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Infection rates and parity of mosquitoes in a Peri-Urban Area of Plateau State, North Central Nigeria

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ABSTRACT

Nigeria has a high burden of vector borne diseases such as malaria and lymphatic filariasis (LF). Thus, a study was carried out to examine the salivary glands of parous mosquitoes for sporozoites and third stage larvae (L3) in order to determine if the mosquito species are infected with malaria or filarial parasite or not. Adult mosquitoes were collected by pyrethrum spray catch (PSC), and were identified and graded according to their abdominal conditions. The ovaries of the mosquitoes were dissected to determine the parity status, while the salivary glands of the parous mosquitoes were dissected to check for third stage larvae of *Wuchereria bancrofti* and the sporozoite of *Plasmodium*. A total of 222 mosquitoes were collected, out of which 58 (26.1%) were *Anopheles gambiae*, while 164 (73.9%) were *Culex quinquefasciatus*. Ovarian dissections showed 23 (57.5%) parous and 17 (42.5%) nulliparous mosquitoes. There was a significant difference in the infection rates of the vectors, 22 (38.6%) female *An. gambiae* were infected with sporozoites, while 45 (28.7%) female *Cx. quinquefasciatus* were infected with L3 of *W. bancrofti*. The high proportion of mosquitoes infected with sporozoites was attributed to the season of collection and lack of adequate sanitary conditions in the area. Thus, health education could help in sensitizing the inhabitants of the area.

Keywords: Infection, parity, malaria, filariasis, mosquitoes

INTRODUCTION

Malaria and lymphatic filariasis (LF) are vector-borne diseases that account for the largest global burden of mortality and morbidity in the world's poorest countries (CDC, 2010; Nematodes.org, 2010). More than half of the world's population is at risk of at least one of these diseases. According to the World Health Organization and the Centre for Disease Control and Prevention, mosquitoes infect a minimum of 216 million people with diseases each year (WHO, 2015; CDC, 2010). It kills about 881,000 people every year of which 90% are in sub-Saharan Africa and 85% are children under five. It is highly endemic in Nigeria with about 97% of the populace at risk of the disease. According to the Federal Ministry of Health, malaria and some other mosquito related diseases are a major public health problem in Nigeria. It accounts for 65% of all hospital admissions, 25% of infant mortality, 30% of childhood mortality and 11% of maternal deaths (Federal Ministry of Health, Nigeria, 2008). Lymphatic filariasis is one of the most debilitating Neglected Tropical Diseases (NTDs) in the world (Brady, 2015). The disease is endemic in 81 countries with an estimated 120 million people infected and 40 million people with clinical manifestations including lymphoedema (elephantiasis) of the limbs and urogenital disorders, especially hydrocele in men. Nigeria bears the highest burden of LF in Africa, with an estimated 80 to 120 million people at risk(Okorie *et al*., 2014). It is common to find malaria andLF in the same human population and sharing the samemosquito vectors. It is therefore common to find co- infections of malaria and LF in a single mosquito vectorin these areas. Malaria and LF are both transmitted by *Anopheles* mosquitoes in Nigeria. The diseases have beenobserved to coexist in some parts of Nigeria, includingNew Bussa, Niger State (Okorie *et al*., 2014). Therefore, any control method geared towards the vector has the capability to control both diseases.

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MATERIALS AND METHODS

Study Area

This study was carried out in Russau village of Jos-North Local Government Area of Plateau State, North central, Nigeria, from August 2018 to May 2019. It lies between Latitude 9°56'N and Longitude 8°53'E (Ajakpo and Okonkwo, 1984). The area is moist, and has a temperature range of 25-30^{0}c and a relative humidity of 60% or more. The study location is surrounded by bushes and stagnant waters; it is also well populated with people and has over 380 residential buildings. The area is also littered all over with domestic water holding containers including gutters and farms; this dirty environment provides breeding sites for mosquitoes.

Method and Instrument of Data Collection

Collection of adult resting mosquitoes (spray sheet collection)

This method involves using a pyrethrum spray to knock down mosquitoes resting inside a house and collect them on white sheets spread on the floor and other flat surfaces. Prior to spraying, all animals, food and small furniture were removed from the room where the collection took place. White sheets were then laid to completely cover the floor and all flat surfaces. All windows and doors were closed. It was then carefully sprayed in a clockwise direction towards the ceiling until the room was filled with a fine mist. The room was then closed for about 10 minutes. Beginning from the entrance, the corners of the sheet were lifted and the sheet was taken outside. All knockdown mosquitoes were collected in the daylight with forceps and placed in a labeled Petri-dish, on top of a layer of damp cotton wool and filter paper. Mosquitoes collected in each house were stored in separate Petri-dishes and appropriately labeled as follows: collection date and hour, village, household number, and name of head of household.

Identification of adult resting mosquitoes, and dissection of ovaries and salivary glands

Adult mosquitoes were identified using mainly their morphological features (WHO, 2000; CDC, 2010). After identification, the knocked down mosquitoes, were prepared immediately (within the next 6 hours) for dissection. The dissection was carried out by first removing the legs and wings of the mosquito and then placing it on a slide with a drop of normal saline. With the help of one needle placed gently on the thorax, a cut was made using the other needle between the 6th or 7th abdominal segment and this detached segment was then teased out carefully exposing the ovaries and the Malpighian tubules. The Malpighian tubules were then cut off and the ovaries were then covered with a cover slip just to flatten the ovaries without breaking it. It was then examined under a compound microscope using first the 10x objective and later confirmed with the 40x objective. The females in which the ovaries have coiled trecheolar skeins are “Nulliparous” while the ones with stretched tracheoles are “parous”. In some females not all developed eggs are laid; if some eggs (usually less than five) are retained in the ovaries, the female is parous. The salivary glands were examined for sporozoites and L3 larvae to determine the mosquito species that carry malaria parasites and filarial worm. The processes involved in the dissection of the salivary glands for sporozoites and L3 larvae are the same. The salivary glands of nulliparous females were not dissected because they are either newly emerged or are yet to find a blood meal, and therefore not infected.

Determination of sporozoite rate

After identification, the legs and wings of the freshly anesthetized mosquitoes were removed, the mosquitoes were placed on the slide lying on its side with the head pointing to the right; a drop of saline solution was placed close to the front of the thorax. The thorax was then held firmly with a blunt dissecting needle in the left hand and the neck held with the other needle in the right hand. Without cutting the neck, the head was gently pulled away from the thorax with the glands coming outof the thorax attached to the head. In cases where the glands did not come out with the head, the thorax was squeezed gently to obtain it. The cover slip was them placed on the already obtained salivary glands crushingthe glands and releasing the sporozoites. The glands were then examined under the high power 40x objectiveand the unstained sporozoites were seen moving.

The sporozoites were stained by placing a drop of adhesive on the top side of the cover slip and using a dissecting needle the cover slip was turned to the other side and fixed to one end of the slide such that the sporozoites stick to the cover slip for staining. A circle was drawn around the salivary glands and sporozoites with a grease pencil on the reverse side to make it easy to locate the specimen later. The preparations were allowed to dry and were protected from ants and flies. The preparation was then fixed by immersing the whole slide for a few seconds in methanol for evaporation of excess water. The slide was stained for 30 minutes with Giemsa stain in buffer solution, being applied with a dropper to flood the specimen and cover slip. The slide was then washed well with buffer solution and examined under the high power of compound microscope to get the clear view of the sporozoites. The sporozoites rate,

which is the percentage of mosquitoes with sporozoites in their salivary glands, is necessary to confirm the role of a particular mosquito species as a vector, to determine the intensity of malaria transmission, and to also evaluate the impact of ongoing malaria control interventions.

Larvae determination

The freshly anesthetized mosquitoes were placed on a petri dish and the wings and legs were removed using two pairs of forceps under low power magnification, the individual mosquitoes were then placed on a microscope slide and with dissecting needles divided into head, thorax and abdomen, placing each portion of the body in a separate drop of saline solution on the same slide. The abdomen was dissected first and ovaries graded for parity. Nulliparous females were immediately registered as uninfected without further dissection. For parous females, the three body segments were teased apart and examined for larval stage worms or microfilaria. The location and number of the parasites were noted. Mosquitoes carrying microfilaria, L1, L2 or L3 larvae were defined as infected while those with the L3 larvae in the salivary glands were termed as infective i.e. vectors with the potential of causing lymphatic filariasis (Barillas-Mury and Kumar, 2005).

Data Analysis

Data were analyzed using R version 3.5.2. Chi-square test was used to compare the number of vectors in relation to sex, type of houses and between nulliparous and parous mosquitoes of the different species. Chi-square test was also used to compare the number of infected vectors with sporozoites and L3. Observed difference was considered significant if $P<0.05$.

RESULTS

A total of 222 mosquitoes were collected and identified from 30 houses. All the vectors belong to theAnophiline and Culicine sub family. Majority, 164 (73.9%) of the 222 mosquitoes collected were *Culex* species (i.e., vector of lymphatic filiariasis), while about aquarter, 58 (26.1%) were Anopheles species (i.e., vector of malaria) as shown in Figure 1. There was no significant difference (χ^2=4.4566, df=1, P=0.1) in the distribution of the vectors collected by sex. Of the 58 *Anopheles* mosquitoes collected, 57 (98.3%) were females,with only 1 (1.7%) male. Similarly, of the 164 *Culex* mosquitoes collected 157 (95.7%) were females, while 7 (4.3%) were males as shown in Figure 2. There was also no significant difference (χ^2=0.339, df=2, P=0.844) in the distribution of the vectors collected by type of house. In this study, 4 Mud houses, 15 Block houses, and 11 Mud and Block (M&B) houses were sampled. Thirty-two female vectors were collected in the Mud houses (9 *Anopheles* and 23 *Culex*), 125 female vectors were collected in the M&B houses (30 *Anopheles* and 95 *Culex*) and 57 female vectors were collected in the Block houses (18 *Anopheles* and 39 *Culex*). Of the 57 female *Anopheles* mosquito collected, 9 (15.8%), 18 (31.6%) and 30 (52.6%) were collected in Mud, Block, and Mud and Block houses respectively. Also, of the 157 female *Culex* mosquitoes collected 23 (14.6%), 39 (24.8%) and 95 (60.5%) were collected in Mud, Block, and Mud and Block houses respectively (Figure 3).

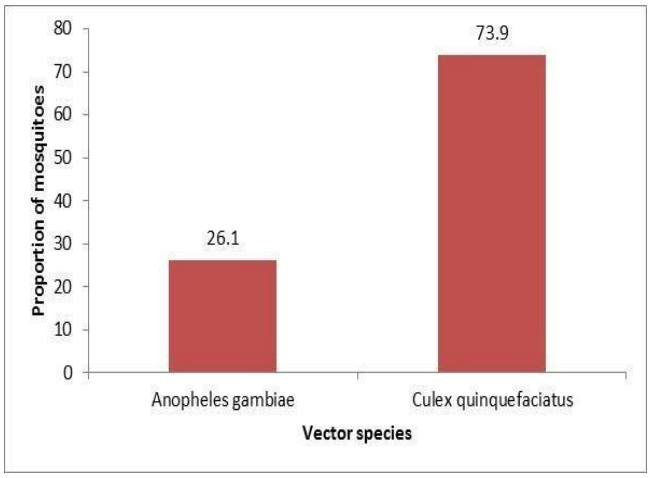

Figure 1: Distribution of vector species of malaria and lymphatic filariasis

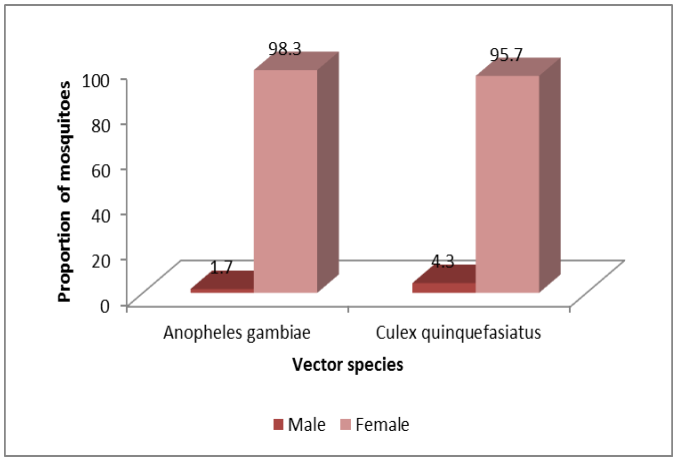

Figure 2: Distribution of the vector species of malaria and lymphatic filariasis by sex

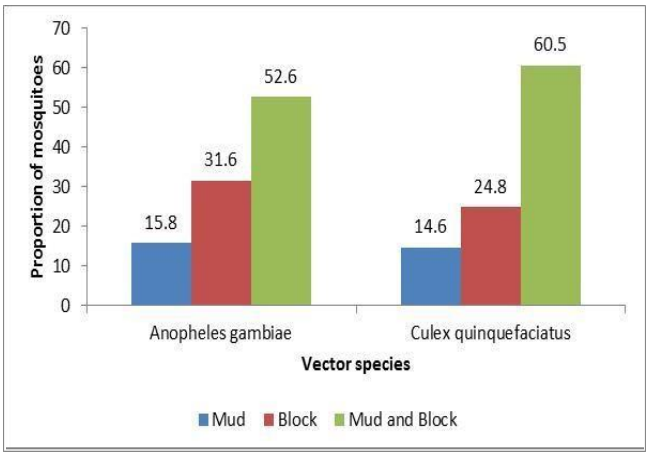

Figure 3: Distribution of vector species of malaria and lymphatic filariasis by type of house

There was no significant difference (χ^2=0.291, df=1, P=0.1) between the parous and nulliparous mosquitoes analyzed. The ovaries of 40 female mosquitoes were dissected for parity, the ovarian dissections showed that there were 23 (57.5%) parous mosquitoes (which comprised 7 *Anopheles* and 16 *Culex* mosquitoes), and 17 (42.5%) nulliparous mosquitoes (which comprised 2 *Anopheles* and 15 *Culex* mosquitoes). Of the 9 *Anopheles* mosquitoes whose ovaries were dissected, 7 (77.8%) were parous while 2 (22.2%) were nulliparous. Also, of the 31 *Culex* mosquitoes whose ovaries were dissected, 16 (51.6%) were parous while 15 (48.4%) were nulliparous (Figure 4).

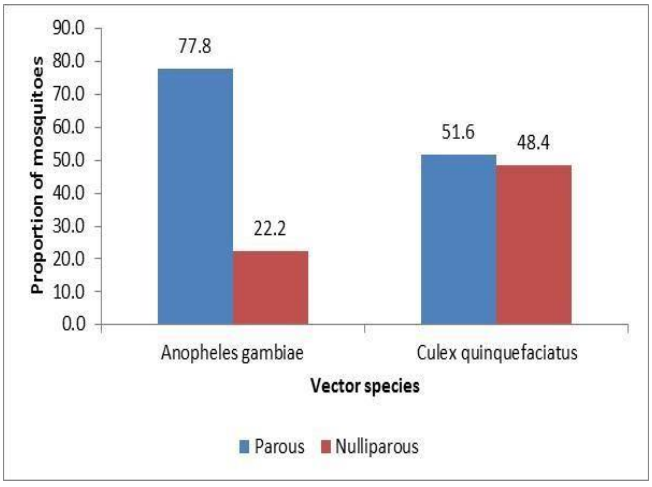

Figure 4: Distribution of vector species of malaria and lymphatic filariasis by parity

The difference between the blood digestion stages of the vectors when analyzed was seen to be non-significant (χ^2=9.143, df=4, P=0.057). Out of the 215 female mosqutoes collected, those with a fed abdominal condition occurs most, which comprised 78 mosquitoes (36 *An. gambiae and* 42 *Cx. quinquefaciatus*), followed by those with half-gravid, which comprised 51 mosquitoes (5 *An. gambiae and* 46 *Cx. quinquefaciatus*), and then 46 gravid mosquitoes (7 *An. gambiae and* 39 *Cx. quinquefaciatus*), and the least in occurence were 40 unfed mosquitoes (9 *An. gambiae and* 31 *Cx. quinquefaciatus*). Of the 57 *Anopheles* mosquitoes, 9 (15.8%), 36 (63.2%), 5 (8.8%), and 7 (12.3%) were Unfed, Fed, Half Gravid, and Gravid respectively, while of the 158 *Culex* mosquitoes, 31 (19.6%), 42 (26.6%), 46 (29.1%) and 39 (24.7%) were Unfed, Fed, Half Gravid and Gravid respectively (Figure 5).

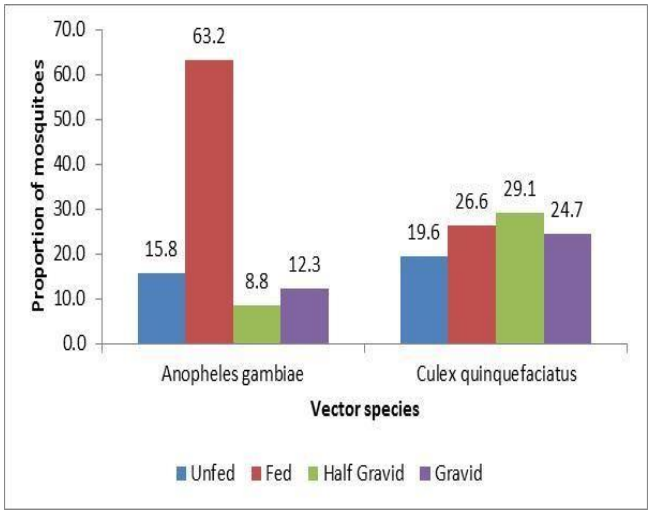

Figure 5: Distribution of vector species of malaria and lymphatic filariasis by blood digestion stages

There was a significant difference (χ^2= 44.3, df=2, P<0.001) in the infection rate between the species of the vector analyzed. A total of 214 female mosquitoes were dissected for infection rate (of which 57 female mosquitoes were examined for Sporozoite rate, while 157 were examined for L3 rate). Twenty-two (38.6%) of the 57 mosquitoes that were examined for sporozoites were seen to be infected, 45 (28.7%) of the 157 mosquitoes that were examined for L3 rate were found to habour the L3 of *W. bancrofti* in their salivary glands, while 147 (68.7%) of all the 214 mosquitoes that were examined (of which 35 were *Anopheles* and 112 were *Culex* mosquitoes) were confirmed to have no infection in their salivary glands when dissected (Figure 6).

An. gambiae was the only vector seen to be infected with Sporozoite, while *Cx. quinquefasciatus* was also the only vector infected with the L3 (Figure 7).

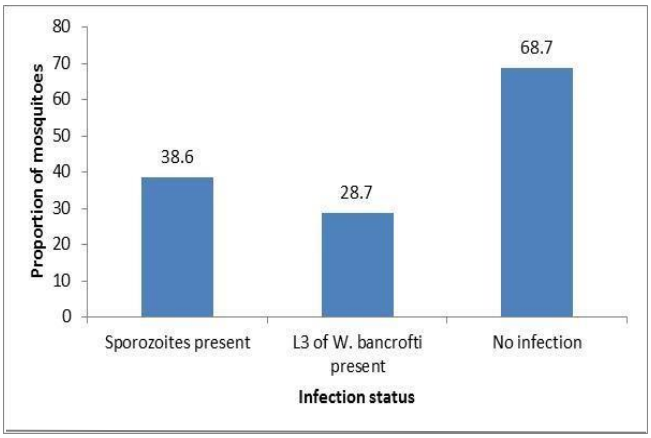

Figure 6: Infection status of mosquitoes

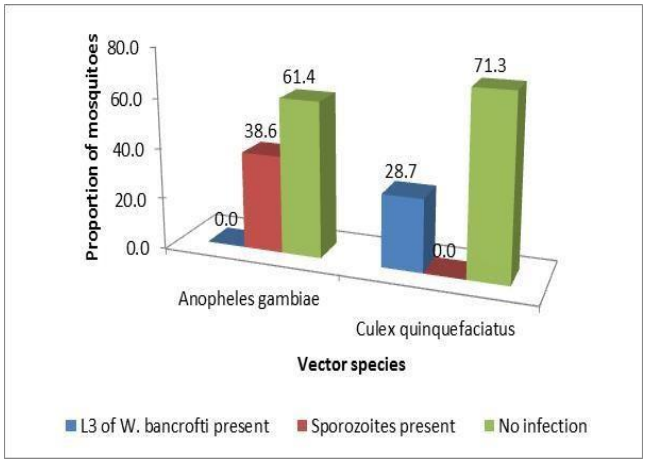

Figure 7: Distribution of infection status by vector species

DISCUSSION

The significant difference in the number of vectors collected in this study may be attributed to the differences in the breeding necessities and habitat characteristics of the vectors (Okorie *et al.*, 2014 and WHO, 2015). *An. gambiae* mosquitoes breed in temporary habitats such as shallow sun lit fresh water pools or human made habitats (paddy farm), hoof prints and tyre tracks, while *Cx. quinquefaciatus* are known to breed in polluted water bodies including open drains, open or cracked septic tanks, and flooded pit latrines (Uttah *et al.*, 2013; WHO, 2015). The study area had limited basic infrastructure and characterized by lack of waste management system that is common to peri-urban areas in Africa which serve as breeding ground for *Cx.* mosquitoes and could explain the high abundance of this species. The low abundance of *An. gambiae* reported in this study is as a result of polluted water bodies which may or not support their breeding (Uttah *et al.*, 2013).

The non-significant difference observed in the distribution of vectors by sex may be due to a uniform influence of physical factors such as temperature and relative humidity in the environment on both male and female mosquito species (Meuti and Short, 2019). However, the higher abundance of females in both species has been attributed to their feeding behavior which makes them highly endophilic, this increases their proximity to man as well as increase their longevity, thus accounting for their higher number (WHO, 2003; WHO, 2004; Brady *et al.*, 2015). The non-significant difference observed in the distribution of vectors by type of house may be attributed to the fact that all the types of houses are found in the same environment (Okorie *et al.*, 2014), however the higher number of vectors seen in Mud and Block type houses (M&B) as well as Mud type houses may be explained on the premise that there is usually absence of window nets and screens that reduce or stop the entry of these vectors (Nematodes.org, 2010).

There was a higher proportion of parous mosquitoes in the area compared to nulliparous ones in both vectors, but the difference in parity between the vector types was not significant. The non-significance difference in parity between the vector types may be attributed to the fact that the population is an older population, where there is high survival rate of the vectors (WHO, 2003), and also, the existence of high fecundity in the mosquito breeding sites which continuously supplies the area with young mosquitoes. The higher proportion of parous vectors may also be a consequence of the suitable climate conditions in the area, such as rainfall and temperature, and also the high relative humidity which largely influence the mosquito biology (Adeleke *et al.*, 2010); this is consistent with the finding in the study conducted by Lutomiah *et al.* (2013) and Arum *et al.* (2015). It was observed that there was no significant difference in the blood digestion stages of the vectors, and this may be attributed to the fact that the collection was conducted within a short period of time with little or no changes in the environmental conditions such as temperature that greatly affects the gonotrophic cycle (Mala *et al.*, 2014).

The relatively low proportion of unfed as well as the relatively high proportion of fed mosquitoes may be ascribed to the fact that many of the residents in the

study area do not sleep under mosquito nets, and they mostly stay indoors at night; and also the absence of any effective vector control intervention in the area; this predisposes the residents to mosquito bite (WHO, 2015).

There was a significant difference between the infection statuses of the vectors analyzed. In the study area, *An. gambiae* was found to be the sole vector of malaria (Okorie *et al.*, 2011). However, *Cx. quinquefaciatus* was seen to harbour only the third stage larvae of *W. bancrofti* showing that it is a vector of filariasis, and this is in tandem with the work done by Anosike *et al.*, (2005) and Udonsi, (1988). The percentage infection rates revealed that the sporozoite rate was more than the L3 rate; the relatively low L3 rate may be attributed to its low transmission in the area (Okorie *et al.*, 2014). It is interesting to note that the high prevalence of malaria in the area as reported in previous studies conducted in the area with malaria parasitemia rates of 38.0 to 41.0% in under-fives could be a consequence of the high sporozoite rate and the low utilization of insecticide treated nets by mothers (Onuh *et al*., 2015; Daboer *et al*., 2010); and this may also be ascribed to the season since the data collection was done in the wet season around August and September. This finding agrees with the finding in a study conducted by Osman (2010), which found that the season of mosquito sample collection greatly affects their sporozoite rates.

CONCLUSION

The lack of good drainage and sewage system in the study area could be one of the factors contributing to the mosquito burden recorded in the area. Health education will go a long way in sensitizing the inhabitants on the importance of environmental and personal hygiene, and self-protection in mosquito control. The vector mosquito species in Russau village is dominated by *Cx. quinquefasciatus*. Control should be targeted at the breeding sites of the juveniles of the Culicine because these mosquitoes transmit other diseases whose burden can rival that of malaria. The findings of this study have helped in achieving a better understanding of the epidemiology of mosquito-borne diseases in Russau Village of Jos-North Local Government Area, and this 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.

Source of support

Nil.

Conflict of interest

None declared.

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