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## Monitoring of Transmission Indices, Antigenemia and Clinical Signs of Lymphatic Filariasis in the Sahel Region of Northern Nigeria

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**Abstract:** Nigeria is home to a high prevalence of lymphatic filariasis (LF), one of the leading causes of lifelong disability globally. Investigating the transmission indices, circulating filarial antigen, and clinical symptoms of LF in five distinct settlements is the aim of this research. Mosquitoes were collected and determined to be *Culex quinquefasciatus*, *Anopheles gambiae* (M and S forms), and *Anopheles funestus* s.s based on molecular and morphological analysis. Biting rate (BR), Monthly biting rate (MBR) and Annual biting rate (ABR) were high in Babbar Ruga village followed by Yarshanya > Kadajji > Gajerar Giwa > Batagarawa town. The entomological results showed that none of the mosquitoes were infected with microfilariae (Mf) and larval stages (L<sub>1</sub>, L<sub>2</sub> & L<sub>3</sub>) of *W. bancrofti*, and then, infective biting rate (IBR), transmission potential (TP) and annual transmission potential (ATP) were found to be zero across the study communities. However, two DNA pools of *Anopheles funestus* s.s 2(4.2%) were tested positive for *W. bancrofti* at approximately 188bps from Gajerar Giwa. The prevalence rates of Circulating filarial antigen (CFA), Microfilaraemia (MF) and Clinical sign (CS) from the study communities recorded; CFA (28%), MF (4%), CS (0.8%) and there were no significant ( $P > 0.05$ ) differences between males and females. Between the ages of 40 and 51, CFA and MF showed greater values, which eventually decreased. Based on these findings, the study communities are qualifying for mass drug administration (MDA).

**Keywords:** *Anopheles gambiae*, microfilaraemia, *W. bancrofti*, *Anopheles funestus* s.s.

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### Introduction

Lymphatic filariasis (LF) is a neglected tropical disease caused by filarial nematodes mostly prevalent in large parts of the tropics and subtropics<sup>1</sup>. In 2018, 893 million

people in 49 countries were living in areas that require preventive chemotherapy to stop the transmission of the disease<sup>2</sup>. Globally, Nigeria was ranked 3rd highest of the disease and prevalence has been determined in 704 out of 774 LGAs of 36 States and FCT<sup>3</sup>.

LF is associated with dermatitis, elephantiasis and hydrocele<sup>4</sup>. Severe complications could include lymphoedema and elephantiasis of the limbs or genitalia, which adversely affect personal and social life, and limit occupational activities which could increase poverty<sup>2</sup>.

Moreover, LF is caused by the filarial parasites; *Wuchereria bancrofti*, *Brugia malayi* or *Brugia timori* and is endemic in 72 countries<sup>5</sup>. The mosquito species that carry the LF parasites are those that belong to the genera *Anopheles*, *Culex*, *Aedes*, *Mansonia*, *Coquillettidia*, and *Ochlerotatus*. *Anopheles* mosquitoes (vectors of malaria) are the principal vectors of LF in West Africa<sup>6,7</sup>. Mostly, *Anopheles gambiae* s.l., and the *Anopheles funestus* s.l. complexes<sup>8</sup>.

Similar to this, these mosquito species are found across the tropical and subtropical areas of the African continent, where they serve as LF parasite vectors. *Culex* spp are also responsible for LF transmission, especially the abundant populations of *Culex quinquefasciatus* that often proliferate in dirty environments of human settlements<sup>9</sup>.

Thus, the disease continues to pose huge challenge and threat to the health and educational sectors as well as the socio-economic development of the affected individuals.

Consequently, identifying the serological and clinical indices from the study communities will go a long way to help the National Lymphatic Filariasis Elimination Programme (NLFEPP) to tailor their control approaches in Katsina central senatorial zone northwest Nigeria using field evidences.

## **Materials and Methods:**

### **Materials**

Analytical grade laboratory chemicals and reagents were used for this study.

Baby fish food (Tetramin), bioassay tubes, cotton wool, distilled water, droppers, filter paper (12cmx 15cm), impregnated papers (12cmx 15cm), mouth aspirator PCR tubes, paper cups, plastic trays, silica gel, phosphate buffer, potassium acetate, ethanol, proteinase K, Taq polymerase, ddH<sub>2</sub>O, agarose gel, 1x TEA buffer, ethidium bromide, 1x HotStar Taq buffer, ATL buffer.

### Study Sites

1. Batagarawa town (12°54'17"N, 7°37'11"E) located in Batagarawa Local Government Area northeast of Katsina state.
2. Gajerar Giwa village (12°05'14"N, 7°05'10"E) located in Rimi Local Government Area northeast of Katsina state.
3. Kadajji village (12°04'15"N, 7°03'9"E) located in Rimi Local Government Area northeast of Katsina state.
4. Babbar Ruga village (12°40'13"N, 7°29'19"E) located in Batagarawa Local Government Area northwest of Katsina state.
5. Yarshanya village (12°40'12"N, 7°29'17"E) located in Batagarawa Local Government Area northwest of Katsina state.

### Indoor Collection of Mosquito

In the early morning hours of 5:00 am to 6:00 am, blood-fed female *Anopheles* and *Culex* mosquitoes were collected from randomly selected residences in five distinct areas within the Sahel savannah region of Katsina state, Nigeria, using a mechanical aspirator.

### Mosquitoes Identification

Using 70% ethanol, the work bench was cleaned. The mosquitoes brought from the study sites were separated using morphological features as described by Gillies and De Meillon (1968); Danilov (1982); Highton (1983); Gillies and Coetzee (1987)<sup>10,11,12,13</sup> to separate the mosquitoes into anophelines (*Anopheles* mosquitoes) and culicines (*Aedes*, *Culex*, and *Mansonia* mosquitoes).

The presence of their plumose (bushy) antennae allowed male mosquitoes in all species to be identified from females. The different species that had been morphologically identified were recorded on appropriate entomological data recording forms/sheets<sup>14</sup>.

### Preparation of Mosquitoes for Dissection

Live blood fed mosquitoes was 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 Manguin et al. (2010)<sup>15</sup>. Following immobilization, each mosquito was set on a slide, gripped by one wing, and its legs were extracted one at a time and the other wing was then removed. 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)<sup>16</sup> and as adopted previously<sup>17</sup>.

**Dissection of the Salivary Glands for Determination of vectorial Potential**

This was carried out on the mosquito vectors in terms of microfilarial infection rates for both *Anopheles* and *Culex* species, using the procedure described by Abeyasingha et al. (2009)<sup>17</sup>. The salivary glands of the nulliparous mosquitoes were not dissected since they are not infected<sup>18</sup>. A drop of saline was injected to keep the specimen fresh after the anterior portion of the mosquito was to be dissected and placed on a slide with its head pointed to the right. Simultaneously, the left dissecting needle was carefully positioned on the thorax, somewhat below the glandular region. At the same location, the proper needle was inserted and then pushed in the appropriate direction to remove the head that contained the salivary glands. However, certain salivary glands were not visible when the mosquito's head was removed; instead, they had to be carefully located by gently nuzzling the lower thoracic cavity and closely inspecting with a dissecting microscope. After the glands were removed from the head, they were put on a different microscope slide, covered with a cover slip, and gently pressed to burst the gland cells. One little drop of saline was added to the cover slip.

To look for microfilariae, the thoracic muscles were also gently teased in a saline solution. In the event that microfilariae were present in the salivary glands, they were observed to emerge as tiny, spindle-shaped entities (ranging in length from 130 to 320  $\mu\text{m}$ ).

**PCR identification of *Wuchereria bancrofti***

*Wuchereria bancrofti* was detected by standard PCR after DNA was isolated from mosquito carcasses using a Qiagen kit.

**DNA extraction**

Twenty mosquito carcasses were combined and placed into 1.5 ml microcentrifuge tubes. Buffer ATL (180  $\mu\text{l}$ ) was added to each pool of carcasses. A volume of 20  $\mu\text{l}$  proteinase K was then added and mixed thoroughly by vortexing using a vortexer (Standard Mini Vortexer, VWR Scientific Products). For ten minutes, this was incubated at 56 °C. For fifteen seconds, each microcentrifuge tube was vortexed. After adding two hundred microliters of Buffer AL to each tube and properly mixing by vortexing, 200  $\mu\text{l}$  of absolute ethanol (96–100%) was added. A DNeasy Mini spin column was filled with the mixture and set within a 2 ml collection tube. This was placed in a centrifuge (Eppendorf Centrifuge 5415D) and spun at  $\geq 6000 \times g$  (8000 rpm) for 1 min. Both the collection tubes and the flow-through were thrown away. Each DNeasy Mini spin column was put into a fresh 2 ml collection tube, 500  $\mu\text{l}$  Buffer AW1 was added, and the mixture was centrifuged for one minute at a speed of 8000 rpm or greater at  $6000 \times g$ . The flow-through and the collection tubes were again discarded. Once more, fresh 2 ml collection tubes were used for the DNeasy Mini

spin columns. To dry the DNeasy membrane, 500µl of buffer AW2 was added to each spin column and centrifuged for 3 minutes at 20,000 x g (14,000 rpm). Once more, the flow-through and collection tubes were thrown away. Finally, each of the DNeasy Mini spin columns was put into a 1.5 ml microcentrifuge tube. Each spin column's DNeasy membrane received 100µl of buffer AE, which was then incubated for one minute at room temperature. To elute the pure DNA, these were centrifuged for one minute at a rate of 6000 x g, or 8000 rpm

### **Identification of *Wuchereria bancrofti* Using PCR**

Using the carcass of infected mosquitoes, filarial parasites were identified molecularly by following established protocol with a few modifications<sup>19,20</sup>. In the identification of *W. bancrofti*, conventional PCR was used to detect the presence of LF parasite given a band size of 188 bp. A final volume of 20 µl was achieved by adding 0.2 µM of each of the primers, NV1 [5'- CGT GAT GGC ATC AAA GTA GCG – 3'] and NV2 [5' – CCC TCA CTT ACC ATA AGA CAA C – 3'] (a mixture of polymerase, buffer, MgCl<sub>2</sub>, and dNTPs in proportions as previously described), ddH<sub>2</sub>O, and 5 µl DNA template to the PCR-mix. Cycle parameters included 10 minutes at 95 °C, 40 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min, culminating in a final extension at 72 °C for 10 min. After loading 10 µl of PCR, 2% agarose gel stained in ethidium bromide was electrophoresed for 30 minutes.

### **Physical Examination and Sample Collection**

A standardized directed physical examination as described by Weil et al. (1999)<sup>21</sup> was conducted by a community health officer. Elephantiasis of the leg, breast, and hydrocele were palpable among the symptoms.

### **Identification of circulating filarial antigens (CFA) and microfilaria (MF) in blood**

Each of the 500 willing participants had their two milliliters (2 ml) of intravenous blood drawn (308 men and 192 women). After being drawn, the blood was tagged and placed into heparinized bottles. In addition to the blood samples, chylurous urine samples were also taken and placed in a sterile bottle. A centrifuging machine was used to concentrate a 10ml portion of the urine samples for 5 minutes at 1500 rpm. The sediments were studied under an oil immersion binocular microscope with an x1000 objective lens after being stained with Geisma. Circulating filarial antigen (CFA) was tested according to the method of Elgeneet al., (2002)<sup>22</sup>. Prior studies have shown that the card detected filarial antigen in 90-97% of sera from microfilaria carrier<sup>23</sup>. Using a large pipette, 100 microliters (100ul) of blood samples were added to the disposable immunochromatographic test (ICT) card well. After 15 minutes,

two lines emerged on the kit well, indicating a positive sample. The concentration of microfilaria in each human participant was ascertained using a modified version of Knott's (1939)<sup>24</sup> concentration technique. A 15 mm<sup>3</sup> centrifuging tube was filled with one milliliter (1 ml) of the blood sample and ten milliliters of 2% formalin. The tube was centrifuged for five minutes at 1500 rpm. Phosphate buffer Geisma (pH 7–7.2) was used to dye the sediments after they had been spread out on a slide. After being cleaned with distilled water, stained slides were allowed to air dry. The slides were inspected microscopically for the presence of microfilaria in an oil immersion setting using an x1000 objective.

### **Statistical Analysis**

The statistical analysis was conducted using SPSS software (Version 21.0; SPSS Inc., Chicago, IL, USA), and the connection between circulating filarial antigen (CFA), microfilaraemia (MF), clinical sign (CS), and sex was evaluated using a chi-square test at a  $P < 0.05$  level of confidence.

### **Ethical Clearance**

The Ministry of Health in Katsina State provided ethical clearance through the Director of Primary Health Care (PHC). Heads of the communities were visited and given a thorough explanation of the study's significance. The heads of the communities gathered their people to the homes. The people who answered the phone served as the sample population. The research areas consisted of five villages.

### **Results**

#### **Microfilaria Load and identification of *W. bancrofti* larval stages in the captured mosquitoes**

Microscopy revealed that none of the mosquitoes retrieved following dissection had microfilariae or larval stages of *W. bancrofti*. For DNA extraction, 20 dissected *Anopheles gambiae* s.l., *Anopheles funestus* s.l., and *Culex quinquefasciatus* mosquitoes per species were combined into tubes. Using traditional PCR, extracted DNA was utilized to find *W. bancrofti*.

### **Transmission Indices**

Biting rates, infection rates, infectivity rates, worm load, infectious biting rate, annual infectious biting rate, transmission potential, and annual transmission potential are among the transmission indices. These indexes are useful in figuring out how an endemic area is transmitting.

### Biting Rate

The predicted number of vector mosquitoes that are likely to bite a single exposed human during the sampling night is referred to as the biting rate. The number of mosquitoes collected or caught divided by the total number of people residing in the room during the sampling and catch nights is the biting rate (BR). The male biting rate is multiplied by 30 or 365, respectively, to determine the monthly biting rate (MBR) or annual bite rate (ABR). The biting rates in Batagarawa, G/Giwa, Kadajji, B/Ruga, and Yarshanya were 0.45, 1.18, 1.24, 3.68, and 3.49 bites person<sup>-1</sup> night<sup>-1</sup>, respectively. The ABR for the five communities therefore translated to 164.25, 430.70, 452.60, 1343.20 and 1273.65 bites person<sup>-1</sup> year<sup>-1</sup> respectively.

**Table I:** Estimation of biting rate from the study communities

Communities	NM	NS	NCN	BR	MBR	ABR
Batagarawa	2462	456	12	0.45	13.5	164.25
G/Giwa	3149	268	10	1.18	35.25	430.70
Kadajji	3051	308	8	1.24	37.20	452.60
B/Ruga	508	46	3	3.68	110.44	1343.20
Yarshanya	607	58	3	3.49	104.66	1273.65

NM: Number of mosquitoes, NS: Number of sleepers, NCN: Number catch night, BR: Biting rate, MBR: Monthly biting rate, ABR: Annual biting rate.

### Infection Rate

The ratio of mosquitoes with any stage of the *W. bancrofti* parasite to all mosquitoes tested for these parasites is known as the infection rate. Usually, a percentage is used to describe this. Upon microscopy, none of the parasite stages were visible. Thus, there was no infection rate in this investigation.

### Infectivity Rate

The infectivity rate refers to the ratio of the number of mosquitoes carrying the infective stage (L3) of the *W. bancrofti* parasite and the total number of mosquitoes screened for the parasites. A percentage is another way to put this. Since no infections were found using microscopy, there was also no infectivity rate.

### Worm Load

The ratio between the amount of L3 parasites that are infectious and the number of mosquitoes that carry these parasites is known as the worm load. No infectious mosquitoes were present. Thus, there was no worm load.



### Infective Biting Rate (IBR) and Annual Infective Biting Rate (AIBR)

The biting rate multiplied by the infectivity rate yields the infective biting rate. That amounted to nil. The result of the IBR and 365 days is the AIBR. Additionally, this was zero.

### Transmission Potential (TP) and Annual Transmission Potential (ATP)

IBR multiplied by the worm load equals transmission potential. Person-1, night-1, and the unit are infectious bites. However, the unit(s) is infectious bites person-1 year-1, and the Annual Transmission Potential is the product of the TP and 365 days. They were also zero.

**Table II:** Estimation of entomological indices from the studied communities

Communities	NM	Microscopy				WL	BR	IBR	TP	ATP
		MF	L1	L2	L3					
Batagarawa	246 2	0	0	0	0	0	0.45	0	0	0
G/Giwa	314 9	0	0	0	0	0	1.18	0	0	0
Kadajji	305 1	0	0	0	0	0	1.24	0	0	0
B/Ruga	508	0	0	0	0	0	3.68	0	0	0
Yarshanya	607	0	0	0	0	0	3.49	0	0	0

NM: Number of mosquitoes, WL: Worm loaded, BR: Biting rate, IBR: Infectivity biting rate, TP: Transmission potential, ATP: Annual transmission potential.

### Presence of *W. bancrofti* in Mosquitoes Using Conventional PCR

All of the mosquitoes recovered following dissections did not have microfilariae or larval stages of *W. bancrofti*, according to microscopy. For DNA extraction, a pool of 20 dissected mosquitoes per specie was placed in each tube. Conventional PCR was utilized to detect *W. bancrofti* from extracted DNA. Only two (4.2%) of the 48 pools of *Anopheles funestus* s.l complex from G/Giwa village tested positive and produced a PCR product of about 188 bp.

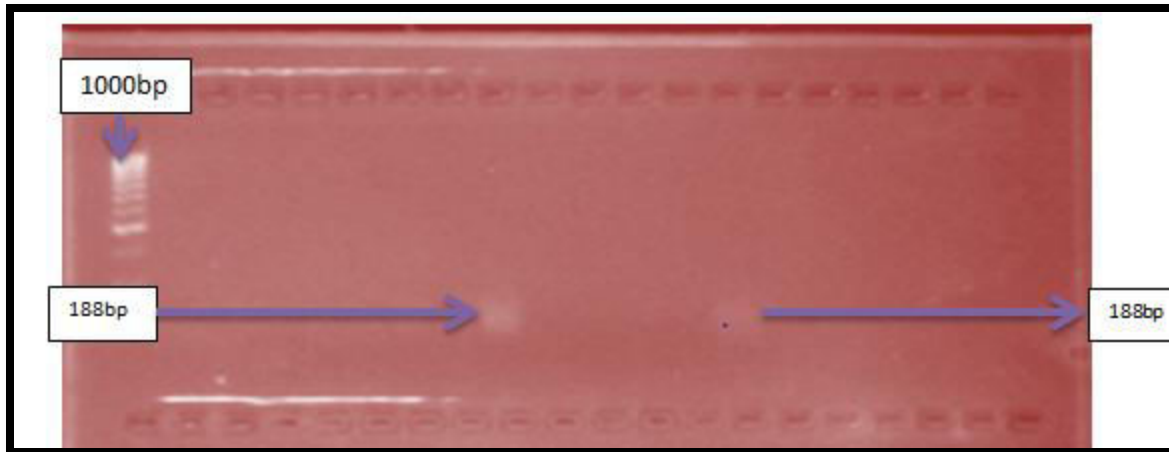


Figure I: Gel electrophoregram for the detection of *W. bancrofti* with the site band of 188bp

### Prevalence of Lymphatic Filariasis in the Study Communities

A total of 500 subjects (308 males and 192 females) were sampled in 5 communities along Sahel Savannah region of Katsina state. The total prevalence rates of circulating filarial antigen (CFA), microfilaraemia (MF) and clinical sign (CS) from the study communities recorded; CFA (28%), MF (4%), CS (0.8%).

Gender specific prevalence rates of CFA, MF and CS showed that male recorded highest, (CFA; 28.6%, MF; 5.2%, CS; 1.3%) than female (CFA; 27.8%, MF; 2.6% and CS; 0). The differences were not significant ( $P > 0.05$ ) Table 4.8.

The age specific prevalence rates of CFA and MF decreases with age, but for CS increases with age. The age specific- prevalence of CFA and MF was significantly higher among age less than 40 years than in older subjects, ( $P < 0.05$ ), while CS was significantly ( $P < 0.05$ ) higher among age greater than 40 years than in younger subjects. The age prevalence rates for CFA and MF recorded higher values in age below 40 years which became relatively stable at middle age between 21-30 years and finally declined in older age above 51 years, (Figure 2.0).

Few clinical Signs of filariasis were observed in male only from Batagarawa, B/Ruga and Yarshanya communities. The symptoms of the clinical filariasis increased with age and became more stable at the age above 51 years. In male, the clinical signs recorded were hydrocoele and elephantiasis of the leg recorded 0.8% and 0.2% respectively, while in female there was no clinical sign observed from the study communities.

**Table III:**Prevalence of CFA, MF and CS in the study communities

Communities	Number Examined	CFA	MF	Clinical Sign (CS) Elephantiasis of leg Hydrocele	
Batagarawa	300	86 (28.7%)	12 (4%)	1 (0.3%)	1 (0.3%)
G/Giwa	50	10 (20%)	1 (2%)	0	0
Kadajji	50	13 (26%)	1 (2%)	0	0
B/Ruga	50	15 (30%)	3 (6%)	1 (2%)	0
Yarshanya	50	16 (32%)	4 (8%)	1 (2%)	0
Total	500	140 (28%)	21 (4%)	4 (0.8%)	1 (0.2%)

G/Giwa: Gajerar Giwa; B/Ruga: Babbar Ruga; CFA: Circulating Filarial Antigen; MF: Microfilaraemia; CS: Clinical Signs.

**Table IV:** Sex Prevalence of Lymphatic Filariasis in the study communities

Sex	Number Examined	CFA	MF	CS
Male	308	87 (28.6%)	16 (5.2%)	4 (1.3%)
Female	192	53 (27.6%)	5 (2.6%)	0
Total	500	140 (28%)	21 (4.2%)	4 (0.8%)

CFA: Circulating Filarial Antigen; MF: Microfilaraemia; CS: Clinical Signs.  $X^2$  test at  $P<0.05$

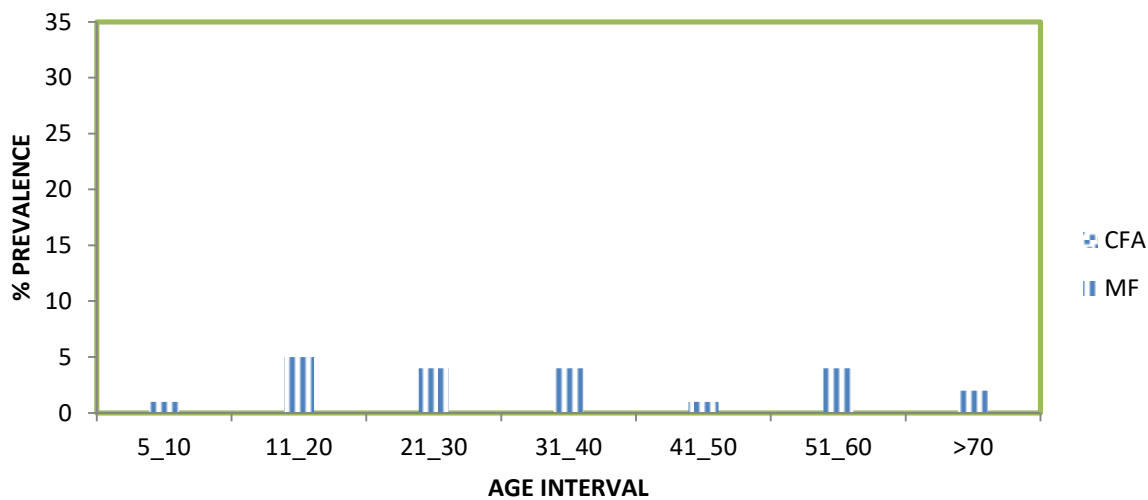


Figure II: Age specific prevalence rates of circulating filarial antigen (CFA) and microfilaraemia (MF).

## Discussion

After multiple rounds of MDA, monitoring *W. bancrofti* infections in mosquito vectors is advised to track the development of control and elimination efforts. In endemic communities that had taken part in rounds of MDA, this study used xenomonitoring to evaluate LF transmission. This made it possible to determine the transmission status of the research locations by providing the information needed to calculate the entomological indices.

According to the study's entomological findings, none of the mosquitoes that were collected were carriers of *W. bancrofti* microfilariae (Mf) or larval stages (L1, L2, and L3). Since *Anopheles* mosquitoes are known to exhibit "facilitation" at very low mf levels, which allows these mosquito species to pick up *W. bancrofti* parasites at high mf rates in the human population and develop them to the infectious stage, the low rate of *W. bancrofti* infections in the human population is likely to result in the absence of microfilaria and larval stages in vectors despite the large numbers of effective vectors in the study communities<sup>25,26,27</sup>. This could assist to explain why the many *Anopheles* vectors that were gathered and examined in the hamlets of Yarshenya, B/Ruga, Kadajji, and G/Giwa were not all infected. Even at low parasite numbers, *Culicine* demonstrates "limitation," allowing this species to consume and produce mf to the infective stage<sup>25,26</sup>. Given the massive amount of *Culex quinquifasciatus* identified in Batagarawa Town, G/Giwa, Kadajji, B/Ruga, and Yarshenya Village, it is implausible that this species did not carry any *W. bancrofti* parasite stages (L1, L2, and L3). This might be because *Culex quinquefasciatus* doesn't appear to be a factor in the spread of LF in West Africa<sup>28,29,7</sup>. Patricia et al, (2015)<sup>30</sup> discovered that no mosquitoes in Ogun Central and Ogun West, Nigeria, were infected with microfilariae (mf) or larval stages (L1, L2, and L3) in LF vectors. Their entomological survey produced similar results.

*W. bancrofti* was found molecularly in mosquitoes, which helped to either support or contradict the results of the entomological investigation. A weak positive result for *W. bancrofti* at 188bp was found in two of the forty eight mosquito DNA pools. These pools of mosquitoes were identified from G/Giwa village and predominantly *Anopheles funestus* s.l 2(4.2%). The molecularly identified *Anopheles funestus* s.l were all *Anopheles funestus* s.s which confirms findings from another study that suggested the *Anopheles funestus* s.s to be more involved in the transmission of LF in Gomaa District of Ghana which is more likely to be a low to medium LF zone<sup>31</sup>.

The BR, MBR and ABR were found to be high in Babbar Ruga village (3.68, 110.44 and 1343.20) respectively followed by Yarshanya> Kadajji> G/Giwa> Batagarawa town. Finding of higher biting rates in B/Ruga village could be due to high human–vector contact. Similar observation was reported in Ahanta West district by Sellaseet al. (2019)<sup>32</sup>. ATP was found to be zero across the study communities and this could be

attributed to the absent of microfilariae (mf) and larval stages ( $L_1$ ,  $L_2$  &  $L_3$ ) of *W. bancrofti* in LF vectors.

It is interesting to note that higher prevalence rate of CFA and MF was found in Yarshenya village 16 (32%) and 4 (8%) respectively, followed by B/Ruga > Kadajji > G/Giwa > Batagarawa town. This is in accordance with the result of a study performed in 2009 at Epie creek communities, Niger Delta, Nigeria<sup>33</sup>, which also reported a higher prevalence using the same diagnostic approach. Based on these results and WHO recommendations to treat only those endemic areas where the prevalence is above 1 %, the Sahel Savannah zone of Katsina state qualifies for MDA.

Males are more prevalence with CFA and MF than females, and this could explain the greater exposure of males to the biting of infected mosquitoes. This observation is in agreement with Martha et al. (2000)<sup>34</sup>. Most males in the study areas always clustered within open places in the night discussing about their businesses and farming activities which always correspond with the peak hours of the host seeking behaviors of LF vectors. On the contrary, females remain around the kitchen where smokes repel the mosquito vector to minimal level before bedtime; hence, they are less bitten by the bloodsucking mosquito vector.

The pattern of age specific prevalence of CFA and MF revealed that it is age and exposure dependent.

Each program to manage lymphatic filariasis must include an investigation of infection rates in humans and vectors in order to determine when to begin and end mass drug administration (MDA) and to certify the disease's elimination. Since the introduction of MDAs, the majority of endemic nations have successfully stopped LF transmission<sup>35,36,37</sup>. However, even in endemic nations that have had numerous MDA cycles, there is still some residual transmission<sup>38</sup>. Systemic noncompliance, population coverage of less than 65%, migration of Mf-positive carriers between endemic and non-endemic areas following administration of MDAs, poor sanitation leading to an increase in the number of breeding sites for LF vectors, dug-outs created by construction projects adding to the number of breeding sites for LF vectors already present, and the rapid rate of urbanisation have all been cited by various research teams as contributing factors for the maintenance of residual transmission<sup>35,39,37,40</sup>.

The higher CFA and MF among younger subjects age 20 and to 49 years than adult age 50 years and above supports the hypothesis that humans develop immunity to filariasis after years of exposure to the parasites. Similar scenario was reported by Ebenezer et al. (2011)<sup>33</sup> from Epie creek communities, Niger Delta, Nigeria. Lower prevalence of clinical sign was observed only in males from Batagarawa town, B/Ruga and Yarshanya village is an indication that *W. bancrofti* is still endemic in

these three study locations. Higher clinical signs (elephantiasis of the leg and hydrocele) among individuals above the age 50 years support the hypothesis of immunological tolerance to parasitic infections at high transmission<sup>41</sup>. Feinsod et al. (1987)<sup>42</sup> also observed that *Wuchereria*-induced clinical signs increased in older subjects. This suggested that both microscopic and rapid diagnostic test be used to establish endemic status of *W. bancrofti* infection. This will maximize the chances of understanding infectious diseases in human population.

### Conclusion:

Despite the absence of Mf and larval stages (L<sub>1</sub>, L<sub>2</sub> & L<sub>3</sub>) of *W. bancrofti* in mosquitoes but, this study revealed the presence of *W. bancrofti* DNA in *Anopheles funestus* and infection in the human population. Based on these results and WHO recommendations to treat only those endemic areas where the prevalence of circulating filarial antigen (CFA) is above 1 %, the Sahel region of Northern Nigeria is qualified for MDA.

**Conflict of Interest/Competing Interests :** Author declared no competing of interest.

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