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SPOROZOITE INFECTION RATES OF FEMALE ANOPHELINE MOSQUITOES IN MAKURDI, AN ENDEMIC AREA FOR MALARIA IN CENTRAL NIGERIA

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A B S T R A C T

This study presents the infection rates of *Plasmodium* species in *Anopheles* mosquito populations within Makurdi, Benue State, Nigeria using four malaria endemic localities: High-level, Wurukum, North- bank and Wadata, between July, 2011 and June, 2012 to cover dry and wet seasons. A total of one thousand, nine hundred and two (1,902) adult female mosquitoes, comprising anopheline mosquitoes were morphologically identified and dissected using standard keys and procedures. Of these, 1,040 (54.7%) were *Anopheles gambiae* sensu lato (s.l.); 641 (33.7%) were *Aonpheles funestus* Giles and the remaining 221 (11.6%) were tagged 'unidentified' species of *Anopheles* mosquitoes. Chi-square statistic showed a significant difference between the mosquito species and their abundance (X^2 *test* $P < 0.05$). An overall sporozoite infection rate of 54.9% was obtained in the study with *Anopheles gambiae* s.l. having the highest sporozoite rate of 31.5%, followed by *Anopheles funestus* with 17.9% while the 'unidentified *Anopheles* species had the lowest sporozoite infection rate of 5.5%. The sporozoite infection rates differed significantly across the *Anopheles* species and also across the four localities (X^2 *test* $P < 0.05$). Human behaviour, some environmental factors, including availability of breeding sites may explain the findings of this study.. The findings indicate that Makurdi is potentially endemic for malaria and that *Anopheles gambiae* s.l. and *Anopheles funestus* are the major malaria vectors in the area. The findings in this study provide the first systematically documented entomological baseline data required for implementation and evaluation of vector control interventions in the Makurdi area of Benue State. Hence the urgent need to initiate serious vector control measures in this area. The results of the studies have been discussed with respect to their epidemiological and public health as well as economic implications.

Keywords: Sporozoites, Infection Rates, *Anopheles* mosquitoes, Makurdi, Nigeria.

INTRODUCTION

According to Dandalo (2007), several mosquitoes belonging to the genera *Anopheles, Culex and Aedes* are vectors for pathogens of various diseases such as malaria, filariasis, yellow fever, dengue, Japanese Encephalitis and haemorrhagic fever. The World Health Organization (2002) reported that even when mosquitoes do not transmit diseases, they may cause great annoyance, making areas originally suitable for human and animal occupation quite uninhabitable. Vector borne diseases are worldwide and exert enormous burden on the continent of Africa (Coetzee, 2000). Malaria and lymphatic filariasis are two of the most common mosquito-borne parasitic diseases worldwide which can occur as concomitant human infections while also sharing common mosquito vectors (Manguin *et al*., 2010). For malaria, pregnant women and children under five years constitute one of the most important risk groups (Adefioye *et al.,* 2007). Amar and Jeffrey (1999) reported that malaria constitutes an urgent threat to global health. Out of the estimated 300 million cases of malaria worldwide and annual deaths of 1 million, 90% occur in Africa (FMOH, 2009). It is estimated that about 2,100 million people live under the threat of malaria in 103 countries, and about 445 million of these are in areas where there is no control (Tuteja, 2007). In terms of adverse economic effects, malaria is

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estimated to cost US$1.8 billion per annum in Africa alone (Oladepo *et al*., 2010). Although since the 1940s, there have been some spectacular anti-malaria campaigns malaria unquestionably remains the most important vector-borne disease (Ayanda, 2009) as control campaigns have not covered all malarious areas of the world. The understanding of the dynamics of malaria vector populations in sub-Saharan Africa, their behaviour and general ecology, and how these affect transmission of disease is still minimal and the current malaria situation is critical, development of alternative control strategies is slow, and existing methods are rapidly losing their efficacy (FMOH, 2009). Although several efforts have been made to effectively control the high incidence of malaria in Nigeria, these have been largely unsuccessful due to a number of factors such as irrigated urban agriculture which can be the malaria vector's breeding ground in the city, stagnant gutters and swamps in our environment where mosquitoes breed in millions, and lack of political will and commitment of the government in its disease management program, lack of awareness of the magnitude of malaria problem, poor health practices by individuals and communities as well as resistance to drugs (Okonko *et al.,* 2009). The Federal Ministry of Health in Nigeria posited that malaria has great impact on the nation's economy as about N132 billion is lost to the disease in form of treatment cost and loss of man hours (FMOH, 2009). For instance, Adeyemo *et al*. (2013) reported that the incidence of malaria accounted for 75% of clinical attendance among students of University of Benin-Nigeria and concluded that the disease was a major public health problem among the students. Similarly, Okonko *et al.* (2009) reported a high malaria prevalence rate of 81.5% in Abeokuta, southwestern Nigeria and attributed it to lack of adequate accommodation and poor sanitary conditions in the area. Igbeneghu and Odaibo (2012) similarly reported high prevalence of malaria due to *Plasmodium falciparum* as 93.3% in southwestern Nigeria. Previous studies in Benue State at large and Makurdi in particular, have shown that malaria is a public health problem in the area. Sanganuwan and Adelaiye (2007) reported high malaria incidence rate of 78.25% in children under 9 years in University of Agriculture, Makurdi health centre and attributed this to environmental conditions transmission of vector diseases in the selected localities (High-level, Wurukum, North-bank and Wadata) of the area. Amuta *et al*. (2014) reported a similar high malaria infection rate of 68.3% in pregnant women in Makurdi, which was also similar to those reported in Gboko (76.9%) and Otukpo (42.4%) by Houmsou *et al*. (2010) and Jombo *et al.* (2010) respectively, which are all Local government areas in Benue State, Nigeria. Jombo *et al.* (2010) also reported an overall malaria prevalence of 32.35% in under five children in Makurdi-central Nigeria, with a high prevalence of 88.955 in those that had fever. It is pertinent to mention here that no work has been published on the infection rates of *Plasmodium* species in mosquito populations in Makurdi and Benue State at large but attention has been concentrated on human infection by *Plasmodium* species, despite the numerous breeding sites and large mosquito population that abound in the area. This study therefore, aimed at ascertaining the *Plasmodium* sporozoite infection rates of *Anopheles* populations in the malaria endemic localities of Makurdi. This study will serve as a reference source for more research work and as contribution to the mosquito vector control strategy for effective malaria control in Central Nigeria.

MATERIALS AND METHODS

Study Area: Makurdi is the capital of Benue State and is located in the middle belt, North of Central Nigeria. It is located between longitude 8°35'E and 8°41'E and latitude 7°45'N and 9°52'N, characterized by undulating rolling plain with irregular river valley and ridges with steep slopes. According to the federal republic of Nigeria official gazette of 2006 population census, published in 2010, Makurdi had the population of 297,398 people (comprising 157,295 males and 140,103 females); and the town is placed 106.4m above sea level (National Meteorological Agency, 2011). Makurdi is an urban setting which lies within the Benue trough, intersected by the river Benue which is a major source of water with other net-works of streams, standing pools, over filled and blocked gutters and drainages. Over grown bushes and fields, even around residential homes and offices are easily noticeable in Makurdi. These provide suitable breeding sites for mosquitoes throughout the wet season (April-October) and dry season (November-March).There is also characteristic high temperature in Makurdi, (30°C-39°C), which aids in the speedy development and hatching of mosquito eggs. It is suspected that temperature may have an impact on throughout the year. The above localities were selected for mosquito sample collection because they are the

most populated parts of Makurdi town and they have more breeding sites for mosquitoes in the area; they also have a closer proximity to river Benue in the study area (Fig. 1). Other detailed geographical and regional indices of the study area have been provided by Udo (1981) and Nyagba (1995).

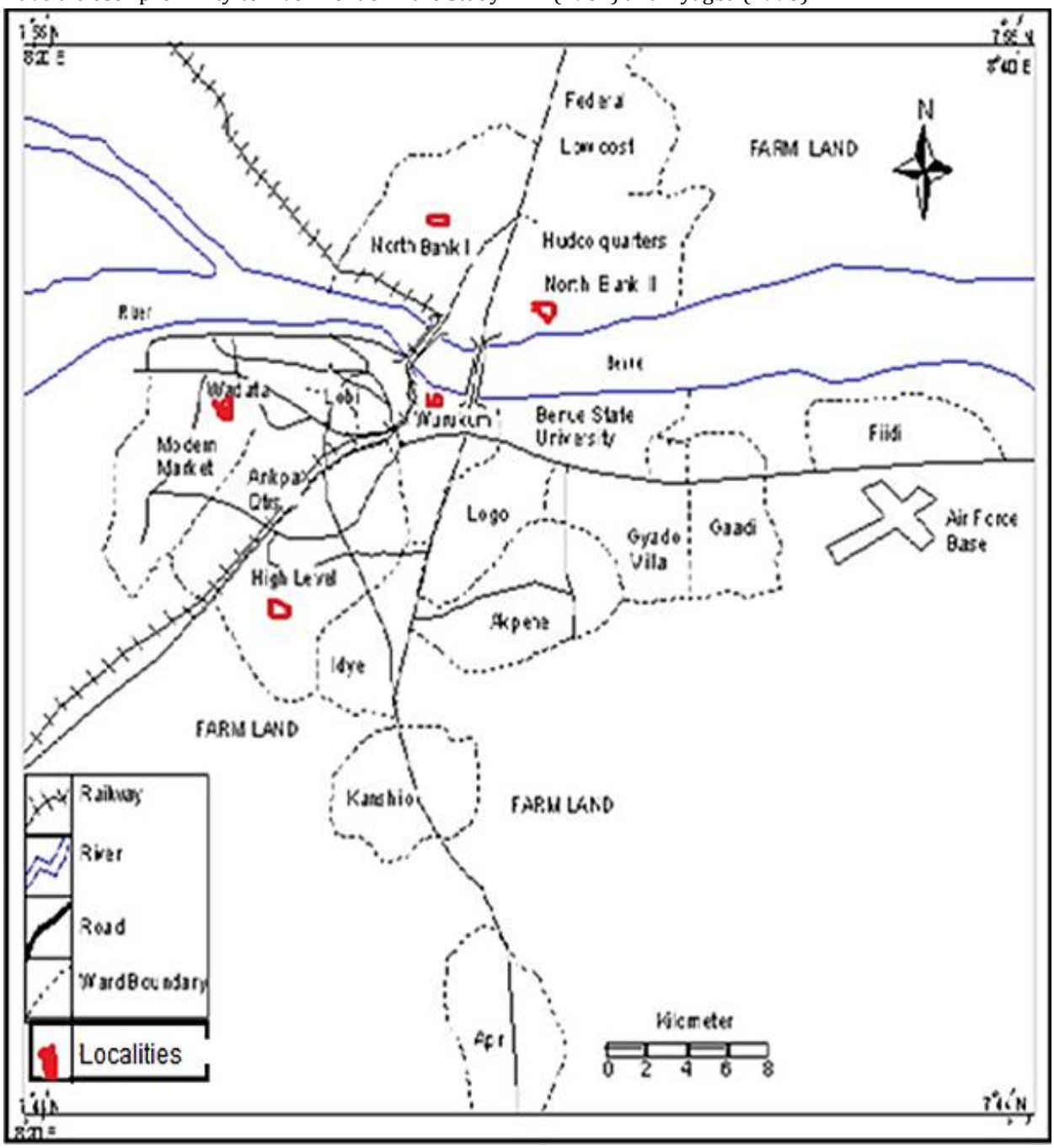

Figure1. Map of Makurdi town Showing the Study Localities.
(Source: *Benue State Ministry of Lands and Survey, Makurdi*, 2012).

Ethical Consideration and Collection of Mosquito Samples: Mosquito samples were collected from a total of forty (40) households, ten (10) from each locality in the study area using a randomised design. Verbal informed consent was obtained from the head of each of the randomly selected households before their houses were accessed for mosquito collection in all the study localities. All mosquito samples were collected using standard procedures as provided by WHO (1975). Sampling units were randomly selected from the four

localities and due to the present security challenges in Nigeria, the mosquito samples were collected with the help of "fly boys" who were recruited from the various study localities where they were well known by the residents of the localities sampled. Mosquito samples were collected between the hours of dawn and dusk. Specifically, the mosquitoes were collected from 6 am to 9 am and 6 pm to 9 pm from living rooms in the study localities, either alive or dead. These periods of sample collection were chosen because previous studies have shown that most mosquitoes enter houses to feed at early hours of the night and struggle to go out in the early hours of the day to rest outdoors (Laumann, 2010; Service, 2012). The mosquitoes were collected from dark corners, walls, ceilings, clothing and other objects inside living rooms using mouth-aspirators (sucking tubes) with the help of battery-operated torch-lights (Service, 1976; Dandalo, 2007); pyrethrum spray collection (PSC) was also used for mosquito collection, which involved the laying of white cloth on the floor and on surfaces of immovable furniture in the houses. The houses were then sprayed using BAYGON (1.0% propoxum, 0.1% imiprothrin and 98% propellant/solvent) as described by Dandalo (2007). After 10 minutes, the cloth was removed and inspected outdoors for knocked down mosquitoes. Window trap method was also used where applicable: The trap consisted of a cage made of 1 ft^3 framework of wire which was covered with mosquito netting. A narrow entrance funnel of ¼ in diameter was made at one end and a string was tied from its narrow end to the four corners of the trap to support the funnel (Service, 1976; Dandalo, 2007).The window traps were now installed in the houses and inspected on daily basis for mosquito collection. The suitability of the sampling methods was determined based on the nature of the houses to be sampled. The mosquito specimens collected from the different capture methods were sorted out separately using forceps and kept in holding tubes, inside cooling boxes, and carried to the laboratory on the same day or the following day for characterization, identification, dissection and examination as in Ungureanu, (1972); WHO,(1975); Goodman *et al.* (2003); Aigbodion and Nnoka, (2008) and Abeyasingha *et al.* (2009). Those mosquito samples that could not be processed on the same day were refrigerated and dissected on the following day according to the methods of Ungureanu (1972). Even though, the mosquito population for this study was only drawn from indoor-resting mosquitoes, which were expected to be only females, some male mosquitoes were also caught along with the females. Male mosquitoes were therefore, distinguished from the females using key morphological features as described by Service (2012).

Identification of Mosquito Samples: Dissecting microscope was used for detailed observation and identification of the mosquitoes with particular reference to the head, thorax, wings and hind-legs according to Gilles and Coetzee, (1987) and Coetzee (1989). Morphological characteristics such as length of maxillary palps, wing spots, leg shape, mouthparts and abdominal end model as presented by Coetzee (2000), Oguoma *et al.* (2010) and Service (2012) were used to identify the *Anopheles* species that co-exist in Makurdi. Observations of the morphological features were made at X40 magnification of the microscope.

Preparation of Mosquitoes for Dissection: Live blood fed mosquitoes were killed with chloroform, ether or carbon (IV) oxide while unfed mosquitoes were collected in a test tube and while at the bottom, the end of the tube was rubbed sharply against the palm of the hand to stun the mosquitoes according to the WHO standard of 1975. After immobilization, each mosquito was placed on a slide and held by one wing while the legs were being removed one at a time and after wards, the other wing was pulled off. The mosquito was then placed on a fresh dry slide and arranged in a more suitable position for dissection of the stomach/abdominal region and salivary glands as described by WHO (1975) and as adopted by Abeyasingha *et al*. (2009).

Dissection of the Salivary Glands for Determination of Infection Rates: The mosquito samples were dissected using the existing standard procedures provided by Ungureanu (1972); WHO (1975); Goodman *et al.* (2003); Aigbodion and Nnoka (2008); Abeyasingha *et al.* (2009). This was intended to incriminate the mosquito vectors and establish the sporozoite infection rates. The anterior part of each mosquito was placed on a slide with the head pointing to the right hand side and a drop of saline was added to keep the specimen fresh throughout the dissecting period. Meanwhile, the left dissecting needle was placed gently on the thorax, just below the region where the glands lie and the right needle was placed at the same but opposite side and pulled towards the right direction to bring out the head with the salivary glands attached. The glands were detached from the head and then placed on another

microscope slide with a little drop of saline and covered with a cover slip after which a gentle pressure was exerted on the cover slip to rupture the gland cells. Some salivary glands however, did not come out with the head of the mosquito but these were located by carefully teasing the lower part of the thorax and examining it carefully with a dissecting microscope (× 40 objective). A drop of 90% absolute alcohol was applied and left for one minute, after which a drop of Giemsa's stain was also added and left for another 40 minutes as recommended by WHO (1975). The sporozoites seen were identified as very minute needle- like forms ($\approx 15\mu$ in length) and the sporozoite Infectivity rates were calculated using the formulae adopted by Kasili *et al. (*2009) as follows:

$$\text{Infectivity Rate} = \frac{No\ of\ Mosquitoes\ carrying\ sporozoites}{No\ of\ Mosquitoes\ dissected} \times 100$$

Statistical Analyses of Data: The Predictive Analytical Software (PASW) Version 18 was used in running Chi-square (X^2) statistic on the data collected. Significant levels were measured at 95% confidence level with significant differences considered at $P < 0.05$. This software, (PASW), is the latest version of the Statistical Package for Social Sciences (SPSS) and was found to include the aforementioned statistics and also, provides better data input and output formats. The Chi-square (X^2) statistic was used because majority of sample data for this study is discrete, which can be referred to as frequency counts. In addition, Chi-square (X^2) statistic was considered the best statistic for test of homogeneity across sample localities so as to determine whether or not the nature of the sample localities affected the distribution of data across them.

RESULTS

A total of one thousand, nine hundred and two (1,902) adult female mosquitoes, comprising anopheline mosquitoes were morphologically identified and dissected using standard keys and procedures. Of these, 1,040 (54.7%) were *Anopheles gambiae* sensu lato (s.l.); 641 (33.7%) were *Aonpheles funestus* Giles and the remaining 221 (11.6%) were tagged 'unidentified' species of *Anopheles* mosquitoes. The overall sporozoite infection rate in the *Anopheles* population dissected in this study was 54.9%. Distribution of infection rates amongst the localities was North-bank (61.4%)> High-level (59.7%) > Wadata (51.9%) > Wurukum (51.3%). Statistically there was a significant difference between the infection rates across the localities (X^2 *test* $P < 0.05$). There were also significant differences in the sporozoite infection rates with respect to the months of study (X^2 *test* $P < 0.05$), as well as with respect to the *Anopheles* species dissected (X^2 *test* $P < 0.05$)for all the study localities (Tables 1-4). Results contained in Table 1 present the total sporozoite infection rate in the *Anopheles* mosquitoes dissected from High-level locality as 59.7%.

Table 1. Sporozoite Infection Rates in *Anopheles Mosquitoes* Dissected from High- level Locality in Maurdi.

Month of Study	No of *Anopheles* Mosquitoes dissected	Species of *Anopheles* Mosquitoes/Infection Rates (%)			
		An. gambiae	*An. funestus*	Unidentified *An. species*	TOTAL Infection
July, 2011	42	10/22(45.5)	4/16(25.0)	2/4(50.0)	16(38.1)
August, 2011	44	11/25(44.0)	7/14(50.0)	1/5(20.0)	19(43.2)
September, 2011	52	29/34(85.3)	14/16(87.5)	3/4(75.0)	46(88.5)
October, 2011	48	24/30(80.0)	5/11(45.5)	2/7(28.6)	31(64.6)
November, 2011	12	3/8(14.1)	3/4(75.0)	-	6(50.0)
December, 2011	10	-	2/10(20.0)	-	2(20.0)
January, 2012	6	-	1/6(16.7)	-	1(16.7)
February, 2012	11	1/6(16.7)	2/5(40.0)	-	3(27.3)
March, 2012	23	10/14(71.4)	4/6(66.7)	1/3(33.3)	15(65.2)
April, 2012	42	14/26(53.8)	6/9(66.7)	4/7(57.1)	24(57.1)
May, 2012	56	21/40(52.5)	7/10(70.0)	3/6(50.0)	31(55.4)
June, 2012	61	31/38(81.6)	13/16(81.3)	5/7(71.4)	49(80.3)
TOTAL	407	154(37.8)	68(16.7)	21(5.2)	243(59.7)

(a) Months: $X^2 = 147.469$, $d.f = 11$, $p = 0.000$ (b) Species: $X^2 = 112.321$, $d.f = 2$, $p = 0.000$

(Data shown are the number of infected mosquitoes while the between brackets are the infection rates)Comparing the sporozoite infection rates within the sample months also showed a significant variability (X^2 *test* $P < 0.05$). There were higher infection rates in the wet season months than in the dry season months for all the species of *Anopheles* mosquitoes dissected in this study. The variability in the infection rates between species of mosquitoes was also statistically significant (X^2 *test* $P < 0.05$). The findings also show that only *Anopheles funestus* had sporozoite infection rates across all the 12 month study period with 16.7% total infection rate; *Anopheles gambiae* had no infection rates for December, 2011 and January, 2012, although it had the highest sporozoite infection rate of 37.8% while the 'unidentified' species of *Anopheles* mosquitoes had no infections in November and December, 2011 and in January and February, 2012 respectively, with the least infection rate of 5.2%. It was observed that the sporozoite infection rates differed significantly across the months during which the study was carried out and also across the species of mosquitoes (X^2 *test* $P < 0.05$) at High-level locality in Makurdi. Table 2. specifically shows total sporozoite infection rate at Wurukum locality as 51.3%. There was a significant variability in terms of sporozoite infection rates within the sampled months (X^2 *test* $P < 0.05$), with the infection rates occurring only in the wet season months apart from January, 2012 where two (2) *Anopheles gambiae* specimens were infected with sporozoites. Although, there was minimal or no sporozoite infections in the mosquitoes during the dry season months across the mosquito species, there was a significant difference in the infection rates within the species of *Anopheles* mosquitoes (X^2 *test* $P < 0.05$) dissected at Wurukum. *Anopheles gambiae* had a total sporozoite infection rate of 28.2%; *Anopheles funestus* had 19.8% infection while the unidentified species of *Anopheles* had 3.3% infection respectively.

Table 2. Sporozoite Infectiion Rates in *Anopheles* Mosquitoes Dissected from Wurukum Locality in Makurdi.

Month of Study	No of *Anopheles* Mosquitoes dissected	Species of *Anopheles* Mosquitoes/Infection Rates (%)			
		An. gambiae	*An. funestus*	Unidentified *An. species*	TOTAL Infection
July, 2011	49	19/34(55.9)	1/10(10.0)	1/5(20.0)	21(42.9)
August, 2011	53	11/24(45.8)	10/19(52.6)	2/10(20.0)	23(43.4)
September, 2011	65	14/31(66.7)	15/29(51.7)	3/5(60.0)	32(49.2)
October, 2011	26	-	-	-	-
November, 2011	13	-	-	-	-
December, 2011	8	-	-	-	-
January, 2012	29	2/29(6.9)	-	-	2(6.9)
February, 2012	33	-	-	-	-
March, 2012	68	24/30(80.0)	21/28(75.0)	9/10(90.0)	54(79.4)
April, 2012	99	36/51(70.6)	26/40(65.0)	1/8(12.5)	63(63.6)
May, 2012	92	39/48(81.3)	31/39(79.5)	2/5(40.0)	72(78.3)
June, 2012	42	18/21(85.7)	10/18(55.6)	1/3(33.3)	29(69.0)
TOTAL	577	163(28.2)	114(19.8)	19(3.3)	296(51.3)

(a) Months: $X^2 = 155.581$, $d.f = 8$, $p = 0.000$ (b) Species: $X^2 = 108.655$, $d.f = 2$, $p = 0.000$

(Data shown are the number of infected mosquitoes while the between brackets are the infection rates)

Table 3. presents the total sporozoite infection rate at North-bank locality as 61.4%. For all the species of *Anopheles* mosquito, the infection was predominant in the wet season months with significant variability within the *Anopheles* species (X^2 *test* $P < 0.05$) and also within the months of study (X^2 *test* $P < 0.05$) respectively. Specifically, *Anopheles funestus* had the highest sporozoite infection rate of 26.3%, followed by *Anopheles gambiae* which had 23.4% sporozoite infection rate while the 'unidentified '*Anopheles* species had the least infection rate of 11.7%. In terms of months during which the study was conducted, December, 2011 through February, 2012 had few mosquito numbers with none of them being infected with *Plasmodium* sporozoites in the North-bank locality (Table 3).

Table 4 presents the *Plasmodium* sporozoite infection rates in *Anopheles* mosquitoes dissected from Wadata locality with a total infection rate of 51.9%. Statistically, there were significant variabilities in the infection rates across the species of *Anopheles* mosquitoes dissected in this study locality (X^2 *test* $P < 0.05$). *Anopheles gambiae* had the highest total sporozoite infection rate of 34.4% without any infections in December, 2011; February and March, 2012 respectively. This was followed by *Anopheles funestus* with 12.8% total infection rate with no infections in both December, 2011 and January, 2012, while the 'unidentified species of *Anopheles* had the least total sporozoite infection rate of 4.7%, without any infections in September, October and November, 2012 consecutively and then February, 2012. Although, all the study months had *Anopheles* mosquitoes that were infected with *Plasmodium* sporozoites, there was a significant difference in the distribution of infection rates with respect to the months during which the study was conducted (X^2 *test* $P < 0.05$), with 100% infection in December, 2011.

Table 3. Sporozoite Infection Rates in *Anopheles* Mosquitoes Dissected from North-bank Locality in Makurdi.

Month of Study	No of *Anopheles* Mosquitoes dissected	Species of *Anopheles* Mosquitoes/Infection Rates (%)			
		An. gambiae	*An. funestus*	Unidentified *An. species*	TOTAL Infection
July, 2011	21	3/12(25.0)	1/5(20.0)	2/4(50.0)	6(28.6)
August, 2011	49	8/13(61.5)	21/26(80.8)	7/10(70.0)	36(73.5)
September, 2011	80	20/31(64.5)	26/32(81.3)	12/17(70.6)	58(72.5)
October, 2011	6	1/6(16.7)	-	-	1(16.7)
November, 2011	4	2/3(66.7)	1/1(100.0)	-	3(75.0)
December, 2011	1	-	-	-	-
January, 2012	2	-	-	-	-
February, 2012	1	-	-	-	-
March, 2012	20	7/7(100.0)	5/7(71.4)	4/6(66.7)	16(80.0)
April, 2012	31	8/19(42.1)	3/12(25.0)	-	11(35.5)
May, 2012	44	11/17(64.7)	14/20(70.0)	3/7(42.9)	28(63.6)
June, 2012	49	12/21(57.1)	10/18(55.6)	8/10(80.0)	30(61.2)
TOTAL	308	72(23.4)	81(26.3)	36(11.7)	189(61.4)

(a) Months: $X^2 = 155.581$, $d.f = 8$, $p = 0.000$ (b) Species: $X^2 = 108.655$, $d.f = 2$, $p = 0.000$
(Data shown are the number of infected mosquitoes while the between brackets are the infection rates).

Table 4. Sporozoite Infection Rates in *Anopheles* mosquitoes Dissected from Wadata, Makurdi.

Month of Study	No of *Anopheles* Mosquitoes dissected	Species of *Anopheles* Mosquitoes/Infection Rates (%)			
		An. gambiae	*An. funestus*	Unidentified *An. species*	TOTAL Infection
July, 2011	126	50/70(71.4)	31/40(77.5)	10/16(62.5)	91(72.2)
August, 2011	210	89/118(75.4)	8/49(16.3)	9/43(20.9)	106(50.5)
September, 2011	83	32/51(62.7)	21/32(65.6)	-	53(63.9)
October, 2011	12	3/9(33.3)	1/3(33.3)	-	4(33.3)
November, 2011	11	3/7(42.9)	2/4(50.0)	-	5(45.4)
December, 2011	2	-	-	2/2(100.0)	2(100.0)
January, 2012	7	1/3(33.3)	-	2/4(50.0)	3(42.9)
February, 2012	6	-	1/6(16.7)	-	1(16.7)
March, 2012	10	-	1/6(16.7)	2/4(50.0)	3(30.0)
April, 2012	34	6/16(37.5)	4/10(40.0)	2/8(25.0)	12(35.3)
May, 2012	50	11/22(50.0)	4/19(21.1)	1/9(11.1)	16(32.0)
June, 2012	59	15/33(45.5)	5/18(27.8)	1/8(12.5)	21(35.6)
TOTAL	610	210(34.4)	78(12.8)	29(4.7)	317(51.9)

(a) Months: $X^2 = 165.886$, $d.f = 2$, $p = 0.000$ (b) Species: $X^2 = 562.407$, $d.f = 11$, $p = 0.000$
(Data shown are the number of infected mosquitoes while the between brackets are the infection rates).

DISCUSSION

If the findings of Koella *et al*. (1998) and Abeyasingha *et al.* (2009) are true, then the overall high sporozoite infection rate of 54.9% found in the present study would imply that the mosquito vectors in the study area will take up larger blood meals and bite several people thus confirming their role in malaria transmission and its intensity in the study area. In the present study, only *Anopheles gambiae s.l.* and *Anopheles funestus* were found to be the major malaria vector groups involved in malaria transmission in the study area as they were found to be significantly infected in this area; an overall sporozoite infection rate of 54.9% was obtained in the study with *Anopheles gambiae* s.l. having the highest sporozoite infection rate of 31.5% followed by 17.9% in *Anopheles funestus* while the 'unidentified' *Anopheles* species had the least sporozoite rate of 5.5%. Similar results of only 2 major vector species (*An. gambiae* and *An. funestus*) confirmed to be infected with *P. falciparum* have also been reported in Western Cameroon (Tchuinkam *et al.,* 2010). In the present study, *Anopheles gambiae* remains the dominant vector, well distributed and infected with sporozoites in all the 4 localities surveyed except North-bank locality where on average, *Anopheles funestus* was more infected (26.3%) than *Anopheles gambiae* (23.4%). *Anopheles funestus* also showed great potential for malaria transmission in all the study localities. The results of this study have shown the transmission potentials of *Anopheles funestus* which is in agreement with the reports from different parts of Sub-Saharan Africa (Hargreaves *et al.,* 2000; Awolola *et al.,* 2002). The sporozoite infection rates in all the four localities in the study area for all the *Anopheles* species were higher in the wet season months as compared to the dry season periods. This is completely in agreement with the findings of Wanji *et al.* (2003) who found higher sporozoite rates for both *Anopheles gambiae* and *Anopheles funestus* in the wet season than in the dry season in the mount Cameroon region. However, the overall sporozoite rate recorded in the present study (54.9%) is far above that recorded by Wanji *et al.* (2003), Moawia and Osman (2010), who separately found sporozoite infection rates of 14.01% and 16.0% in Cameroon and central Sudan respectively. Dia *et al.* (2009) found as low as 0.17% *Plasmodium falciparum* infection rate for species of the *Anopheles gambiae* complex in Mauritania. Again, Shililu *et al.* (1998) working on the sporozoite rates of *Anopheles gambiae* and *Anopheles funestus* in Western Kenya, found *Plasmodium falciparum* sporozoite infection rates of 6.3% for *Anopheles gambiae* s.l. and 9.5% for *Anopheles funestus* respectively. And Salako (1997) reported sporozoite rate of 100% in his studies on malaria problems in Africa. He added that sporozoite rates tend to be higher in the rural areas than urban areas. The high sporozoite rate recorded in our study therefore comparatively shows that Makurdi is not yet fully urban but may best be described as being semi-urban. In Nigeria Oduola *et al*. (2012), working in Oyo, south-western Nigeria, found that *Plasmodium falciparum* sporozoite infection rate of *Anopheles gambiae* s.s. varied between 1.9% and 3.1% in the study communities as compared to 1.5% and 4.5% in *Anopheles funestus*. In this study, the highest sporozoite rate (61.4%) was observed in North-bank locality and this compared contrarily with 3.6% and 4.3% reported in coastal Lagos and Aba Onilu (Awolola *et al.,* 2002). Another study conducted in the tropical rain forest also reported a sporozoite rate of 2.5% for *Anophele gambiae s.s* (Oyewole *et al.,* 2005). Sporozoite rates in the present study were higher compared with sporozoite rates of 5.9% (Bruce-Chwatt, 1951) and 5.3% (Hanney, 1960) earlier reported in Northern Nigeria. Again, lower sporozoite rates of 7.6 and 1.4% as compared to those reported in the present study, were reported in *Anopheles gambiae s.s.* and *Anophele arabiensis* respectively during the Garki Malaria Control Programme (Molineaux and Gramiccia, 1980). However, Ajegbo (1983) gave sporozoite infection rates of 42.4% and 18.8% for *Anopheles gambiae* and *Anopheles funestus* respectively in the Jos area, as against the 31.5% and 17.9% obtained in the present study. The differences in the sporozoite rates recorded in this study as compared to other works elsewhere can be explained by the fact that sample collections were done majorly during the malaria season (2 wet seasons) and only one dry season was surveyed. This also agrees with the findings of Moawia and Osman (2010) who found that the season of mosquito sample collection greatly affects their sporozoite rates. Moawia and Osman (2010) also emphasized that the most important parameters in understanding malaria transmission by mosquito vectors is the identification of sporozoite infection rate. The detection of *Plasmodium* sporozoites in mosquitoes' salivary glands in this study is therefore, important to determine the vector status of identified mosquito

species in Makurdi. Although, both *Anopheles funestus* and *Anopheles gambiae* have similar feeding and resting behaviour (both feed on man, both enter houses to feed, and both rest inside houses after feeding hence the terminologies: anthropophilic, endophilic and endophagic respectively), *Anopheles funestus* is reportedly less susceptible to infection with *Plasmodium* species than *Anopheles gambiae* (Chariwood *et al.,* 1997). However, because of the large number with which it occurred in the present study, it may also be considered as a very important vector in this study area. The combination of anthropophily and endophily puts both *Anopheles gambiae* and *Anopheles funestus* in a special place in the study area. This is corroborated by the findings of Coetzee (2000) who found that high survival rate coupled with anthropophily and endophily, ensure that across Africa, both species are responsible for most of the malaria transmission. There have not been any significant mosquito control project in Makurdi in recorded literature, despite the numerous and suitable breeding sites that are found in the area. It is therefore, convincing to experience the high population of mosquitoes and the corresponding sporozoite infection rates as recorded in this study. Moreover, Moawia and Osman (2010), in their report on the species identification and infectivity rate of malaria vectors in two endemic malaria areas in Sudan, attributed their low sporozoite infection rate of 16.0% to their small sample size. They further recommended large sample size in order to accurately determine the sporozoite positivity in the areas. The large sample size in the present study therefore, may be one of the strong reasons for the high sporozoite infection rate in the area. In the central area of Nigeria, scanty literature exists on the infection rates of mosquitoes with *Plasmodium* sporozoites. Inyama *et al.* (2003) reported *Plasmodium* sporozoite infection rate of 2.3% in Jos, Plateau State, which is still far below the sporozoite rate obtained in the present study. If the recommendation of large sample size by Moawia and Osman (2010) is accepted, then the large variations in the sporozoite infection rates in the 4 localities surveyed in this study and other findings elsewhere may be attributed to differences in sample sizes and availability of human hosts as well as suitable breeding sites for the vector species in question. The large population of indoor-resting/biting *Anopheles* mosquitoes caught in living rooms in the study area at the time of this study may be due to the steady rains and subsequent flood that was witnessed during the study period which led to the proliferation of more breeding sites, and lack of personal protection practices against mosquito bites by the inhabitants of the area. The high temperature generally experienced in the study area may also have predisposed humans to mosquito bites since a good number of people were compelled by the hot weather to sleep outdoors; thus making it possible for those anthropophilic mosquitoes that could not enter houses to feed to take their blood meals outdoors and transmit or pick-up *Plasmodium* parasites to maintain and sustain the transmission cycle in the study area. Several studies elsewhere in Nigeria and other parts of Africa (Awolola *et al.,* 2000 in Lagos; Rishikesh *et al.,* 1985 in Kaduna and Oyewole *et al.,* 2007 in southern Nigeria; Masaninga *et al.,* 2012 in Zambia and Shililu *et al.,* 1998 in western Kenya), have reported seasonal variability in malaria transmission. In the present study, monthly sporozoite rate across the vector species from all the four localities increased progressively from July to October, 2011 (wet season 1), then dropped gradually between November, 2011 to March, 2012 (dry season) with decreasing rainfall amounts, and showed another increase between April to June, 2012 (wet season 2), giving a double peaked pattern. This is similar to the findings of Shililu *et al.* (1998) who also established two peaks of sporozoite infection rates with a progressive increase in the sporozoite rate following increased amount of rainfall and vice versa. Cook *et al.* (2007) stated that vector life span is the most sensitive determinant of a vector population's capacity for pathogen transmission. As a consequence, only a small fraction of the vector population that is oldest is of epidemiological importance. This follows that the overall high sporozoite rate (54.9%) obtained in the present study may not necessarily imply infectivity by the vector species in the study area as some of them may die before having access to the human or vertebrate hosts. Koella *et al.* (1998) found that two mechanisms, namely: enhanced probing and increased blood meal volume, act together to increase the biting frequency and the number of host contacts of mosquitoes infected with malaria sporozoites. This will, by implication, lead to increased transmission only if parasites are injected into the host during successive bites. Shute (1945) reported that mosquito's salivary glands are not depleted of sporozoites even in vectors that have fed up to 15 times. The high sporozoite rates recorded in the present study

therefore, only indicate the vectorial capacity of the vectors in the study area for malaria transmission but the actual infectivity may not be the same, considering all other factors interplaying in the transmission process. This is also corroborated by the works of Koella *et al.* (1998) and Ribeiro *et al.* (1985), who separately reported that even though, sporozoite infected mosquitoes have the tendency to bite more frequently than uninfected ones, a higher number of bites on the same host is not effective to enhance transmission. They further stated that increased biting on a single host may have only little influence on the parasite's rate of transmission; increased biting rate is more likely to increase transmission, if it is coupled with a large number of people that are bitten

CONCLUSION/RECOMMENDATION

The respective Sporozoite Infection rates of 31.5%, 17.9% and 5.5% reported in the present study has confirmed the potential roles of *Anopheles gambiae, Anopheles funestus* as well as the unidentified species respectively as malaria vectors. This has also translated to the intensity of malaria transmission in the Makurdi area. The breeding sites of *Anopheles* species in this area should be cleared or eliminated to prevent them from building up their populations in the various localities. Hence the use of Insecticide Treated bed Nets (ITBNs), insecticidal sprays, effective mosquito repellent creams, screening of windows and doors, wearing of long sleeves and other personal protection practices against mosquito bites should be employed by the inhabitants of Makurdi.

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