IJABR Vol. 15(2): 147 - 152 (2024)

Original article

Xenomonitoring of Lymphatic Filariasis, post evaluation of Mass Drug Administrationin selected of Kaduna North LGA of Kaduna State, Nigeria

Bala, G. W., Dikwa, K. B., Omalu, I. C. J., Luka, N. E., Lawal, B. N. and Mamuda, F.,

Department of Biological Sciences, Nigerian Defence Academy, PMB 2109, Kaduna

Submitted: September 2024; Accepted: December 2024; Published: December 2024

ABSTRACT

Lymphatic filariasis (LF) is a parasitic disease transmitted by mosquitoes, specifically those carrying the causative parasite *Wuchereria bancrofti*. While there have been elimination programs targeted at eradicating LF among other vector-borne diseases, this disease remains endemic in many countries. The aim of the study is to employ molecular xenomonitoring (MX) in detecting the prevalence of lymphatic filariasis in selected study sites in Kaduna North. Mosquitoes were collected using the Pyrethrum Spray Collection method in selected houses across four communities (Ungwan Gwari, Kabala Doki, Rafin Guza, and Badarawa Kwaru). Morphological identification of these mosquitoes was conducted using standard keys, while the detection of the microfilaria in mosquitoes was carried out using polymerase chain reaction (PCR). The molecular testing indicated that one of the processed mosquito pools tested positive for *W. bancrofti*. MX is a sensitive and effective method for post-MDA surveillance of lymphatic filariasis, emphasizing the importance of MX in identifying residual transmission areas and assisting with the lymphatic filariasis eradication campaign.

Keywords: Lymphatic filariasis, Molecular xenomonitoring, Polymerase Chain Reaction, *Wuchereria bancrofti*.

***Corresponding author's email**: jacobgofup6@gmail.com

INTRODUCTION

Mosquitoes are vectors of medical importance; they are responsible for transmitting many infection-causing agents, from bacteria to parasites [1,2]. Lymphatic filariasis (LF) is a mosquito-borne neglected tropical disease (NTD) that transmits filarial parasites like *Brugia timori, Brugia malayi,* and *Wuchereria bancrofti*. These parasites are usually borne and transmitted by mosquito species in the genera of *Culex, Anopheles, Aedes, Ochlerotatus*, and *Ansonia*, depending on the biological peculiarities of each species and the geographical location [3]. Over the years, there have been various elimination programs targeted at eradicating vector-borne diseases, including LF, and the World

Health Organization (WHO) has facilitated mass drug administrations (MDA) since 2000 across several countries [4].

Nevertheless, this disease remains endemic in many countries, with most LF infections being asymptomatic. Thus, it is imperative to identify residual LF infections for effective decision-making with the goal of eliminating the disease and knowing when to stop MDAs. It is known that repeated rounds of MDAs have significantly decreased the infection rates, but it has become more challenging to choose an appropriate method for surveying transmission interruption [5]. LF programmatic surveys usually employ a standard diagnostic test that detects the presence of filarial antigens in the blood. However, the microfilariae, which is usually produced by the adult worm, may not be detected in certain cases; for instance, detecting the microfilariae may be difficult when the worms have not mated or if they are too young or extremely old [6].

Nevertheless, molecular xenomonitoring (MX) plays a huge role in determining how effective the various control measures against this infection have been. This technique uses polymerase chain reaction (PCR) to reveal the presence of positive pools and may serve as a potentially sensitive measure in detecting the transmission levels of LF compared to the diagnostic antigen testing in humans. Nigeria has a significant number of people affected with NTDs, including LF, and Kaduna State has several recorded cases of this infection [7]. Also, Kaduna State has limited surveillance routines in the efforts to decrease the transmission levels of this vector-borne disease, and the absence of such control strategies keeps hampering the accurate estimation of the rate of infection within the state, especially in Kaduna North Local Government Area (LGA).

MATERIALS AND METHODS

Study Area

The study was conducted across some selected communities in Kaduna North LGA, located in Kaduna State. This LGA has its headquarters situated in Doka, with its secretariat sited at Magajin Gari. Kaduna North is between longitudes 7:25 East and latitudes 10:35 North. It is bordered with the Local Government Areas of Igabi to the north and west, Kaduna South to the southwest, and Chikun to the east. Kaduna North LGA is approximately 72 km^2 in size, and its density is 5,883.1 inh/km^2. An estimated number of 364,575 people were living in Kaduna North as per the 2006 Nigerian population census [8].

Mosquito Collection and Examination

The collection of mosquitoes was conducted in Kaduna North, across four selected communities: Ungwan Gwari, Kabala Doki, Rafin Guza, and Badarawa Kwaru. Mosquito collection was conducted for a period of nine months, from May 2023 through April 2024. Using the entomological survey method employed by Pi-Bansa *et al.* [9]., with some major modifications, resting adult mosquitoes were captured using the pyrethrum spray catch (PSC) method during monthly collections in selected communities within Kaduna North LGA. Mosquitoes were collected over seven consecutive days each month, with three households sampled daily in each community. This methodology resulted in a total sampling effort of 756 sampling days, calculated as follows:

1 (no. of LGAs) x 4 (number of communities) x 9 (no. of months) x 7 (no. of days per month) x 3 (no. of households per sampling day).

The PSC method involved applying pyrethroid insecticidal sprays to the ceilings and walls of designated "enclosed sleeping rooms." Prior to the spraying, household occupants were instructed to cover their food items within the space. After the application of pyrethrum, the insecticide was allowed to circulate for approximately fifteen minutes. Subsequently, mosquitoes were collected from white sheets that had been spread across the floor of the sleeping room. The mosquitoes collected in the field were preserved using silica and stored in microcentrifuge tubes to prevent sample degradation. These tubes were then placed in larger sample bags, organized by the month of collection, and properly labeled [10].

Sample Processing and Analysis

Morphological identification of mosquitoes was conducted using proper taxonomic keys as described by Farag *et al.* [11]. The genomic DNA was extracted from these captured mosquitoes; the mosquitoes were first removed out of the Eppendorf tube and placed into different extraction tubes, which were designated N1 to N12 to represent the monthly collections. The AccuPrep Genomic DNA Extraction Kit (K-3032) was then used to extract the DNA in accordance with the manufacturer's instructions. The purity of the DNA was next examined by NanoDrop absorption spectroscopy [12].

Polymerase chain reaction (PCR) for the amplification of *Wuchereria bancrofti* gene in the isolated genomic DNA extracted from the sampled mosquitoes was conducted. This was carried out in a total volume of 25 µl reaction, consisting of 1 µl of template DNA, 5 µl PCR premix containing Taq polymerase, and 1 µl (each) of forward and reverse primers: ITS1 primers (ITS1-F: 5'-GGTGAACCTGCGCGGAAGGATC-3' and ITS1-R: 5'-GCGAATTGCAGACGCATTGAG-3'). Other components included 2.5 mM DNTPs, 17 µl distilled water, and reaction buffer. The reaction was carried out in optimal thermal conditions, and the resulting products visualized in 1.5% agarose gel electrophoresis [11,13].

Ethical Clearance

Ethical clearance for the survey was granted by the Kaduna State Ministry of Health (MOH/ADM/744/VOL.1/954), following approval from the board of the ethical committee. In addition, permissions were secured from the leaders of the chosen communities and the residents of the selected households. All these were instrumental in facilitating a seamless execution of the survey throughout its duration.

RESULTS

Prevalence of Lymphatic Filariasis Using Molecular Xeno Monitoring

Over the course of the nine months, a total of 1,915 mosquitoes were captured. The mosquitoes that were collected belonged to the species *Culex* (993) and *Anopheles* (922). All the collected mosquitoes were pooled in nine tubes (according to their monthly collection) and molecularly analyzed to check for *W. bancrofti* genes, and only one positive case was recorded. This result of the molecular analysis of the

mosquitoes is presented in Table 1 and Plate I.

Table 1: Molecular Xenomonitoring for Surveillance of Lymphatic Filariasis in Collected Mosquitoes

Areas; Kaduna North	Number of Mosquitoes Processed	Positive
N-1	378	0
N-2	180	0
N-3	171	0
N-4	186	1
N-5	189	0
N-6	233	0
N-7	241	0
N-8	153	0
N-9	184	0

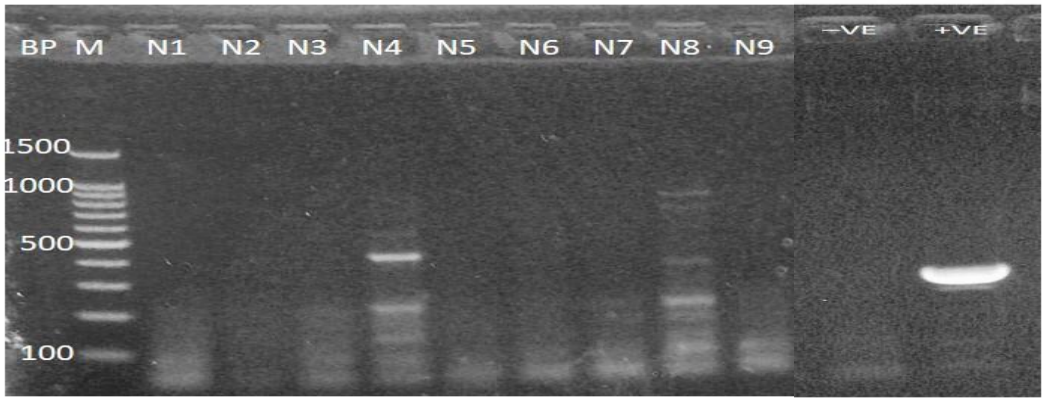

Plate I: Amplified *W. bacrofti* DNA. Lane M: Molecular Ladder (1,500bp), Lane N1 – N9, Gene Lane N4, *W. bancrofti* gene (500bp), Lane -ve control, and Lane +ve control.

DISCUSSION

The molecular analysis of Culex and Anopheles species conducted in this study showed one positive case (Badarwa Kwaru- Kaduna North) of *Wuchereria bancrofti* in the processed pools of mosquitoes. The presence of fewer numbers of positive or infected mosquitoes identified may be primarily due to an MDA conducted in the selected areas. Other factors affecting mosquito infection prevalence include the mosquito biting rate, the presence of infected humans, and the likelihood of trapping

infected or uninfected mosquitoes [6]. Efforts to eliminate lymphatic filariasis are ongoing, particularly through Mass Drug Administration as recommended by the World Health Organization.

According to the WHO [4], for post-MDA surveillance and endpoint assessment using PCR on mosquito pools, it is recommended that molecular xenomonitoring and mosquito sampling assessments focus on individual villages or clusters of small villages rather than larger implementation units. In this study, mosquitoes were collected from several houses across four different communities and processed based on monthly vector collection (N1 – N9). Although MX has been shown to be an effective tool in the fight against LF, there is still a need for constant refinement of this method. Validating this highly sensitive detection technique would further improve accuracy and reliability, and this becomes more efficient with a standardized protocol effected across varying epidemiological settings.

Acknowledgements

The authors acknowledge the staff of the Department of Biological Sciences at the Nigerian Defence Academy, Echo Labs, and DNA Labs, as well as the community heads of sampled communities in Kaduna North LGA.

Author Contributions

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

Conflict of Interest: The authors declare no conflict of interest

REFERNCES

1. **Álvarez-Hernández, D.A., and Rivera, A.S.** (2017). An Introduction to Vector-Borne Diseases. *Austin Journal of Vector Borne Diseases, 1*(1), 1-2.

2. **Ogunmodede, A. F.** (2020). Mosquitoes and their medical importance. *Western Journal of Medical Biomedical Sciences, 1*(1), 115-120.

3. **Famakinde, D. O.** (2018). Mosquitoes and the lymphatic filarial parasites: Research trends and budding roadmaps to future disease eradication. *Tropical Medicine and Infectious Diseases, 3*(1), 1-10.

4. **World Health Organization** (2023). Lymphatic filariasis. Available at https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis (Accessed on 18th May, 2023).

5. **Subramanian, S., Jambulingam, P., Krishnamoorthy, K., Sivagnaname, N., Sadanandane, C., Vasuki, V., Palaniswamy, C., Vijayakumar, B., Srividya, A., and Raju, H. K. K.** (2020). Molecular xenomonitoring as a post-MDA surveillance tool for

global programme to eliminate lymphatic filariasis: Field validation in an evaluation unit in India. *PLOS Neglected Tropical Diseases, 14*(1), e0007862.

6. **McPherson, B., Mayfield, H. J., McLure, A., Gass, K., Naseri, T., Thomsen, R., Williams, S. A., Pilotte, N., Kearns, T., and Graves, P. M**. (2022). Evaluating molecular xenomonitoring as a tool for lymphatic filariasis surveillance in Samoa, 2018–2019. *Tropical Medicine and Infectious Disease, 7*, 203.

7. **Salam, S. P., Ameh, C., Dahiru, T., Umeokonkwo, C. D., and Dauda, A.** (2022). Evaluation of the neglected tropical diseases surveillance system in Kaduna State, Nigeria, 2019. *Journal of Medicine and Public Health, 3*(1051), 161-165.

8. **City Population.** (2022). Kaduna North LGA in Nigeria. Available at https://citypopulation.de/en/nigeria/admin/kaduna/NGA019009__kaduna_north/ (Accessed 20th May, 2024).

9. **Pi-Bansa, S., Nyarko Osei, J. H., Frempong, K. K., Elhassan, E., Akuoko, O. K., Agyemang, D., Ahorlu, C., Appawu, M. A., Koudou, B. G., Wilson, M. D., Dadzie, S. K., Utzinger, J., and Boakye, D. A.** (2019). Potential factors influencing lymphatic filariasis transmission in "hotspot" and "control" areas in Ghana: The importance of vectors. *Infectious Diseases of Poverty, 8,* 9.

10. **Silva, B. E., Zingoni, Z. M., Koekemoer, L. L., and Moss, Y. L. D.** (2021). Microbiota identified from preserved Anopheles. *Malaria Journal, 20*, 230.

11. **Farag, E. A. B. A., Bansal, D., Mardini, K., Sultan, A. A., Al-Thani, M. H. J., Al-Marri, S. A., Al-Hajri, M., Al-Romaihi, H., and Schaffner, F.** (2021). Identification and characterisation of mosquitoes from different locations of Qatar in 2017–2019. *Parasite*, 28, 84. https://doi.org/10.1051/parasite/2021079

12. **Gracia-Alegria, A. M., Anduri-Corona, I., Perez-Martinez, C. J., Corella-Madueno, M. A. G., Rascon-Duran, M. L., and Astiazaran-Garcia, H.** (2018). Quantification of DNA through Nanodrop spectrophotometer: Methodological validation using standard reference material and Sprague Dawley rat and human DNA. *International Journal of Analytical Chemistry, 2020*, 1–9.

13. **Dagher, I. J., and Shaher, K. W.** (2023). Isolation and identification of Lactobacillus bacteria in the stomach of honey bees and recording a type of bacteria. *Euphrates Journal of Agriculture Science, 15(*1), 335–341.