Original article

Evaluating the Effectiveness of Molecular Xeno Monitoring for Lymphatic Filariasis Surveillance in Low-Prevalence Areas of Kaduna South LGA, Kaduna State, Nigeria

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ABSTRACT

Lymphatic filariasis is a notable neglected tropical disease spread by mosquitoes, causing severe disfigurations and, eventually, high levels of psychological trauma. The implementation of mass drug administration (MDA) has been effective in the control of this infection. Still, there is a need for continuous surveillance to prevent the resurgence of this infection within communities. To determine the suitability of molecular xenomonitoring (MX) in areas of low lymphatic filariasis prevalence across Kaduna South LGA. Mosquito vectors were collected from selected communities in Kaduna South local government area (LGA) for nine months using pyrethrum spray catch (PSC). Then, a polymerase chain reaction (PCR) analysis was conducted to detect the presence of the genomic DNA of the filarial parasites using *Wucheria bacrofti*-specific primers. Exactly 1,611 mosquitoes were analyzed (processed in nine tubes based on the monthly collection), with no detection of *W. bancrofti* DNA. This suggests a low prevalence of LF in the surveyed communities, indicating the effectiveness of previous MDA efforts and a low rate of human infection. MX is a sensitive and effective method for post-MDA surveillance of lymphatic filariasis. This study highlights a low frequency of this parasite in the human population due to the absence of *W. bancrofti* gene in mosquito vectors, further emphasizing the importance of MX in identifying residual transmission areas and assisting with the lymphatic filariasis eradication campaign.

Keywords: Lymphatic filariasis, Molecular xenomonitoring, Mosquito vectors, *Wuchereria bancrofti*.

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INTRODUCTION

More than half of the world's population is at risk of contracting illnesses spread by mosquitoes, making them, without a doubt, the most significant vectors of infectious diseases that substantially impact global health. Besides malaria, over a billion mosquito-borne infections are reported yearly [1,2]. Lymphatic filariasis (LF) is also a mosquito-borne disease that has been of great concern to medical entomologists and other experts as they yearn to eliminate this infection as soon as possible. Human lymphatic filariasis remains a neglected tropical disease (NTD) that is characterized by persistent disfiguring such as elephantiasis and lymphoedema of the legs, the breasts and vulva in females, and the scrotum (hydrocoele) in males [3]. About 120 million people worldwide are infected with this disease, with almost 40 million suffering from psychological trauma (stigmatization) and disabilities [4].

Following the initiation of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) in 2000 and their annual mass drug administration (MDA), various regions achieved great success. However, according to WHO guidelines, transmission assessment surveys support stopping MDAs when the prevalence of microfilariae is less than 1% or the antigen prevalence is less than 2% [4].Tools employed for transmission assessment include an immunochromatographic test (this includes the use of a filarial test strip [FTS]), enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and a microscopy detection of the microfilariae. However, these assessment tools require blood samples from many community members [5]. Nevertheless, the use of vector monitoring (xenomonitoring) has grown to be another key assessment tool for lymphatic filariasis elimination programs, as it provides real-time data that estimates the level of microfilariae found across community members [6].

Xenomonitoring broadly refers to the detection of human pathogens present in arthropods. Experts have practiced this in estimating the risk of human exposure to the transmission of varying vector-borne diseases [7]. In this case, xenomonitoring can be achieved via the dissection of mosquitoes to find filarial larvae or by using various molecular methods to detect the DNA of these worms. Although entomological surveillance for many vector-borne infections involves the detection of infective or infected vectors in the assessment of disease transmission, molecular xenomonitoring (MX) is the detection of pathogenic nucleic acids (DNA/RNA) in a vector, serving as a proxy for the pathogen's presence within the human population. Also, MX indicates current infections, complies with integrated surveillance systems for multiple diseases, and is relatively inexpensive [8].

Hence, WHO [9] recommends the incorporation of MX in the continuous surveillance of lymphatic filariasis, as it is aimed at supporting various programs' decisions. Multiple studies show that MX monitors MDA impacts, defines elimination endpoints, and further maps lymphatic filariasis occurrence [10,11]. Lymphatic filariasis has a profound impact in Kaduna State, Nigeria, as there have been recorded cases across various communities. However, the surveillance and control efforts enacted within the state, especially in Kaduna South Local Government Area (LGA), are limited.

There is a need for proper surveillance to keep up with MDAs and also effectively control vectors transmitting this infection [11]. Thus, this study would effectively evaluate MX as an effective method for surveilling the elimination of lymphatic filariasis.

MATERIALS AND METHODS

Study Area

The study was conducted across a few selected Kaduna South Local Government Area (LGA) communities. Kaduna South is an LGA within Kaduna Metropolis, headquartered in Makera. It is sited on latitude 10.468 and longitude 7.241 (100, 28' 5" North, 70 25' 16" East), with approximately 5,900 hectares and an altitude of 1,988ft. This local government is around 46.2 km2 in size and has a warm tropical savanna climate. Kaduna South LGA has an estimated population density of 12,879/Km^2 [12]. The mosquito collection/survey was conducted across selected Kaduna South communities, including River Side Down Quarters, Tudun Ilu, Ungwan Barde, and Angwan Rami.

Mosquito Collection and Examination

Mosquitoes were collected for nine months across selected communities from May 2023 through January 2024. Entomological surveys were conducted using the procedure explained by Pi-Bansa *et al.* [13], with some major modifications. Resting adult mosquitoes were trapped using pyrethrum spray catch (PSC) for a monthly collection across the study areas. These vectors were collected for seven consecutive days each month, with three households sampled daily within each community. This yielded a total sampling effort of 756 sampling days [1 (number of LGAs) x 4 (number of communities) x 9 (number of months) x 7 (number of days per month) x 3 (number of households per sampling day)].

The PSC method consisted of insecticidal sprays (pyrethroid) applied to ceilings and walls of selected "enclosed sleeping rooms." Before the collection, the occupants of each household were asked to cover their food items within the space. Following a spray with pyrethrum, the insecticide was allowed to circulate for about fifteen minutes, after which the mosquitoes were carefully collected on a white sheet covering placed across the floor space of the sleeping room. The field-collected mosquitoes were preserved on silica and kept in microcentrifuge tubes to prevent likely degradation of the samples; then, they were packed in larger sample bags (according to the month of collection) and properly labeled [14].

Sample Processing

Collected mosquitoes were properly identified morphologically, categorized into groups of species and sex (females and males), using standard keys as described by Farag *et al.* [15]. Then, identified mosquitoes were pooled in nine tubes, labeled S1 to S9, indicating the monthly collections. Then, the DNA was extracted using AccuPrep Genomic DNA Extraction Kit (K-3032), following the manufacturer's instructions. Afterwards, the DNA quality was checked using NanoDrop absorption spectroscopy [16,17].

PCR amplification with *W. bancrofti* Primers

Wucheria bancrofti was amplified using polymerase chain reaction (PCR) in a total volume of 25µl reaction using ITS1 primer (ITS1-F; 5'-GGTGAACCTGCGGAAGGATC-3' and ITS1-R; 5'-GCGAATTGCAGACGCATTGAG-3'). The reaction mixture consisted of one 1µl of the template DNA, PCR premix consisting of Taq polymerase (5µl), forward and reverse primer (1µl each), and distilled water (17µl), DNTPs (2.5 Mm), and reaction buffer. The reaction was carried out in thermal conditions of an initial denaturation temperature of 95°C for 5 minutes (45 seconds for subsequent denaturation), annealing temperature of 58°C for 45 seconds, and extension temperature of 72°C for 45 seconds. The amplified products were visualized in 1.5% agarose gel in the presence of a reference DNA to determine the size of the DNA fragments produced from the PCR process [18,19].

Ethical Clearance

Ethical clearance (with reference number: MOH/ADM/744/VOL.1/958) was obtained from the Kaduna State Ministry of Health, as approved by the board of ethical committee prior to this study. Also, permission was also obtained from heads of the selected communities, along with the residents of selected houses. All these were further helpful in ensuring a smooth flow of work during the duration of this survey.

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RESULTS

Prevalence of Lymphatic Filariasis in Kaduna South from MX Surveillance

A total number of 1,611 mosquitoes were collected over the entire period of 9 months. The species composition of the mosquitoes collected were *Culex Spp* (51.33%). and *Anopheles* (48.67%). All the collected mosquitoes were processed to check for *W. bancrofti* infection and out of 1,611 mosquitoes molecularly analyzed (processed in 9 tubes), none (0%) were found positive. Table 1 below vividly presents the information of the mosquitoes analyzed. Also, the autoradiographed image of the molecular xeno monitoring surveillance using agarose gel electrophoresis and *W. bancrofti* primers for S1-S9 is shown in Plate I and II

Table 1: Molecular Xeno Monitoring for Surveillance of Lymphatic Filariasis in Selected Communities of Kaduna South Local Government Area.

Processing Tubes	Number of Mosquitoes Processed	Positive
S-1	133	0
S-2	144	0
S-3	164	0
S-4	208	0
S-5	233	0
S-6	255	0
S-7	266	0
S-8	122	0
S-9	86	0

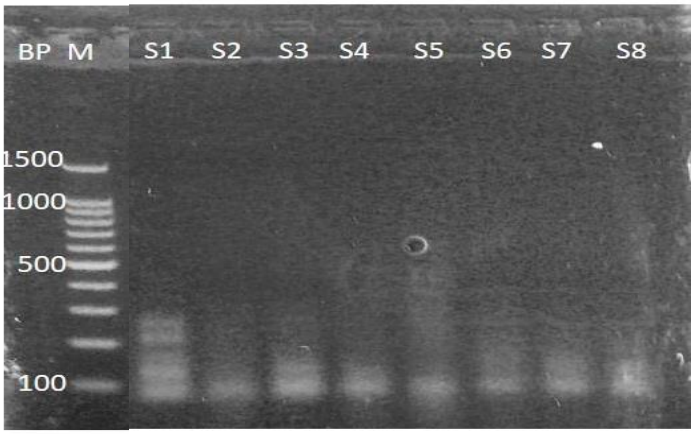

Plate I: Amplified *W. bancrofti* DNA Products. Lane M, Molecular Ladder (1500 BP), Lane S1 – S8.

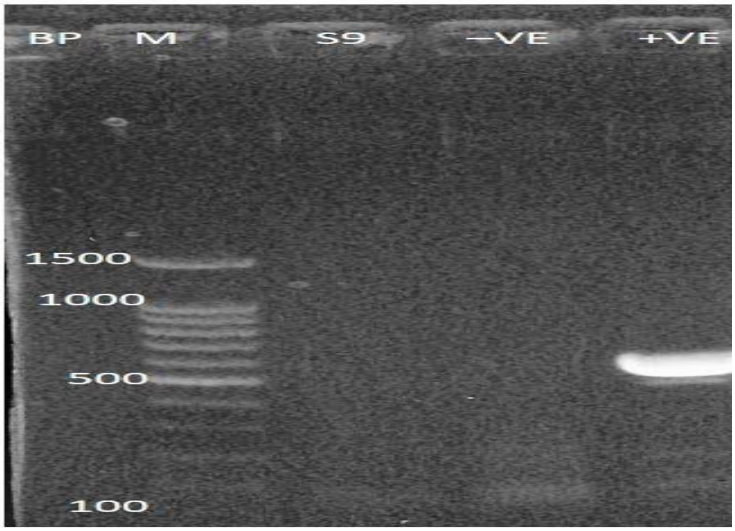

Plate II: Amplified *W. bancrofti* DNA Products. Lane M, Molecular Ladder (1500 BP), Lane S9 , +ve and –ve controls.

DISCUSSION

The molecular analysis of *Culex* and *Anopheles* species in this study revealed no infection *of W. bancrofti.* It is known that these mosquito vectors mostly spread the filarial nematodes. Ongoing efforts are geared towards eliminating lymphatic filariasis through MDA, as recommended by WHO. The detection of filarial parasites from infected vectors (mosquitoes) using molecular methods has been reported to be highly useful in surveillance studies [6]. Pryce *et al.* [8] further highlighted that MX has been very effective as a proxy indicator for measuring the amount of parasitic infection across the human population. Notably, the number of PCR-negatives is potentially related to the abundance of the mosquito vector; thus, a wider range of collection may have influenced this study further. Nevertheless, other factors that affect mosquito infection prevalence include the mosquito biting rate, the prevalence of infected humans, and the likelihood of trapping infected/uninfected mosquitoes [6]. Thus, this research proposes that the absence of infected mosquitoes in this diagnostics is most likely due to fewer cases of infected humans.

In conclusion, this research aligns with several other studies that show that molecular xenomonitoring emerges as a more sensitive and efficient method of detecting filarial transmission. This study has further validated this post-MDA tool as an effective measure for detecting resurgence of lymphatic filariasis infection. This surveillance technique helps reveal transmission risks and exposes residual transmission hotspots [21]. While there have been several speculations of seasonal or behavioral variations in mosquitoes driving changes

in transmission intensity, this was not proven during this investigation, despite the possibility that they do [6]. Forthcoming research endeavors must address this particular inquiry and enhance the interpretation of molecular xenomonitoring outcomes.

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Author Contributions

Conceptualization: LNE, DKB, OI.C.J Data Collection/Processing: LNE, EEN, GWB, LNE, BLN and MF. Data Curation: OICJ and DKB.. Analysis/Interpretation: LNE, OICJ, DKB, and EEN. Literature Research/Writing: LNE, OICJ, and DKB.

Conflict of Interest: The authors declare no conflict of interest

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