A REVIEW UPDATE ON DETECTION AND MOLECULAR CHARACTERIZATION OF *Wuchereria Bancrofti*

Sanusi Bello Mada [1,2*], Nasir Anka Garba [1], Ahmad Balarabe Hamza [1], Yila Lakabra David [2], Rilwan Abdullahi [1]

[1]Department of Biochemistry, Federal University Gusau, Zamfara State-Nigeria
[2]Department of Biochemistry, Ahmadu Bello University Zaria, Kaduna State-Nigeria
[*]Corresponding Author's Email address: sbmada@fugusau.edu.ng and Contact Number: +2348038152143

Abstract:

Lymphatic filariasis (LF) is classified as Neglected Tropical Disease (NTD) which are transmitted to humans through repeated mosquito bites. The disease is caused by filarial worms; Wuchereria bancrofti, Brugia malayi, and Brugia timori. Among the three main parasites causing lymphatic filariasis, Wucereria bancrofti principally accounts for about 90% of lymphatic filariasis whereas the remaining 10% are caused by Brugia malayi and Brugia timori. The disease has no obvious symptoms at an early stage however, if left untreated, eventually leads to several clinical manifestations like hydrocele, recurrent adenolymphangitis, lymphedema, elephantiasis, or tropical pulmonary eosinophilia. In addition LF affects both males and females adults as well as children below the age of 15 years. According to World Health Organization (WHO), globally LF affect more than 72 countries, in Africa about 39 African countries are affected which represent a third of the global burden. Global Program for the Control and Elimination of Lymphatic Filariasis (GPLELF) was later established to provide sustained delivery of drugs to affected communities for the purpose of stopping mass transmission of the disease, and ultimately eliminate this burden on public health. Hence, this review mainly used Google scholar, ISI, SCOPUS and PubMed Indexed Journals reporting various studies on Lf involving both mosquito-vectors and infected human population. Accordingly, this review enhances our understanding on the detection, diagnosis pathogenesis and molecular mechanism of the Lf as well as the host-pathogen relation.

Key words: Detection, Epidemiology, Molecular Characterization, *Wuchereria bancrofti.*

Introduction

Lymphatic filariasis (LF) is classified as neglected tropical disease (NTD) caused by filarial worms such as Wuchereria *bancrofti*, *Brugia malayi*, and *Brugia timori* (De-Souza *et al.*, 2014) which is transmitted to humans through repeated mosquito bites (WHO, 2011; Joshi, 2018). The disease has no obvious symptoms at an early stage but, from an immunological perspective, there is a subclinical condition associated with high levels of circulating microfilariae or parasite antigen (Nutman & Kumaraswami, 2001) which are fundamental to the pathogenesis of the disease. However, if left untreated, the infection eventually leads to several clinical manifestations like hydrocele and tropical pulmonary eosinophilia (Michael, 2000). Among the three main parasites causing lymphatic filariasis, *Wucereria bancrofti* principally accounts for about 90% of lymphatic filariasis in 72 countries of the world, and 39 African countries which represent a third of the global burden (WHO, 2010; Taylor, 2010; Manguin *et al.*, 2010). The remaining 10% are caused by *Brugia malayi* and *Brugia timori.* LF is transmitted in rural African communities by the Anopheles species (Merelo-Lobo *et al.*, 2003), whereas in many urban areas of the world, it is transmitted by *Culex* mosquitoes (Buck,

1991; Ramaiah & Das, 1992). According to World Health Organization, LF affects about 120 million people and mostly in tropical African and Asian countries (Ottesen, 2006), and the disease affects both males and females adults, as well as children below 15 years of age (Mandal *et al.*, 2010). Global Program for the Control and Elimination of Lymphatic Filariasis (GPLELF) was later established to provide sustained delivery of drugs to affected communities for the purpose of stopping transmission of the disease, and ultimately eliminate this burden on public health (Taylor *et al.*, 2010). Thus, World Health Organization (WHO) recommends mass anthelminthic drugs administration (MDA) to all eligible persons living in endemic areas (WHO 2012; WHO 2006). Hence, this review is designed to highlights recent update on the molecular detection, diagnosis pathogenesis and molecular mechanism of the lymphatic filariasis.

Life Cycle of *Wuchereria bancrofti*

The morphologically, *Wuchereria Bancroft* is a white, cylindrical, threadlike worm, with both ends tapering (Fig. 1). Under the microscope, it appears translucent with a smooth external cuticle. It has a blunt rounded head end and pointed tail end with two spicule-like structures (Khokhar *et al.*, 2015). It appears to have dark-spotted nuclei which are dispersed throughout the body cavity, with no nuclei at the tail tip (Fig. 1). Males and females can be differentiated by the size and structure of their tail tips. In terms of sex, the male worm is smaller, ranging from 2.5-4.0 cm long and 100 μm wide. While the female is 5-10 cm long and 300 μm wide, nearly three times larger in diameter than the male (CDC, 2006). Through nocturnal periodicity (Manguin *et al.*, 2010), *Wuchereria bancrofti* have been described to have a biphasic life-cycle (Michael, 2010), involving mammalian host and various genera of mosquitoes (*Anopheles, Aedes, Culex, and Mansonia*). The parasite appears to be exclusively a human parasite. Man is the Primary host (definitive) whereas, mosquitoes act as the secondary host (Bockarie *et al.*, 2009). During a blood meal, an infected mosquito introduces third-stage filarial larvae onto the skin of the human host, where the parasite penetrates through the bite wound (Chandy, *et al.*, 2011). Thereafter, they develop into adult larvae and reside in the lymphatics (Fig.2). These adult larvae developed into tiny microfilariae of about 244 to 296 μm by 7.5 to 10 μm, which are sheathed and also have nocturnal periodicity (CDC, 2017). The microfilariae move into lymph and blood where mosquito ingests it during a blood meal. After that, the microfilariae lose their sheaths and move through the wall of the proventriculus and cardiac portion of the mosquito's midgut and reach the thoracic muscles (CDC 2017).

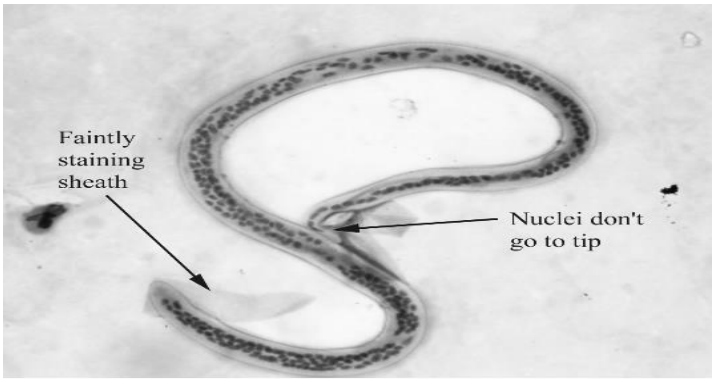

Fig 1: Microscopic image of *Wuchereria bancrofti* (Westmann, 2018)

As shown in Fig. 2, microfilariae develop into first-stage larvae and subsequently into second and third-stage infective larvae called L3 (Lee *et al.*, 2010). The L3 stage of the parasite is the infective stage that hides in wait in the buccal cavity before transmission into a human host (Joshi, 2018). The process is completed when the L3 migrate through the hemocoel to the mosquito's proboscis to infect another human when the mosquito takes a blood meal (CDC 2017). The microfilariae have clear, distinct nocturnal periodicity, appearing between 12 midnights and 4 am before receding from peripheral circulation almost completely during the day (Nwoke *et al.*, 2010).

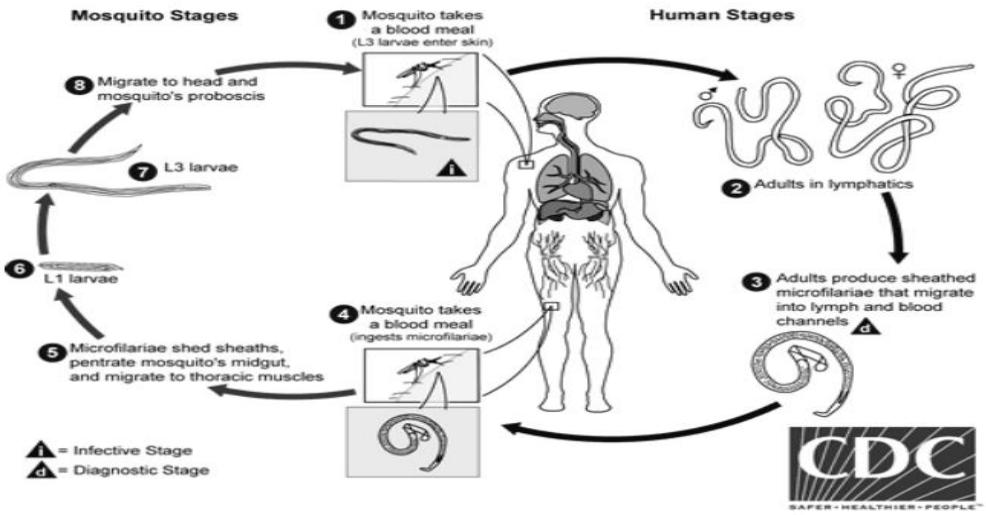

Fig 2: Life cycle of *Wuchereria bancrofti* (CDC 2017)

Genome of *Wuchereria bancrofti*

Although *Wuchereria banccrofti* has been identified more than 100 years ago, but information on its genome has not been reported until 2013 (Desjardins *et al.*, 2013). The sequenced of the whole genome from the mosquito vector was reported to have 81.51mbp, 29.70% GC content of scaffolds, 19,327 genes number of predicted protein-coding genes, and 6.2% repetitive sequences (Desjardins *et al.*, 2013; Small *et al.*, 2016). In addition, previous study also reported the actin gene of *W. bancrofti* to have 5 exons that encode 376 amino acids, and four introns with at least 109 to 190 base pairs (Saverimuttu, *et al.*, 2000). The mitochondrial genome of *W. bancrofti*, was having approximately 13,637 nucleotides length and contains two ribosomal RNAs (rRNA), 22 transfer RNAs (tRNA), 12 protein-coding genes, and 74.6% AT content (Ramesh *et al.*, 2012). The table below is the unanointed sequence obtained from a previous study conducted in Egypt (Abdel-Shafi *et al.*, 2017) and submitted in NCBI with the accession number: KP114615.

Table 1: Nucleotide Sequence of a *pWb12* Repeat

>KP114615.1 *Wuchereria bancrofti* isolate CU/ISCIII/Giza-1 repeat region
CACCGCTATCGAGATTAATTAGAGAAAAAGTTTCATTTTTATCAGAAGCG ATGCAAAGCAGAAGANTGAGCCAAATGATGGGTGTCATAGGTATAGTGG AGAACATGGTGAAATATAGAATTTTATGAAATGACAGAAATGCTCAAAT GAAAGTTTTTTATTTCAGATTGAAATTAAGGACCAAAGGTTCAAACAATT CTTAATCCGTCCATTGTTTGTCATATTTTTACATCCTCTCCCTCAACTGTTA GTTTATCAATGNTCTTTTGAAAGCATTAAGGGAAGCATTAAAGTAGAATG TACGTGCTACTGATAGTATCACTGGTGGAATTTCATCGGATGAACGCATC ACCTGAAATTTGGTAATTGTTGCTTTTTGACATACATCAA

Pathogenesis of Lymphatic Filariasis (LF)

Clinical manifestations of LF usually vary but are commonly grouped as either those with only a subclinical condition of circulating microfilariae or those with significant lymphatic damage (Nutman, 2013). The latter is characterized by the development of lymphangiectasia which can remain subclinical with no apparent inflammatory reaction for uncertain periods of time before evolving into chronic disease (Babu & Nutman, 2012). The second category is called elephantiasis or acute dermatolymphangioadenitis (ADLA), which is a chronic condition that is not curable at moment but the symptoms can be alleviated by appropriate management otherwise, it can progress and become difficult to manage (Medeiros *et al.,* 2021). In LF infection, the host-parasite relationship is presumably dynamic; the host defense system, mainly immunological, constantly produce both cellular and secretary molecules in an attempt to kill, or at least contain the parasite, whereas the parasite mounts evasive mechanisms to continue its survival and production of the appropriate stage for transmission (Medeiros *et al.,* 2021). This complex interplay of immunologic factors plus endothelial factors, and genetic factors determine the emergence of lymphangiectasia and consequently, elephantiasis (Varghese, 2021). Meanwhile, the later development leading to elephantiasis is not entirely caused by filarial worms or the host's immunologic responses, but, secondary bacterial infections are suspected to be a major factor intensifying the condition (Medeiros *et al.,* 2021). This is because, the damaged lymphatics with leaky lymphatic endothelium act as a potential nidus for bacterial translocation thus showing increased bacterial and fungal loads at such locations (Nutman, 2013). Hence, more studies are required on the etiology of lymphedema are needed to explore and enhance our understanding of the types of tissue changes across the pathological stages and causes of lymphedema for development of a novel therapeutic approaches for this chronic and debilitating disease (Varghese, 2021).

Epidemiology of Lymphatic Filariasis (LF)

Presently more than 120 million are infected with LF globally (Joshi, 2018). One-third of the people with LF infection are living in India, one-third in Africa, and most of the remainder are in South-east Asia, the Pacific region, and the Americas (Quah, 2016). The highly infected countries are, India, Indonesia, Nigeria, and Bangladesh which account for 70% of all infections worldwide (WHO, 2000). Nigeria has a significant burden of LF (Nwoke *et al.,* 2010; Okorie *et al.,* 2013), and is positioned first in Africa and third according to global ranking (Hotez *et al.,* 2012). So far, about 106 million people in Nigeria

are at risk of the disease (FMoH, 2012). This prevalence stretched to all states and geopolitical zones across Nigeria and a total of 241 lymphedemas, and 205 patients with hydrocele cases have been reported from mapping surveys conducted in the country (FMoH 2012; Okorie *et al.,* 2011).

Social Impact of Lymphatic Filariasis (LF)

Chronic LF has several serious negative, social and economic effects on individual affected. For instance those with elephantiasis and hydrocele are often socially marginalized and poor (Wynd *et al.,* 2007). Although the degree of social disability varies between cultural settings, stigmatization appears to be directly correlated with the severity of the disease (Evans *et al.,* 1993; Mujinja *et al.,* 1997). Similarly, among many consequences, one direct social impact of lymphatic filariasis is school drop-out by affected individuals due to stigma received from fellow pupils leading to low or no formal education (Eneanya *et al.,* 2019). Another resulting implication of filariasis was the likelihood to remain unmarried by affected persons particularly women (Eneanya *et al.,* 2019). Also men suffer from physical and psychological burden which hurts their marriages and employment prospects (Gyapong *et al.,* 2000). Therefore it is imperative to understand that people affected by filariasis are relegated to social gatherings and constantly faced various kinds of stigmatizations from friends, neighbors, and many others. Thus, proper awareness about filariasis can educate community members and help reduce the severity of stigmatization and stop transmissions.

A. Conventional Diagnosis of Lymphatic Filariasis

Indeed, in areas where *W. bancrofti* or *B. malayi* are endemic, the overwhelming majority of infected individuals have few overt clinical manifestations of their filarial infection despite the presence of circulating microfilariae or parasite antigen in the peripheral blood (Gordon *et al.,* 2018). Although they may be clinically asymptomatic, virtually all persons with *W. bancrofti* or *B. malayi* infection have some degree of subclinical disease that includes microscopic hematuria or proteinuria (Dreyer, 1992). A blood smear is a simple and accurate diagnostic tool, provided the blood sample is taken during the day when the juveniles are in the peripheral circulation (Van Hoegaerden *et al.,* 1982). Some infected people do not have microfilaria in their blood. As a result, tests aimed to detect antigens from adult worms are used. In addition, an ultrasonography test can be used to detect the movements and noises caused by the movement of adult worms (Amaral *et al.,* 1994).

i. Microscopy

Giemsa-staining of the blood smear for microscopic examination is recognized as the standard method for detecting microfilaria with thick or thin smear (Tan *et al.,* 2016). This technique basically relies on key morphological features to distinguish and identify parasite species at specific magnifications. These morphological parameters considered include body length, cephalic space length and width, length of the head to nerve ring and body width at nerve ring length (Jongthawin *et al.,* 2020). However, caution for this procedure is that blood samples should be obtained at a time consistent with the known periodicity of microfilariae in the specific geographic region, which is between 10 pm and 2 am for nocturnally periodic forms of *brugian* and *bancroftian* filariasis (Chavarkar, 2016). This makes the microscopic method for the detection of microfilariae in night blood samples time-consuming, laborious, and not feasible for large-scale epidemiological surveys, apart

from being less sensitive (Yasin *et al.*, 2020). When using an electron microscope, the tail of the microfilariae of *Wuchereria bancrofii* tapers to a point and exhibits no terminal nuclei, and can be easily distinguished from the microfilariae of *Brugia malayi* and *Loa loa*, the other sheathed microfilariae of clinical importance (Leventhal & Cheadle, 2019) The microfilariae of *Wuchereria bancrofii* are stains pink with Giemsa (Simonsen *et al.,* 2013) and usually, the slide is examined under a microscope for microfilariae at 100 X-magnification level (Mallawaarachchi *et al.*, 2015). To improve microscopic sensitivity, Concentration procedures, such as Knott's technique and membrane filtration technique, can be used for the enhanced detection of microfilariae in blood or other body fluids (Mathison *et al.,* 2019). In Knott's test, 2 milliliters of venous blood is mixed with 2% formalin to dilute the blood sample before it is centrifuged and finally examined under a microscope (Kalyan, 2018). On the other hand, the membrane filtration method for microfilaria detects early stages of the disease before clinical manifestations and is effectively used to determine microfilarial density than as a means of microfilaria identification (Kalyan, 2018).

i. Immunochromatographic Test (ICT)

The detection of parasite antigens in biological fluids such as serum or urine, by using ICT (Fig. 3), is more reliable and useful in the early diagnosis of filariasis (Harinath, 1986). Circulating filarial antigen (CFA) testing is also more sensitive than thick smear microscopy for detecting *W. bancrofti* infections, and it is also more convenient because it can be performed with blood collected during the day or night in the field with no requirement for electricity, special equipment, or skilled microscopists (Weil & Ramzy, 2007). The same applies to urine samples (Itoh *et al.,* 2001). CFA test detects a 200 kDa parasite antigen that is a sensitive and specific biomarker for the presence of adult *Wuchereria bancrofti* (Michael *et al*., 1996). The principle is by antigen-antibody reaction of the polyclonal antibodies raised against *W. bancrofti* microfilariae antigens in sera of filarial patients (Cheirmaraj *et al.,* 1992). Antibodies against filarial proteins are known to be sensitive biomarkers of transmission intensity and can provide evidence of continued exposure to filarial infection, even before or after antigenemia or microfilaria detection (Pastor, 2021). Individuals living in endemic regions have been reported to have a high proportion of immunoglobulin G4 (IgG4) antibodies against known filarial antigens, even if they do not have circulating microfilaria or detectable filarial antigens (Damgaard *et al.,* 2016).

Figure 3: A typical Antigen Detection Card, Source: (Damgaard *et al.,* 2016)

iii. Ultrasonographic Detection Using a Transducer

Ultrasonography has been used to locate and study the movements of active adult filarial worms of *Wuchereria bancrofti* **(**Ibrahim *et al.,* 2015; Rocha *et al.,* 2009). The basic identification is described by a fast and distinctive pattern of worm movement called the filarial dance sign (Khan *et al.,* 2021). The detection of the filarial dance sign has been used to diagnose *W. bancrofti* infection in both men and women. (Vyas *et al.,* 2020; Khan *et al.,* 2021), and to directly assess the efficacy of antifilarial drugs against the adult worm (Dreyer *et al.,* 1996).

B. Molecular Diagnosis of Lymphatic Filariasis

Although, the conventional methods for diagnosis of LF are simple, sensitive and specific. However, the limitations of the antigen tests are cost, limited availability and cross-reactivity with other filarial parasites (Nuchprayoon, 2009). Fortunately, DNA-based techniques have been developed to diagnose and differentiate filarial parasites in humans, animal reservoir hosts, and mosquito vectors (Khatri *et al.,* 2019; Sakthidevi *et al.,* 2010). These include DNA hybridization, polymerase chain reaction (PCR) amplification using specific primers; for targeting: Ssp I repeat, pWb12 repeat, pWb-35 repeat, and LDR repeat for *Wuchereria bancrofti* and Hha I repeat, glutathione peroxidase gene, mitochondrial DNA for *Brugia malayi*, and universal primers, multiplex-PCR, PCR-restriction fragment length polymorphism (PCR-RFLP), PCR enzyme-linked immunosorbent assay (PCR-ELISA), as well as quantitative PCR (Ximenes *et al.,* 2014).

Quantitative Polymerase Chain Reaction

PCR-based diagnostic assays are promising tools for the monitoring and evaluation of the Global Programme for Elimination of Lymphatic Filariasis and are cost-effective when employed for pool screening (Fischer *et al.,* 2003). Pooled samples can be from venous blood, urine, or even mosquito vectors when carrying xenomonitoring surveys, especially during monitoring and post-MDA surveillance (Pryce & Reimer, 2021; Vasuki, *et al.,* 2021; Subramanian, *et al.,* 2020; Irish *et al.,* 2018). This is particularly useful when an infection is at a level lower than that detectable by antigen or microfilariae-testing (Subramanian, *et al.,* 2020). Vector infection of <0.25% for *Culex*, <1% for *Anopheles*, and <0.1% for *Aedes* are suggested as provisional threshold levels to decide on stopping MDA (WHO, 2010).

Global Program for Elimination of Lymphatic Filariasis

In 1993, the International Task Force for Disease Eradication (ITFDE) identified Lymphatic Filariasis as one of the six potentially eradicable infectious

diseases and in 1997, the World Health Assembly passed a resolution to eliminate LF as a public health problem by 2020 (Behbehani, 1998; WHO, 2010). In 1998, WHO initiated a Global Program for Elimination of Lymphatic Filariasis (GPELF), with two main objectives namely: interruption of active transmission through mass drug administration (MDA) once a year, a single-dose two-drug regimen with DEC and albendazole (or Ivermectin and albendazole) to the endemic population (at least 80% coverage of areas where the prevalence of microfilaremia (Mf) or antigenemia is ≥ 1%) for at least five years (Modi *et al.,* 2017), and alleviation of disability by management of clinical cases (Modi *et al.,* 2017). A Global Alliance for Elimination of Lymphatic Filariasis (GAELF) comprising donors, Ministries of Health of endemic Countries, and NGOs were formed to tackle the wide-ranging and complex process of science and practice to achieve the goals of GPELF (Noordin, 2007). Before the establishment of GPELF in 1999, epidemiological surveys on filariasis showed that Japan and Australia have already achieved the elimination of lymphatic filariasis (Kimura & Itoh, 2011; Ichimori & Graves 2017). But the majority of countries worldwide were letter declared free of active infection (De-Jian *et al.,* 2013). Although broad declines in prevalence are observed globally, focal areas in Africa and Southeast Asia remain less likely to have attained infection prevalence thresholds proposed to achieve local elimination (Cromwell *et al.,* 2020). Thus, the 2020 deadline to achieve elimination was not met because some endemic countries are yet to complete mapping or launch MDA (Kamgno & Djeunga, 2020). So far, 23 African countries are still implementing MDA with 100% geographical coverage, 6 countries still implement MDA only in part of the geographical area considered, and only Malawi and Togo were verified to have eliminated LF (Sodahlon *et al.,* 2013; Deribe *et al.,* 2021).

Phylogenic Analyses

Understanding of the major transitions in evolution, and is key to inferring the origin of new genes, detecting molecular adaptation, understanding morphological character evolution, and reconstructing demographic changes in recently diverged species (Kapli *et al* ., 2020). It gives an estimate of the relationship among taxa or sequences and their hypothetical common ancestors (Hall, 2013). To describe the importance of phylogeny, it is important to know that organisms of bacterial, viral, and fungal have all been well understood and variants of species that could be more deathly than the wild type have been elucidated. Parasite populations causing lymphatic filariasis have been studied using phylogenic analysis by targeting sequences of conserved DNA regions or genes (Small *et al.,* 2016). Such analysis shows the complex phylogeography of *W. bancrofti* populations and determines the levels of genetic divergence and gene flow among populations (Thangadurai *et al.,* 2006). Other researchers have molecularly characterized and analyzed *Wuchereria bancrofti* in human blood samples by phylogeny (Mutanu *et al.,* 2020). One notable DNA target analysed is the Wb repetitive DNA region that is peculiar to *Wuchereria bancrofti.*

Conclusion

Lymphatic filariasis (LF) is classified as one of the Neglected Tropical Disease (NTD), which is mainly transmitted to humans through mosquito-bites. The disease is majorly caused by *Wuchereria bancrofti.* The present review describe the recent update on the identification, detection, diagnosis and molecular characterization of *Wuchereria bancrofti and* further enhance our understanding on the pathogenesis and

molecular mechanism of Lf as well as host-pathogen relation. Thus, our research group is presently conducting a study on the detection and molecular epidemiology of *Wuchereria bancrofti* in Anka and Talata-Mafara Local Government Areas, Zamfara State-Nigeria.

Conflict of Interest

The authors declared no conflict of interest

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