

## Detection Of *Wuchereria Bancrofti* And *Onchocerca Volvulus* Antigens Using Igg4 Antibodies Among Residents Of Bakassi Local Government Area Of Cross River State, Nigeria

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**Abstract:** Onchocerciasis and lymphatic filariasis have remained a public health problem, despite several efforts over the years to eliminate them. The prevalence of human lymphatic filariasis and onchocerciasis was determined amongst 400 residents of Ekpri-Ikang, Ikang and Ebot-20 community of Bakassi Local Government Area, Cross River State, Nigeria. The subjects were 140 males and 260 females within the age range of 18-50 years and above who were involved in various occupations including farming, fishing and trading. Onchocerciasis and lymphatic filariasis infections were diagnosed by the detection of Wb123 and Ov16 antigens using IgG4 antibodies in the SD bioline filariasis and Onchocerciasis kit. Knott's concentration technique and Skin snip test were also performed using blood and skin biopsies respectively. A total of 64(16%) and 20(5%) of the study subjects harboured antigen of *W. bancrofti* and *O. volvulus* respectively. In Ikang community, the prevalence rate of *W. bancrofti* was 12(5.7%). In terms of gender 28(20%) male and 36(13.9%) female subjects were positive for *W. bancrofti* whereas 8(5.7%) male 12(4.6%) female subjects were positive for *onchocerca volvulus* respectively. For both infections although the male subjects were more infected than the female counterparts, the difference was not statistically significant ( $X^2 = 0.96$  and  $0.93$ ;  $P > 0.05$ ). The highest infection rate of 16(14.8%) and 12(13.6%) for *W. bancrofti* was observed in persons within the age range of 26-33 years and 34-41 years respectively. The highest infection of 12(11.1%) for *O. volvulus* was observed in persons within the age range of 26-33 years. However, there was no statistically significant difference in infection rate with to age-groups. Also there was no statistically significant difference in the prevalence of infection with respect to the people occupations ( $X^2 = 0.96$  and  $0.97$ ;  $P > 0.05$ ). However farmers fishermen were more infected i.e 28(23.3%) and 20(35.7%), and 8(6.7%) and 8(14.3%) for *W. bancrofti* and *O. volvulus* respectively. The study has revealed the presence of *Wuchereria bancrofti* and *Onchocerca volvulus* antigens and circulating microfilariae in the blood of residents in Bakassi Local Government Area of Cross River state, Nigeria. It is hereby recommended that efforts should be intensified towards a complete eradication of these diseases in the study area. This can be achieved through massive distribution of ivermectin and albendazole to the local dwellers as well as health education on the mode of transmission of these diseases.

**Keywords:** Antigens, Detection, Lymphatic filariasis, Microfilariae, Onchocerciasis

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### I. Introduction

Lymphatic filariasis commonly known as elephantiasis is a vector borne parasitic disease caused by the filarial worms, *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* which live in the lymphatic vessels and lymph nodes (Cheesbrough, 2006).

Lymphatic filariasis, the second most common vector-borne parasitic disease after malaria, is found in 81 tropical and sub-tropical countries (Ottesen *et al.*, 2008). The World health Organization (WHO) estimates that 120 million people are infected with this parasite and 1.3 million are at risk of infection. It is estimated that 40 million people are suffering from the long term complications of the disease. One third of people infected with lymphatic filariasis live in Africa, another one third live in India and the remainder live in the Americas, The Pacific Islands, Papua New Guinea and South East Asia (WHO, 2008).

Chronic filarial disease has serious social and economic effects. Those afflicted with elephantiasis and hydrocele are often socially marginalized and poor. Acute attacks and disability cut economic output and increase poverty (Ottesen, 2000). This is evident from the observation that 94% of the countries with the lowest human development index (HDI) are endemic for lymphatic filariasis (Durrheim *et al.*, 2004). The chronic manifestations of filariasis can have significant and often very negative, social impact. Lymphatic filariasis has

traditionally been considered to be a disease associated with poverty, inadequate sanitation and underdevelopment (Galvez Tan, 2003).

While the infection may be acquired during childhood, its visible manifestations may occur later in life, causing temporary or permanent disability. In endemic countries, lymphatic filariasis has a major social and economic impact, with an annual loss of one billion dollars and impairing activity up to 88% (Katarbarwa M. *et al.*, 2008).

Among the above listed parasites, *Wuchereria bancrofti* is responsible for 90% of the cases (WHO, 2002). *Brugia malayi* and *Brugia timori* are responsible for the remainder of the cases.

The mosquito vectors of *W. Bancrofti* have a preference for human blood; humans are apparently the only animal naturally infected with *W. bancrofti*. There is no reservoir host and the disease could therefore potentially be eradicated (King *et al.*, 2000).

Onchocerciasis is an infection caused by a filarial parasite known as *Onchocerca volvulus* and transmitted by black flies belonging to the genus *Simulium*. It is commonly known as river blindness (Murray, 2013). He also classified it as the second most common cause of blindness due to infection after trachoma. These flies breed in fast flowing streams and rivers, increasing the risk of blindness to individuals living nearby. Another pathological condition of onchocerciasis is skin disfigurement (Abegunde *et al.*, 2016).

The worm is spread by the bites of a black fly of the *Simulium* species. Usually many bites are required before infection occurs. These flies live near rivers, hence the common name of the disease (WHO, 2014). Once inside a person, the adult female worms produce larvae that reside in the skin (WHO, 2014) where they can infect the next black fly that bites the person.

There are a number of ways to make the diagnosis, including: placing a biopsy of the skin in normal saline and watching for the larvae to come out, looking in the eye for larvae, and looking within the bumps under the skin for adult worms (Abegunde *et al.*, 2016). A vaccine against the disease does not exist: prevention is by avoiding being bitten by flies (WHO, 2014)

About 15.5 million people are infected with river blindness (GBD, 2015). Approximately 2.8 million people have some level of loss of vision from the infection. Most infections occur in sub-Saharan Africa, although cases have also been reported in Yemen and isolated areas of Central and South America. (Reddy *et al.*, 2007).

The failure to contain various infectious diseases biologically or environmentally usually results in the wide spread of such diseases. Also, ignorance regarding their effect and how to control them play a key role in their prevalence. In the case of lymphatic filariasis and onchocerciasis, there is need to understand the roles they play in affecting the social and economic variables of communities, populations and the world at large.

This study seeks to create awareness on their prevalence and pathogenicity and how to control them. This study is providing information on the prevalence of lymphatic filariasis and Onchocerciasis, among the people of Bakassi Local Government Area of Cross River State using SD bioline Oncho/Lf bplex test kit in the detection of Onchocerciasis/lymphatic filariasis

## **II. Materials And Methods**

This study was conducted in Bakassi Local Government Area of Cross River State, Nigeria. The ethical consideration was obtained from the Cross River State Ministry of Health and the consent of the participants was sought. Four hundred subjects resident in the study area were enrolled in the study. The subjects were males and females aged 18 to 50 years.

Structured questionnaires were administered to get information about age, history of the diseases, educational background and social life from the consented subjects.

Two millimetres of blood sample was collected from each participant through venepuncture between 10-11pm on each visit to test for *W. bancrofti* *wb123* and *Onchocerca volvulus* *ov16* antigens.

### **Immunochromatographic method**

Immunochromatographic method using the SD bioline Oncho/LF bplex test kit.

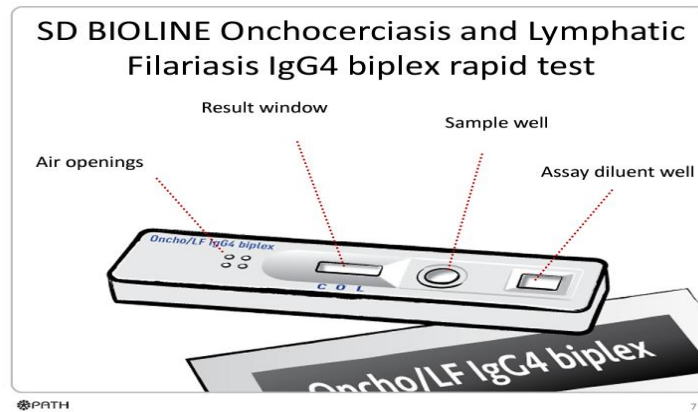
### **Principle**

SD Bioline oncho/LF test cassette contains a membrane strip which is precoated with a monoclonal antibody. The test lines are precoated with polyclonal antibodies specific to the *Onchocerca volvulus* *ov16* and *Wuchereria bancrofti* *Wb123* antigens in human serum, plasma or whole blood. The test utilizes the principle of immunochromatography. As the specimen flows through the membrane after the addition of the assay diluent, the colored colloidal gold conjugates of anti *Ov16* and *Wb123* move to the test line region where they are immobilized by the polyclonal anti *Ov16* and *Wb123* which indicates a reactive (positive) test result. The absence of a coloured band in the test regions indicates a non reactive (negative) test result. The control line is

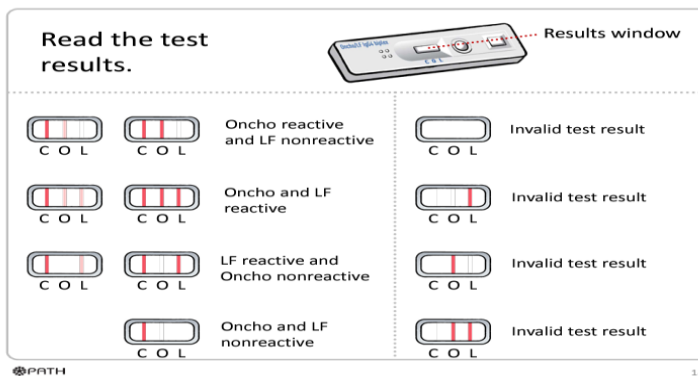
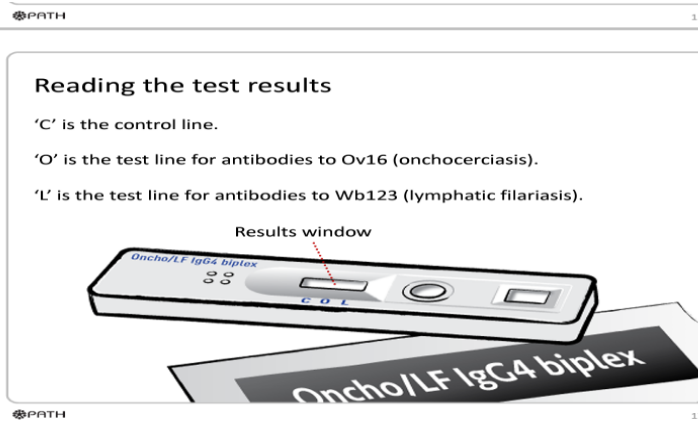
used for procedural control and should appear when blood is added to the test device and the test reagents are stable.

**Procedure**

- The participant identification numbers were written on the test cards
- The area of collection was swabbed with alcohol
- Using sterile syringes 2ml of blood was gotten from each participants
- The blood was dispensed into Ethylene Diamine Tetracetic Acid (EDTA) bottles
- The capillary pipette was used to collect blood up to the 10ul mark
- The blood was added into the sample well of the test kit and the blood allowed to absorb into the pad.
- Four drops of assay diluents were put into the square assay diluents well.
- The test results were read through the viewing window after 20 minutes
- Results were recorded .



**Fig.1:** Showing the SD Bioline Onchocerciasis and lymphatic filariasis IgG4 biphase and rapid test



**Fig.2:** Showing how results are read

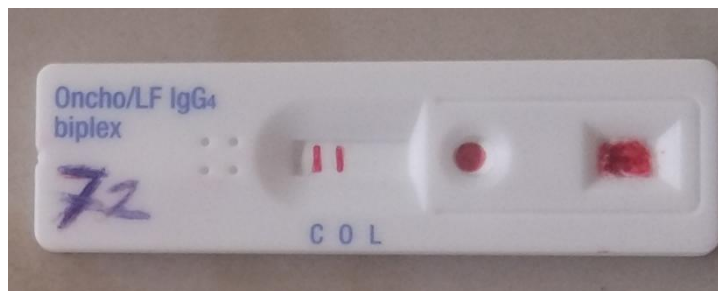


Fig.3: Showing positive result for *Onchocerca volvulus* antigen

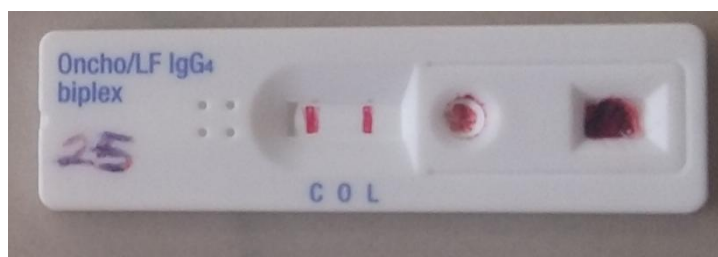


Fig.4: Showing positive result for *Wuchereria bancrofti* antigen

#### Knotts concentration method

- 1ml of venous blood was collected and placed in a tube containing the anticoagulant Ethylene Diamine Tetracetic Acid (EDTA)
- 9ml of 1% formalin was placed in the tube and mixed thoroughly
- The tube was centrifuged at 3,000 rpm for 5 minutes.
- The supernatant was discarded.
- A wet smear from the deposit was made on a clean grease free slide.
- The smear was allowed to dry in air
- It was stained with 3% Giemsa solution for 30 minutes
- The slide was rinsed in water and allowed to dry in air
- The stained smear was examined with X10 objective lens to detect any microfilariae. Identification of microfilariae was done by examination under X100 objective lens with immersion oil applied.

#### Skin snip test

- The back of the participant was observed for nodule or patches and was swabbed with an antiseptic
- A fold of the skin was squeezed and a tiny slice of the skin was removed with a sterile razor blade
- The skin snip was teased and was placed in saline and was viewed under X10 objective lens to look for microfilaria of *Onchocerca volvulus*
- The microfilaria load was estimated by counting the emerging microfilaria.

### III. Results

Table 1 shows the prevalence of *W. bancrofti* Wb123 and *Onchocercavolvulus* Ov16 antigens detected according to the sample location. Out of the 400 persons examined, the prevalence of *W. bancrofti* Wb123 was 64(16%) while that of *Onchocercavolvulus* Ov16 antigens was 20(5%). Ekpri-Ikang had the highest prevalence for *W. bancrofti* with 24(22.2%), while Ikang community had the least prevalence with 28(13.2%). Ikang had the highest prevalence for *O. volvulus* with 16(7.6%) followed by Ekpri-Ikang 4(5.9%) while Ebot-20 had no infection. There was no statistical significant difference in the prevalence of *W. bancrofti* and *O. volvulus* antigens according to locations ( $X^2=0.76$ ,  $P>0.05$ ).

The prevalence of *Wuchereria bancrofti* and *Onchocerca volvulus* in the study area by the presence of microfilaria is presented in Table 2. Out of the 400 persons examined in the study areas, 20(5%) people were positive for *Wuchereria bancrofti* microfilaria while 8 (2%) people were positive for microfilaria of *Onchocerca volvulus*. Ikang people were more infected with *W. bancrofti* 12 ( 5.7% ) followed by Ebot-20 4(5%) and lastly by Ekpri Ikang with 4(3,7%). Ekpri Ikang had the highest prevalence for *O. volvulus* with 4 ( 3.7%) while Ebot-20 had no prevalence for *O. volvulus*. There was no statistically significant difference in the distribution of both parasites according to location. ( $X^2=0.88$ ;  $P>0.05$ ).

The prevalence of *W. bancrofti* and *O. volvulus* antigens detected according to age-range of the individuals is shown in Table 3. The age group ranging from 42-49 had more prevalence for *W. bancrofti*

antigens with 20( 55.6%) while the age group ranging from 18-25 had the least prevalence for *W. bancrofti* with 4(4.2%). The age group 26-33 years had more prevalence for *O. volvulus* with 12( 11.1%) while 34-41 had no parasites. There was no statistical significant difference in the distribution of parasites according to age groups ( $X^2=0.87$  and  $0.51$ ;  $P>0.05$ ).

The prevalence of Antigenaemia of *W. bancrofti* and *O. volvulus* antigens by gender is shown in Table 4. Male subjects were more infected with *W. bancrofti* 28(20%) while females had the least prevalence for *W. bancrofti* with 36(13.9%). The prevalence of *O. volvulus* among males was 8(5.7%) while females had 12(4.6%). Although the infection rate was higher in males than females in both cases, there was no statistical significant difference in the distribution of parasites according to gender ( $X^2=0.96$  and  $0.93$ ;  $P>0.05$ ) respectively

The prevalence of *W. bancrofti* and *O. volvulus* by presence of microfilaria by gender is shown in Table 5. Out of the 140 males and 260 females examined respectively, the males had more prevalence for *W. bancrofti* and *O. volvulus* microfilaria with 12(8.6%) and 4(2.9%) respectively while females had the least with 8(3.1%) and 4(1.5%). There was no statistically significant difference in the presence of microfilaria in both females and males ( $X^2=0.93$ ;  $P>0.05$ ).

The prevalence of *W. bancrofti* antigen and *O. volvulus* antigen according to occupation is shown in Table 6. Out of the 400 subjects examined, fishermen had the highest number of infection for *W. bancrofti* and *O. volvulus* antigen with 20(35.7%) and 8(6.7%) respectively followed by farmers 28(23.3%) and 8(6.7%) and lastly the students with 4(3%) infection with *W. bancrofti*. There was no statistical significant difference ( $X^2=0.96$  and  $0.97$ ;  $P>0.05$ ) according to the occupations of the people in the study area for both *W. bancrofti* and *O. volvulus* antigens respectively.

The prevalence of *W. bancrofti* and *O. volvulus microfilariae* by occupation of subjects using Knott's concentration method and skin snip test is shown in Table 7. Out of the 120 farmers examined, 8(6.7%) and 4(3.3%) were positive using Knott's concentration in both cases. Among 56 fishermen, 4(7.1%) were positive for *W. bancrofti* only. Among 92 traders examined, 8(8.7%) and 4(4.4%) reacted positive to Knott concentration technique and skin snip test for *W. bancrofti* and *O. volvulu* respectively. Among 132 students examined, no infection was recorded. There was also no statistical significant difference ( $X^2=0.93$  and  $0.85$ ) according to the subjects' occupation using Knott's concentration techniques and skin snip test.

**Table 1: Prevalence of *Wuchereria bancrofti* Wb123 and *Onchocerca volvulus* Ov16 antigens by sample location**

Location	Number Examined	No. (%) positive by <i>Wuchereria bancrofti</i> Wb123	No. (%) positive by <i>Onchocerca volvulus</i> Ov16
Ekpri-Ikang	108	24(22.2)	4(5.9)
Ikang	212	28(13.2)	16(7.6)
Ebot 20	80	12(15)	0(0)
<b>Total</b>	<b>400</b>	<b>64(16)</b>	<b>20(5)</b>

**Table 2: Prevalence of *Wuchereria bancrofti* and Onchocerciasis in the study area by presence of microfilaria**

Location	Number examined	No. (%) of positive by <i>Wuchereria bancrofti</i>	No. (%) of positive by <i>Onchocerca volvulus</i>
Ekpri-Ikang	108	4(3.7)	4(3.7)
Ikang	112	12(5.7)	4(1.9)
Ebot 20	80	4(5)	-
<b>Total</b>	<b>400</b>	<b>20(5)</b>	<b>8(2)</b>

**Table 3: Prevalence of *Wuchereria bancrofti* antigen Wb123 and *Onchocerca volvulus* antigen Ov16 by Age**

Age range (years)	Number Examined	No.(%) positive for <i>Wuchereria bancrofti</i> Wb123	No.(%) positive for <i>Onchocerca volvulus</i> Ov16
18-25	106	4(4.2)	4(4.2)
26-33	108	16(14.8)	12(11.1)
34-41	80	12(15)	0(0)
42-49	76	20(55.6)	4(5.3)
>50	30	-	-
<b>Total</b>	<b>400</b>	<b>64(16)</b>	<b>20(5)</b>

**Table 4: Prevalence of Antigenemia of *Wuchereria bancrofti* antigen *Wb123* and *Onchocerca volvulus* antigen *Ov16* by gender**

Gender	Number examined	No.(%) positive for <i>Wuchereria bancrofti</i> <i>Wb123</i>	No.(%) positive for <i>Onchocerca volvulus</i> <i>Ov16</i>
Male	140	28(20)	8(5.7)
female	260	36(13.9)	12(4.6)
<b>Total</b>	<b>400</b>	<b>64(16)</b>	<b>20(5)</b>

**Table 5: Prevalence of *Wuchereria bancrofti* and *Onchocerca volvulus* by presence of microfilaria by gender**

Gender	Number examined	No.(%) positive for <i>Wuchereria bancrofti</i>	No.(%) positive for <i>Onchocerca volvulus</i>
Male	140	12(8.6)	4(2.9)
Female	260	8(3.1)	4(1.5)
<b>Total</b>	<b>400</b>	<b>20(5)</b>	<b>8(2)</b>

**Table 6: Prevalence of *Wuchereria bancrofti* antigen *wb123* and *Onchocercavolvulus* antigen *ov16* by occupation**

Occupation	Number Examined	No.(%) positive for <i>Wuchereria bancrofti</i> antigen <i>Wb123</i>	No.(%) positive for <i>Onchocercavolvulus</i> antigen <i>Ov16</i>
Farmers	120	28(23.3)	8(6.7)
Fishermen	56	20(35.7)	8(14.3)
Traders	92	12(1.3)	4(4.4)
Students	132	4(3.0)	-
<b>Total</b>	<b>400</b>	<b>16(16)</b>	<b>20(5)</b>

**Table 7: Prevalence of *Wuchereria bancrofti* and *Onchorcerca volvulus microfilariae* by occupation of subjects using Knott Concentration method and skin snip test**

Occupation	Number Examined	No.(%) positive for <i>Wuchereria bancrofti</i>	No.(%) positive for <i>Onchocerca volvulus</i>
Farmers	120	8(6.7)	4(3.3)
Fishermen	56	4(7.1)	-
Traders	92	8(8.7)	4(4.4)
Students	132	-	-
<b>Total</b>	<b>400</b>	<b>20 (5)</b>	<b>8(2)</b>

#### IV. Discussions

The prevalence of lymphatic filariasis and onchocerciasis has been established in different parts of Nigeria by different authors. The rate of occurrence as reported by different researchers varies from one geographical area to another.

The results of this study in three communities in Bakassi Local Government Area of Cross River State has demonstrated a low prevalence for Bancroftian filariasis and onchocerciasis with 16% and 5% respectively, which is lower than the 48% recorded by (Mbah *et al.*, 2010) who carried out research studies on the diagnosis and transmission dynamics of lymphatic filariasis in Biase local government area of Cross River State.

This study also shows a lower prevalence of the endemicity of onchocerciasis in Bakassi Local Government Area. The prevalence rate observed in the study for onchocerciasis is 5% which is lower than the 83% recorded in Ovia Northeast Local Government area of Edo State by (Akimbo and Okaka, 2010).

Similar studies were carried out by Udoidung *et al.*, (2008) on “The current status of bancroftian filariasis in rural communities of the Cross River basin” and they recorded a 6.5% prevalence which is lower than the result gotten in this study. But Akinboye *et al.*, 2010 on the other hand recorded a higher prevalence of 52% in their research work on onchocerciasis in Ibarapa Local Government Area of Oyo State, Nigeria.

Infection rates observed in this study showed that subjects between the ages of 26 and 49 had higher prevalence of filariasis and onchocerciasis than the ages below and above them. The results were generally observed lower than that by (Uttah *et al.*, 2004); Nmorsi (2002) who recorded a very high statistical significance, with subjects within the ages of 30 and 50 having a high prevalence of filariasis and onchocerciasis.

The low prevalence of the infections in younger and older individuals must have been associated with reduced exposure and a long time of enhanced immunity respectively (Uttah *et al.*, 2004). The low prevalence

of lymphatic filariasis and onchocerciasis recorded in the areas of this study is due to the probable efficacy of the mass drug administration of ivermectin to the local dwellers by the onchocerciasis control programme of the Cross River State Ministry of Health.

The prevalence of lymphatic filariasis and onchocerciasis in this study was similar to that of Atu, 2003, Nnaji and Ozor, 2001 with males infection higher than females although there was no statistically significant difference in the occurrence of diseases by gender ( $X^2=0.96$  and  $0.93$ ;  $P>0.05$ ).

The prevalence of filariasis in this study showed that although infection rates were more common with farmers and fishermen than with traders and students, there was no statistically significant difference in the occurrence of lymphatic filariasis and Onchocerciasis by occupation ( $X^2=0.96$  and  $0.97$ ;  $P>0.05$ ). The high infection rates between farmers and fishermen could be explained by the work done by Wogu and Okaka. (2008), who observed that, engagement in outdoor activities close to breeding sites by farmers and fishermen exposed them to a high risk of infection.

The relationship between man and his environment is symbiotic. The environment influences human activities; those activities in turn influence the nature of the environment. Physical, social and economic factors are very important factors that determine the severity of lymphatic filariasis and onchocerciasis in a given society (Oye, 2008).

## V. Conclusion

The result of this study has shown the prevalence of *W. bancrofti* as 64(16%) and *Onchocerciasis* as 20(5%). Lymphatic filariasis and Onchocerciasis still remain public health problem, and government should intensify efforts for their complete eradication through mass drug chemotherapy.

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