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ORIGINAL ARTICLE

TRANSMISSION DYNAMICS OF MALARIA IN FOUR SELECTED ECOLOGICAL ZONES OF NIGERIA IN THE RAINY SEASON

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Abstract

Background: Two of the problems of malaria parasite vector control in Nigeria are the diversity of Anopheline vectors and large size of the country. Anopheline distribution and transmission dynamics of malaria were therefore compared between four ecotypes in Nigeria during the rainy season.
Methods: Polymerase chain reaction (PCR) was used in molecular identification after morphological identification microscopically. Enzyme linked immunorsorbent assay (ELISA) was used for the blood meal analysis and sporozoite detection.
Results: Five species were identified out of 16,410 anophelines collected. *An. gambiae s.s* made up approximately 29.2%-36.6% of the population in each zone. All five species acted as vectors for *P. falciparum*. *An. gambiae s.s* had the highest sporozoite rate. The most infected mosquitoes were found in the rain forest. More blood meals were taken from bovids, except the savannah forest, where 73.3% were on humans and Human Blood index (HBI) was 57.3%. The Entomological inoculation rate (EIR) was a mean of 13.6 ib/p but was highest in the rainforest zone.
Conclusions and limitations: This study demonstrates the complex distribution of anophelines and the considerable variations in the intensity of malaria transmission in Nigeria. We highlight the need to consider diverse epidemiological situations when planning countrywide control programmes.

***Key words**: Malaria; vectors;* Plasmodium falciparum*; transmission dynamics; ecotypes*

Résumé

Fond: Deux des problèmes de la commande de vecteur de parasite de malaria au Nigéria sont la diversité des vecteurs d'Anopheline et grande taille du pays. La distribution d'Anopheline et la dynamique de transmission de la malaria ont été donc comparées entre quatre ecotypes au Nigéria pendant la saison des pluies.
Méthodes: La réaction en chaîne de polymérase (PCR) a été employée dans l'identification moléculaire après identification morphologique au microscope. L'analyse immunorsorbent liée par enzyme (ELISA) a été employée pour la détection d'analyse et de sporozoite de repas de sang.
Résultats: Cinq espèces ont été identifiées sur 16.410 anophelines rassemblés. *gambiae s.s* composés approximativement 29.2-36.6% de la population dans chaque zone. Chacune des cinq espèces a agi en tant que vecteurs pour *P. falciparum*. *gambiae s.s* a eu le taux de sporozoite le plus élevé. Les moustiques les plus infectés ont été trouvés dans la forêt tropicale . Plus de repas de sang ont été pris des bovids, excepté la forêt de la savane, où 73.3% étaient sur des humains et l'index humain de sang (HBI) était 57.3%. Le taux entomologique d'inoculation (EIR) était un moyen de 13.6 ib/p mais était le plus haut dans la zone de forêt tropicale.

Conclusions et limitations: Cette étude démontre la distribution complexe des anophelines et des variations considérables de l'intensité de la transmission de malaria au Nigéria. Nous accentuons la nécessité de considérer des situations épidémiologiques diverses en projetant des programmes de gestion nationaux.

***Mots clés**: Malaria, vecteurs,* Falciparum de Plasmodium, *dynamique de transmission, ecotypes*

Malaria is holoendemic in Nigeria, accounting for 25% of infant mortality and 30% of childhood mortality.[1] Ninety five percent of malaria infections in Nigeria are caused by *Plasmodium falciparum* and five percent by *Plasmodium malariae.* [2] According to Gallup and Sachs, [2] malaria transmission is however geographically specific. De Mellion[3] also reported that malaria vectors exhibit behavior variations in different localities. The knowledge of major vectors and their bionomics in Africa remains a problem.[4] As a focal disease, malaria will therefore differ in its characteristics from place to place, since all malaria vectors do not exhibit identical behavior and ability to transmit parasites.

Hay et al[5] reviewed entomological inoculation rates (EIR) across Africa but there was no data from Nigeria. Nigeria is a large and diverse country and transmission dynamics will vary according to climatic, geographic and socio-economic conditions. Large areas of the country have no reliable data on the presence or absence of vectors and there is little information on sporozoite rates in southern Nigeria.[6,7] Most of the original information on the transmission of malaria in Nigeria comes from isolated and intermittent studies in the northern parts of the country,[8] In southern Nigeria, a few localized and short-term studies of mosquito populations were made[9] and more recently Awolola et al [7, 8] studied population dynamics and biting behavior of anophelines in this area. Onyabe and Conn[10] reported the distribution of two major malaria vectors, *An. gambiae s.s* and *An. arabienisis*, in southwestern Nigeria and most recently Awolola et al[11] focused on the *An. funestus* group. In Nigeria, large numbers of *Anopheles* species are thought to be involved in transmission, but their distribution and vectoral capacity is not fully understood.

Species complexes that vary in behavior and vectorial capacity present a real problem to malaria control.[12] All the vectors belong to species complexes whose members vary widely in their vectorial capacity and competence.[13] Correct analysis of the distribution of specific malaria vectors is one of the prerequisites for meaningful epidemiological studies and for planning and monitoring of successful malaria control or eradication programmes.[14] A regular assessment of each country's malaria situation is worthwhile because control measures can only be effective if the abundance, behavior and proportion of the species are known. It is therefore very important to understand the dynamics of the transmission of malaria in a large country like Nigeria with different ecological zones.

The aim of this study was to provide information on, and compare, species distribution, blood meal sources, sporozoite rates and entomological inoculation rates (EIR) of vectors in four ecological zones of Nigeria. This knowledge will facilitate better understanding of the dynamics of malaria transmission and could lead to development of early warning systems and species-specific vector control activities.

Materials and Method

Study areas

Nigeria is approximately 923,768 sq. km (Figure 1). There are seven ecological zones in Nigeria, the arid savannah in the north gradually turning to humid forest in the south.[10] Rainfall is the real climatic variable in Nigeria with June to September the rainiest months throughout the country. Mosquitoes were collected from the following four ecotypes based on accessibility, logistics, personnel and ethical reasons. The study sites were selected randomly based on the cooperation of people.

Northwestern focus (Northern Guinea Savannah zone)

Bungudu-Gusau, Zamfara State, where the Hausa is the predominant ethnic group, falls within the Northern Guinea Savannah zone of northern Nigeria. The rural population is mainly agricultural. Rainfall is approximately 500-700 mm per year. The numerous burrow pits; cattle dips, quarries, communal laundries and water storage containers provide small standing water sources that contribute to perennial breeding sites. Cattle are mainly found in compounds together with goats, sheep, dogs, fowl and bats

Southwestern focus (Rainforest zone)

Badagry, Lagos State, is a coastal suburban town, close to the Atlantic Ocean. The predominant ethnic groups are the Eguns and Yorubas. Fishing, poultry farming and trading are the basic occupation. The area is characterized by swampy sandy soil, which becomes waterlogged during the rainy season. Rainfall is approximately 2000- 3000 mm per year. Goats, sheep, pigs, fowls and dogs were common animals. Cattle are also found in the abattoirs and ranches.

Southeastern focus (Savannah - forest zone)

Onitsha, Anambra State, is in a sub-urban inland region. It falls within the savannah - forest zone in east central Nigeria. The predominant ethnic group is the Igbos. They are mainly involved in trading. The area is overpopulated because of the commercial activities. Rainfall is approximately 1900 mm per year. Dogs, fowl, goats, sheep, rabbits and cats are the common animals. Cattle were found at the abattoirs and cattle ranches.

South-southern focus (Mangrove forest zone)

Bonny, Rivers State is also a coastal suburban town that falls within the mangrove rainforest zone. It is within the Niger delta area very close to the Atlantic Ocean. The predominant ethnic groups are the Ijaws, who are mainly fishermen. Rainfall is approximately 2000- 4000 mm per year. Cats, fowl, dogs and goats are common animals. Cattle were found at the abattoirs and cattle ranches.

Mosquito collection

Collections were made during the rainy season (once a month) between July and October 2005 in the four zones. Female mosquitoes were caught outdoors between 1800-0600 hours by two human bait collectors who worked alternatively. Outdoor collections were made around open gutters, pools of water, open containers and abandoned tires and vehicles. They interchanged after 6 hours. An aspirator was use to collect the mosquitoes that landed and attempted to feed. Mosquitoes were also caught by pyrethrum spray indoors in the evenings between 1800- 2000 hours by floor sheet collections. The Mosquitoes were preserved in 70% ethanol.

Morphological identification of mosquitoes

Anopheline mosquitoes were distinguished from Culicines according to the morphological characteristics of their maxillary palps using the morphological keys of Gilles and Coetzee.[15] The distinguishing feature of *An. m. nigeriensis* being a fringe spot opposite vein 6 on the wing was used to distinguish it from other anophelines.[16]

Dissection of mosquitoes

All the female anophelines collected were dissected. They were cut transversely at the thoraxes between the 1st and 3rd pairs of legs under a dissecting microscope (x20). The abdomens of blood-fed anophelines were used for blood meal analysis while the heads and thoraxes were used for sporozoite detection. The wings and legs were used for species identification following DNA extraction.

Molecular identification of Anophelines

DNA Extraction

Genomic DNA from whole male mosquitoes and wings and legs of female mosquitoes were extracted according to the standard procedures of Collins et al[17] Extracted DNA was resuspended in 50 μl of PCR grade water.

Polymerase chain reaction

PCR was performed with universal and species specific primers for the *An. gambiae and An. funestus* species complexes. Molecular identification of *An. gambiae* species complex is based on the species-specific nucleotide sequences in the ribosomal DNA intergenic spacers (IGS) as described by Scott et al[18] The sequences of the *An. gambiae* complex primers used were as follows: Universal 5′-GTGTGCCCCTTCCTCGATGT-3′, *An. gambiae* 5′-CTGGTTTGGTCGGCACGTTT-3′, *An. melas* 5′-TGACCAACCCACTCCCTTGA-3′ and *An. arabiensis* 5′-AAGTGTCCTTCTCCATCCTA-3′. PCR for *An. gambiae* complex consisted of one cycle of initial denaturation at 94°C for 5 minutes, 30 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for 30 seconds and final extension at 72°C for 7 minutes.

For the *An. funestus* species complex, molecular identification was based on species–specific primers in the internal transcribed spacer region (ITS2) on the ribosomal DNA as described by Koekemoer et al[19] The sequences of the *An. funestus* complex primers used were: Universal 5′-TGTGAACTGCAGGACACAT-3′, *An.funestus* 5′-GCATCGATGGGTTAATCATG-3′, *An. vaneedi* 5′-TGTCGACTTGGTAGCCGAAC-3′, *An.rivulorum* 5′-CAAGCCGTTCGACCCTGATT-3′, *An.parensis* 5′-TGCGGTCCCAAGCTAGGTTC-3′ and *An. leesoni* 5′-TACACGGGCGCCATGTAGTT-3′.

PCR performed for *An. funestus* complex consisted of one cycle of initial denaturation at 94 $^{\circ}$C for 2 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 45°C for 30 seconds and extension at 72°C for 40 seconds, with additional final extension at 72°C for 5 minutes. Laboratory strains of *An. gambiae* and *An. funestus* were used as controls. Amplification was performed in a Gene AMP PCR system 9700 using Sigma (U.K) reagents throughout. Oligonucleotides were produced by Proligo (France). All PCR products were electrophoresed on a 1.4% ethidium bromide agarose gel. The amplified fragments were then visualized by UV transilluminator and documented using Syngene bio-imaging system.

Identification of blood meal origin

A direct ELISA using anti-phosphatase conjugates; anti-human IgG (Fab specific); anti-bovine IgG (whole molecule) and anti-goat IgG (whole molecule) [Sigma], were used to identify human, cattle (bovine) and goat (ovine) host blood respectively, based on the procedures of Beier et al[20] A total of 4160 mosquitoes with blood meals were assayed for each host in 96-well flat-bottomed well plates with

absorbance read 30 minutes after the addition of substrate (pNPP) at 405nm on a Labsytem Multikans Multisoft Type 349 ELISA reader. Samples were considered positive if the optical densities (OD) were at least twice the mean of four negative wells on the same plate.[21] Positive controls were female mosquitoes with known blood meals. Negative control contained male mosquito triturates.

Human blood index

The human blood index (HBI), which is the proportion of female anophelines giving a positive reaction for human blood alone or multiple blood feeds with human blood, was calculated.[22]

This is the percentage of female anophelines that had human blood or mixed blood that contained human blood, i.e., No. of female anophelines with human blood or mixed human blood/No. of female anophelines tested

Plasmodium falciparum sporozoite rate

The heads and thoraxes of individual mosquitoes were tested for *P. falciparum* circumsporozoite protein (CSP) using *Pf2A10* monoclonal antibody, as recommended and modified by Wirtz et al.[23] The monoclonal and peroxidase-conjugated antibodies were obtained from Centre for Disease Control (CDC, Atlanta, Georgia). A sandwich ELISA was carried out on dried mosquitoes. The heads and thoraxes were ground individually in 50 μl grinding buffer containing, bovine serum albumin (2.5g), boiled casein (1.25g) in phosphate buffered saline (250mls), phenol red (100ul) and Nonidet P-40 (1,250ul) and the final volumes of each triturate brought to 250 μl. These triturates (50 μl) were then used in the ELISA test according to the standard protocols of Beier et al.[20] Five negative controls (male mosquitoes) and three positive controls (the appropriate synthetic peptide) were included in each 96-well flat-bottomed microtiter plate. The OD was read at 415nm, 60 minutes after the addition of substrate (ABTS). Any sample giving an OD that was at least twice that of the mean OD for the negative controls on the same plate was considered CSP positive.[21] Positive samples were retested for confirmation.

Sporozoite and entomological inoculation rates

Sporozoite rates were determined as the percentage of mosquitoes carrying *P. falciparum* CSP antigen. Combined data from mosquitoes collected by human bait and pyrethrum spray during the whole study were used to calculate sporozoite rates of each species in each area. However, only data from the human bait collections were used to calculate the entomological inoculation rates (EIR) as the product of the sporozoite and the man-biting rate; the man-biting rate being the number of mosquitoes biting per person per night .i.e. per human bait. EIR is expressed as infective bites per person for the rainy season (ib/p).

Statistical analysis

χ^2 was used to determine the differences in results between the study sites and among species. $P<0.05$ was regarded as an acceptable level of significance.

Results

Species distribution

A total of 16, 410 anophelines were collected from the four foci. Pyrethrum sprays collected more mosquitoes. Five species were identified**;** three were from the *An. gambiae* complex but of the *An. funestus* complex, only *An. funestus s.s* was present. Overall, *An. gambiae s.s* was the most abundant species and this was followed by *An. moucheti nigeriensis*. The least common species identified was *An. melas,* which was only found in two zones. In addition, 7% of the collection was un-identified (Figure 2, Table 1).

In the Guinea savannah, out of 2870 anophelines, only three species were identified. There were more *An. funestus s.s* than *An. gambiae s.s* or *An. arabiensis* and no *An. m. nigeriensis* or *An. melas* were found, but there were other secondary species.

In the coastal rainforest, all five species were identified in a collection of 5580 anophelines. *An. gambiae s.s* and *An. m. nigeriensis* were the most abundant; both 31.8% of the collection. *An. funestus s.s, An. arabiensis,* and *An. melas,* were also identified and 7.31% were unidentified species.

In the savannah-forest, out of 4050 anophelines, four species were identified; *An. melas* being absent, whereas, in the mangrove–forest four species were also identified but *An. funestus s.s.* was not found and 5.82% were unidentified. See Table 1 for values. Species distribution between zones differs significantly $X^2 = 487.8$ df $= 5$ $P < .05$.

Biting females

Of the 1110 biting females collected by human bait outdoors, *An. gambiae s.s* predominated at 37% of the total catch; *An. m. nigeriensis* represented 28%, *An. funestus s.s* 15.3%, *An. arabiensis* 11% *An. melas* 3.6%. In terms of distribution, the highest numbers of biting species were found in the Guinea savannah where biting *An. gambiae s.s* were 41.7% of the catch for that area. This was followed by the rainforest where biting *An. moucheti nigeriensis were* 24.6% of the catch, savannah-forest with *An. gambiae s.s at* 40% and Mangrove–forest where biting *An. moucheti nigeriensis* were 48.6% of the catch (Figure 3).

Blood meals

A total of 4160 (25.3%) anophelines were blood fed. Overall, 1360 (32.7%) had fed on bovids, 820 (19.7%) on humans and 300 (7.21%) had mixed feeds with human blood as a component. Only 150(3.6%) goat and 40(0.96%) bovine/goat meals were identified (overall human blood index of 27%) (Table 2). Generally, more blood meals had been taken from bovid. However, in the savannah forest 440 (73.3%) of blood meals were taken on humans alone.

The highest proportion of bovid blood meals was in the Guinea savannah (79.6%) of the 1230 blood fed females examined. In this zone, 140 (58.3%) of outdoor caught-females contained blood meals and of these 120 (86%) contained blood meals of bovid origin and 20 (14.3%) mixed bovid and human meals. Indoors in the Guinea savannah, 390 (78%) blood meals were of bovid origin and 40 (8%) of human origin. There were no multiple blood feeds (Figure 4). A human blood index of 4.87% was recorded here (Table 2).

In the rainforest, 40% of the 1240 blood fed females was caught outdoors by human bait catches and 50% of these had blood meals of bovid origin only, whereas only 20% were of human origin, 20% human/bovid origin and 10% from bovid/goat origin. A similar picture emerged for indoor pyrethrum catches in this zone (Figure 4). A human blood index of 32.2% was obtained (Table 2*).*

In the Savannah-forest, from 890 blood fed females, few females were found outdoors and they were all bovine fed, whereas 76% blood meals from indoors-caught anophelines were of human origin and only 50 (8.6%) of the blood meals were taken on bovid. There were few multiple blood feeds of human/bovid 20 (3.45%), human/goat 30 (5.2%) and human/bovid/goat 20 (3.45%). Human blood index of 57.3% was recorded in the rain forest (Table 2*).*

In the mangrove- forest, there were 800 blood fed females. Seventy (19%) were caught by human bait outdoors, out of which 20 (28.5%) had taken blood meals from a human, 10(4.2%) from human/bovid and 40 (57.1%) from bovids. indoors, 330 (73.3%) of the blood meals were taken from bovids and 90 (20%) on human. A human blood index (HBI) of 18.75% was recorded (Table 2*).* HBI between zones differs significantly (p <.05).

Sporozoite rates

Out of a total of 11 990 female anophelines examined for *Plasmodium falciparium* sporozoites, 670 (5.6%) were positive and all species identified contained sporozoites and thus acted as malaria parasite vectors in at least one zone (Table 3). A higher proportion of anophelines caught by the pyrethrum spray method contained sporozoites than by human bait. In the Guinea savannah, 50 (2.6%) female anophelines caught indoors and 20 (8.3%) caught outdoors had sporozoites. This gave an overall sporozoite rate of 3.24% out of the 2160 females examined. *An. funestus* had the highest sporozoite rate of 2.3% in this zone. In the rainforest, a sporozoite rate of 12% outdoors and 9.71% indoors was recorded for the female anophelines examined. This indicated an overall sporozoite rate of 9.9%, which was the highest among the four zones with *An. gambiae*, *s.s* having the highest sporozoite rate of the five species identified (4.3%). In the savannah- forest, a sporozoite rate of 20% outdoors and 3.1% indoors was recorded from 3510 female anopheles examined. This gave a sporozoite rate of 4.3%, with *An. gambiae s.s* again having the highest sporozoite rate of 2.3%. In the mangrove-forest, there was a sporozoite rate of 8.11% outdoors and 2.3% indoors. This gave an overall sporozoite rate of 3.11%, which was the lowest obtained among the zones. *An. gambiae s.s* and *An. moucheti nigeriensis* had the highest sporozoite rates of 1.2% each for this zone. (Table 3). Sporozoite rates between species differs significantly (p<.05). Sporozoite rates between zones differs significantly (p<.05).

Entomological inoculation rates (EIR)

Overall for the whole of Nigeria, mean EIR calculated from human bait catches alone was 13.6 ib/p for the rainy season. The EIR was highest in the southern rainforest (24.7 ib/p) and lowest in the drier northern Guinea savannah with 7.7ib/p (Table 4).

Table 1. Species composition and abundance of anophelines collected in the four ecological zones of Nigeria in the rainy season

Species	GS zone (%)	RF zone (%)	SF zone (%)	MF zone (%)	All zones (%)[a]
An. gambiae. s.s.	840(29.2)	1870 (31.8)	1400 (34.5)	1320 (36.6)	5430 (33.1)
An. arabienisis	560 (19.5)	560 (9.52)	840 (20.7)	150 (4.2)	2110 (12.8)
An. melas	-	450 (7.65)	-	890 (24.6)	1340 (8.16)
An. funestus	1050 (36.6)	700 (12)	800 (19.75)	-	2550 (15.5)
An. moucheti	-	1870 (31.8)	920 (22.71)	1040 (28.8)	3830 (23.3)
Others	420 (14.6)	430 (7.31)	90 (2.2)	210 (5.82)	1150 (7)
Total	2870	5880	4050	3610	16410

[a] Species distribution between zones differs significantly; X^2=487.8 ; df=5; p<.05; GS = Guinea Savannah; RF = Rainforest; SF = Savannah-forest; MF = Mangrove- forest

Table 2. Host preferences of blood fed malaria vectors and their human blood index in four zones

Blood meal origin	Guinea savannah (%)	Rain forest (%)	Savannah forest (%)	Mangrove forest (%)	All zones (%)
Human	40 (6.25)	230 (25.3)	440 (73.3)	110 (1.75)	820 (19.7)
Bovine	510 (79.6)	410 (45.0)	70 (11.7)	370 (71.1)	1360 (32.7)
Goat	70 (10.9)	60 (6.6)	20 (3.33)	0	150 (3.6)
Human/bovine	20 (3.12)	120 (13.2)	20 (3.33)	40 (7.7)	200 (4.8)
Human/goat	-	-	30 (5)	-	30 (0.72)
Bovine/goat	-	40 (4.4)	-	-	40 (0.96)
Human/bovine/goat	-	50 (9)	20 (3.33)	-	70 (1.68)
Samples analyzed positive	640 (52)	910 (73.3)	600 (67.4)	520 (65)	2670 (64.2)
Total with blood meal	1230	1240	890	800	4160
Human blood index[a]	(4.87)	(32.2)	(57.3)	(18.75)	(27)

[a] Human blood index (HBI) between zones differs significantly; $X^2 = 47.26$; df = 3; $p<.05$; HBI is the proportion of anophelines with human blood and multiple blood feeds with human out of the total blood meals taken

Table 3. Proportion of female anophelines with sporozoites in their salivary glands in the four zones

Species with sporozoites (%)	Zones and number of female anophelines examined				
	GS (n =2160)	RF (n=3750)	SF (n=3510)	MF (n=2570)	Total (n=11990)
An. gambiae s.s	10 (0.46)	160 (4.3)	80 (2.3)	30 (1.2)	280 (2.33)[a]
An. arabiensis	-	40 (1.1)	20 (0.6)	-	60 (0.50)
An. melas	-	20 (0.53)	-	10 (0.4)	30 (0.25)
An. funestus s.s	50 (2.3)	110 (2.9)	10 (0.3)	-	170 (1.42)
An. moucheti	-	30 (0.8)	30 (0.85)	30 (1.2)	90 (0.75)
Others	10 (0.46)	10 (0.3)	10 (0.28)	10 (0.4)	40(0.33)
[b]Total with sporozoites	70 (3.24)	370(9.9)	150 (4.3)	80 (3.11)	670 (5. 6)

[a] Sporozoite rates between species differs significantly; $X^2 = 41.9$; df = 5; $P<.05$

[b] Sporozoite rates between zones differs significantly; $X^2 = 34.9$; df = 3; $p<.05$; GS = Guinea savannah; RF = Rainforest; SF = Savannah-forest; MF = Mangrove- forest

Table 4. Entomological inoculation rates (EIR) of female anophelines obtained by human bait (HB) in the four zones in the rainy season July-October, 2005

Ecological zones	Number of biting females	Man biting rate	Sporozoite Rate (%)	EIR Ib/p/rs[a]
GS	240	240/1hb	3.24	7.77
RF	250	250/1hb	9.9	24.7
SF	250	250/1hb	4.3	10.7
MF	370	370/1hb	3.11	11.5
Mean	1110	1110/1hb	7.1	13.6

[a]infective bite per person per rainy season; GS = Guinea savannah; RF = Rain forest; SF =Savannah forest;
MF = Mangrove forest; HB = Human bait; Man biting rate is the total number of mosquitoes caught per human bait

Figure 1. Map of Nigeria showing the seven ecological zones and four localities sampled for malaria vectors between July – October 2005 (1: Sahel savannah; 2: Sudan savannah; 3:Northern Guinea savannah; 4: Southern guinea savannah; 5:Savannah- forest; 6:Rainforest; 7:Mangrove-forest; *= Study sites)

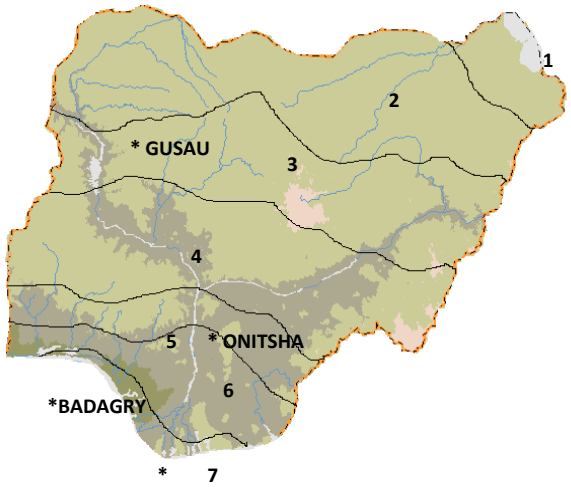


Figure 2. Species composition and abundance of the malaria vectors collected in the four zones

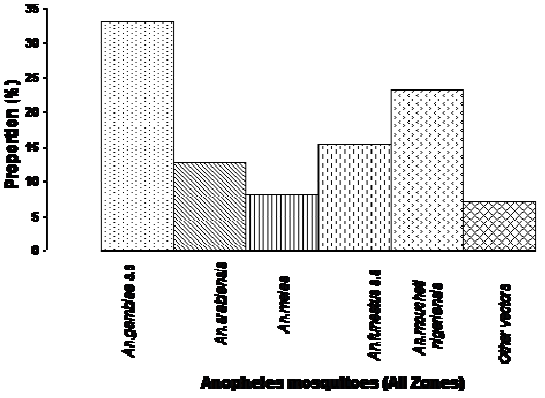


Figure 3. The blood meal origin of female anophelines collected indoors and outdoors in the four zones (GS = Guinea savannah; RF = Rainforest; SF = Savannah-forest; MF = Mangrove-forest; in = indoors; ot = outdoors)

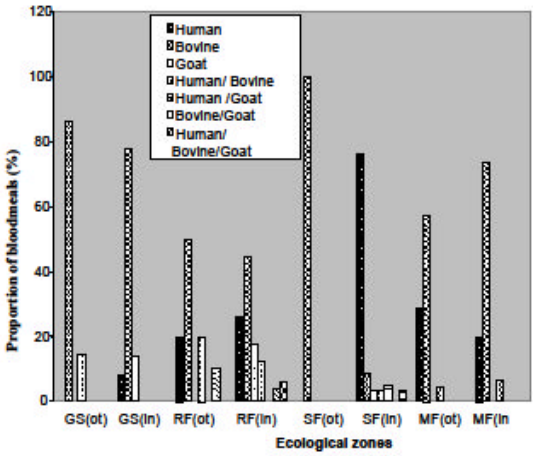
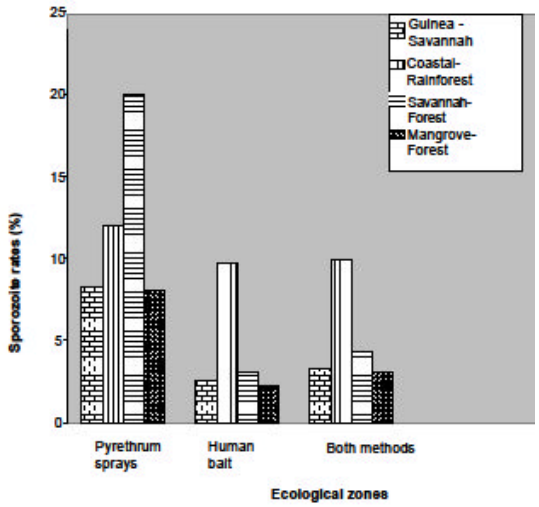


Figure 4. *Plasmodium falciparum* sporozoite rates of female anophelines obtained by human bait and pyrethrum sprays in the four zones



Discussion

This study investigated the principal vectors of the malaria parasite in Nigeria and their role in malaria transmission. Species distribution in the four areas suggests that members of *An. gambiae*, *An. moucheti nigeriensis* and *An. funestus* complexes can be found in sympathy. Several authors[15, 16] also reported this sympatric distribution. In this study, *An. gambiae s.s* was distributed over the four zones, and was most abundant, which is consistent with findings of Bruce-Chwatt[24] who concluded that *An. gambiae s.s* is omnipresent in Nigeria, because of its indiscriminate breeding habitats. They described it as highly endophilic, anthropophagous, wet season vector, but can occasionally be zoophilic and exophilic. Although we observed that *An. gambiae s.s* fed on non-human hosts, it had the highest *P. falciparum* sporozoite rate of 2.33%.

An. arabiensis has been described as a savannah vector, in isolated populations, deforested areas and predominant in the dry season.[8] In this study, *An. arabiensis* was found in considerable numbers in the rainforest zone. Wherever *An. arabiensis* occur in the rainforest, it is associated with a history of extensive land clearance.[12] The coastal sampling sites in this study were areas where deforestation is increasing due to urbanisation. Onyabe and Conn[10] reported that there has been an extension in the range of *An. arabiensis* in Nigeria, prevailing in arid zones, but also in some forest zones. Puzzling shifts in species composition of *An. arabiensis* and *An. gambiae s.s* have been observed in Nigeria.[10] Githeko et al[25] reported that *An. arabienisis* could be anthropophagous, where there are less animal hosts, as we observed, in the Savannah- forest, where *An. arabiensis* was responsible for 34.1% of human blood meals. *An. arabiensis* appears to be a good vector of malaria, especially in the Savannah-forest.

An. melas occurred only in the Coastal rainforest (7.65%) and Mangrove forest (24.6%). According to Bruce-Chwatt, [24] this is the dominant vector in the west coast being related more to sea tides. De Mellion[3] first reported it from Lagos. Until recently, *An. funestus s.s*, has received scant attention.[11] This is inconsistent with its obvious major role in malaria transmission, with a sporozoite rate of 1.42%, second only to *An. gambiae s.s*. Most research has focused on the members of *An. gambiae* complex. According to Gilles and Cozetee, [16] the *An. funestus* group may be as complex and problematic as the *An. gambiae* group with different biology and vectorial capacity. In some areas of Nigeria, it has been projected that it could replace *An. gambiae s.s* as the major vector of endemic malaria. Three members of this group have been found to date in Nigeria: *An. funestus s.s, An. leesoni* and *An. Rivulorum*.[7, 11] However, we only identified *An. funestus s.s* in three ecotypes. We did not observe *An. funestus* in the mangrove forest focus. This could be because of the more restricted habitat choice of *An. funestus s.s* preferring very clean fresh shaded water. This explains, in part, why malaria transmission was less intense in the mangrove forest than in the rainforest.

An. moucheti nigeriensis was identified only in the Southern zones in this study, but was more abundant in the rainforest. *An. moucheti* as a species complex found in sympatry with *An. gambiae* complex and a major or only human malaria vector in villages and towns situated in forest areas. *An. moucheti* was reported as an important primary vector in Nigeria, [15] but played a lesser role when compared to *An. gambiae s.s* and *An. funestus s.s.*). This is consistent with the present study in which *An. moucheti nigeriensis* has a lower sporozoite rate of 0.75%. Despite this epidemiological importance, very few studies have been carried out on this vector. PCR identification should be extended to other important anopheline species complexes such as *An. moucheti*.

Bruce-Chwatt[24] reported 28 anopheline species in Nigeria and in the same year, De Mellion[3] reported an increase in number of anophelines, infected in nature and mainly exophilic. Recently, Awolola et al[6] stated that the diverse species of anophelines involved, confounds studies on malaria transmission in Nigeria. We obtained a sporozoite rate of 0.33% for secondary vectors, showing their role in transmission.

In Northern Nigeria with the shortest transmission period, one expects to get epidemic malaria instead of holoendemic, but there is no evidence of such to date. The presence of cattle within compounds must have attracted *An. arabiensis* to the vicinity of humans. This was reflected by low a HBI and EIR in the Guinea savannah. In a coastal region of southwestern Nigeria, Awolola et al[6] reported the presence of the following vectors*: An. gambiae s.s, An. moucheti, An. melas and An. arabiensis*. We also identified all of these vectors in the southwestern focus in addition to *An. funestus s.s*. In this study, *An. moucheti* is an important vector in the mangrove with a sporozoite rate of 8.1% outdoors. The highest HBI (57. 3%) was recorded in the savannah- forest zone, which was overpopulated with humans.

On several occasions, we detected multiple blood meal sources. These multiple blood meals suggest that female anophelines take successive bites to complete a blood meal. To complete their gonotrophic cycle, female anopheline mosquitoes usually require a second blood meal one day after the first to mature the first egg batch.[15] Shililu et al[22] and Githeko *et al*,[25] in two different studies in Kenya, also reported that anophelines took multiple blood feeds of human, bovid and avian origin. In their study, some blood meals proved negative for all tested hosts, as was also found in this study. According to Bruce- Chwatt, [24] the mean overall sporozoite rate in Nigeria is 6%. This is close to the estimated sporozoite rate of 5.6% that we obtained. We obtained overall sporozoite rates for *An. gambiae s.s.* (2.33%) and *An. funestus s.s.* (1.42%). Beier [27] also reported that sporozoite rate of *An. gambiae s.s* and *An. funestus s.s* in tropical Africa is about 1%-5%. The mean EIR indicates that in Nigeria, about 13 infective *Anopheles* could transmit malaria parasite successfully in the rainy season (July –October).

The malaria problem in Africa south of the Sahara represents a peculiar case because the vectorial system is the most complex anywhere. Beier [27] also suggested that malaria transmission dynamics is variable throughout Africa with huge variability in transmission patterns even within villages few kilometres apart. This vectorial system diversity will impact on malaria epidemiology and control. The *An. gambiae* complex is not the only vector in the field. Targeting only this species by whatever method is nonsense. This study expands the view that the malaria vectorial system in Nigeria is more complex than expected, looking at the combined contribution of these mosquito species to malaria transmission. The diversity of the epidemiological situation within the country ecotypes presents differing malaria situation. Comprehensive knowledge of behavior and heterogeneities that exist within, and among these vectors, will benefit the whole country. Any strategy aiming at control will have to account for this heterogeneity.

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