for further analysis.

Identification of mosquito was carried out by morphological and PCR methods. The keys of Gillies and De Meillon (1968), Gillies and Coetzee (1987) were used as guides for morphological identification. Molecular assays used were the species-specific PCR techniques for confirmatory identification of the species within *An. gambiae* complex, and a cocktail PCR technique of Koekemoer *et al*. (2002) to identify the species within *An. funestus* group.

ELISA Tests

Sporozoite ELISA

The circumsporozoite proteins of Plasmodium species present on the head and thorax of 1500 Anopheles mosquitoes were tested following the method of Wirtz *et al.* (1987). Sporozoite rates were determined photometrically at 405nm using an ELISA plate reader (Anthos 2010, Anthos Labtec GmbH, 5017 Wals/Salzburg, Austria), 30 min after the addition of substrates (Beier *et al*., 1990). Positive ELISA reading was taken as the absorbance value greater than twice the mean of negative control.

Blood Meal ELISA

The blood meal source of 830 blood-fed samples caught outdoor and indoor was determined using the direct ELISA method of Beier *et al.* (1988).

Statistical Analysis

The relative abundance of the species was expressed as the percentage of the total number of Anopheles collected. Chi-Square tests were used to analyze differences in indoor and outdoor biting activities and sporozoite rates.

Results

A total of 1500 adult female Anopheles mosquitoes were identified by molecular assays.

The *An. gambiae* species-specific PCR identified 52.7% as *An. gambiae s. s* and 37% as *An. arabiensis* while the multiplex PCR assay identified 7% as *An. funestus s.s* and 3.3% as *An. rivulorum.*

The indoor catch 807(53.8%) predominated over the outdoors 693(46.2%). *An. gambiae* constitute the highest indoor biting species followed by *An. funestus* while *An. arabiensis* and *An. rivulorum* were mainly outdoor species (Table 1).

Overall biting activity observed indoor was significantly higher than outdoor ($P<0.05$) with a ratio of 10.1: 9.60, indoor to outdoor. Although, outdoor biting activity was higher in the dry season during which *An. arabiensis* was the predominant species.

Table 1: Abundance of species within An. gambiae complex and An. funestus group caught indoors and outdoors

Species	Indoor collection No (%)	Outdoor collection No (%)	Total caught/ location No (%)	Indoor /Outdoor Ratio
An. gambiae	500(62)	290(42)	790(52.7)	1.48
An. arabiensis	200(24.8)	355(51.2)	555(37.0)	2.06
An. funestus	85(10.5)	20(2.8)	105(7.0)	3.75
An. rivulorum	22(2.7)	28(4)	50(3.3)	1.48
Total	807(53.8)	693(46.2)	1500	

Table 2: Seasonal variation in man-vector contact

Season	Total no collected	*An. gambiae*		*An. arabiensis*		*An. funestus*		*An. rivulorum*	
		Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
Wet Season (April – Oct)	1013	382	105	114	164	131	13	43	60
Dry Season (Nov – March)	487	141	48	54	91	81	7	31	34
Total	1500	523	153	168	255	213	20	74	94

The wet season biting activity was more pronounced indoor and often started as early as 2000h (pre-bed time). In most of the species found indoor, biting activity lasted throughout the night from 2400-0600h (post-bed time). There was no significant difference in the seasonal biting activity (P>0.01). The species of *An. gambiae, An. funestus* were more of endophilic than exophilic as characterized by An. *arabiensis and An. rivulorum* irrespective of the seasonal changes (Table 2).

Blood Meal Source

The ELISA results of 830 anopheles mosquitoes that appeared blood -fed showed that 84.7% An. *gambiae* s.s, 68.8% *An. funestus* s.s, 41.6% *An. arabiensis* and 8.2% *An. rivulorum* fed on man (Table 3). The mosquito species that fed exclusively on man were significantly higher than those with mixed blood (P< 0.05). The preference for blood meal has no relationship with the season of the year.

Table 3: Percentages indoor-outdoor blood-fed Anopheles mosquitoes and their respective hosts

Positive blood meal ELISA test							
Species	No	Indoor	Outdoor	Human	Bovine	Mixed	Other sources
		No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
An. gambiae	360	253 (70.2)	107 (29.8)	305 (84.7)	10 (2.8)	5 (1.4)	40 (11.1)
An. arabiensis	245	65 (26.5)	180 (73.5)	102 (4106)	90 (36.7)	40 (16.4)	13 (5.3)
An. funestus	128	88 (68.8)	40 (31.3)	88 (68.8)	18 (14.1)	7 (5.5)	15 (11.2)
An. rivulorum	97	23 (23.7)	74 (76.3)	9 (8.2)	55 (56.7)	24 (15.5)	9 (9.3)
Total	830	429 (51.7)	401 (48.3)	504 (60.7%)	173 (21.0)	76 (9.2)	77 (9.3)

Sporozoite ELISA

Out of 1374 Anopheles mosquitoes examined, only 34(2.5%) tested positive for *P. falciprum* circumsporozoite antigen (CSA). The overall sporozoite rates were 10/245(2.3%) for *An. arabiensis*, 12/360(2.5%) for *An. gambiae* s. s. and 16/552(2.9%) for *An. funestus* and 0/97(0%) for *An. rivulorum* (Table 4). There was no statistical difference in the overall sporozoite rates for *An. gambiae s. s., An. arabiensis* and *An. funestus* s. s. in all study sites (P >0.05). Although the overall sporozoite rates dropped from 2.5% in the wet season to 1.6% in the dry season.

The major malaria vectors implicated in malaria transmission in the study areas are *An. gambiae, An. arabiensis* and *An. funestus*.

Table 4: Percentages indoor-outdoor catches of Anopheles mosquitoes infected with Plasmodium falciprum

Species	Total no examined		Total no positive		
	Indoor	Outdoor	Indoor n (%)	Outdoor n (%)	Total n (%)
An. gambiae s.s	267	213	8 (3.0)	4 (1.9)	12 (2.5)
An. arabiensis	100	145	2 (2.0)	4 (2.8)	6(2.3)
An. funestus	370	182	10 (2.7)	6 (3.3)	16 (2.9)
An. rivulorum	23	74	0	0	0
Total	429	401	22 (5.1)	16 (3.9)	34 (2.5)

Discussion

The abundance of mosquito is closely related to the prevalence of malaria most especially in the endemic area. The mosquitoes attracted to man are the major vectors implicated in the transmission of malaria. Out of the total amount of mosquitoes collected in this study, Anopheline species account for about 60% indicating a high density. Previous studies have also shown that the density of Anopheles mosquitoes is usually very high in the tropical rainforest perhaps due to the environmental friendly conditions (Awolola *et al.*, 2003).

The correct identification of the species within the taxa is very important in recognizing those

that actually fed on man with vectorial capacity to transm-it malaria parasite. In this study we used molecular techniques to complement morphological identificati-on of Anopheline species caught feeding on man. Molecular assays have been of tremendous help in solving the problem of misidentification of species especially those that are non-vectors and those that occur in sympatry but with different behaviour (Scott *et al.*, 1993; Koekemoer *et al.*, 2002, Weeto *et al.*, 2004).

Two of the members within *An. gambiae* complex which are the major vectors of malaria and which occur sympatric were *An. gambiae* s. s. and *An. arabiensis*. Our findings showed that both *An. gambiae* and *An. arabiensis* were often found in the same vicinity, although more of *An. gambiae* were found feeding on man indoor than outdoor. The biting activities of these species have been found to complement each other such that while one is endophagic, the other is exophargic (Gillies & Coetzee 1987). The population of *An. arabiensis* was significantly high compared to other species in our dry season collections. *An. arabiensis* had been reported to persist even during the dry season while *An. gambiae* were more abundant during the wet season (Rishikesh *et al.*, 1985; Awolola *et al.*, 2002). This study also showed that biting activity of Anopheline species especially those that fed indoor continues through the day and night and all year round. Although *An. arabiensis* has been described as opportunistic but largely more of zoophilic behaviour (White, 1974; Gillies & Coetzee, 1987). Other findings showed that *An. arabiensis* readily fed on both humans and cattle (Gillies & De Meillon 1968; White, 1974). This was also confirmed in this study as we found mixed blood meal in *An. arabiensis*. Species within the *An. funestus* group occurred in sympatry, but, *An. funestus* s. s. was more endophilic and anthropophagic than *An. rivulorum*. Earlier findings also showed that some members of *An. funestus* group were found in sympatry in West Africa but of different feeding behaviour (Gillies & Coetzee, 1987; Kamau *et al*., 2003).

The present study showed that sporozoite rates in *An. gambiae s. s.*, *An. arabiensis* and *An. funestus s. s.* were statistically insignificant (P>0.05). This was an indication that malaria transmission was not an exclusive role of the endophilic mosquitoes. The exophilic species also play specific role in the transmission process though they showed less anthropophilic behaviour (Haridi, 1972; Ameneshewa & Service, 1996; Fetttene *et al.*, 2004). The 0% sporozite rate recorded for An. rivulorum showed that it is not a vector of malaria despite the fact that it fed on man. Previous findings had also showed that *An.rivul-*not a vector of malaria despite the fact that it fed on man. Previous findings had also showed that *An. rivulorum* and *An. leesoni* played no tangible role in malaria transmission in West Africa (Costantini *et al.*, 1999).

The knowledge of the biology and behaviour of the Anopheline species attracted to man is vital to control and prevention of malaria. This study has made scientific attempt to provide information on the anthropophilic mosquitoes and their malaria epidemiology which is pivotal to the formulation of effective control programmes.

Acknowledgements

We gratefully acknowledged the efforts of our field assistants during the field activities. This study was partly supported by the grant MIM project A30026 through the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) awarded to T. S. Awolola.

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