



Mosquito Vectors Associated with Lymphatic Filariasis in some Endemic Rural Communities of Katsina State, Nigeria

Lawal, Saadatu Bagiwa, Dalhatu, Hadiza Umar, Jubril, Jamila
Department of Biology,
Federal College of Education, Zaria

ABSTRACT

Lymphatic filariasis (LF) is a chronic nematode infection transmitted by mosquitoes, in sub-Saharan Africa the causative agent is *Wuchereria bancrofti*. Despite the success of MDA, the national and global LF elimination slated for the year 2020 are now practically unattainable as Mass Drug Administration (MDA) has not commenced in all of the endemic implementation units. The study was conducted in three endemic local governments of Katsina state to detect the presence of microfilariae of *W. bancrofti* in the mosquito vector and blood samples of the participants. Indoor resting mosquitoes were collected using the pyrethrum knock-down (PSC) method in randomly selected houses, morphologically identified and *Anopheles* mosquitoes confirmed using molecular technique. Mosquito samples were assessed using polymerase chain reaction (PCR). Two genera of mosquitoes; *Anopheles* and *Culex* were identified in the study. Two species of *Anopheles*, *An. gambiae* s.s and *An. funestus* were isolated of which the former were more predominant, constituting 61% of the *Anopheles* population in the study area. Both species of *Anopheles* were found in all the communities except from Sabon G/Nadungu which had only *A. gambiae*. Highest number of *An. gambiae* s.s and *An. funestus* (28/70 and 13/45 respectively) were collected from Garhi in Dutsin-Ma LGA. In conclusion the result presented in this study gave an insight into the mosquito population of the study area, with *Anopheles gambiae* s.s as the dominant species. Ssp1-PCR assay detected no infection in *Anopheles funestus* and *Culex* species, but only *Anopheles gambiae* s.s was positive with *Wuchereria bancrofti*. It is important to understand vector-parasite relationships and mode of transmission of LF through mass awareness campaigns in order to gain support of control programmes by the communities.

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INTRODUCTION

Lymphatic filariasis (LF), also referred to as elephantiasis, is a neglected, mosquito-borne parasitic disease caused by three species; *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* thread-like nematode worms of the Super family Filarioidea (WHO, 2010). Transmission is acquired through the bites of infective female mosquito vectors belonging to the genera *Anopheles*, *Culex*, *Aedes* and *Mansonia*, depending on the geographic region. *Anopheles* species are considered the principal vectors in

West Africa (White, 1989). Lymphatic filarial infections are predominantly found in humid tropical areas of Asia, Africa, the Western Pacific and scattered areas of the Americas with an estimated 1.4 billion people at risk for developing new active infection globally (WHO, 2010). It is considered as the second leading cause of physical disability worldwide by the World Health Organization (WHO); its infection being profoundly painful and disfiguring, leading to severe psychosocial consequences. The disease is endemic in 52 countries (Small *et al.*, 2018) and



current estimates indicate that about 67 million people are infected; 36 million exhibiting clinical symptoms of the disease, 19 million with genital hydrocele and 16 million with lymphedema (Ramaiah and Ottesen, 2014). The disability adjusted life year (DALY) burden due to LF is about 5 million DALYs (WHO, 2017).

The epidemiology of LF is influenced by host-parasite, vector-parasite interaction and also resurgence emanating from new or residual infections disseminated by the vectors. Evidence indicating insecticide toxicity to humans (Lushchak *et al.*, 2018) and emergence of resistant strains among mosquito populations has also caused setbacks in effectiveness of control measures. In Africa, the scale up of programmatic activities have been slow especially in countries with logistical challenges, conflict, instability and fragile infrastructures (Okorie *et al.*, 2013). The World Health Organization classifies lymphatic filariasis as a vulnerable infectious disease meaning that it could potentially be completely eradicated. *Wuchereria bancrofti* is the main causative agent of lymphatic filariasis. In order to eliminate lymphatic filariasis we must drive *W.bancrofti* to extinction. To increase success of LF elimination we need to understand several parameters which include; the effectiveness of drug regimens, the optimal time-course of drug administration, the potential for drug resistance, vector characteristics and human host migration dynamics. Chemotherapy is considered as the most potential and cost effective tool to interrupt the transmission of Lf (Wijegunawardana, *et al.*, 2013) which is normally carried out through MDA; also control of the mosquito vector is important.

A PCR assay developed by Zhong *et al.*, (1996) has successfully amplified the *SspI* repeat region on the *W.bancrofti* genome and it's a highly sensitive method for detecting *W.bancrofti* DNA in both individual and pooled mosquitoes (Chanteau *et al.*, 1994). Although LF is endemic within communities in the study area, there is no documented study about the mosquitoes that act as vectors in the region. This study was conducted in three endemic LGAs to identify the mosquito vectors of LF in the study area and detect the presence of microfilariae in the mosquitoes using PCR.

METHODS

Mosquito collection was carried out in each of the communities by dividing them into four approximately equal sections. Households in each section were randomly selected and consent sought from the head of the household. If any household refused to participate, a different household was selected. New households were selected for each sampling day and mosquitoes were collected only once from each household.

In each of the villages, mosquitoes were collected for two days with the help of community volunteers who were trained for mosquito collection and storage. Mosquito collections were done using the pyrethrum knock-down spray sheet collection (PSC) method (WHO, 2013). The method is considered the 'gold' standard for collecting in-door resting, blood-fed and gravid mosquitoes (Dorkenoo *et al.*, 2018; Onyango *et al.*, 2013) and it is also widely used for sampling *Anopheles* in malaria intervention programs (Onyango *et al.*, 2013). On each mosquito collection day, rooms were randomly selected (one room/house), and mosquitoes collected from the rooms. The day before the collection, Consent was obtained from occupants of the households, they were asked to keep bedroom doors and windows closed the following morning. Mosquitoes were collected early in the morning between 06:00 h and 08:00 h by the personnel. The occupants were asked to remove or cover all food items in the room. Potential mosquito hiding places (under the bed, corners) were searched and disturbed to displace any resting mosquito. White sheets were laid on the floor and other surfaces in the rooms. The room was then sprayed with pyrethrum- based insecticidal spray and left for about 15 mins, after which the white sheets were inspected for any dead or knocked down mosquitoes. They were then picked with forceps and placed in labelled Petri dishes.

Mosquito Sample Processing

Mosquito genera and species identification were conducted at the Parasitology and Entomology laboratory of the Department of Zoology, Ahmadu Bello University, Zaria using morphological identification keys (Gillies and Coetze, 1987). The engorged or gravid female



mosquitoes were separated and transferred into microcentrifuge tubes. All collected female *Anopheles* mosquitoes were pooled in pools of 20-25 according to species while the mosquitoes in the *Culex* genus were stored in a separate container with silica gel desiccant at room temperature until needed for analysis.

Preparation of Mosquito DNA Template (DNeasy Blood and Tissue Kit, Qiagen)

DNA extraction was performed following the protocol provided by the manufacturer for animal tissue with slight modification. Briefly, dried pools of mosquitoes were homogenized with a disposable polypropylene pestle (Kimble/Kontes, Vineland, NJ, USA) in 90µL buffer ATL and 10µL Proteinase K and incubated at 55°C for 2h. Prior to homogenization, the dried mosquito pooled samples were rehydrated at room temperature in 20µL of double distilled water for 20min with subsequent removal of the supernatant. 100µL of buffer AL (lysis buffer) was added to the 100µL slurry and vortexed thoroughly. It was incubated at 56°C for 10min and 200µL of ethanol (96-100%) added to the sample and thoroughly mixed by vortexing. The mixture was pipetted into the DNeasy Mini spin column placed in a 2ml collection tube and centrifuged at 6000x g (8000 rpm) for 1 minute using a refrigerated centrifuge (Eppendorf). The flow-through and collection tube were discarded.

The DNeasy Mini spin column was placed into a new 2ml collection tube and 500µL buffer AW1 added, and centrifuged for 1 minute at 8000 rpm. The eluent and collection tube were discarded. The DNeasy Mini spin column was placed in a new 2ml collection tube, and 500µL buffer AW2 was added, and the tube centrifuged for 3 minutes at 14,000 rpm to dry the DNeasy membrane. The DNeasy Mini spin column was again placed in a clean 1.5ml microfuge tube, and 200µL buffer AE (Elution Buffer) pipetted directly onto the DNeasy membrane. The tube was incubated at room temperature for 1 min, and then centrifuged for 1 min at 8000 rpm to elute. Finally, the DNA was visualized on 1% agarose gel in 0.5X TBE buffer using DNA stain G currently used in molecular biology research to replace ethidium

bromide because of its less carcinogenic effect and UV-transilluminator.

Genotyping Mosquito Species

Manual primer design was used to design an *Anopheles gambiae* specific oligonucleotide primer for the mtDNA cytochrome B (cytb) gene and amplification was achieved. The forward primer sequence was 5'-TAT GTA CTA CCA TGA GGA CAA ATA TC-3' (26 base pairs) and the reverse primer sequence was 5'-ATT ACA CCT GCT AAT TTA TTA GGA AT-3' (26 base pairs).

Molecular Identification of *Wuchereria bancrofti*

The molecular identification of *Wuchereria bancrofti* as described by Ramzy *et al.* (1997) was used with some modification. The PCR-based method was done as described with the following primers and the 188-bp *SspI* repeat of *W. bancrofti* was amplified using generic primers:

NV-1, 5'-CGT GAT GGC ATC AAA GTA GGG-3', and
NV-2, 5'-CCC TCA CTT ACC ATA AGA CAA C-3' (Zhong *et al.*, 1996).

RESULTS

Mosquito Occurrence and Identification

The occurrence and distribution of mosquito species associated with LF in endemic areas of Katsina State, Nigeria is depicted in Table 1. The two (2) genera of mosquitoes isolated in the study were *Anopheles* and *Culex*; though *Culex* was found only in Magam, Kankia LGA. Two species of *Anopheles*, *An.gambiae* s.s and *An.funestus* were isolated of which the former were more predominant, constituting 61% of the *Anopheles* population in the study area. Both species of *Anopheles* were found in all the communities except from Sabon G/Nadungu which had only *A. gambiae*. Highest number of *An. gambiae* s.s and *An. funestus* (28/70 and 13/45 respectively) were collected from Garhi in Dutsin-Ma LGA, then those collected from Magam in Kankia LGA (11/70 and 10/45 respectively), while the lowest number of the two species were collected from Jataka in Matazu LGA (1/70 and



1/45). There were more female *Anopheles* mosquitoes than males.

Table 1: Occurrence and distribution of mosquito species associated with lymphatic filariasis in endemic areas of Katsina

Location	Mosquito sp.	Number	Percentage abundance (%)			
			Male	female	male	female
Dutsinma						
Garhi	<i>An.gambiae</i> s.s	28	6	22	4.88	17.89
	<i>An. funestus</i>	13	3	10	2.44	8.13
Takatsaba	<i>An.gambiae</i> s.s	8	6	2	4.88	1.63
	<i>An. funestus</i>	3	1	2	0.81	1.63
Makera	<i>An.gambiae</i> s.s	5	0	5	0	4.06
	<i>An. funestus</i>	4	1	3	0.81	2.44
Matazu						
S/Garin Nadungu	<i>An.gambiae</i> s.s	3	1	2	0.81	1.63
	<i>An. funestus</i>	0	0	0	0	0
Jataka	<i>An.gambiae</i> s.s	1	0	1	0	0.81
	<i>An. funestus</i>	1	0	1	0	0.81
Rinjin Idi	<i>An.gambiae</i> s.s	2	1	1	0.81	0.81
	<i>An. funestus</i>	1	0	1	0	0.81
Kankia						
Magam	<i>An.gambiae</i> s.s	11	2	9	1.63	7.32
	<i>An. funestus</i>	10	2	8	1.63	6.5
	<i>Culex</i> sp.	8	0	8	0	6.5
Akutai	<i>An.gambiae</i> s.s	7	1	6	0.81	4.88
	<i>An. funestus</i>	8	1	7	0.81	5.69
U/Fulani	<i>An.gambiae</i> s.s	5	0	5	0	4.07
	<i>An. funestus</i>	5	2	3	1.63	2.44
Total		123	27	88	21.95	78.04

Molecular Genotyping of *Anopheles* Mosquitoes

Plate 1 shows the result of gel electrophoresis for the molecular identification of *Anopheles gambiae* species complex. The 390 bp product in lanes 1 and 2 represent the mitochondrial cytochrome B gene of *Anopheles gambiae* s.s mosquitoes.

PCR Based Diagnosis of *W. bancrofti* DNA from Mosquito DNA

Amplification using generic primers of the *SspI* repeat region of genomic DNA of *W. bancrofti*, amplified the *SspI* repeat of *W bancrofti* from the DNA extracted from the mosquito pools (Plate 2).

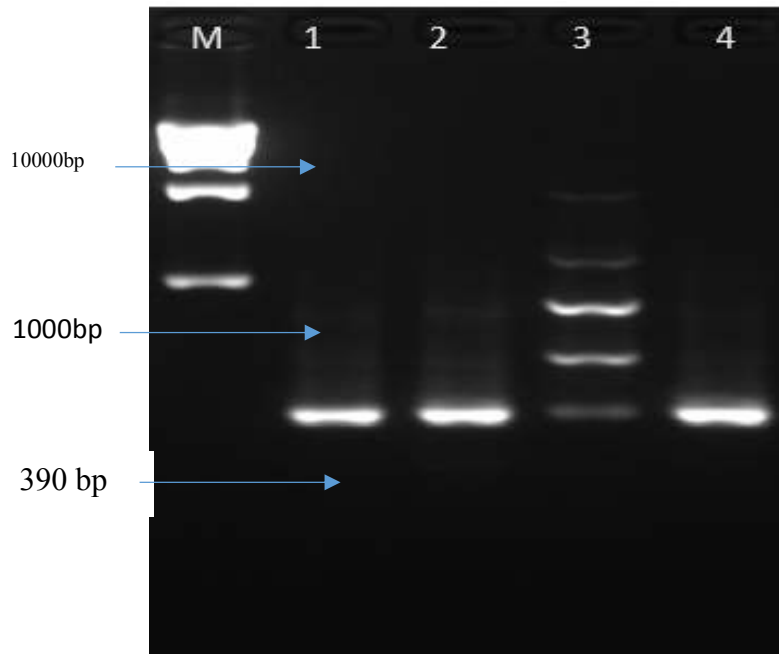


Plate 1: Gel electrophoregram for molecular genotyping of *Anopheles gambiae* s.s mosquitoes. Lane M = 100bp molecular weight marker, lanes 1 and 2 = *Anopheles gambiae* s.s lane 3 = -ve control while lane 4 = +ve control. Arrow indicates size in base pair of the amplification product.

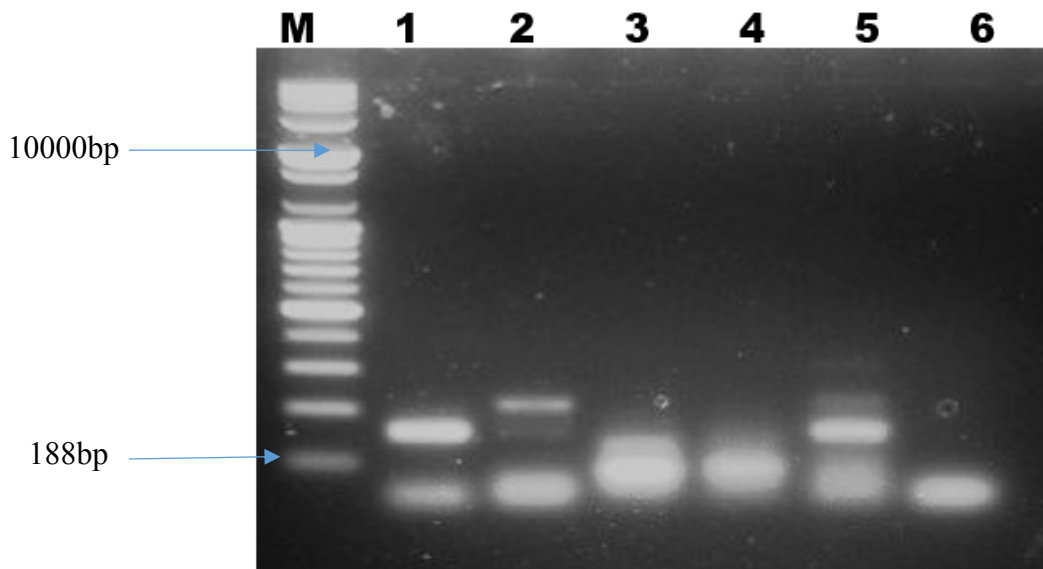


Plate 2: Gel electrophoregram showing amplification of *W.bancrofti* DNA in mosquito pools. Lane M = Marker; lane 1 was positive control; lanes: 2, 3 and 4 were negative while lane 5 was positive. Lane 6 was the negative control. Arrow indicates the amplified 188 base pair of the PCR product.



DISCUSSION

The entomological results presented here (Table 1) showed that three mosquito species were encountered during the sampling period which included; *An. gambiae* s.s; *An. funestus* and *Culex* species. The abundance of female *Anopheles* (71.54%) compared to male (21.95%) in this study. This may be attributed to females being gravid and requiring blood meal for egg maturation and are always found near human hosts which was also reported by Olayemi *et al.*, (2010). It was evident that the abundance of *Anopheles* mosquitoes compared to *Culex* species in the study area (Table 1) could be attributed to unpolluted breeding sites in the study area as previously reported (Dorkenoo *et al.*, 2018). Polymerase chain reaction assay was used to detect the parasite instead of dissection and this method was chosen because it is often difficult to detect mf and L₃ larvae using dissection (Pederson *et al.*, 2009). Overall, PCR becomes more cost effective than dissection when mosquito infection levels are low. As infection levels drop, it becomes increasingly difficult and more costly and time consuming to dissect large numbers of mosquitoes with little or no infection ever detected (Bockarie *et al.*, 2000). Microfilariae was detected in the mosquito vector by PCR assay to detect *Ssp1* gene which is species-specific and the assay is sensitive enough to detect as little as 0.1 pg of *W.bancrofti* genomic DNA. This specific assay is also more cost effective since mosquito pools can be used (Ramzy *et al.*, 1997). Only one mosquito pool was positive in this study, and that is the pool containing *A.gambiae* s.s mosquitoes collected from Magam a community in Kankia LGA. The reason for detection of microfilariae in the mosquito vectors from this region might be due to high prevalence of microfilaremia in the human population of the area. This observation might also result in a higher transmission potential in Kankia LGA. The *Culex* mosquitoes were not positive with mf which supports the evidence that they are not efficient vectors of LF in West Africa (de Souza *et al.*, 2014). Although *Culex* mosquitoes are widely distributed in the region, they are considered refractory to infection with microfilaria (Dorkenoo *et al.*, 2018). In addition to morphological identification of the mosquito vector, molecular

genotyping was conducted where all the pools containing the mosquitoes were confirmed as *Anopheles gambiae* s.s with visible strong bands.

CONCLUSION

In conclusion the result presented in this study gave an insight into the mosquito population of the study area, with *Anopheles gambiae* s.s as the dominant species. *Ssp1*-PCR assay detected no infection in the *Anopheles funestus* and *Culex* species, but only in *Anopheles gambiae* s.s was positive with *Wuchereria bancrofti*. Control strategies can be enhanced through a better understanding of the epidemiological and pathological dynamics of the disease in order for the GPELF programme to succeed.

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