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Comparative assessment of the Vector Competence and transmission of malaria and filariasis in Makurdi, Benue State, Nigeria

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Abstract
Studies on the vector competence and their role in the transmission of malaria and filariasis in Makurdi, Nigeria were carried out over a 6 month period in four localities. A total of 475 adult female mosquitoes were morphologically identified and dissected using standard procedures to determine the parity rates, insemination rates, age structure, oocyst, sporozoite and microfilarial worms. 156(32.84%) were *Anopheles gambiae*, 79(16.63%) were *A. funestus* and 240(50.53%) were *Culex quinquefasciatus*. Of these, 407(85.68%) were parous, while 68(14.32%) were nulliparous. The findings indicate that Makurdi is endemic for malaria with *A. gambiae* contributing a higher overall transmission potential when compared with *A. funestus* and *C. quinquefasciatus*. The occurrence of *A. gambiae*, *A. funestus*, and *C. quinquefasciatus* is suggestive of the prevalence of vector-borne diseases such as malaria and filariasis in the area. Therefore, intensive vector control programmes and public enlightenment especially on human activities that encourage mosquito breeding are recommended.

Keywords: Comparative assessment, Vector competence, Malaria, Filariasis, Makurdi, Nigeria

1. Introduction
For any vector control measures to be successful, good knowledge of the breeding ecology of mosquitoes including, the types and preferences for larval habitats, spatial and temporal distribution of breeding sites, as well as, the physical, biological and chemical characteristics of the habitats are required [1]. Studies have also revealed that convenient aquatic breeding sites for certain mosquito species may be inconvenient for other species [2, 3].

Anopheles species are known to change their ecological range, behavior, adapting to new climatic, ecological and human induced changes [4]. This may not be frequent but it does occur particularly in this era of global warming and varied land use practices, and may have serious public health implications and on the epidemiology of malaria. Man-made environmental changes therefore, can have a limiting and or enhancing factor on the diversity of the vectorial system of an area and invasion by new vectors.

Malaria is a mosquito-borne infectious disease caused by protozoa of the genus *Plasmodium*. The disease is wide spread in the tropical and sub-tropical regions of the world including parts of the Americas, Asia and Africa [5]. The establishment and spread of malaria within a geographical area can vary greatly between villages and households [6, 7].

Bancroftian filariasis, caused by *Wuchereria bancrofti* is widespread in Nigeria. It is a serious public health problem as well as a major cause of acute and chronic morbidity in Nigeria. *A. gambiae* and *A. funestus* are the main vectors in rural Nigeria while *Culex quinquefasciatus* is the vector in the urban and semi-urban areas. Although these mosquito vectors breed and transmit Bancroftian filariasis in Nigeria, human behaviour and activities, urbanization and overcrowding as well as industrialization in Nigeria have created abundant breeding sites. The availability and proximity of human settlement to these numerous breeding sites for the vectors play important role in the disease transmission and intensity in both rural and urban areas [8].

Studies have also reported malaria parasites with Lymphatic filariasis in humans and that mosquitoes carrying malaria-parasites (*A.* species) also transmit human lymphatic filariasis [9-11]. These diseases are largely distributed throughout the warmer regions of the world where the vectors breed. In Ebonyi State (South East, Nigeria) the vectors are considered to be endophagic and bite mostly at night [12].

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Makurdi, the Benue state capital, located in the Benue valley, is characterized by stagnant gutters, marshes and fadama areas that provide good breeding sites for mosquitoes, making them to be abundant throughout the wet and dry seasons, unlike most other drier parts of the country where mosquito breeding occurs predominantly during the rainy season. The high temperature in Makurdi also help speed up the reproductive cycle of mosquitoes thus, giving them a chance to flourish, hence increase in their numbers and also the chances of biting man and transmit malaria and filariasis. Therefore, the present investigation was carried out to study the comparative assessment of the vector competence and transmission of malaria and filariasis in Makurdi, Benue State, Nigeria.

2. Materials and Methods
2.1 Study Area
This study was carried out in Makurdi town, using four major sites: Wurukum, North- Bank, Wadata and High level, for a period of 6 months (that is, between April and September, 2013). Makurdi is the capital of Benue State, Nigeria popularly called Food Basket of the Nation. Makurdi is located between longitude 8º35'E and 8º41'E and latitude 7º45'N and 9º52'N. Benue State experiences two distinct seasons, the wet/rainy season and the dry/summer season. The rainy season lasts from April to October with annual rainfall in the range of 100-200mm. The dry season begins in November and ends in March. Temperatures fluctuate between 23-37 degrees Celsius in the year.

2.2 Ethical Consideration and Collection of Mosquito Samples
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 sites. All mosquito samples were collected using standard procedures adopted by [13]. Sampling units were randomly selected from the four sites and the mosquito samples were collected with the help of "fly boys" who were recruited from the various study sites where they were all known by the residents of the localities sampled. Each sampling locality was subdivided into four (4) sites for convenience, making a total of 16 sample sites altogether.

Samples were collected mainly from dawn to noon (0600-1200hours) and also at dusk (1700-1900hours) from living rooms in the study sites alive or dead, most of which were blood fed. The mosquitoes were collected from dark corners, walls, ceilings, clothing and other objects inside living rooms with the aid of mouth-aspirators, mosquito nets, test tubes and or pyrethrum spray sheets. The mosquito specimens were kept in holding tubes, inside cooling boxes, and carried to the laboratory on the same day for characterization, identification, dissection and examination as described by [14-16].

2.3 Preparation of Mosquitoes for Dissection
Live mosquitoes were killed either with chloroform, ether or carbon (IV) oxide or the mosquitoes were collected in a test tube and while at the bottom, the end of the tube was raped sharply against the palm of the hand to stun the mosquitoes. 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 and salivary glands as described by [13].

2.4 Extraction of Spermathecae for Determination of Insemination Rate
The method described by [13] was adopted. The seventh abdominal segment of the mosquitoes was teased with the dissecting needle under a dissecting microscope (X6) to isolate the tiny spermathecae. These were isolated and transferred to another slide with a drop of normal saline to avoid drying up of the specimen. A cover slip was then placed on the slide and viewed with a X40 objective of a dissecting Olympus inverted microscope. A gentle pressure was applied to the cover slip with the dissecting needle and the spermathecae were crushed to view for spermatozoa. The thread-like spermatozoa were seen to exhibit a rotational movement in an inseminated female mosquito while no such movements were observed in those female mosquitoes that were not inseminated.

2.5 Stomach Dissection for Determination of Oocyst and Parity Rates
After the extraction of the ovary, a gentle pressure was exerted on the abdomen to bring out the Malpighian tubules and the stomach. The stomachs of the female mosquitoes were dissected to confirm the parity or nulliparity of the mosquitoes by examining the tracheolar skeins on the surface of the stomach walls for both *Anopheles* and *Culex* species. The stomach walls of the female *Anopheles* mosquitoes only was closely examined to estimate the oocyst rate. Although, this was not easy with blood fed mosquitoes, care was taken to avoid contamination of the medium with blood. When the stomach was partially extracted, the Malpighian tubules were severed from around the stomach as close as possible without tearing the gut wall, while the rectum was cut off from the stomach just below the pyloric ampulla. The stomach was then transferred to another slide containing a drop of saline and covered with a cover slip and was viewed under a compound microscope (X40) for the condition of tracheolar skeins on the surface of the stomach wall. Tracheae with terminal coilings signified nulliparity while stretched tracheae signified parity. Examination of the stomach for oocysts began from the posterior end, proceeding towards the anterior end. This is because the oocysts are usually located in the posterior half of the stomach in low and moderate infections [13]. The presence of oocysts was detected with a low power magnification and young oocysts were detected because of their refractiveness and characteristic parasite pigment.

2.6 Extraction of the Ovary for Parity and Physiological Age-grading.
The anaesthetized mosquito was placed on it's back on a microscope slide and a drop of diluted Carnoy's fixative (2 parts alcohol, 1 part glacial acetic) was added. This was then placed under a dissecting microscope and dissected. After two (2) minutes, the preparation was examined under an Olympus inverted microscope (X 40) where the tracheolar skeins, Christopher's stages and dilatations were looked for. Dilatations were located by isolating the ovarioles so that the stalks were straightened, making possible the examination for the presence or absence of dilatations, follicular sacs and follicular relics. The number of dilatations on the stalk of the ovarioles were seen clearly and counted. The dilatations represent the number of times the mosquito had laid eggs and hence, the potential risk of disease transmission.

2.7 Dissection of the Salivary Gland for Incrimination Rate

This is intended to incriminate the mosquito vectors and establish the sporozoite rate (for *Anopheles* species only) and microfilaria rate (for both *Anopheles and Culex* species). The anterior part of the same mosquito was placed on a slide with the head pointing to the right. A drop of saline was added to keep the specimen fresh. Meanwhile, the left dissecting needle was placed gently on the thorax, just below the region where the glands lie. The right needle was pulled towards the right direction to bring out the head with the salivary glands attached. Some salivary glands may not come out with the head of the mosquito but these were located by carefully teasing the lower part of the thorax. 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. A gentle pressure was exerted on the cover slip to rupture the gland cells. The sporozoites were seen (if present) and identified as very minute needle like forms. A drop of 90% alcohol was applied and after one minute, a drop of Giemsa's stain was also added and left for 40 minutes [13]. Afterwards the thoracic muscles were teased carefully in a saline solution to look for microfilariae. If the salivary glands contained microfilariae (as in *Culex* species and some species of *Anopheles)*, the microfilariae were seen to emerge from the glands.

2.8 Statistical analysis

Analysis of Variance (ANOVA) was used to assess the significant difference in the vector competence of the three species of mosquitoes and the transmission potential of malaria and filariasis. While P-values less than 0.05 indicated significance.

3. Results

A total of 475 mosquitoes were dissected for parity from the four study sites in Makurdi. Out of these, 407 (85.68%) were parous, while 68(14.32%) were nulliparous. There was a significant difference in the parity rate of mosquitoes dissected from the study sites ($P<0.05$) (Figure, 1).
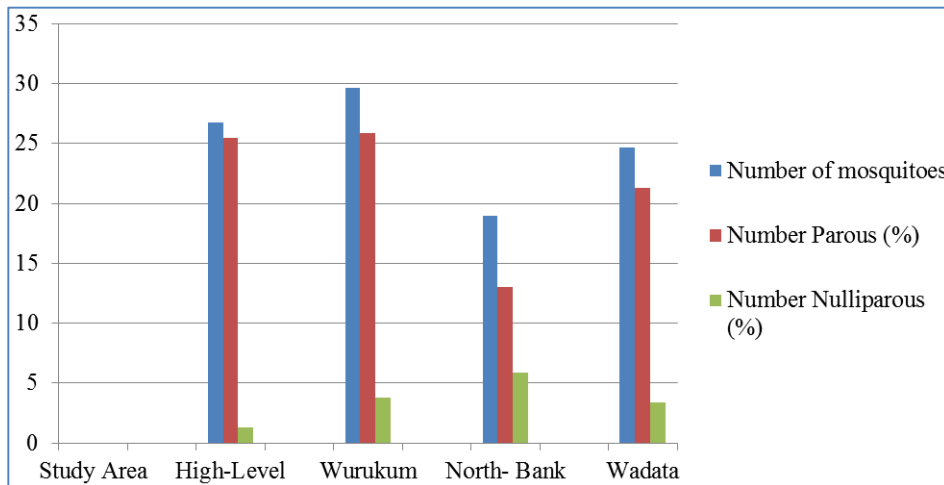

Fig 1: Parity Rates of the Mosquitoes Dissected from different study sites in Makurdi.

Results from the spermathecal dissections to determine insemination rates in the populations of *Anopheles* and *Culex* mosquitoes from the different study sites is presented in Figure 2. A total of 475 mosquitoes were dissected for insemination rates in the populations of *Anopheles* and *Culex* mosquitoes from the four study sites. Out of these, 117 (27.40%) were from High level, 113(26.46%) were from Wurukum, 88(20.61%) were from North-Bank, and 109(25.53%) were from Wadata. There was a significant difference in insemination rates of *Anopheles* and *Culex* mosquitoes dissected from the study sites ($P<0.05$).
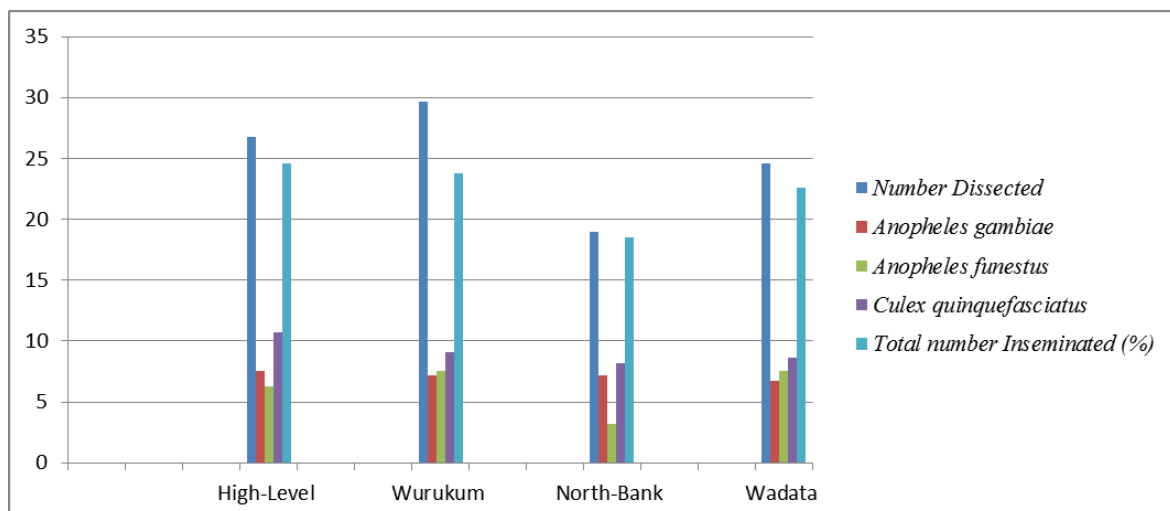

Fig 2: Insemination Rates of *Anopheles* and *Culex* Mosquitoes from the different Study site in Makurdi.

The oocyst loads were determined from the *Anopheles* population across four study sites within Makurdi, the results of which are presented in Table 1. A total of 235 *Anopheles* Mosquitoes were dissected for *Plasmodium* oocyst load from the study sites from April to September. Out of these, the total oocyst load of *Anopheles gambiae* was 66(28.08%), while *Anopheles funestus* was 27(11.48%). There was a significant difference in the oocyst rates of mosquitoes species dissected from the study sites (*P*<0.05).

Table 1: Oocyst rates of mosquito species dissected from the Study sites.

		Study Sites (%)								
Months	**Total dissected**	**High Level**		**Wurukum**		**Northbank**		**Wadata**		**Total**
		Anopheles gambiae	*Anopheles funestus*	*Anopheles gambiae*	*Anopheles funestus*	*Anopheles gambiae*	*Anopheles funestus*	*Anopheles gambiae*	*Anopheles funestus*	
April	34	3(8.82)	1(2.94)	4(11.76)	1(2.94)	1(2.94)	1(2.94)	3(8.82)	1(2.94)	15(44.12)
May	42	2(4.74)	2(4.76)	3(7.14)	2(2.38)	2(4.76)	1(2.38)	2(4.76)	1(2.38)	14(33.33)
June	31	1(3.22)	1(3.22)	2(6.45)	2(6.45)	2(6.45)	-	3(9.68)	1(3.22)	12(38.71)
July	41	2(4.88)	1(2.44)	3(7.32)	4(9.76)	3(7.32)	1(2.44)	2(4.88)	-	16(39.02)
August	42	3(7.14)	1(2.38)	2(4.76)	1(2.38)	3(7.14)	-	3(2.38)	1(7.14)	14(33.33)
Sept.	45	4(8.89)	2(4.44)	3(6.67)	1(2.22)	2(4.44)	1(2.22)	8(17.78)	1(2.22)	22(48.89)
Total	235	15(6.38)	8(3.40)	17(7.23)	10(4.25)	13(5.53)	4(1.70)	21(8.94)	5(2.13)	93(39.57)

The *Plasmodium* sporozoites load in the *Anopheles* mosquito populations from the four localities in Makurdi is presented in Table 2. A total of 235 *Anopheles* Mosquitoes were dissected for *Plasmodium* sporozoites load from the study sites from April to September. Out of these, the total sporozoite infection rates of *Anopheles gambiae* was 65(27.65%), while *Anopheles funestus* was 23(9.78%). There was a significant difference in Sporozoite rates of mosquitoes species dissected from the study sites (*P*<0.05).

Table 2: Sporozoite rates of mosquito species dissected from the Study sites.

		Study Sites (%)								
Months	**Total dissected**	**High Level**		**Wurukum**		**Northbank**		**Wadata**		**Total**
		Anopheles gambiae	*Anopheles funestus*	*Anopheles gambiae*	*Anopheles funestus*	*Anopheles gambiae*	*Anopheles funestus*	*Anopheles gambiae*	*Anopheles funestus*	
April	34	2(5.88)	1(2.94)	3(8.82)	1(2.94)	1(2.94)	1(2.94)	2(5.88)	1(2.94)	12(35.29)
May	42	3(7.14)	2(4.76)	5(11.90)	2(4.76)	2(4.76)	1(2.38)	3(7.14)	1(2.38)	19(45.24)
June	31	1(3.22)	1(3.22)	2(6.45)	2(6.45)	1(3.22)	-	2(6.45)	-	9(29.03)
July	41	2(4.88)	1(2.44)	6(14.63)	-	2(4.88)	-	4(9.76)	1(2.44)	16(39.02)
August	42	3(7.14)	1(2.38)	3(7.14)	1(2.38)	1(2.38)	1(2.38)	3(7.14)	1(2.38)	14(33.33)
Sept.	45	4(8.89)	-	5(11.11)	2(4.44)	-	2(4.44)	5(11.11)	-	18(40.00)
Total	235	15(6.38)	6(2.55)	24(10.21)	8(3.40)	7(2.98)	5(2.13)	19(8.08)	4(1.70)	88(37.45)

A total of 475 *Anopheles* and *Culex* Mosquitoes were dissected for microfilarial load from the study sites from April to September. Out of these, the total microfilarial load of *Culex quinquefasciatus* was 178(37.48%), *Anopheles gambiae* was 38(7.99%), while *Anopheles funestus* is 26(5.46%). There was a significant difference in microfilarial rates of mosquitoes species dissected from the study sites (*P*<0.05) (Table 3).

Table 3: Microfilarial rates of mosquito species dissected from the study sites.

		Study Sites (%)											
Months	**Total dissected**	**High Level**			**Wurukum**			**Northbank**			**Wadata**		
		C.q	**A.g**	**A.f**	**C.q**	**A.g**	**A.f**	**C.q**	**A.g**	**A.f**	**C.q**	**A.g**	**A.f**	**Total**
April	74	13 (17.57)	2 (2.70)	-	9 (12.16)	-	1 (1.35)	3 (4.05)	1 (1.35)	-	1 (1.35)	2 (2.70)	1 (1.35)	33 (44.59)
May	78	11 (14.10)	-	1 (1.28)	6 (7.69)	2 (2.56)	1 (1.28)	6 (7.69)	2 (2.56)	1 (1.28)	7 (8.97)	-	1 (1.28)	38 (48.72)
June	97	4 (4.12)	-	1 (1.03)	13 (13.40)	2 (2.06)	1 (1.03)	4 (4.12)	1 (1.03)	1 (1.03)	3 (3.09)	4 (4.12)	1 (1.03)	35 (36.08)
July	69	9 (13.04)	1 (1.45)	-	9 (13.04)	1 (1.45)	-	8 (11.59)	1 (1.45)	1 (1.45)	3 (4.35)	1 (1.45)	-	34 (49.27)
August	75	19 (25.33)	1 (1.33)	3 (4.00)	10 (10.33)	1 (1.33)	2 (2.67)	9 (12.00)	1 (1.33)	3 (4.00)	5 (6.67)	1 (1.33)	3 (4.00)	58 (77.33)
Sept.	82	8 (9.76)	6 (7.32)	1 (1.22)	4 (4.88)	4 (4.88)	1 (1.22)	2 (2.44)	-	1 (1.22)	12 (14.63)	4 (4.88)	1 (1.22)	44 (53.66)
Total	475	64 (13.47)	10 (2.10)	6 (1.26)	51 (10.74)	10 (2.10)	6 (1.26)	32 (6.74)	6 (1.26)	7 (1.47)	31 (6.53)	12 (2.53)	7 (1.47)	242 (50.95)

A total of 475 Mosquitoes were dissected for physiological age-grading from four study sites between April and September (Fig. 3). Out of these, a total of 68 (14.32%) were nulliparous, while 407(85.68%) were parous. Out of the parous mosquitoes, 350(73.68%) were 1-parous, 49(10.31%) were 2-parous, while 8 (1.68%) were 3-parous. There was a significant difference in the age structure of mosquitoes dissected from the study sites (*P*<0.05).
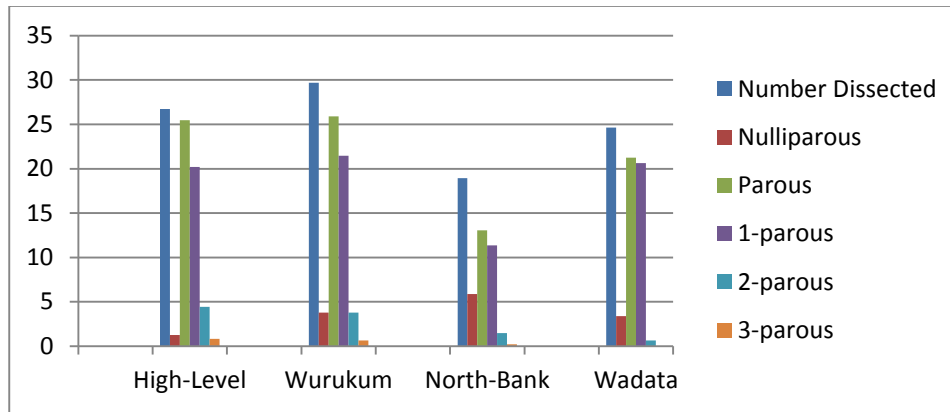
Fig 3: Age Structure of Dissected Mosquitoes from four study sites in Makurdi.

4. Discussion

Anopheles gambiae and *Anopheles funestus* mosquitoes is the vector of malaria and lymphatic filariasis in Nigeria [17]. However, *Culex quinquefasciatus* have been reported to be infective with *Wucheraria bancrofti* in Nigeria [18, 19]. The possible involvement of *C.* species in the transmission of lymphatic filariasis in Makurdi, Benue State has not been substantiated. This is linked to the fact that *C.* species is known to breed in poorly sanitized areas with filthy and foul smelling water collections which are eminent in the Makurdi area, [20]. This may explain why [21] reported *W. bancrofti* infection to be a major public health problem in Benue State.

The overall insemination rate in this study was found to be 89.89%. Similar insemination rates have been reported elsewhere by various authors: [22] observed insemination rates of 88.9% in some *Anopheline* and *Culicine* populations in the Makurdi area of Benue State [23] also found high insemination rates of 73% in *A. gambiae* and 90% in *A. funestus* in the Jos area.

The high insemination rates of the mosquitoes reported in the present study, irrespective of the species, imply that their activity in terms of biting and flight would be greatly enhanced at night than during the dusk. This is in accordance with the findings of [24] who reported that once inseminated, the flight and biting activity of female mosquitoes would change, shifting the peak of activity from dusk to a later time in the night [25] stated that high insemination rate in a mosquito population would mean high parity rate, therefore the high parity rate observed in this study and the subsequent high infection may be due to the age of the mosquitoes and changing habits of the different mosquito species.

In the present study, only *A. gambiae* and *A. 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 37.43% was obtained in the study with *A. gambiae* having the highest sporozoite infection rate of 27.65% and 9.78% in *A. funestus*. Similar results of only 2 major vector species (*A. gambiae* and *A. funestus*) confirmed to be infected with Plasmodium falciparum have also been reported in Makurdi [26]. In the present study, *A. gambiae* remains the dominant vector, well distributed and infected with sporozoites in all the 4 localities surveyed. The results of this study have shown the transmission potentials of *A. funestus* which is in agreement with the reports from different parts of Sub-Saharan Africa [27, 28]. 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 during the wet season. This also agrees with the findings of [29] who found that the season of mosquito sample collection greatly affects their sporozoite rates.

The microfilarial rates of 16.83%, 14.1%, 9.47% and 10.53% respectively obtained across the localities in the present study are comparable to reports by [30] in a similar study on vectorial potential of *A.* and *C.* species in the transmission of bancroftian filariasis in the Localities of Makurdi, North Central Nigeria. However, the variation in the microfilarial rate may be attributed to differences in sample sizes.

The incrimination of *C. quinquefasciatus* in this study is in line with the earlier observations by [10] on the possible involvement of *C.* Species in the transmission of lymphatic filariasis in Northern Nigeria. It is also in consonance with the findings of [11] that *C. Quinquefasciatus* is a potential vector of bancroftian filariasis in most West African cities.

There were more parous mosquitoes in the Study area compared to nulliparous ones. This indicates that the population is an older population and signifies the high survival rate of the mosquitoes [31]. Likewise, the high number of nulliparous mosquitoes could be an indication of high productivity of the mosquito breeding sites which continuously supplies the area with young mosquitoes. The monthly variation in the parity rate of *A. gambiae*, *A. funestus* and *C. quinquefasciatus* mosquitoes is probably associated with the seasonal abundance of mosquito breeding sites. The parity rate is likely influenced by environmental variables such as Temperature, Rainfall, Relative Humidity and availability of breeding site [32].

5. Conclusion

Vector mosquito species in Makurdi is dominated by *Culex quinquefasciatus*. Thus, the ongoing anti-anopheline control interventions, to reduce malaria burdens, in the country should be broadened to target the breeding sites of the Culicines as well, as these mosquitoes transmit other disease burdens that can rival malaria.

The findings of this study had help in achieving a better understanding of the epidemiology of mosquito-borne diseases in Makurdi, which is a pre-requisite for sustainable disease control.

Therefore, intensive vector control programmes and public enlightenment especially on human activities that encourage mosquito breeding are recommended.

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