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# ASSESSMENT OF THE PRESENCE OF SIMULIUM SPP BLACKFLIES AND ONCHOCERCIASIS IN SELECTED COMMUNITIES AROUND AWHUM WATERFALLS, A TOURIST SITE IN UDI LGA OF ENUGU STATE, NIGERIA

**[1]Chikezie, C. I., *[2]Uzoka, N. B., [2]Dalla, C. V., [3]Monday-Chukwu, O., [2]Iyam, O. I., [2]Onwe, D. A., [1]Machina, I. B., [2]Edeh, G. C., [3]Onyebuchi, F., [2]Maduforo, A. S., [2]Awam, C. O. and [3]Ani, N. M.**

[1]Nigerian Institute for Trypanosomiasis Research (NITR), Surami Road, Kaduna State, Nigeria.
[2]Nigerian Institute for Trypanosomiasis Research (NITR), South East Zonal Office, Enugu State, Nigeria.
[3]Nigerian Institute for Trypanosomiasis Research (NITR), Liaison Office, Orra-Ozziza, Ebonyi State, Nigeria.

*Corresponding authors' email: ngozi.uzoka@yahoo.com Phone: +2348023757033

## ABSTRACT

The study is an assessment of the abundance of blackflies (*Simulium spp*.) and Onchocerciasis in selected communities around Awhum waterfalls, a tourist site in Udi LGA of Enugu State, Nigeria. For the entomological aspect of this work, traps were set to catch and collect black flies in and around the Awhum Monastery which does not only serve as a tourist site but also a religious pilgrimage ground. Serological screening using Standard diagnostics test kits for Onchocerciasis (SD-Bioline OV16 kits) was used to screen 125 participants, who were members of the communities ranging from ages 5 – 70 years and comprising of 50 males and 75 females. The result revealed that only 28 (22.4%) of the participants were positive while majority 97(77.60%) were negative. Nineteen (19) positive cases were confirmed by molecular analysis of mitochondrial cytochrome oxidase subunit I (COI) gene. Of the total positive cases, more males 16(57.1 4%) than females 12(42.86%) were infected with *Oncocerca volvulus*. The findings confirmed the presence of blackflies and the endemicity of Onchocerciasis (River Blindness) in communities around the Awhum waterfalls, a tourist site in Awhum, Udi Local Government Area of Enugu State.

**Keywords**: Onchocerciasis, Blackfly, *Onchocerca volvulus*, Awhum, Tourist

## INTRODUCTION

Onchocerciasis also known as River Blindness is caused by a parasitic filarial worm known as *Onchocerca volvulus* (Nworie *et al.,* 2014; WHO, 2022). The disease is transmitted to humans when bitten by an infected *Simulium spp.* (black fly) of the family Simulidae (Eezzuilemoi and Wilson, 2017). The black flies breed in environments with fast flowing water bodies, thereby increasing the possibility of infection among people living around these water bodies (Ikpo *et al.,* 2016).

The disease affects more than 120 million people in 37 countries in African with Latin America and Yemen also at risk and over 37 million of the infected people are mostly rural communities dwellers (Nworie *et al.,* 2014; WHO 2017; Pukuma *et al*., 2023). Nigeria accounts for 40% of all onchocerciasis cases with greater frequency more than any other country in the world with about 50 million persons in about 40, 000 communities in 32 States at risk (Bump *et al.,* 2004; Ikpo *et al.,* 2016). Nigeria is among countries that are most endemic to Onchocerciasis (Noma *et al*., 2014) with prevalence in all her states except Bayelsa, Katsina, Lagos and Rivers as reported in the Nigeria onchocerciasis elimination plan (Busari *et al*., 2022).

In Enugu State Onchocerciasis is endemic in 16 Local Government Areas out of the 17 Local Government Areas of the state. About 1377 villages affected, 818 villages are hyper-endemic while 559 villages are meso-endemic (FMOH, 2017).

Onchocerciasis causes intense itching, lesions, altered skin manifestation in the form of leopard skin or lizard skin and irreversible blindness (Eneanya and Nwaorgu, 2001; Eyo *et al.,* 2013). Onchocerciasis can be diagnosed through clinical manifestation and microscopic examination of skin samples for the presence of microfilaria. This technique has some limitations because it has a high incidence of false-negative as in most cases microfilaria are not present in the skin samples of infected patients under examination which makes it difficult to accurately determine the prevalence rate of the disease (Ikani, *et al*., 2022).

Onchocerciasis control programs have recently utilized the serological method, Ov16 antibody testing by enzyme-linked immunosorbent assay (ELISA) to detect IgG4 antibodies to the *Onchocerca volvulus* Ov16 antigen (Lipner *et al*., 2006; Rodrıguez-Perez *et al*., 2011; Schmidt *et al*., 2019).), together with entomological surveillance according to WHO guidelines to demonstrate elimination of Onchocerciasis transmission, and also the use of rapid diagnostic tests (WHO, 2016). Ov16 assay is a more sensitive method for the detection *Onchocerca volvulus* parasites than parasitological surveys and also more useful in the of elimination programs at the advanced stages of the disease. The serological methods are less invasive and requires only a blood sample from a finger prick from individuals (Rodrıguez-Perez *et al*., 2011).

The detection of *Onchocerca volvulus* DNA in infected individuals and in the *Simulium spp*. black fly vectors of onchocerciasis using molecular technique can serve as a proxy for the detection of parasite presence in the humans and black flies (Pryce *et al.,* 2021). The Polymerase Chain Reaction (PCR) is a molecular method that makes use of body cells from skin-snips to confirm parasitic presence. It is more sensitive and specific than the other conventional methods of parasite detection (Boatin *et al.,* 1998). The use of Polymerase chain reaction (PCR) technology, has helped the in early diagnosis of the disease as well as expression of the parasite genome (Cotton *et al*., 2016).

Onchocerciasis has quite a number of implications on the people, the economy and on the areas where it is endemic. In tourist areas, its effect results in reduction in the usefulness of the tourist site and consequent economic loss. Visitors who may want to come to the tourist centers might be discouraged upon their knowledge of the high density of black fly population in the area. This may affect economic activities in that area (WHO, 2017). Tourists who visit endemic areas for

a prolonged period of time are at a higher risk of infection. Also, it has been reported that among the 85 million people living in endemic areas, 18 million estimated people are infected, 4 million people suffer skin diseases, while 2 million are blind or visually impaired as a result of Onchocerciasis (Marie, 2010).

It is against this backdrop that emanates the need for serious and continuous examination of such tourist areas harboring breeding grounds for black flies, so as to achieve control of onchocerciasis and its vector, and subsequent elimination of the disease. Therefore, this study was done to carry out an entomological and a serological assessment of the presence of blackflies (*Simulium spp*.) and Onchocerciasis in selected community dwellers around Awhum waterfalls, a tourist site in Udi LGA of Enugu State, Nigeria for future Onchocerciasis remediation in this endemic area hosting many tourists from different parts of Nigeria and beyond.

## MATERIALS AND METHODS

### Study Area

This study was carried out at Awhum in Udi Local Government Area Enugu State where Awhum waterfall, a tourist center is located (Usifo. 2018). The study area is located on a Longitude of $7^0$25'0" East and Latitude of $6^0$32'0" North with altitude of 30 meters high. The weather condition around the site consists of rainy season from April to September, and dry season from December to March (Duke, 2017). The Awhum town has a population of about 100,000 people according to the 2006 census with most of this population made up of farmers, traders, students, teachers, artisans and civil servants. The valley town can boast of various lakes and waterfalls. It is famous for its capability of dispelling satanic or evil forces, and this was the reason the site became a place of Christian pilgrimage, a prayer center and a tourist site. Visitors from different parts of the country and overseas flock to the site all through the year. The waterfalls and lakes are managed by the Abbey, which is a Roman Catholic Monastery situated close to the waterfall (Duke, 2017).

### Study Design

The research was a cross sectional and qualitative study. It was done in two phases; the pre-survey and the main survey. The study population constituted of young and adult males and females who have resided in the community for not less than a year, and are between 5 – 70 years of age. Demographics of participants were collected using a specially designed form. Questions on knowledge of the disease and its vector were also elicited from the study subjects and entered in the form. Information was also collected from participants in the Focused Group Discussion (FGD) and in-depth interviews conducted (ESMH, 2005; Okafor *et al*., 2016). Traps were set to catch and collect black flies in and around the Awhum Monastery while Serological screening for the disease was done using OV16 diagnostic kits for Onchocerciasis and positive cases were confirmed using Polymerase Chain Reaction (PCR) amplification of mitochondrial cytochrome oxidase subunit I (COI) gene.

### Collection of Blackflies

Blackfly traps were set at the Awhum Monastery and waterfalls. The trap consists of Soda Cups that contained a mixture of yeast, distilled water and sugar, meant to attract and capture the flies (Denis *et al*., 2017). The collectiom of blackflies followed the protocols by Service, (2007) and Hernández-Triana, (2007). Some flies were also captured using human baits (Opara *et al*., 2005; Hendy, A. *et al*. 2021). The captured black flies were counted and preserved in accordance with Opara *et al*., (2005). The blackflies caught were morphologically identified to be of Simulium family with an optical microscope using standard keys for identification of *Simulium spp* (Service, 1977).

### Serological Rapid Diagnostic Testing

The bodies of the participants were examined physically by palpating to detect clinical signs of the Onchocerciasis. The upper parts of their bodies were felt for the presence of nodules (sites of adult female worm activities) and their eyes were examined to check for impaired sight or blindness. After the physical examinations, the middle fingers of the participants were pricked with lancets and blood sample collected and diagnosed using the Standard Diagnostic OV16 Bioline Kits according to methods by Pukuma *et al*., (2023), Lipner *et al*., (2006) and Weil *et al*., (2000). Blood samples were also taken from the participants on filter papers that were stored for further confirmatory analysis.

### Molecular Analysis

Twenty (20) samples that were positive using the OV16 Standard BIOLINE Onchocerciasis test strips were subjected to PCR amplification of the mitochondrial cytochrome oxidase subunit I (COI) gene of *Onchocerca volvulus.* DNA was extracted from the blood spotted on filter paper using Accuprep® Genomic DNA extraction kit (Bioneer, South Korea) following the manufacturer's instructions. Briefly, the spotted portion of the filter paper was cut into smaller pieces and was transferred into a 1.5 ml tube, 20 µl of proteinase K, 10 µl of RNase A and 200 µl of tissue lysis buffer was added to the tube and was incubated at 60 $^oC$ for 45mins, after the incubation 200 µl of GB buffer was added and mixed by vortexing, then 400 µl of chilled ethanol was added and mixed by pipetting, the contents was then transferred into binding column attached to a collecting tube and centrifuge at 8000 rpm for one minute, the manufacturer's procedure is then followed to the end.

PCR was carried out with Accupower® PCR Premix (Bioneer South Korea) in a total reaction volume to 20µl containing 2µl of the extracted DNA, 1µl each of the forward and reverse primers and 16µl of nuclease free water. Amplification was done with primers; Ov F 3' TGTGGAAATTCACCTAAATAT 5'; Ov R 5' AATAACTGATGACCTATGACC 3'. The samples were transferred to the thermal cycler and amplification was performed under the following conditions; 95$^oc$ for 5 min followed by 35 cycles (94 $^oC$ for 30s, 55 $^oC$ for 45s, 72 $^oC$ for 1min) and final extention for 10mins at 72 $^oC$. The PCR amplicons were further separated in 1% agrose gel stain with ethidium bromide at 100 volts for 1 hour. Amplicons size of approximately 305bp were observed as shown in the figure 1.

### Ethical Approval and research consent

Ethical clearance was obtained from the State Ethical Committee, Enugu State Ministry of Health. Prior to the commencement of study, a pre-survey visit was made to the Guest Master representing the Roman Catholic Monastery near the waterfall, in order to be granted access to the waterfall and the environment of the monastery. The research team also met with the Awhum community acting paramount ruler, other leaders and members of the community where research benefits explained to them. Informed consent to participate in the study was also gotten.

**Data Analysis**

Data collected from this study were analysed using SPSS 26 and Excel 2016. Descriptive statistics was used and results were presented in percentages, tables and graphs.

## RESULTS AND DISCUSSION

**Entomological catch and fly identification**

A total of 32 blackflies were caught during the survey and were identified morphologically to be of the Simulium family.

Figure 1: Image of blackfly feeding on exposed skin
Source: Purdue, 2025.

**Interview and Focused Group Discussion**

The interview and focused group discussion mainly centered on the knowledge and therapeutic measures of the participants towards Onchocerciasis and its vector. It was discovered that majority of them knew nothing about the disease and its cause but could only attest to the bites of a strange fly in their farms and streams which they termed ‘‘Otaokunenye” (bite and fly away) in their local dialect that leaves them debilitated after series of bites. However, some of them thought it to be ‘‘Ose’’ (tsetse fly), or ‘‘Kpishikpishi’’ (sunfly). Though they admitted the routine administration of ivermectin and its efficacy against Onchocerciasis through the Community Directed Treatment with Ivermectin (CDTI) intervention in their communities, they were still skeptical about the drug as most of them especially the men complained about general body weakness, rashes and the fact that they would have to abscond from alcohol intake after taking the drug.

**Serology (Rapid Diagnostic testing with OV16 test kits)**

**Table 1: Onchocerciasis prevalence among individuals around Awhum waterfalls**

| | Male | Female | Total |
|---|---|---|---|
| Positive | 16 (57.14 %) | 12 (42.82 %) | 28 (22.40 %) |
| Negative | 34 (35.05 %) | 63 (64.95 %) | 97 (77.60 %) |
| | 50 (40.00 %) | 75 (60.00 %) | 125 (100.00 %) |

A total of 125 samples were analyzed, all samples gotten randomly from participants who presented themselves ranging from 5 – 70 years and above. The OV16 of the samples showed that 28 persons (22.4 %) out of the total 125 participants tested positive to onchocerciasis, while 97 persons (77.6 %) tested negative to the disease (table 1). The positive cases comprise of 16 (57.14 %) males and 12 (42.86 %) females. For the negative cases the number of the males was 34 (35.05 %) and the females were 63 (64.95 %) (figure 1 and 2).

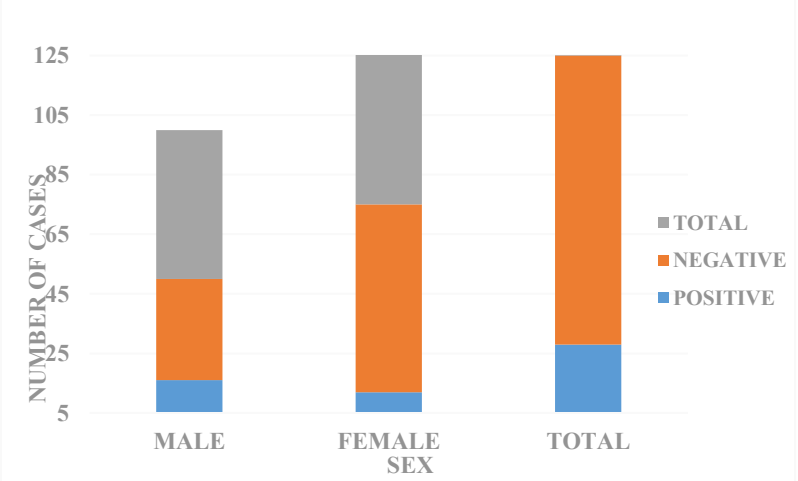



Figure 1: Sex prevalence rate of Onchocerciasis of individuals around Awhum waterfalls

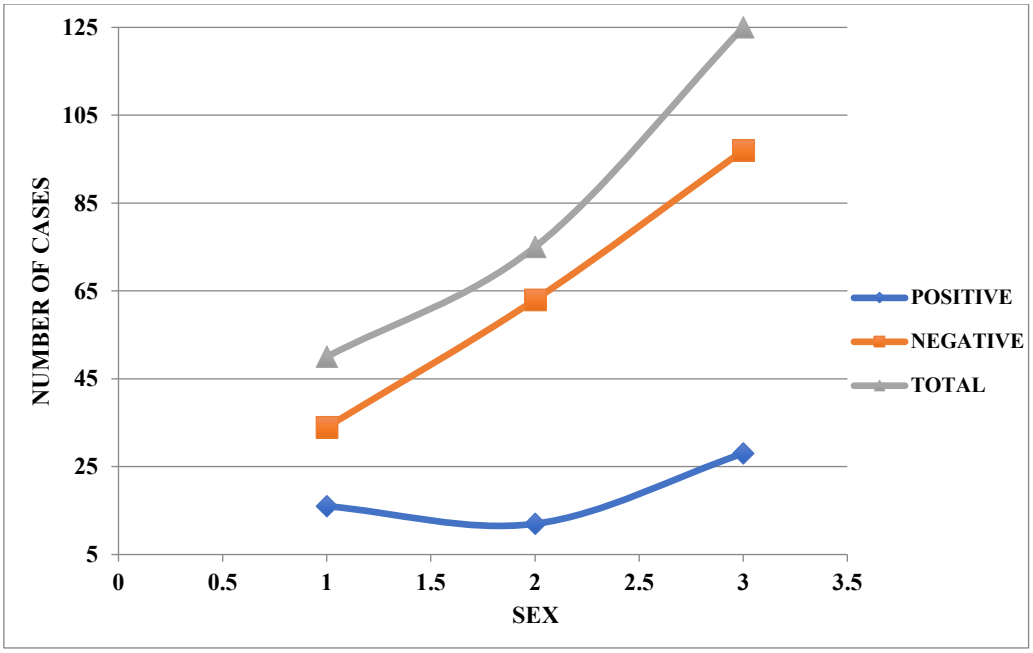
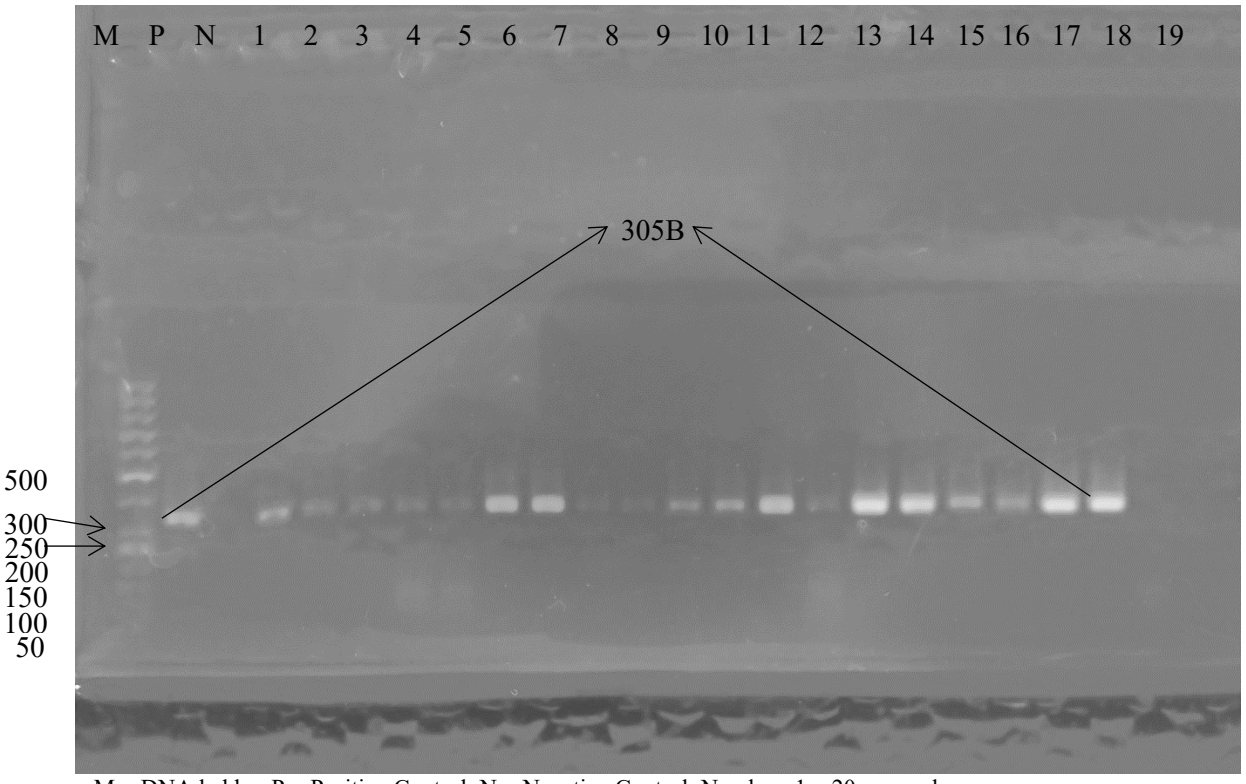



Figure 2: Graph plot of prevalence rate of Onchocerciasis among individuals around Awhum waterfalls

**Molecular Analysis and Gel Electrophoresis**

Nineteen positive cases (1-19) from the twenty positive cases recorded from the Standard Diagnostic OV16 rapid tests showed marked amplification of mitochondrial cytochrome oxidase subunit I (COI) gene. Visualisation of the gel image performed on the obtained sequences showed amplification of 305bp DNA sequence (figure 3).




M = DNA ladder; P = Positive Control; N = Negative Control; Numbers 1 – 20 = samples

Figure 3: Agarose gel visualized image showing the amplified DNA products.

**Discussion**

The study revealed a substantial presence of blackflies and a total Onchocerciasis infection rate of 22.40 %, which were clear indications of the presence of the disease vector and a marker of the endemicity of Onchocerciasis among community dwellers around the Awhum waterfalls, a tourist site in Udi Local Government Area, Enugu State, Nigeria. This might be as a result of the fact that Awhum is a valley town with many lakes and waterfalls that can serve as good oviposition sites and habitats for blackflies. This is in analogy with situations reported in Agbokim, Aningeje, Ekong Anaku and Orimekpang communities in Cross River State (Chikezie

*et al*., 2023), Ahani-Achi community in Enugu State (Chikezie *et al*., 2015 and Wanji *et al*., 2015) in South West Cameroun. This is also similar to the study carried out by Ikani *et al*. (2022), which reported a 30% prevalence rate in Benue and 37% Cross River prevalence when expressed against the 150 patients sampled from each state. The positive molecular identification of *Onchocerca volvolus* among residents of Awhum is also in agreement with Ikani *et al*. (2022), where positive cases for *Onchocerca volvulus* following the amplification of the gene, CO1 subunit of the mtDNA of *Onchocerca volvulus* were reported among people of Benue and Cross River States of Nigeria. The cases of infection tend to be higher among people of this community because these are riverine areas and favour the breeding of black flies (Dumas *et al*., 1998).

Also, the attraction of many people to these waterfalls makes them more exposed to the bites of black flies and consequently leading to the widespread distribution of onchocerciasis in this area. This is in agreement with the previous studies of Nwoke *et al*. (1987), Akogun and Onwuliri (1991); Nwoke (1992) and Ikpo *et al.* (2016), which noted that onchocerciasis was more prevalent in communities with abundant fast flowing rivers and streams.

This study also showed a low prevalence level of Onchocerciasis when compared to other previous studies by Eyo *et al*. (2013) that reported an overall onchocerciasis prevalence rate of 39.02 % in Opi-Agu community, a tropical semi-urban community in Enugu State. Again, in Oji River Local Government Area of Enugu State, Ikpo *et al.* (2016), showed a mesoendemic Onchocerciasis status of 43.58%. Nworie *et al*. (2014) also reported a 32.8 % rate of infection in Ebonyi Central Senatorial Zone and 29.2 % prevalence rate in Ebonyi North Senatorial Zone in Ebonyi State. This observed low level prevalence rate from this study may be attributed to the people's diligence to ivermectin treatment through the CDTI intervention. However, this study is in contrast with the previous work of Ikpo *et al.* (2016), where a hypoendemic onchocerciasis infection rate of 12.20 % was reported in Awgu Local Government Area of Enugu State.

The infection rate of Onchocerciasis based on the gender was seen to be higher in the males (57.14 %) than in the females (42.82 %). This is in conformity with the work of Uzoigwe *et al*., 2012 in Nasarawa State, Nigeria, where the males recorded a prevalence rate of 8.7 % and the females 5.1 %. Eyo *et al*. (2013) noted that the male prevalence rate was higher with an infection rate of 43.24% than that of the females with (35.03 %). Also, Okorie *et al*. (2014), in a study from two geopolitical zones in Ebonyi State found out that there were 38.71 % males and 23.16 % females Onchocerciasis infection rate in Ebonyi Central Zone, while in Ebonyi North Zone, it was 32 % and 25 % for the males and females respectively. According to Okoye and Onwuliri (1997), a higher prevalence rate of onchocerciasis was recorded in the males more than the females among the inhabitants of Hawel River valley, Nigeria.

The observed increase in the onchocerciasis infection rate of the males over the females may stem from the fact that men are usually the breadwinners and family providers. They engage more in some outdoor activities such agricultural production, which make them more exposed to the bites of the black fly and onchocerciasis infection (Anderson *et al*., 1974; Ottensen, 1984; Nwoke, *et al*., 1991; Iroha *et al*., 2010). Again, since Awhum is a center for tourists, the men tend to attract and link up more with visiting tourists who they always tour around the vicinity of the waterfalls and this constitutes another serious disposing factor to the bite's blackfly. These findings nevertheless, were not in tandem with the report of Akinboye *et al*., (2010) who indicated in a study conducted in Ovia North East Local Government Area of Edo State Nigeria, that the onchocerciasis infection rate was higher in the females with 93 % than the males with 74.5 %. The qualitative survey carried out from the study showed that there was still a diminutive knowledge about onchocerciasis and its vector blackfly together with the skepticism of the community dwellers on ivermectin chemotherapy. The findings from this study are pointers that this wonderful center of tourists attraction is yet to be immuned from the menace of Onchocerciasis and its disease vector and so more strategic measures still need to be adopted especially in the areas of community based health re-orientation, ivermectin administration and vector control.

## CONCLUSION

The study confirmed the presence of infected blackflies and showed that Onchocerciasis is still endemic among the community dwellers around the Awhum waterfalls, a tourist site in Awhum, Udi Local Government Area of Enugu State. This is indication that the war against Onchocerciasis and blackly, the disease vector is yet to be won despite the huge investments that have been channeled towards their elimination. Therefore, more concerted efforts are needed by the government, international organizations, the individuals and other key stakeholders to fight this disease to a finish through a more proactive and intentional community directed education, treatment and proper vector control measures.

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