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Research Article

Molecular xenomonitoring of lymphatic filariasis transmission in Okobo, Akwa Ibom State, Nigeria

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ABSTRACT

Lymphatic filariasis (LF) is still a significant public health disease of great burden in endemic regions. Molecular xenomonitoring (MX) is crucial to evaluate elimination efforts during mass drug administration (MDA) and post-MDA settings. This study investigated *Wuchereria bancrofti* prevalence in mosquitoes using molecular techniques in Okobo LGA, an endemic area that has undergone rounds of MDA. Entomological survey involved capturing mosquitoes using CDC light traps during wet (May-July, 2024) and dry (November 2024 - January 2025) seasons. A total of 518 female mosquitoes were captured and morphologically identified comprising of *Culex spp.* (95.95%, 497) and *Anopheles spp.* (4.05%, 21). PCR was used to detect *W. bancrofti* DNA in ≤ 30 mosquito pools. This protocol amplified a segment of the *Ssp* I repeat found in all larva stages of *W. bancrofti* enabling detection of parasite's DNA. Results showed 82.22% pool positivity. PoolTestR was used to estimate prevalence of *W. bancrofti* in mosquitoes throughout the LGA using Maximum Likelihood Estimates (MLE). The overall estimated prevalence (MLE) of *W. bancrofti* DNA in *Culex spp.* mosquitoes was 9.99% (95% CI: 6.49%-14.69%) while *Anopheles spp.*, was 63.91% (95% CI: 39.59%–84.67%). The high infection rate revealed by this study begs the need for continued MDA in Okobo as high estimated prevalence is suggestive of ongoing human infection. These findings provide a baseline data for LF monitoring and elimination strategies and emphasizes the importance of molecular surveillance in transmission assessment surveys in endemic LGAs.

KEYWORDS: PCR; Lymphatic filariasis; Molecular xenomonitoring; Mosquitoes; *Wuchereria bancrofti*; Ssp I

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INTRODUCTION

Lymphatic filariasis (LF) also called Elephantiasis is a neglected tropical parasitic disease (NTD), a parasitic disease endemic in Nigeria. The World Health Organization (WHO) reports that over 882 million people in 44 countries still facing the risk of the disease at a global scale [1]. This disease accounts to be one of the major causes of disability across the world as it results in very painful swelling of the limbs, breasts, and even genitals [2]. As a result of the disabilities caused by this disease, it has been ranked as the fourth (4th) leading contributor to Disability Adjusted Life Years [DALYs] [3]. Lymphatic filariasis is globally caused by three major pathogens namely, *Wuchereria bancrofti, Brugia malayi*, and *Brugia timori* [4]. Studies have shown that LF is transmitted by species of mosquitoes from 3 major genera, namely: *Culex, Aedes* and *Anopheles* [5]. According to WHO [1], more than one billion people still require intervention which could be for one or more neglected tropical

diseases (NTDs) every year. This drives this research to determine and evaluate the prevalence of lymphatic filariasis (LF), a well-established NTD endemic in Nigeria using mosquitoes.

In the year 2000, in a bid to eliminate the disease the World Health Organisation (WHO) launched the Global Programme to Eliminate Lymphatic Filariasis (GPELF) with a control strategy which involved Mass Drug Administrations (MDA) to all eligible endemic communities. Nigeria had aligned to this vision using the National Lymphatic Filariasis Elimination Programme (NLFEP) to ensure MDA was also extended to her endemic communities [5-7]. The goal of MDA approach has always been to reduce circulating parasites to a minimal level hence, causing an interruption in the transmission cycle of the disease. Once MDA is stopped there is a need for monitoring and evaluating the effectiveness of the control approach in order to assess and provide a progress report for the state, country and international public health bodies. The provisional mosquito positivity thresholds carried out by molecular xenomonitoring (MX) for stopping MDA are < 0.25 % for *Culex spp.*, < 1% for *Anopheles spp.*, and < 0. 1% for *Aedes spp.* [8]. Despite progress in Nigeria, Akwa Ibom state still reflects a high burden of Lymphatic filariasis affecting 11 local government areas in Akwa Ibom, Okobo inclusive, with Akwa Ibom alone accounting for a reported estimate of 238,460 antigenemia cases [9, 10].

Lymphatic filariasis needs constant surveillance as well as development of new strategies for controlling the spread until it is completely eliminated. The management of lymphatic filariasis in Akwa Ibom faces is still a problem with the presence of numerous challenges still being faced, including limited awareness in rural communities to lymphatic filariasis, inadequate surveillance systems, and the problem of socio-cultural barriers which reduces the effectiveness of intervention [11, 12]. Another challenge to its elimination is the growing and alarming mosquito insecticide resistance with mosquitoes showing full resistance to pyrethroids (permethrin, deltamethrin) and also some partial resistance to pirimiphos-methyl [13, 14].

Molecular xenomonitoring (MX) has become an essential technique in the transmission assessment surveillance of vector-borne diseases like lymphatic filariasis (LF) as it can bridge surveillance gaps [15, 16]. The WHO has recommended this tool to be incorporated into national programmes as a means to drive entomological surveillance of lymphatic filariasis and malaria [17]. This tool focuses on the vector to evaluate the success of interventions or control programmes. Molecular xenomonitoring detects pathogen's DNA in the vector, and this in turn acts as an indicator of pathogen circulation among people indirectly [18, 19]. Molecular xenomonitoring has not been implemented in Okobo to evaluate control efforts. This research bridges this gap by the introduction of molecular xenomonitoring to estimate prevalence of the parasites in mosquito vectors. This research was aimed at showing the prevalence of the disease parasite in mosquitoes using molecular techniques. This therefore forms a useful approach to validate the extent and progress of MDA control programmes in Okobo, Akwa Ibom state Nigeria.

MATERIALS AND METHODS

STUDY AREA

The study was carried out in 5 selected communities (Table 1) in Okobo Local Government Area (LGA) of Akwa Ibom State, Nigeria between the months of May 2024 and January 2025. Okobo LGA is an endemic LGA for lymphatic filariasis (LF) in Akwa Ibom state presenting itself as a suitable study area [10]. The study area is geographically located in latitude 4° 4' 59.98"N and longitude 7° 50' 59.99" E.

MOSQUITO COLLECTION

Informed written consent was be obtained from the house owners to allow setting of traps. Collection of mosquitoes from selected villages was done using CDC light traps placed indoor and outdoor randomly in a combined total of 10 selected homes. Weekly collection was carried out for 12 weeks in the rainy season and

for 12 weeks during the dry season. Collections were done within the months of May to July 2024 for wet season and November to January 2025 for dry season. Traps were set by 6:00 pm and retrieved by 6:00 am following the peak feeding periods of female mosquitoes [20-22]. Trap containing all caught mosquitoes were removed and properly sorted by village collected without damaging the mosquitoes and placed in an insulated container containing silica gel and were transported to the Department of Animal and Environmental Biology Postgraduate Malaria Research Laboratory, University of Uyo, for identification and processing.

Table 1. Selected Sample Sites and their GPS Coordinates.

S/N	Sampling Sites (Location_ID)	GPS Corrdinates (Latitude|Longitude)	
1	Nung Atai Eta (LOC_01A)	4.839827	8.125908
2	Nung Atai Eta (LOC_01B)	4.842307	8.135499
3	Okopedi (LOC_02A)	4.852183	8.119849
4	Okopedi (LOC_02B)	4.850172	8.131279
5	Odobo (LOC_03A)	4.827932	8.107037
6	Odobo (LOC_03B)	4.813294	8.103722
7	Amammong (LOC_04A)	4.859379	8.133396
8	Amammong (LOC_04B)	4.872016	8.138209
9	Ekeya (LOC_05A)	4.882662	8.139958
10	Ekeya (LOC_05B)	4.884461	8.143445

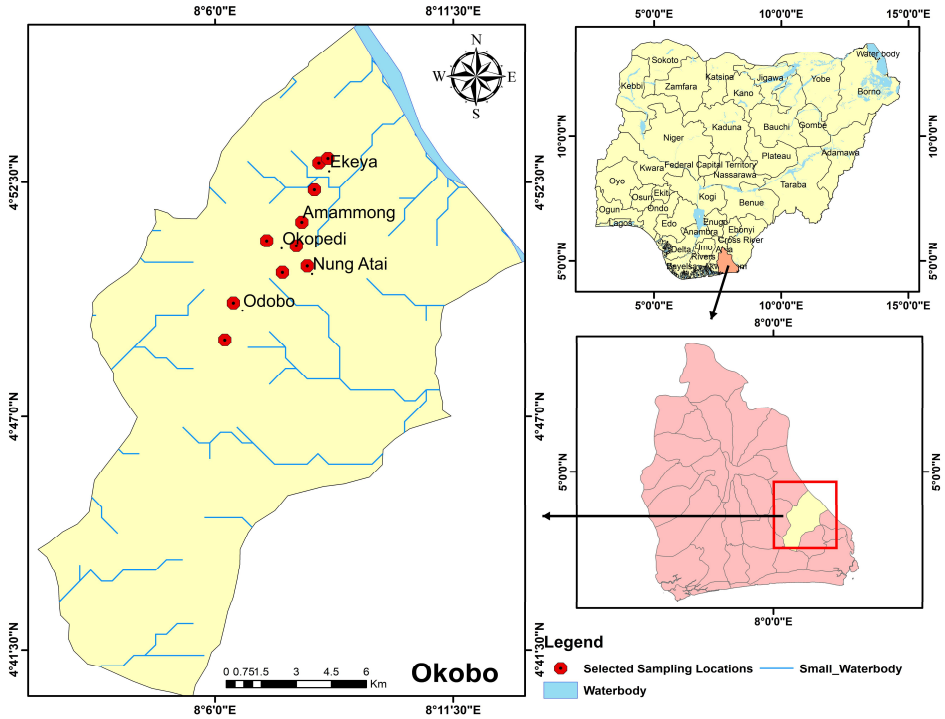

Figure 1. Map of Study Area Showing Selected Study Sites for mosquito trapping (Created by Researcher, 2025).

MOSQUITO SAMPLE PROCESSING AND IDENTIFICATION

In the laboratory, counting and identification and sorting of mosquitoes were carried out morphologically to genera and species using morphological identification keys under a dissecting microscope [23-26]. Female mosquitoes were sorted by species into pools and by community and put in an Eppendorf tube containing silica gel and taken to Osun State University Molecular Parasitology research laboratory for molecular analysis.

DNA EXTRACTION IN MOSQUITOES

The mosquitoes were prepared for extraction by separating the head and thorax from the abdomen. All heads and thorax were put in 1.5 mL Eppendorf bottles in mosquito pools of ≤ 30 mosquitoes pooled by location and month. Extraction of DNA was carried out in the molecular lab using the commercial DNeasy Blood and Tissue Kit from Qiagen [27]. The modified procedure briefly involved grinding of each pool using sterile pestle to break down the tissues releasing the cells. Next 180 µL of Animal Tissue Lysis (ATL) buffer was added to each tube to lyse the cells. 20uL of proteinase K (enzyme digesting protein) was added to the mixture. Thereafter it was mixed by vortexing and incubating for about an 1hr afterwards at 56˚C until completely lysed. After incubating 200 µL of AL buffer was added (to help bind DNA to spin columns membrane) and mixed thoroughly by vortexing. After this, 200 µL of ethanol (96-100%) was added (to facilitate DNA precipitation) and mixed thoroughly. The entire volume is pipet into the DNeasy Mini spin column placed in a 2 ml collection tube. It is centrifuged at 8000 rpm (≥6000 g) for 1 min. The flowthrough and collection tube were then discarded. Thereafter the spin column was placed into a new 2 ml collection tube and 500uL buffer AW1 is added and centrifuge for 1min at 8000 rpm (≥6000 g). Discard the flow-through and collection tube. The spin column again was placed in another new 2 ml collection tube and this time 500 µL buffer AW2 was added and centrifuged for 3 mins at 14000 rpm (20000 g) to dry the DNeasy membrane. Then the flowthrough and collection tube were discarded again. The spin column was then transferred into a new 2 ml microcentrifuge tube and to it was pipetted 200 µL Buffer AE directly unto the DNesay membrane and was then incubated for 1min at room temperature (15-25˚C) and then centrifuged for 1 min at 8000 rpm (≥6000 g) to elute it. The extracted DNA was then stored in the freezer at -70˚C for subsequent PCR analysis to detect DNA of *Wucheraria bancrofti*.

PCR DETECTION OF *WUCHERARIA BANCROFTI* DNA

Following extraction, the DNA of *W. bancrofti* was identified by using modified PCR protocol from Zhong et al. [28]. Briefly, the procedure involved utilizing the extracted DNA from mosquitoes to amplify to amplify a unique segment of the *Ssp* I repeat found in all larva stages of *W. bancrofti* enabling detection of parasite's DNA. Primers NV-1 (5' CGTGATGGCAT-CAAAGTAGCG 3' [21-mer] and NV-2 (5' CCCTCACTTAC-CATAAGACAAC 3' [22-mer] were used to yield and amplify a 188-bp segment of this *Ssp* I repeat. The process involved preparation of the PCR stock solution (reaction mixture) in a total volume of 20 µL. This constituted 2 µL of the extracted DNA template, 2 µL of NV-1 and NV-2 primers each, 4 µL of the 2x Taq PCR master mix (containing buffer, $MgCl_2$, dNTPs, and Taq DNA polymerase), and 10 µL of nuclease-free distilled water. This final volume was added to PCR reaction tubes. The tubes were then placed in the PCR thermal cycling machine and thermal cycling conditions were set. This involved initial denaturing at 95˚C for 10 minutes and 40 cycles of denaturing at 94°C for 1 minute, annealing at 55°C for 1 minute, extension at 72°C for 1 minute and final extension at 72°C for 10 minutes. After successful PCR reactions, electrophoresis was carried out using 1.5% agarose gel prepared using gel-green fluorescent dye. The PCR product was added to the agar block inside the consort tank and then electrophoresis was left to take place for one hour. After Electrophoresis, the agar block was placed in the UV illuminator to visualize amplification and identify DNA base pair for detecting *W. bancrofti*.

STATISTICAL ANALYSIS

Geographic coordinates for each trap location were recorded using Arc GIS Android mobile app [29]. This result was used to map the trapping points and visualized using Arc GIS Desktop Version 10.4.1 [30]. The prevalence of *Wuchereria bancrofti* in mosquitoes was estimated by using Maximum Likelihood Estimates (MLE) calculated using PoolTestR [31] with 95% confidence interval. The percentage positive pools of mosquitoes containing *W.*

bancrofti DNA was calculated based on the total number of pools screened, the number of positive pools observed, and the pool sizes.

RESULTS

A total of 518 female mosquitoes were collected and sorted into 45 pools of ≤ 30 individual mosquitoes each for PCR testing. PCR results for both *Culex spp.* and *Anopheles spp.* are shown in Table 2. The 188bp segments of the *Ssp* I was seen to be amplified during visualization as seen in Figure 2. 26 Pools out of 33 pools of *Culex spp.* were positive for *W. bancrofti* DNA by PCR (78.79% pool positivity). This also showed an estimated overall prevalence of 9.9% with a 95% confidence interval of 6.49-14.69%. In the case of *Anopheles spp.* only one out of twelve pools were negative (91.67% pool positivity). This showed a higher estimated prevalence of 63.91% with a 95% confidence interval at 39.59-84.67%.

The relative abundance of the mosquitoes species captured is shown in Table 3. *Culex quinquefasciatus* (72.97%) was the most abundant mosquitoes species captured across all study areas followed by *Culex pipiens* (22.97%) and the least being *Anopheles gambiae* (4.05%).

Table 2. Overall Prevalence Estimates of *W. bancrofti* DNA in Okobo LGA mosquitoes by PCR.

Species	Total Pools	Total Female Mosquitoes	Positive Pools	Pool Positivity (%)	Prevalence (MLE)	95% Confidence Internal
Culex spp.	33	497	26	78.79	9.99%	6.49-14.69
Anopheles spp.	12	21	11	91.67	63.91%	39.59-84.67

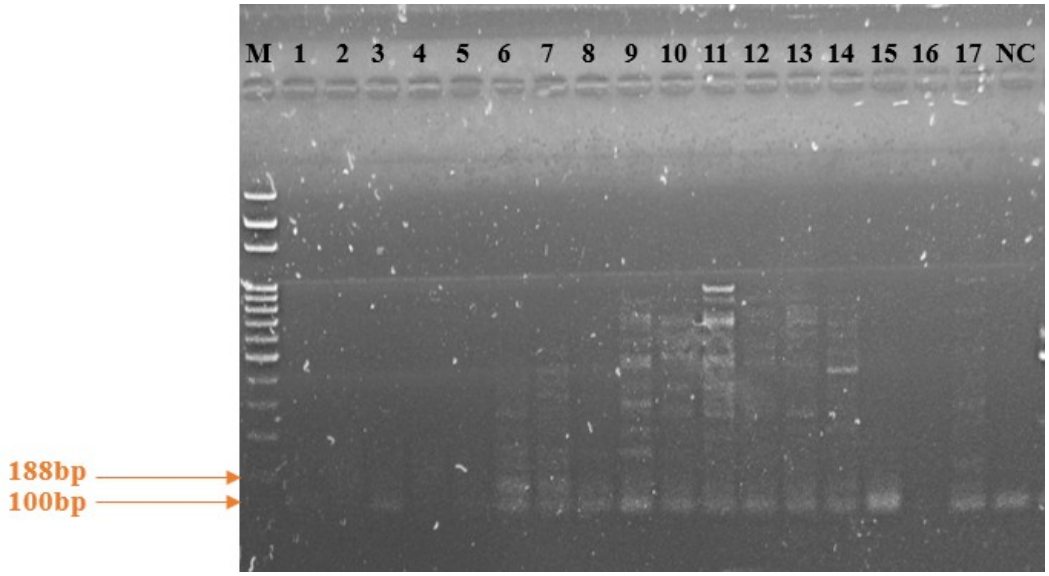

Figure 2. Agarose gel electrophoresis showing detection of PCR for *W. bancrofti* DNA. Lane M = molecular DNA ladder; Lane 1-5 = *Culex spp.* negative for LF; 15 and 16 = *An. gambiae s.s.* negative for LF; Lane 6,7,8, 9, 10, 11, 12 = *Culex spp.* positive for LF; Lane 13, 14 and 17 = *An. gambiae s.s.* positive for LF; Lane 18 = Negative Control.

Table 3. Relative Abundance of Mosquito species caught during the study.

Species	LOC_01 (Nung Atai Eta)	LOC_02 (Okopedi)	LOC_03 (Odobo)	LOC_04 (Amammong)	LOC_5 (Ekeya)	Total (%)
Anopheles gambiae	6 (28.57)	7 (33.33)	4 (19.05)	2 (9.52)	2 (9.52)	21 (4.05)
Culex quinquefasciatus	83 (21.96)	125 (33.07)	68 (17.99)	48 (12.70)	54 (14.29)	378 (72.97)
Culex pipiens	31 (26.05)	36 (30.25)	14 (11.76)	19 (15.97)	19 (15.97)	119 (22.97)
Total	120 (23.17)	165 (31.85)	86 (16.60)	69 (13.32)	75 (14.48)	518 (100)

Positive pools were detected across all the five selected sampling sites as seen in Table 4. Comparatively across the other villages, Ekeya (LOC_05) showed higher pool positivity and prevalence estimates as all pools were positive both for *Culex spp*. and *Anopheles spp.* In all five locations the prevalence estimate is still high with a high 95% confidence interval for both mosquito species.

Table 4. Prevalence Estimates of *W. bancrofti* DNA by PCR across selected study sites in Okobo LGA mosquitoes by PCR.

Location	Species	Total Pools	Total Female Mosquitoes	Positive Pools	Pool Positivity (%)	Prevalence (MLE)	95% Confidence Interval
LOC_01 (Nung Atai Eta)	*Culex spp.*	7	114	2	28.57	2.23%	0.37-6.86
	Anopheles spp.	3	6	3	100.00	100.00%	34.99-100.00
LOC_02 (Okopedi)	*Culex spp.*	8	161	7	87.50	9.03%	3.67-18.96
	Anopheles spp.	3	7	2	66.67	28.57%	5.42-64.98
LOC_03 (Odobo)	*Culex spp.*	6	82	6	100.00	100.00%	12.34-100.00
	Anopheles spp.	2	4	2	100.00	100.00%	21.43-100.00
LOC_04 (Amammong)	*Culex spp.*	6	67	5	83.33	14.60%	5.06-32.32
	Anopheles spp.	2	2	2	100.00	100.00%	38.28-100.00
LOC_05 (Ekeya)	*Culex spp.*	6	73	6	100.00	100.00%	10.66-100.00
	Anopheles spp.	2	2	2	100.00	100.00%	38.28-100.00

DISCUSSION

The results of the study showed a successful detection of *W. bancrofti* DNA in mosquitoes captured across sampling sites in the study area using PCR. Female *Culex spp.* and *Anopheles gambiae* showed the presence of *W. bancrofti* DNA. This confirms with studies that report *Culex spp.* and *Anopheles spp.* to be the most recognized vectors of the disease Lymphatic filariasis in sub-Saharan Africa including Nigeria [4, 32-36].

The abundance of *Culex quinquefasciatus* (72.97%) as the dominant mosquito species reaffirms its potential public health significance as a vector of lymphatic filariasis understudy and as well other diseases like arboviruses in Nigeria and elsewhere. It can be suggested that numerous breeding sites abound influencing its ecological success [37, 38]. The second most abundant reported in this study was *Culex pipiens* (22.97%). This is another vector of public health importance as it can also distribute filarial diseases and mosquito-borne arboviral diseases (such as Sindbis virus, West Nile Virus, Equine encephalitis and more), hence more ongoing surveillance is necessary [39, 40]. Whereas the lower abundance of *Anopheles gambiae* (4.05%), suggests the influence of its unique ecological requirements, like favouring cleaner transient water bodies [41]. The report of its abundance in the study area warrants consistent integrated vector management to control importantly malaria and lymphatic filariasis being the focus of this study for which it is a vector.

The overall result of molecular xenomonitoring showed that *Culex spp.* had an estimated prevalence of *Wuchereria bancrofti* infection to be 9.99% (95% CI: 6.49-14.69%) while in *Anopheles gambiae,* the estimated prevalence was 63.91% (95% CI: 39.59-84.67%) across all the study sites. The result shows a significantly higher estimated prevalence of *W. bancrofti* infection in *Anopheles spp.* (63.91%) compared to *Culex spp*. (9.99%). Although a higher estimated prevalence was seen in *Anopheles spp.*, this had least number of mosquitoes per pool when compared to *Culex spp.* and as such the high infection rates for *Anopheles spp*. During MDA and post-MDA settings it is important that very high number of mosquitoes should be analysed especially when there is a low level of infection in the human population [42]. The wide confidence intervals for *Anopheles spp.* (39.59-84.67%) observed in this finding suggests the hyper endemicity of the study area. It may also suggest possible sampling limitations due to lesser number of *Anopheles gambiae s.s.* captured during the

study. However, for *Culex spp.* (6.49-14.69%) this data is more consistent. This finding has important implications for understanding the elimination efforts of lymphatic filariasis in the study area, Okobo LGA.

Comparatively, global studies on molecular xenomonitoring of lymphatic filariasis reports varying infection rates in different regions as a reflection of transmission intensity. This study comparatively reports notably higher prevalence rates than other regions globally. Rao et al. [43] for instance, reports a *Culex* infection rate of 0.36% (95% CI: 0.29-0.45) in Galle district, Sri Lanka, post-MDA. This low rate was attributed to effective MDA coverage. Another report in Egypt shows a lower estimated prevalence than this study after 5 MDA rounds [44]. It showed that parasite DNA rates in Culex mosquitoes were significantly lower 0.19% (95% CI: 0.076-0.382%) and 0% (95% CI: 0-0.045%) in high- and low-prevalence areas respectively [44]. Kouassi et al. [45] reports respective prevalences of 0% and 0.19% using conventional PCR method to test 14,135 *Culex spp.* and 161 *Anopheles spp.* Similarly, another study in Sri Lanka shows that out 600 pools of *Culex spp.* analysed only 179 (27%) pools were positive for *Wuchereria bancrofti* DNA by qPCR with a resulting estimated infection rate of 1.26% [CI: 1.0-1.5%] in mosquitoes [46]. These low reports are reflection of the elimination efforts post-MDA. Schmaedick et al. [47] used poolscreen to infer territory wide prevalence of *W. bancrofti* in mosquitoes as seen in their reports in American Samoa. Their findings also align with this study reports as they detected positive pools of *W. bancrofti* DNA in mosquitoes belonging to *Culex quinquefasciatus, Ae. polynesiensis, Aedes aegypti* and *Ae.* (Finlay) *spp.*

The prevalence of *W. bancrofti* DNA in *Anopheles gambiae s.s.* (63.91%) in this study is significantly high compared to global data and this could be a reflection of the need for more effective MDA rounds. Again, this high prevalence is not uncommon. It suggests that there may be a hyperendemic foci with intense human-microfilaria loads, compounded by high vector density and limited control. In Tanzania, Materu [48] reports a high estimated *W. bancrofti* infection rate of 3.3% among captured mosquitoes. This comparatively is less than the findings in this study. In Kenya, a similar high prevalence was recorded Kilifi (35.4%; 95% CI 28.0-43.3%) and lower one in Taita Taveta (5.3%; 95% CI 3.3-8.0%) when *Anopheles spp.* were tested [49]. Contrastingly, reports in Togo, using MX, reveals that in the pool screen analysis of 9191 caught *An. gambiae* mosquitoes in 629 pools no mosquitoes were infected with *W. bancrofti* (0%; CI: 0-0.021) [50]. This is lesser than the reported high prevalence in this study 63.91% (95% CI: 39.59-84.67%).

The current findings in Okobo which depicts a 9.96% prevalence estimates for *Culex* and higher prevalence rates of 63.91% prevalence estimates in *Anopheles* spp., suggest active ongoing transmission in the study area. The PCR method used measures infection rate as it detects a unique segment of the *Ssp* I repeat found in all larva (L1, L2 and L3) and does not specifically test infectivity rate (L3 infective larva unique DNA). Hence it cannot directly reflect infectivity rates in the study area. However, the observed estimated prevalence aligns with other global reports and WHO call for introduction of molecular xenomonitoring to ascertain control efforts. These observed rates align more closely with baseline studies data that indicate or represent a hotspot of continued transmission.

The significant difference seen in infection rates between *Culex spp.* (9.96%) and *Anopheles* spp. (63.91%) in the studies reveals the basic differences in both vector competence and transmission dynamics. Studies have shown that *Anopheles* mosquitoes are more efficient vectors of *W. bancrofti* in Africa, while *Culex quinquefasciatus* being more common as vectors of *W. bancrofti* in many urban regions [51, 52]. This finding is aligned to the superior vector competence in anopheline mosquitoes. It has been revealed that *Anopheles gambiae s.l.* can achieve infection rates which may range from 4.2% to 100% depending on the density of microfilarial parasite exposure [53]. This findings from supports the high prevalence estimates of this study [53]. They revealed that at variating high-low filarial density exposures the prevalence of infective stage larvae (L3s) ranged from 24.5% to 68.6% in *An. farauti s.s.* and 61.9% to 100% in *An. hinesorum*.

Several studies in Nigeria have also reported varying estimated prevalence (MLE) of *W. bancrofti* DNA in mosquitoes. This has been used as an indication of both pre- and post-MDA transmission assessment as well as control and elimination progress evaluation. For example, in Kano, mosquitoes were collected from three sampling urban sites and tested using conventional PCR for *W. bancrofti* DNA and this showed a low estimated prevalence in *An. gambiae s.l.* between 0.96% and 6.78% and also in *Culex* mosquitoes between 0.19% and 0.64% yet federal ministry of health recommended two rounds of MDA [54]. In Kaduna South, a similar study was conducted to evaluate the effectiveness of control programs using molecular xenomonitoring in a low prevalence area and showed 0 estimated prevalence [55].

Prevalence estimates of mosquito infection rates may serve as an indicator of *W. bancrofti* transmission intensity and this may even suggest and successfully predict future transmission potential [56]. This is why the World Health Organization also accepts molecular xenomonitoring to be used as a surveillance tool, especially in post-MDA settings [57]. The result of prevalence estimates for both species when compared with the threshold associated with transmission interruption, is well above the threshold. It has been suggested that estimated mosquito infection rates that fall below 0.25% are suggestive of an interrupted transmission [58]. While those above 1% may suggest an active transmission. [59]. Therefore, this study has been able to estimate the prevalence rates of 9.96% for *Culex* and 63.91% for *Anopheles* an indication of all *W. bancrofti* larva (L1, L2 and L3) DNA in the study area. This study clearly suggests the presence of active transmission that requires further larger sampling site estimates to confirm the need for immediate intervention. This suggestion implies there may be the presence of infected humans within the study area. However, further confirmatory studies using sensitive protocols that test for the *W. bancrofti* L3 DNA is of great importance.

CONCLUSIONS

The study affirms the estimated prevalence of *W. bancrofti* DNA (L1-L3) in mosquitoes, measuring the infection rates of 9.99% (CI: 6.47 - 14.66) in *Culex spp.* and 63.91% (CI: 39.59-84.67) for *Anopheles spp*. This is a predictor of significant high ongoing LF transmission in Okobo. However, the infectivity rate was not measured as L3 specifically was not tested to show this actual position. Notwithstanding the findings of this study showing high estimated prevalence (infection rate) in mosquitoes and suggests the need for continued rounds of MDA and transmission assessment in the study area. Okobo LGA presents itself as a suitable entomological landscape of LF transmission in the study area confirming earlier reports of its mapped hyper endemicity. There is also the need for molecular xenomonitoring to be integrated in routine surveillance and transmission assessment surveys. This will establish the true position of control efforts within the LGA.

Author Contributions: IN, OK, AM, UN, CF, and YA conceived the idea and designed the study. IN carried out field survey. IN and IZ carried out molecular bench work. IN, OK, UN, CF and YA analyzed the data and interpreted the results. IN revised, proofread and prepared the final manuscript. All authors read and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

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Ethical Statement: This study does not contain any studies with human subjects or animals.

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Data availability: All the data supporting the results of this study are included in the article itself and any supplementary data can be requested.

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