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Survey and Detection of Circumsporozoite Protein of Anopheles Mosquito Vectors in Some Selected Wards in Kaduna North Local Government Area of Kaduna State, Nigeria

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ABSTRACT

Malaria is a life-threatening disease caused by parasites, transmitted to humans through the bite and subsequent inoculation of sporozoites by infected female Anopheles mosquitoes. The study aimed to survey and detect the Circumsporozoite antigen of Anopheles mosquito vectors in some selected wards in Kaduna North Local Government Area of Kaduna State. Three hundred and eighty-four (384) indoor and outdoor female Anopheles mosquitoes were randomly trapped and collected using spray sheet and modified light trap methods, respectively. Mosquitoes were examined under an entomological stereomicroscope and identified through morphological features using the standard morphological Identification Key. Mosquitoes were dissected transversely under a dissecting microscope. Circumsporozoite protein was detected using the head and thorax, while the Abdomen was used for blood meal source analysis, both using ELISA. Result revealed that female Anopheles mosquitoes were identified by their spotted wings, hairy antennae and long maxillary palpi as the proboscis. Circumsporozoite protein was detected in 28 of the 384 mosquitos examined with a positive sporozoite rate of 7.3% while 92.7% were negative for the protein. The highest sporozoite rate of 9.4% was recorded in Unguwan Shanu. The highest proportion (10.2%) of human blood-fed mosquitoes were also detected in UnguwanShanu, with 13 out of 128 mosquitoes positive for human blood meal. The result showed that the dominant Anopheles mosquito species with high sporozoite rate detected in UnguwanShanu is an indication of an area with high malaria transmission. Measures aim at eliminating mosquito breeding sites, and reducing contact with mosquito vectors be adopted by community members.

Keywords: *Malaria; Anopheles mosquitoes; sporozoites; Circumsporozoite; Plasmodium.*

1.1 INTRODUCTION

Malaria is a life-threatening infectious parasitic disease transmitted during blood meal to humans by an infected female Anopheles mosquito. Five species of Plasmodia namely; *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale. Plasmodium vivax and Plasmodium knowlesi* cause malaria in humans [1]. Among these species, *Plasmodium falciparum* and *Plasmodium vivax* pose the greatest threat [2]. The World Health Organization estimates that there are 300-500 million cases of clinical malaria per year, with 1.4-2.6 million deaths, mainly among African children [3]. Malaria is a major cause of infant mortality and is the only insect-borne parasite disease reported to be the World's major killer transmissible diseases comparable to diarrhea, acute respiratory infections, tuberculosis and AIDS [4].

In Nigeria, up to 60% of outpatient attendance in health facilities is due to malaria as well as 30% of all hospital admissions with an estimation that malaria is responsible for nearly 110 million clinical cases, 300,000 deaths per year, and the disease is responsible for 25% infant mortality, 30% childhood mortality, 11% maternal death, in addition to the economic burden on Nigerian being equally estimated to be N132 billion loss annually in terms of treatment costs, prevention and loss of man hours [5]. Most malaria deaths occur at home hence are not reported. The power of *Anopheles gambiae* as a malaria vector, for instance, is well illustrated by its accidental introduction into Brazil, where in 1938 after a series of small but intense local outbreaks, it caused the worst epidemic of malaria ever recorded there, with over 14,000 deaths in less than 8 months [6]. In Africa alone, the economic burden is about $12billlion annually. Malaria is estimated to slow economic growth in African countries by about 1.3% per year. The disease thus constitutes a great burden on the already depressed Nigerian economy, thereby imparting more hardship amidst the current economic realities faced by Nigerians [7]. Malaria causes great misery to sufferers, and adversely affects the social and psychological well-being of individuals, families, and the nation at large [8].

As estimated, about 40% of the world population is at risk of malaria, of this number 500 million are in Sub-Saharan Africa.

240-400 million cases are recorded yearly with children experiencing between 1-9 attacks per child per year and adults experiencing between 1-4 attacks. Deaths due to malaria are between 1-2 million; at least 90% of this figure is in Africa. Malaria is directly responsible for 10% of deaths in children under five years and indirectly responsible for 25% of all childhood deaths [9]. Despite all attempts made to eradicate malaria burden, the disease is still on the rise as reports estimated that, without the development of new methods of control, the number of cases could double in the next twenty years [10].

Circumsporozoite protein (CSP) is a secreted protein of the sporozoite stage of the malaria parasite (*Plasmodium* Species) and is the antigenic vaccine target currently undergoing clinical trials [11]. Circumsporozoite protein is found in the salivary gland of the mosquito, it's a protein found in the sporozoites that coated the corresponding antibodies targeting the antigen of the circumsporozoite. The various stages leading to the development (production) of sporozoite take place in the mosquito guts as explained in the mosquito life cycle. Circumsporozoite (CS) is present on the sporozoites surface and early liver forms, and plays an important role in penetration, attachment, and entry into hepatocytes, modulating intracellular biochemical pathways. Circumsporozoite CS was an early target of malaria vaccine research, as passive transfer of Circumsporozoite specific antibodies or T-cell effector was demonstrated to protect rodents from experimental infection but the initial construct targeting the central repeat region of the CS failed to provide conclusive protection [12]. Circumsporozoite is a multifunctional protein required for sporozoites development and likely mediates several steps. Circumsporozoite has two conformational states, an adhesive conformation in which the C- terminal cell adhesive domain is exposed and a non-adhesive conformation in which the N terminus masks this domain. Between these two events the sporozoites must travel from the mosquito midgut to the mammalian liver, and masking of the cell adhesive domain maintains the sporozoites in a migratory state [13].

2.1 MATERIALS AND METHODS

2.2 Study Area

Kaduna North, often referred to as the pioneer Local Government, is one of the Local Government Areas (L.G.A.) of Kaduna State, Nigeria (Figure 2.1). Kaduna North is the oldest Local Government in the State. It is located between Longitudes $7^0$24'26"E to $7^0$24'42"E of the equator and latitudes $10^0$33'14"N north of Greenwich. It is bordered by Igabi Local Government to the South, West, and Southeast, by Kaduna South, Chikun, Kajuru and Kaura Local Governments to the Northeast. It has an area of 72.2km^2 and a density of 5,883.1 inch. /km^2. Kaduna North L.G.A has an estimated population of about 423,580 [14]. The geology of Kaduna North is predominantly metamorphic rocks of the Nigerian basement complex, consisting of biotite gneisses and older granites [15]. It is characterized by two distinct seasonal regimes, oscillating between cool to hot dry and humid to wet seasons. Kaduna North Local Government Area consist of 12 electoral wards, namely: Badarawa, DadiRiba, HayinBanki, Kabala, Kawo, Maiburji, Sardauna, Shaba, UnguwarDosa, UnguwarRimi, UnguwarSarki, UnguwanShanu [15].

2.3 Sampling sites

Three sampling sites were randomly selected namely, UnguwanShanu ward, Kawo ward, and Unguwan Rimi ward.

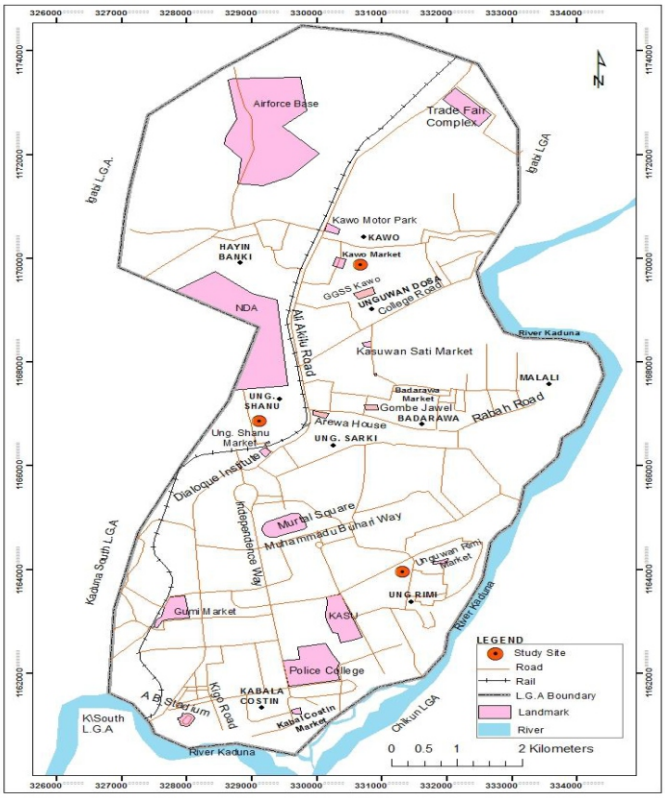

Figure 2.1: Map of Kaduna North Local Government Area Showing the Sampling Sites (Image Source: GIS Laboratory, Department of Geography, and KASU 2022).

2.4 Sample Size Determination

Sample size was determined using the most recent prevalence by [16] and calculated as follows: $N = \frac{Z^2 P(1-P)}{d^2}$

Where:

N =sample size

Z =statistics for a level of 95% confidence level (1.96).

P = a Standard deviation of 0.5

d =precision (margin error) of 5% (0.05).

To substitute the value in the formula:

$$N = \frac{1.96^2\ 0.5(1-0.5)}{(0.05)^2}$$

$$=384.16$$

2.5 Mosquito Collection

Resting female mosquitoes were collected from human habitations following the methods of [17]. Indoor mosquito collection was carried out using spray sheet collection method with pyrethrum spray catchers. A method considered most successful in capturing anthropophagic (human biting) and Endophilic Mosquitoes [18]. It is also considered far less ethically objectionable in areas of multidrug resistance. While the outdoor mosquito collection was conducted using modified light traps. Mosquito collection was conducted in three (3) selected wards in Kaduna North L.G.A namely: UnguwanShanu ward, Kawo ward and UnguwanRimi ward, within Kaduna Township using a mechanical aspirator with the aid of flashlight and Mosquito insecticide.

Thereafter, the Mosquito samples were preserved in an Eppendorf tube. The samples were stored dry on silica gel prior to analysis. All samples were later transferred to the laboratory.

2.6 Morphological Identification of Mosquito Samples

All mosquitoes collected were identified and sorted out under a stereomicroscope (Leica model NSW series IMNS 210 Germany) and (Olympus Tokyo VT-II 225329 Japan) Entomological microscope. The Anopheles mosquitoes were identified as far as possible using morphological keys by Sexes. The male anopheles are recognized by their long proboscis as long as palpi, they are smaller in size than the female and has hairy antennae, while the female Anopheles mosquito was identified by their spotted wings, maxillary palpi as long as the proboscis and less hairy antennae The following key features were considered for the identification of Anopheles [19].

2.6.1 Palps: Smooth with three pale rings, including a wide ring at the tip occasionally divided into two.
In adult males, the palps are as long as the proboscis and swollen at both ends while in adult females the palps are as long as proboscis (females have non-plumose antennae very conspicuous; in others, only just visible).
2.6.2 Tibiae: Narrow pale ring at the tip.
2.6.3 Tarsi: Segmented.
2.6.4 Legs: Irregularly speckled (speckling in some specimens 1-4 pale at tips; segments 2-4 also pale at the base.
2.6.5 Wings: Pale areas are very variable in extent (sometimes greatly reduced). Pale scales creamy yellow pale patches on costa and vein, 1 usually long, two pale interruptions at the base of costa, pale spot in 3rd dark area of vein 1, vein 3 pale except at each end. Main branch of vein 5 pale except at each end, fringe spots present at tips of veins 2-6.
2.6.6 Scutella: Are single lobed Body size: Anopheles is one of the three mosquito genera easily identified from other genera. Anopheles mosquitoes vary in size ranging from small size such as *An. nili*, *An. moucheti* and *An. funestus*; through medium size such as *An. squamosus*, *An. Pharoens is*, *An. maculipalpis*, *An. gambiae* complex, *An. wellcomei* and *An. rhodesiensis*; to a very large size such as *An. implexus* [19].

2.7 Dissection of Mosquitoes

All female Anopheles Mosquitoes collected were dissected transversely at the thorax between the 1st and 3rd pairs of legs using a dissecting microscope. This separated the mosquitoes into two parts for ELISA test. The abdomen of blood-fed Anopheles was set aside for blood meal analysis while the head and thorax were used for Sporozoites detection [20].

2.8 Detection of *Plasmodium falciparum* Circumsporozoite Protein Using ELISA.

Detection of *Plasmodium falciparum* sporozoites was carried out through the detection of plasmodium Circumsporozoite protein (CSP), the major surface protein of the sporozoites [21]. The involvement of each species in malaria transmission was assessed using ELISA tests for *Plasmodium falciparum* sporozoites detection. The head and thorax of each mosquito were crushed in PBS (pH7.4) and tested for circumsporozoite antigen using a double-antibody ELISA. The sporozoites infection rate was determined as follows:
The sporozoites rate mosquito is defined as;

$$\frac{\text{Number of Mosquito positive for sporozoite}}{\text{Total number of mosquitoes dissected}} \text{ X } 100\%$$

2.9 Detection of Blood Meal Source in Mosquitoes by Direct ELISA.

The freshly fed Anopheles mosquitos were counted and sorted based on their abdominal condition before identification of blood meal sources using direct ELISA. These were generally freshly fed and were morphologically identified under a stereomicroscope using morphological identification. The abdomen of freshly blood-fed Anopheles mosquitoes was used for blood meal analysis [22]. Freshly blood-fed mosquitoes were cut transversally between the thorax and abdomen beneath a dissecting microscope 10-20 magnification. The freshly fed Anopheles were recognized by the presence of blood in the abdomen. Blood meal sources were then identified using an Enzyme–linked immunosorbent assay (ELISA) [23].

2.9.1 ELISA Procedures

The blood meal sources of freshly fed Anopheles mosquitoes were determined from indoor resting female mosquito using a direct (ELISA). Each mosquito abdomen was crushed in 50ul phosphate-buffered Saline (PBS) Solution (pH 7.4), which was further diluted by adding 950ul PBS. The Microliter wells were coated with 50ul of mosquito triturate. The plate was covered and incubated for 3 hours at room temperature. After incubation, the wells were washed twice with PBS containing Tween 20 Solution, and 50µl host-specific conjugate (either peroxidase or phosphates) were added to each well and incubated for 1 hour at room temperature.
After 1 hour each well was washed 3 times with a PBS – Tween-20 Solution. Finally, 100 µl of peroxidase substrate was added to each well and after 30 minutes, the absorbance at 405nm were recorded with an ELISA Plate reader. Finally, positive samples including positive control, were changed to blue-green color for human blood (peroxidase) and dark yellow reaction (phosphatase) detected visually. Immediately, using the ELISA reader, each blood meal sample was considered positive if the absorbance value exceeded two times the mean of three negative control, The Positive control contained human blood, unfed mosquitoes/ PBS-bland solution.

2.10 Data Analysis.

Data obtained in the study were analyzed using IBM Statistical Product and Services (IBM -SPSS) version 26, Data were subjected to Chi-square to test for association between Sporozoite rate and blood meal source detected in female Anopheles mosquito. P values < 0.05 was considered significant.

3.1 RESULTS AND DISCUSSION

3.2 Morphological Identification of Anopheles Mosquito

A total of 536 Anopheles mosquitoes were collected and identified in the study sites. Out of these, 152 (28.36%) were males and 384 (71.35%) were females (Table 3.1). Female Anopheles mosquito species were identified by their spotted wings, maxillary palpi as long as the proboscis and has less hairy antennae, While the male anopheles are recognized by their long proboscis as long as palpi, they are smaller in size than the female and has hairy antennae.

Table 3.1 Anopheles Mosquitoes Species Collected and Identified in the Study Sites

Sampling sites	No of Mosquitoes Collected (%)	No. of Male identified (%)	No. of Female identified (%)
Kawo ward	176 (32.83)	48 (31.57)	128
UnguwanRimi ward	196 (36.56)	68 (44.73)	128
UnguwanShanu ward	164 (30.59)	36 (23.68)	128
Total	536	152 (28.36)	384

3.3 Detection of Circumsporozoite Protein of female Anopheles Mosquito

The detection of Circumsporozoite protein of the 384 mosquitoes collected shows that, antigen was detected in 28% out of the (384) female *Anopheles* mosquitoes that were dissected. The sporozoites rate were 7.3% and 92.7% for positive and Negative cases in all the samples respectively were recorded. The highest sporozoite rate was recorded among mosquitoes sampled at Unguwan shanu ward with a sporozoites rate of 9.4% as the Circumsporozoite protein was detected in 12 out of the 128 mosquitoes sampled at the location. Among mosquitoes collected at Kawo ward, the sporozoites rate was 7.0% as the antigen was detected in 9 of the 128 dissected mosquitoes collected at the location. The least sporozoites rate (5.5%) was recorded among mosquitoes collected at Ungwan Rimi ward as 7 of the 128 mosquitoes collected at the location were positive for the antigen. Results further shows no significant difference (p> 0.05) in the sporozoites rate detected between the three sampling sites (Table 3.2).

Table 3.2 Occurrence of Circumsporozoite Protein in Female Anopheles Mosquitoes Sampled at the selected Sites

Sampling sites	No. of Female Anopheles collected	No. Positive (+) for Sporozoites (%)	No. Negative (-) for Sporozoites (%)
Kawo ward	128	9 (7.0)	119 (93.0)
Unguwan Rimi ward	128	7 (5.5)	121 (94.5)
Unguwan Shanu ward	128	12 (9.4)	116 (90.6)
Total	384	28 (7.3)	356 (92.7)

$\chi^2 = 1.464$; d.f =2; P--value = 0.481 (P > 0.05)

3.3 Blood Meal Sources Detected Using ELISA.

Out of the 384 females Anopheles mosquitoes dissected for blood meal source detection,32 were detected as freshly fed blood meal with sporozoites rate of 8.3%, while blood meal was not detected in 353 (91.7%) of the dissected mosquitoes analyzed. The result of abdominal condition of the flies revealed that majority of the vector species collected were unfed, because most where caught while searching for their blood meals before they took blood. The highest proportion (10.2%) of blood-fed mosquitoes were detected at Unguwan Shanu, of which 13 out of the 128 mosquitoes were positive for human blood meal source. Among mosquitoes sampled at Kawo ward, 10 were positive for human blood meal giving a feeding rate of 7.8%; the least number of blood fed mosquitoes were identified among mosquitoes collected at Unguwan Rimi ward with a feeding rate of 7.0%. The difference in the number of blood fed mosquitoes did not differ significantly (p > 0.05) between the three sampling sites (Table 3.3).

Table 3.3 Anopheles Mosquito Blood Meal Source Detected Using ELISA

Sampling sites	No. of Female Anopheles Examined	No. Positive (+) for Human Blood meal (%)	No. Negative (-) for Human blood meal (%)
Kawo ward	128	10 (7.8)	118 (92.2)
Unguwan Rimi ward	128	9 (7.0)	119 (93.0)
Unguwan Shanu ward	128	13 (10.2)	115 (89.8)
Total	384	32 (8.3)	352 (91.7)

χ^2 0.886; d.f = 2; P-value = 0.648 (P>0.05)

3.4 Relationship between Circumsporozoite Protein and Blood Meal Source Detected in Dissected Mosquitoes.

At Kawo ward, the Circumsporozoite protein was detected in 9 (90.0%) out of the 10 mosquitoes which were positive for blood meal.

At Ungwan Rimi ward, the antigen was detected in 7 (77.8%) out of the 9 mosquitoes that were positive for blood meal. At Ungwan Shanu ward, the antigen was detected in 12 of the 13 mosquitoes that were positive for blood meal. The difference in the distribution of the antigen in blood-fed mosquitoes did not differ significantly (p > 0.05) between the sampling sites (Table 3.4).

Table 3.4: Relationship between Circumsporozoite Protein and Blood Meal Source Detected in the Dissected Mosquitoes

Sampling site	No. of Female Anopheles Examined	No. of Positive (+) for No of positive for Blood meal Source (+) Sporozoite (%) (%)
Kawo ward	128	10 (7.8) 9 (7.0)
Unguwan Rimi ward	128	9 (7.0) 7 (5.5)
Unguwan Shanu ward	128	13 (10.2) 12 (9.4)
Total	384	32 (8.3) 28 (7.3)

$\chi^2 = 1.110$; d.f =2; P-value = 0.574 (P>0.05)

4.0 DISCUSSION

In the study, *Anopheles* species were identified in the three different study sites. *Anopheles* mosquitoes were the most abundant mosquito genera and several major *Anopheles* species were present all year round, which explains the high malaria burden in the region. The unequal distribution of the *Anopheles* species within the communities studied further confirms that mosquito occurrence is influenced by macro- and micro-environmental differences exhibited by different areas. The common mosquito species identified in the study sites were A*nopheles* collected in all three 3 study sites. This could be as a result of the presence of collections of dirty water in sample locations which serve as their breeding grounds. Similarly, the surrounding bushes around the various residential areas of the three 3 selected study sites, which include tufts of grass and other emergent vegetation which could serve as their resting places, this could be responsible for large number of mosquito species observed. These findings agree with the report of [24] who reported the presence of similar species of mosquitoes in Zaria dam in which *Culex quinquefasciatus* and *Anopheles gambiae* were the most dominant species. This finding is in line with the study of [25] who recorded *Culex quinquefasciatus* to be the second predominant species of mosquitoes occurring in Abeokuta [26].

The study revealed that varying sporozoite rates were observed across different study sites, indicating differences in malaria transmission risk. Unguwan Shanu showed the highest sporozoite rate at 9.4% suggesting a higher risk of malaria transmission in the area compared to Kawo and Unguwan Rimi area, which had the rate of 7.0% and 5.5% respectively. However, Statistical analysis revealed that no significance difference (P > 0.05) in the sporozoite rates among the three 3 areas, implying that, the observed variation could be due to random fluctuation instead of actual differences in malaria transmission. The presence of salivary gland sporozoites marks infectiousness to humans, detecting earlier developmental stages may have significant operational benefits as reported by [27].

In the context of public health, the prevalence of mosquitoes that are infectious to humans is the most relevant output for determining the efficacy of ELISA [28]. Oocysts may produce many thousands of sporozoites, the majority capable of invading and establishing themselves in the salivary glands where they await egestion by the blood-feeding mosquito into the human dermis [29].

Since mosquitoes egest very few sporozoites when feeding, a mosquito with any number of sporozoites is probably infectious.

The high percentage of blood-fed mosquitoes in this study site shows that the mosquito genera are not just endophagic, but also endophilic as they were caught while resting indoors. This is not surprising as the species is anthropophilic. This finding was observed by [30] in a similar study in some part of Bayelsa State and [25] in Abeokuta.

In the study, the highest proportion (10.2%) of blood fed mosquitoes detected in Unguwan Shanu could be due to numerous breeding sites for the mosquitoes and high population of people living in the area as well as inadequate drainage system. This agrees with the study of [31] that, stagnant water near houses likely facilitates the outbreak malaria through increases in mosquito breeding sites following rains. The study also showed that least (7.8%) blood-fed mosquitoes were detected in Unguwan Rimi. And may be due to the similarity in the composition of the type of people living in all the study areas as well as the architectural design of all the study areas in respect to their drainage system, layout, and population density. This is in agreement with the reported work of [32] that, species composition, distribution and other biological parameters of mosquitoes in different ecological zones could be due to the differences in the amount of blood detected in different groups of mosquitoes.

5.0 Conclusion

The study revealed that A*nopheles* mosquitoes were identified in all the three different study sites, Unguwan Rimi had the highest number 196 (36.56%) while Ungwan Shanu had the least 164 (30.58%) of Anopheles mosquitoes identified. In the present study, Circumsporozoite protein was detected in 28 of the 384 dissected female anopheles' mosquitoes. The positive sporozoites rates were 7.3%, and 92.7% were negative in all samples analyzed. The highest rate was recorded among mosquito samples in Unguwan Shanu ward with a sporozoites rate (9.4%) and the least sporozoites (5.5%) were recorded in Unguwan Rimi ward. Blood-fed meal analysis revealed that the highest proportion (10.2%) of blood-fed mosquitoes was detected in Unguwan Shanu, and the least (7.8%) blood-fed mosquitoes were detected in Unguwan Rimi. The differences in the distribution of antigen in blood-fed mosquitoes did not differ significantly ($p<0.05$) between the sampling locations.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

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