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Sporadic and Hypoendemicity of Onchocerciasis in Awgu, Area of Enugu State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author UDE conceptualized and designed the work, the questionnaire and validated the questionnaire wrote the initial script. Author ACA carried out the day to day running of the work. Authors ACA and UDE and the rest of authors visited the survey areas, distributed the questionnaire and collected blood samples. Authors NBU, FUO, and NCA contributed in writing the work, in editing and statistical works and prove reading. All authors read and approved the final manuscript.

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Abstract

Despite years of mass drug administration to inhabitants of Awgu in Enugu State Nigeria, onchocerciasis has persisted. Evidently the persistence of onchocerciasis is linked to environmental drivers of recrudescence, occupation and anthropogenic activities. There were 0% prevalence rate of body itching among respondents that do not visit streams. Prevalence of body nodules was same for both male and female that visits streams and rivers 50 ± 0.01%. Prevalence of body nodules among females that don't visit rivers and streams was

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3.82±0.12% and the male counterpart was 0.63±0.03%. Prevalence of red eyes among female folks that visits streams and rivers was 60.58 ± 0.04% and 39.42 ± 0.21% for the males. Female folks that do not visit had prevalence of 4.46±0.09% and the males 1.27±0.02%. Molecular identification using DNA analysis of dried blood and primer sequence of OvFWD 5′-TGT GGA AAT TCA CCT AAA TAT G-3′, OvREV 5′-AAT AAC TGA TGA CCT ATG ACC-3′ in a singleplex reaction was employed in the early identification of *Onchocerca volvulus*. Residents were identified under, traders, farmers, civil servants and students. Farmers, traders and civil servants had highest cases of onchocerciasis. Prevalence of body itching among female folks that visit streams and rivers was 57.25 ± 0.32% while their male had 42.75 ± 0.15%. There were no body rashes and leopard skin among the male and female folks that do not visit streams and rivers. Female folks that visit streams and rivers had rashes prevalence of 58.14 ± 0.05% and males 42.85 ± 0.60%. Leopard skin prevalence was equal for both female and male folks that visits streams and rivers 50 ± 0.01. Results suggests that onchocerciasis is associated with anthropogenic activities. Interventions should be an integrated management approach involving mass drug administration, vector ecology and ecosystem manipulations and on provision of portable water.

Keywords: Endemicity; Filaroid worm; Mectizan; onchocerciasis; river blindness.

1. Introduction

Onchocerciasis is very serious but neglected debilitating tropical diseases caused by a nematode *Onchocerca volvulus* (Boatin, 2008; Lloyd et al., 2015; Ncogo et al., 2025; Enyidi et al., 2026). Onchocerciasis is arthropod borne disease caused by infection of *Onchocerca volvulus* which is an transmitted to humans when one gets bitten by black fly *Simulium damnosum* (Crosskey, 1981; Adler et al., 2004; Uttah et al., 2012; Ekpo et al., 2022). Onchocerciasis is reported to be endemic in 37 countries in West, East and Central Africa; the Arabian Peninsula; and parts of South and Central America. Nigeria has the highest number of onchocerciasis endemic people with estimated endemicity of 40% of global prevalence (Richards et al., 2015). It has been reported that in Nigeria Onchocerciasis is prevalent at different stages in estimated 36,000 communities, in 413 local government areas of 32 states of Nigeria, including Abuja (WHO, 1995; NOCP, 2002)., More than 99% of onchocerciasis infected people live in 31 countries in sub-Saharan Africa. World Health Organization (WHO) reported that 14.6 million people are infected with onchocerciasis globally, while 220 million people needs serious preventive chemotherapy (WHO, 2023; World Health Organization, 2023). Female filaroid worms can live in the subcutaneous nodules for 15 years and would produce millions of young worms that are microscopic called microfilariae that are sent into the blood stream of the victims. The microfilariae that are released by the female *O. volvulus* into the blood, migrate to the skin and the eyes (Kale, 1998). The released microfilariae swim under subcutaneous tissue of the hosts skin and gets picked up by the female *S. damnosum* when it bites the victim during blood meal (WHO, 2023). Microfilariae migrate to different parts of the body like skin ans eyes causing severe health problems like itching, blurred vision, redness of eye, severe dermatitis and leopard skin (Liese et al., 1991; Vlassoff et al., 2000; Diawara et al., 2009; Enyidi et al., 2026). There had been series of onchocerca control programs in Africa and Nigeria in particular (Gemade et al., 1998, Opara et al. 2005; Ityonzughul et al., 2024). Most of the interventions had been through mass drug administration and larvicidal spraying using helicopters (Liese et al., 1991) and MDA, In Nigeria, Tekle et al. (2012), Onah et al. (2020), Richards et al. (2015). Based on the results of rapid epidemiological mapping of onchocerciasis in Nigeria between 1994 to1996, it was noted that about 50 million Nigerians are at risk and Nigeria has highest prevalence of the disease in the World (Gemade et al., 1998; Ogbonna & Ikani, 2020). Nigerian and African governments government have tried to combat onchocerciasis scourge by establishing African Program for Onchocerciasis Control (APOC) and Nigerian onchocerciasis control program (OCP) (Homeida et al., 2002). The African APOC and the Nigerian OCP, aimed at using anti helminthic drug ivermectin (mectizan) for community-directed treatment (CDTI) of onchocerciasis, Homeida et al. (2002) The CDTI was carried out over the years between 1992 to 2017, at Plateau, Nasarawa (Richards et al., 2015), Kaduna state 1991 to 2008, and Adani in Enugu State, from 1991 to 2008 (Onah et al. 2020). In Nigeria there had been a paradigm shift from onchocerciasis control to elimination (Ogbonna, 2015). The Nigerian onchocerca elimination program was done through mass drug administration (MDA) national health intervention put forward in 2004. The Nigerian onchocerca elimination program health policy and intervention targeted 2025 for elimination of onchocerciasis as main target (Ogbonna, 2015; Boakyeet al., 1993; Uttah, 2010; World Health Organization, 1994, 2000).

Nevertheless, there had been reduced cases but generally the disease had persisted in different parts of Nigeria, spreading Ogbonna and Ikani (2018), in Edo state Olusegun and Ehis (2010), Cross River state (Chikezie et al., 2023), in Adamawa state (Usman et al. 2024) in Abia, Imo, Enugu, Anambra (Ityonzughul et al., 2024), In Awgu local government area of Enugu State (Enyidi et al., 2026), and in Kaduna state (Olanrewaju et al., 2025). Control of onchocerciasis through MDA of mectizan, does not eliminate he adult worm but destroys the skin stages and inhibits embryogenesis (Chavasse et al., 1992, Opoku et al., 2018). The continuous administration of mectizan in affected areas have been suggested (Ekpo et al., 2022, Olanrewaju et al., 2025), while some workers suggested that onchocerciasis had been controlled in Abia and Enugu and MDA should be stopped (Ityonzughul et al., 2020). Onchocerciasis is transmitted by *Simulium damnosum* whos larvae develops in fast flowing stream. Awgu in Enugu is a very hilly terrain and head stream of several rivers and streams in the south east. Fast flowing rivers and streams are very common in Awgu and it is a farming community known for production of all food stuff including rice, vegetables, yam and cassava among others.

Against these backdrops this survey on prevalence of onchocerciasis in Awgu in Awgu local government area was carried out to ascertain the true state and prevalent status.

2. Materials and Methods

2.1 Manuscript Content

2.1.1 Geography of Study Area

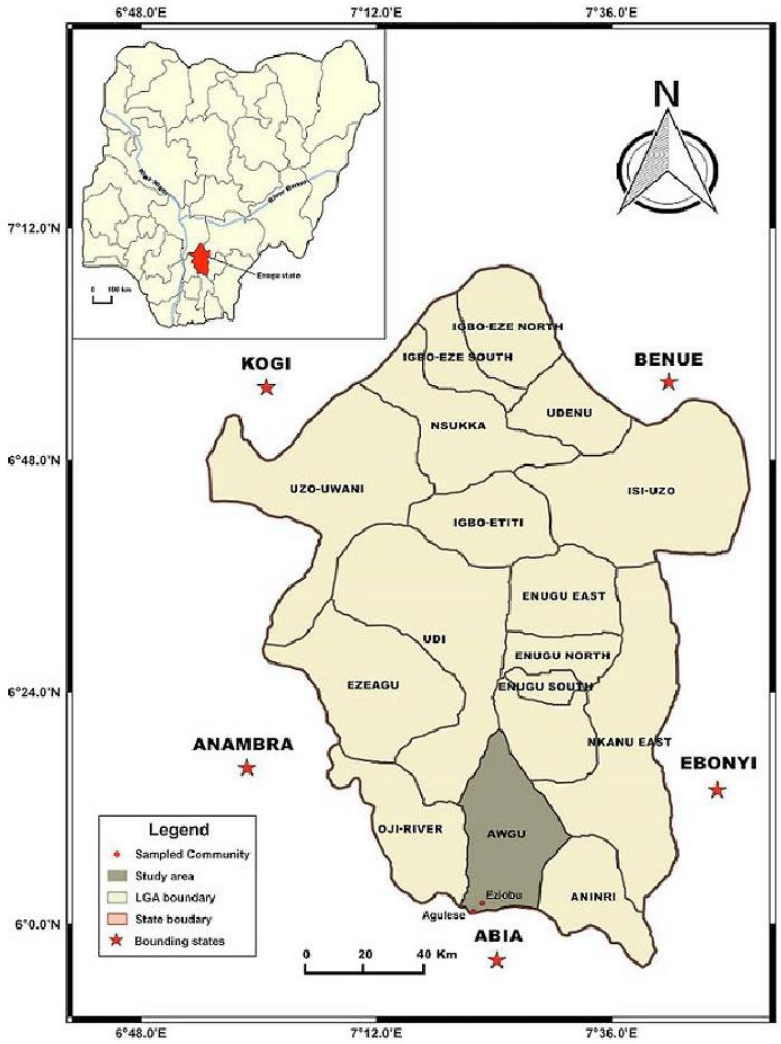

Plate 1. Map of Awgu inset in map of Enugu state inset in map of Nigeria

Study area. This study was carried out in Awgu headquarters of Awgu local government area of Enugu State Nigeria. The geographical coordinates of Awgu LGA is between latitudes 06 00' and 06 19' North of the Equator and longitudes 07 23' and 07 35' East of the Greenwich Meridian. Awgu is the head quarters of the Local government and is topographically a very hilly region with deep valley and rising hills. Awgu LGA is bounded in the north by Udi and Nkanu West LGAs, in the west by Oji River LGA, Aninri LGA and Ivo LGA of Ebonyi State in the East, and shares borders with Umunneochi L.G.A of Abia State. Awgu is home to National Youth Service (NYSC) Camp and the General hospital and Enugu State Nursing School.

2.1.2 DNA Extraction (ZymoResearch Extraction Kit)

DNA extraction from blood samples were done using ZymoResearch extraction kit purchased from Inqaba Biotec West Africa Ltd. The kit is a product of BioLabs England. 800 µl of genomic lysis buffer was added to 200 µl of serum sample in 1.5ml of eppendorf tube and was allowed to incubate for 1minute at room temperature. It was then transferred into Zymo-SpinTM IICR Column1 in a collection tube. It was centrifuged at 10000rpm for 1minute. The flow through was discarded and 200 µl of DNA pre wash buffer was added to the Zymo-SpinTM IICR Column1, centrifugation was done again at 10000rpm for 1minute. Washing was repeated with 500 µl of gDNA wash buffer. After 1minute dry spinning, 80 µl of the extracted DNA was eluted into sterile eppendorf tube and properly stored at -20ºC.

2.1.3 DNA Amplification

The molecular identification of Onchocerca volvulus was done in a singleplex reaction mix. The following set of primer sequence for Onchocerca volvulus was used OvFWD 5′-TGT GGA AAT TCA CCT AAA TAT G-3′, OvREV 5′-AAT AAC TGA TGA CCT ATG ACC-3′. A commercially prepared Master mix were gotten from Inqaba Biotec West Africa and the Amplification conditions were adopted and modified from an already published work by Fanello et al., (2002) as shown in the table below

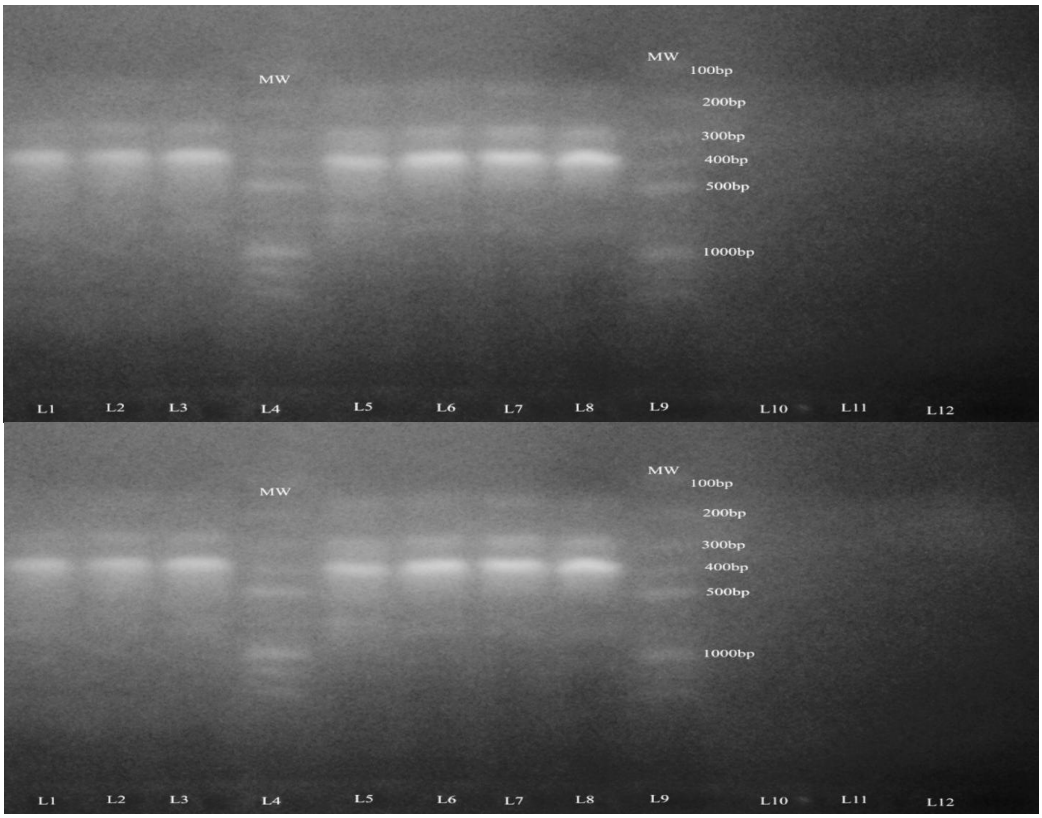

Image 1. L4 and L9 are the 100bp DNA ladder;L1, L2, L3, L5, L6. L7, and L8 are positive samples resolving at 397bp.; L10 L11 and L12 are the negative samples

Table 1. DNA amplification conditions for molecular identification and sequencing of *Onchocerca volvolus* from the blood samples residents of Awgu, in Awgu local

Government area of Enugu State Nigeria	
No of Cycles	**Temperature and Time**
Hold	98ºC for 10 second
	98ºC for15 secods
30 cycles	56ºC for 30 seconds
	72ºC for 30 seconds
Final Extension	72ºC for 45 seconds

2.1.4 Gel Electrophoresis

The amplicons were run on 2% agarose gel for 1hr30mins at 120 volts. Expected band size is 397bp.

2.1.5 Population of Study

The population of study are taken from the following groups in Awgu headquarters, Nursing school students, farmers, workers and Corp members, villagers and patients of Awgu general hospital.

2.1.6 Sample and Sampling Techniques

The individuals were randomly selected from patients of Awgu general hospital from whom blood samples were collected. The blood sample was collected by puncture of the thumbs using well sterilized. The blood sampled were received in a punch card as recommended for use in molecular biology research. Blood samples were randomly collected from 100 patients over a period of two weeks. Well structured and validated questionnaire were administered to randomly selected individuals in nursing school, local government headquarters, general hospital, general hospital outpatient department and the communities around the local government headquarters of Awgu. A total of questionnaire were distributed and collected from respondents. Instruments for data collection and validation.

The data or molecular biology analysis were collected from DNA analysis analysis of the blood samples. Then DNA card were also designed with questionnaire on age, sex, profession. The non DNA questionnaire had seven sections covering name age, community, duration of residence, visit to stream and rivers (No visit, regularly and often), presence of skin itching, skin nodules, redness of eyes or discomfort, hazy or blurred vision, Leopard skin, rashes and skin discoloration. These are clinical signs and symptoms of *O. volvolus* infection. The validation of the questionnaire was done by Prof U.D based on the appropriateness of clinical and asymptomatic signs, appropriateness of language, clarity of instrument and technical correctness.

2.1.7 Statistics and Calculations

Incidence=incidence rate = number of new cases / population at risk × population size

Prevalence =Prevalence = number of existing cases/total population x 100

Abundance=Number of parasites recovered/number examined

Prevalence=Number of people infected /number examined * 100

Mean intensity = Number of parasites recovered/Number of people infested

Null hypothesis: Ho= there is no significant difference in the prevalence, abundance and mean intensity of the parasites

Ha= there is significant difference in the prevalence, abundance and mean intensity of the parasite.

The results were analyzed using one way analysis of variance (ANOVA). Turkey highly significant difference (THSD) 0.05 was used in separating possible differences of treatment means. SPSS version 14.0 statistical package was used for analyses.

3. Results and Discussions

The DNA amplification produced base 397 base pair and the L1, L2, L3, L5, L6. L7, and L8 are positive samples resolving at 397bp.; L10 L11 and L12 are the negative samples. The The results of molecular identification using OvFWD 5′-TGT GGA AAT TCA CCT AAA TAT G-3′, OvREV 5′-AAT AAC TGA TGA CCT ATG ACC-3′ in a singleplex reaction mix identified identification of Onchocerca volvulus.

3.1 Molecular Biology, Serological Analysis and Effects of Sex Ratio

The molecular biology analysis results of the molecular biology analysis and serological examination of respondents Awgu residents showed that *O. volvulus* was endemic in the study area. Blood samples DNA analysis showed that virtually approximately close number of males and females were *O. volvulus* DNA+ve in the research area. The *O. volvulus* DNA +ve prevalence male: female sex ratio was 1.63. The mean intensity *O. volvulu*s DNA+ve male:female sex ratio of the *O. volvulus* was 1.62. However the mean intensity of *O. volvulus* DNA+ve infection among the males population of respondents in Awgu was 0.089 ± 0.04, and this was significantly ($P<0.05$) different that of the female 0.055 ± 0.06 (Table 2). The abundance of the infection among the male population (0.571 ± 0.05) was also significantly ($P<0.05$) higher than in the female population, sex ratio=1.33.

Table 2. Prevalence, abundance of mean intensity of *Onchocerca volvulus* infection among male and female population in Awgu in Awgu local government area

Enugu State	Prevalence	Mean intensity	Abundance
Males	8.88 ± 0.02^{a}	0.089 ± 0.04^{a}	0.571 ± 0.05^{a}
Females	5.47 ± 0.06^{b}	0.055 ± 0.06^{b}	0.429 ± 0.02^{b}
Sex ratio (M:F)	1.63	1.62	1.33

3.2 Clinical Signs and Asymptomatic Symptoms Analysis and Effects of Sex Ratio

The results of respondent questionnaire showed that inhabitants of the experiment area are positive to *O. volvulus* clinical signs and asymptomatic signs. There was higher prevalence of body itching among the females population than male in the study area that visit fast flowing streams and rivers. The prevalence of itching for the female population was 57.25 ± 0.32% while it was 42.75 ± 0.15% for the male. The sex ratio for the prevalence of male and female body itching was 0.74. Nodules were reported among the respondents and the prevalence of nodules among female population was 50 ± 0.01%. There was equal prevalence of nodules for the male and female population of respondents. The male female sex ratio for prevalence of nodules was 1. There was higher prevalence of red eyes for the female population 60.58 ± 0.04% than the male 39.42 ± 0.21%. The male: female sex ratio for occurrence of red eyes was 0.65. There was higher prevalence of blurred vision among female respondents (57.14 ± 0.33%) than men 42.86 ± 0.22%. The sex ratio for the male: female prevalence of blurred vision was 0.75. The prevalence of skin rashes was higher in the female population 57.14 ± 0.05%, than in the male 42.85 ± 0.60 %. The male female sex ratio for skin rashes was 0.75. There were observable prevalence of leopard skin among the respondent population. The prevalent of leopard skin among female and male population that visit fast flowing streams was 50 ± 0.01. The prevalence of the leopard skin was same for both male and female and the male female sex ratio was 1. Conversely the prevalence of skin discoloration was higher in the female population (52.63 ± 0.07%) than the male 47.37 ± 0.08%. The male: female sex ratio for the prevalence was 0.9 (Table 3).

There was a very low prevalence and incidence of *O. volvulus* clinical signs among respondents that do not visit either fast flowing streams or rivers at Awgu (Table 3). The prevalence of body itching among male population was 1.06 ± 0.15% of the total population examined. The prevalence among female was 6.95 ± 0.32% of the total population examined. There was significant difference between the prevalence of body itching among the male population and the female ($P<0.001$). The female: male sex ratio of prevalence of body itching among the

Table 3. Prevalence of *O. volvulus* clinical sings and asymptomatic signs on the male and female populations that visit fast flowing streams at Awgu in Awgu local government area of Enugu state Nigeria

	Males	Females	Prevalence %M	Prevalence %F	Sex Ratio1	Incidence M	Incidence F	Sex Ratio2
Visit Stream	60	80	42.86 ± 0.02b	57.14 ± 0.01a	0.75			
Itching	59	79	42.75 ± 0.15b	57.25 ± 0.32a	0.74	0.37 ± 0.06b	0.5 ± 0.02a	1.33
Nodules	1	1	50 ± 0.01ns	50 ± 0.01ns	1	0.01 ± 0.04ns	0.01 ± 0.05ns	1
Red eyes	54	83	39.42 ± 0.21b	60.58 ± 0.04a	0.65	0.34 ± 0.02b	0.52 ± 0.01a	1.54
Blured V	30	40	42.86 ± 0.22 b	57.14 ± 0.33a	0.75	0.19 ± 0.07b	0.25 ± 0.04a	1.31
Rashes	3	4	42.85 ± 0.60b	57.14 ± 0.05a	0.75	0.02 ± 0.05b	0.03 ± 0.06a	1.33
Tiger skin	9	9	50 ± 0.01ns	50 ± 0.01ns	1	0.06 ± 0.01ns	0.06 ± 0.02ns	1
Discolor	9	10	47.37 ± 0.08b	52.63 ± 0.07a	0.9	0.05 ± 0.11b	0.06 ± 0.11a	1.11

Sex ratio 1 =male/female for prevalence, sex ratio 2= female /male for incidences
Prevalence and the incidences of *O. volvulus* clinical sings and asymptomatic signs on the stream visiting male and female population at Awgu in Awgu local government area of Enugu state Nigeria

Prevalence and Incidence of O. volvulus among population of people that do not visit streams and rivers at Awgu

population was 4.34, showing that for every one male 4.34 females were infected. The incidence of body itching for he male population was 0 while it was 0.07 for the female population. There was 0 cases of body nodules for the male and female populations that visited no streams and rivers in Awgu There were also 0 incidences for the same population. There was very low prevalent rate of red eyes 0.54 ± 0.21% of the total population, among the males, while it was significantly higher for the females 5.88 ± 0.04%. There was more incidence of redness of the eyes among the female than male population (Table 4). The female: male sex ratio of incidence of redness of eye was 6.4. The prevalence of cases of blurred vision among the male population that doesn't visit streams and rivers in Awgu was very low, 1.06 ± 0.22 as against female population of 6.42 ± 0.33. There were similar prevalent cases of body rashes and blurred vision among the male population that do not visit the water bodies 1.06 ± 0.60. However the female population had a significantly higher prevalence case of 6.95 ± 0.05% of the total population examined. There were virtually no cases of leopard skin or skin discoloration among the male population that never visits the rivers or streams. The female population that visited no stream or rivers had 1.60± 0.01% prevalent rate of leopard skin and 3.74 ± 0.07% of skin discoloration.

3.3 Age Group Effects on the *O. volvulus* DNA+ve

The results in this experiment showed that there was age group effects on the *O. volvulus* DNA+ve manifestation. There was no serological DNA +ve manifestation among residents of age group 5 to 37 (Table 4). The highest prevalence rate of the *O. volvulus* infection was recorded in the age of 60years + group. The prevalence rate of *O. volvulus* DNA +ve manifestation among age group 60year + was 25.45 ± 0.07 %. There was significant difference between the prevalence rate of the age 60 + and the rest of the age groups. The prevalence rate of *O. volvulus* DNA +ve among the age group 49-59 was 11.43 ± 0.11%. The age group 49-59 had significantly (P<0.05) higher prevalence rate *O. volvulus* DNA +ve, than all other age groups except 60+. The least prevalence rate was found among the age group of 38-48, prevalence rate 6.85 ± 0.01%. The abundance of the infection was found to be highest among age group 49-59 (0.57 ± 0.08). The abundance of the *O. volvulus* DNA +ve manifestation in age group 49-59 (0.57 ± 0.08) was significantly (P<0.05) higher than that of age group 60+ (0.33 ± 0.06). Nevertheless the *O. volvulus* infection abundance of 60+ age group was much higher than those of age group 38-48 (0.28 ± 0.04). The mean intensity-of the inflection was highest for the age group 60+ (0.25 ± 0.07) % and this was higher than all other age groups mean intensity (P<0.05). The mean intensity was increasing as the age grouping increased. Consequently the mean intensity of *O. volvulus* DNA +ve infection was higher for age group 49-59 (0.11 ± 0.02) than for age group 38-48, *O. volvulus* DNA +ve mean intensity=0.07 ± 0.06 (Table 4).

Table 4. Serological analysis of prevalence abundance and mean intensity of *Onchocerca volvulus* among male and female and age groups in Awgu Enugu state Nigeria

No.		Male	Female	0	38-48	49-59	60 +AB
1	Prevalence (%)	8.88 ± 0.02^{d}	5.47 ± 0.06^{e}	0	6.85 ± 0.01^{c}	11.43 ± 0.11^{b}	25.45 ± 0.07a
2	Abundance	0.57 ± 0.08^{a}	0.42 ± 0.03^{b}	0	0.28 ± 0.04^{d}	0.57 ± 0.08^{a}	0.33 ± 0.06^{c}
3	Mean Intensity	0.09 ± 0.04^{c}	0.05 ± 0.01^{e}	0	0.07 ± 0.06^{d}	0.11 ± 0.02^{b}	0.25 ± 0.07^{a}

3.4 Clinical Signs and Asymptomatic Manifestation of *O. volvulus* Infection (Skin Region)

The results of questionnaire respondents showed that inhabitant of the research area were having clinical signs of the *O. volvulus* infections. There were high prevalence of skin itching, skin rashes, Leopard skin, skin discoloration and presence of skin nodules. The prevalence o skin itching among the residents was 86.78 ± 0.06% and this was significantly (P<0.05) higher than all other clinical and asymptomatic sings on the populace studied. The prevalence of skin discoloration among the people was 13.38 ± 0.05%. The prevalence of Leopard skin among the residents of studied area was 11.57 ± 0.01%. There was significantly (P<0.05) higher prevalence of skin discoloration than that of Leopard skin in thew study area. The prevalence of skin rashes among the residents of Awgu was 15.62 ± 0.08% and this was significantly (P<0.05) higher than the prevalence of Leopard skin. Skin nodules recorded the lowest prevalence rate of 2.75 ± 0.04%. The abundance of symptoms cases of skin itching (26.97 ± 0.06) was most prominent in the study area. There abundance of skin itching cases was significantly (P<0.05) higher than all other O. volvulus clinical sign and asymptomatic cases in the study area. The studied population had 5.011 ± 0.08 abundance of skin rashes and 4.28 ± 0.03 abundance of skin discoloration. Skin discoloration cases was however significantly lower than cases of skin rashes. The lowest

abundant case was that of skin nodules 0.84 ± 0.0 (Table 5). The mean intensity of the clinical symptoms was highest for skin itching 0.89 ± 0.02, followed by the mean intensive cases of 0.16 ± 0.01. The mean intensity of skin itching cases was significantly higher than that for cases of rashes (Table 5). There was however higher mean intensity of Leopard skin 0.12 ± 0.08, compared to skin nodules 0.03 ± 0.09. There was significant differences (P<0.05) between the mean intensity of Leopard skin cases, skin discoloration and skin nodules.

Table 5. Prevalence abundance and mean intensity of clinical signs, symptom and asymptomatic signs of *Onchocerciasis volvulus* infection among residents of Awgu in Awgu Local government area of Enugu state Nigeria

No	Parameter	Itching	Rashes	Leopard skin	Discoloration	Nodules
1	Prevalence %	86.78 ± 0.06[a]	15.62 ± 0.08[b]	11.57 ± 0.01[d]	13.38 ± 0.05[c]	2.75 ± 0.04[e]
2	Abundance	26.97 ± 0.06[a]	5.011 ± 0.08[b]	3.64 ± 0.02[d]	4.28 ± 0.03[c]	0.84 ± 0.05[e]
3	Mean Intensity	0.89 ± 0.02[a]	0.16 ± 0.01[b]	0.12 ± 0.08[d]	0.14 ± 0.05[c]	0.03 ± 0.09[e]

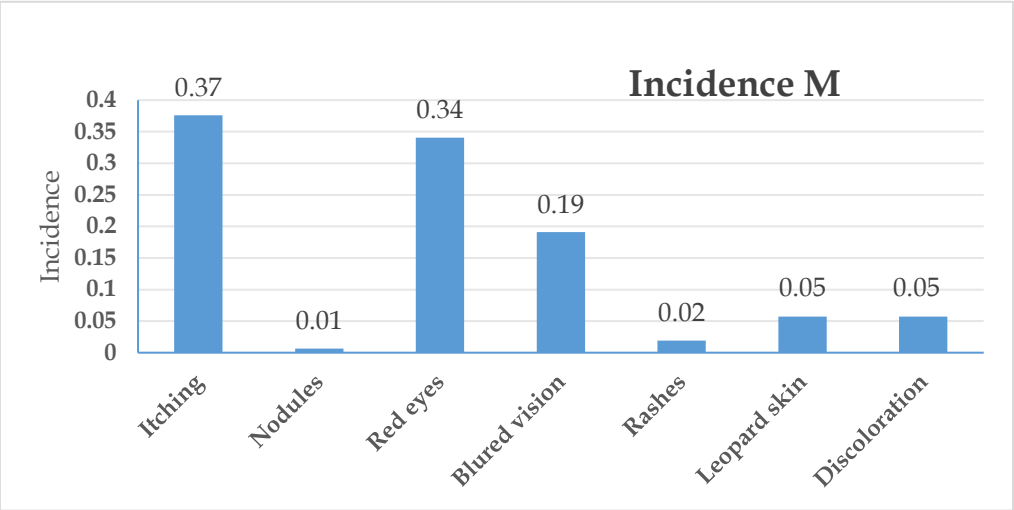

Fig. 1. The incidence of itching, nodules, red eye, blurred vision, rashes, leopard skin and skin discoloration among male population of Awgu in Enugu State Nigeria that visit Fat flowing rivers and streams

3.5 Clinical Signs and Asymptomatic Manifestation of *O. volvulus* infection (Eyes and Itching)

Results of responses from respondents shows that there are cases of eyes problems related to infection of *O. volvolus* in the research area. There was high prevalence of body itching 82.83 ± 0.08% among the population irrespective of sex. The prevalence rate of body itching was was significantly higher (P<0.05) than all other symptoms prevalence (Table 6). The symptom of redness of eyes was reported with prevalent rate of 46.26 ± 0.04%. The prevalent rate of redness of eyes was significantly (P<0.05) higher than other symptoms excepts itching of body and eyes. There was similar cases of prevalence of body nodules 2.70 ± 0.12% and blurred vision 2.69 ± 0.09 (P>0.05). This research did not record cases of blindness 0.834724541. There was higher abundance of skin itching 25.71 ± 0.03, than all other symptoms (P<0.05). There was no significant differences (P>0.05) between the abundance of blurred vision case (0.84 ± 0.01) and nodules (0.83 ± 0.03). There was however higher abundance cases of redness of the eyes (14.35 ± 0.05) than blurred vision or occurrence of nodules (P<0.05). The mean intensity of the symptoms followed same pattern as the prevalence and the abundance. The highest mean intensity (0.85 ± 0.08) was recorded for skin itching symptom and it was significantly higher (P<0.05) than all other symptoms. The mean intensity of redness of the eyes 0.47 ± 0.01, was next to itching but was significantly higher than intensity of blurred vision 0.03 ± 0.11 and nodules 0.02 ± 0.05. The mean intensity of nodules is the least of all the symptoms (Table 6).

The results of this experiment shows a high incidence and prevalent rate of onchocerciasis at Awgu. The sporadic and hypointensive prevalences, shows that the research area is evidently endemic for Onchocerca

volvulus infection. Microfilaremia was prevalent in the population with highest prevalent rate of 25.45 ± 0.07% for age group 60yr +. The high prevalent rate of microfilaremia with age and for age up to 60 yr supports the findings of Mcmahon et al. (1988), who noted the microfilaremia in the eyes increased with age up to 60 years. The higher manifestation of microfilaremia among the age group 60yrs + was in line with the work of Uttah et al. (2004), who noted that microfilaremia was higher among older people than younger in lower Imo River basin. The endemicity of *O. volvulus* has been classified by onchocercha control program as sporadic if the prevalence is up top 10%, Uttah et al. (2012), hypoendemic if the prevalence is 10- 29%, mesoendemic if prevalence is 30-59%, or hyperendemic if prevalence is 60% and above (Akinbo & Okaka 2010), (McMahon et al. 1988). Accordingly, the endemicity *O. volvulus* of in the research area of this experiment Awgu in Awgu local government of Enugu State is hypo endemic based on microfilaremia prevalence of 11.43 ± 0.11 for age group 49 - 59 and prevalence of 25.45 ± 0.07% for age group 60 yrs and above.

The female population had had higher overall microfilaremia manifestation in terms of all clinical manifestations. The endemicity of the research area to onchocerciasis is in line to previous reports of Eyo et al. (2013), Ekpo et al. (2022), Nwoke et al. (2023) Who noted similar trend also for female populations. The DNA analysis of blood samples in this experiment showed *O. volvulus* +ve traits for both males and females. This is inline with Lloyd et al. (2015), who developed and used sensitive qPCR assay was in detecting *O. volvulus* DNA from dried skin snips in Côte d'Ivoire, Abidjan. The use of PCR in this experiment is also in line with similar to Ityonzughul et al. (2024) who used O-150 PCR in identifying positive *O. volvulus* DNA in respondents0 skin-nips at Enugu, Abia and Imo states of Nigeria. The use of dried blood in PCR O. volvulus DNA analysis was not painful like collecting skin nips. The use of PCR in this experiment is better than traditional skin nip because it enhanced early diagnosis. It has been noted that (PCR) is beneficial because it aids identifies infected people before even the emergence from skin nip by diagnosis the expression of the parasite genome through the dry blood. This is inline with Cotton et al. (2016) and the serological analysis shows higher prevalence of *O. volvulus* DNA +ve traits among the males than the females. The prevalence and abundance and mean intensity sex ratio of the serological results were between 1.3 to 1.6. This is in line with Eyo et al. (2013) who noted more male infection at Opi Nsukka Enugu State and Ekpo et al. (2022), who noted microfilariae more in males than females while studying *O. volvulus* infection at Enugu state and Ogun state. The lower prevalence level of *O. volvulus* DNA +ve in this experiment than Ekpo et al. (2022), could be due to previous MDA in Awgu Enugu State. The lower prevalence rate of infection in this experiment than the investigation could be as result of previous intervention and MDA in the research area. It is noteworthy that Ekpo et al. (2022) Worked in Nkanu local government areas of Enugu state and this work was done at Awgu local government area of Enugu state. This could be because the microfillariae were more released in the males than females at the point of collection. The males were engaged in harder works and this could have affected tn release. There was age group effect on the serological results. There were more *O. volvulus* DNA +ve traits in age group 60years +. The results of age group is similar to previous findings of Otache et al. (2022), who noted higher rate of infection among age group 55 years and above. This could also be due to long incubation of the parasite in them hence *O. volvulus* DNA +ve. The low incidence and prevalence level of level of body nodules suggests that there was previous control in the area by mass drug administration. Enugu state of Nigeria is among the states mapped and treated for onchocerciasis (Gemade et al. 1998, NOCP 2009, Ekpo et al. 2022). This research serves as a proof of the persistence and resurgence of onchocerciasis in Enugu state. The high prevalence of *O. volvulus* DNA +ve traits among residents of the research area suggests lapses in previous treatments that depended solely on skin microfilariae emergence as prove of infection. Molecular biology analysis proved to be useful tool in identifying *O. volvulus* infection in the population. It had been noted that molecular biology analysis can be used in replacing painful skin snip collection (Lakwo et al. 2020). The detection of PCR-amplified parasite DNA in this experiment proved versatile and valuable tool in extricating early diagnosis of infection in the population. This is inline with Cotton et al. (2016), Mekonnen et al. (2017), Prince-Guerra et al. (2018) and Ogbonna and Ikani (2020), who advocated the use of PCR identification of *O. volvulus* DNA in onchocerchiasis survey. Most identification assays specifically target the O–150 repeat tandemly repeated sequence in the *O. volvulus* genome. The use of dry blood makes this method of analysis environment friendly and doesnt necessitate preservation or chemical addition. Blood analysis was easy to collect than skin snip which is painful and would be costlier to obtain. The manifestation of specific clinical signs like skin nodules was more in the female population 28. 48% in the female as against 14.12 % in the male. Epidemiology of the parasite shows that adult worms lives in the nodules from where they produce millions of microfilariae that attack the host. These shows that incubation of *O. volvulus* larvae is higher in the female than thew males. The microfilariae usually emerges from the subcutaneous nodules and invades the blood and resides in the skin produce skin and eyes. Consequently the higher endemicity of clinical signs on the female than males shows

that developmental stages microfilariae, which are found throughout the body but preferentially reside in the skin and eye (Fig. 2 and Fig. 3). The mean intensity represents quantity of the parasites in their developmental stages in the individuals. Repeated exposures to infected flies increase the number of adult worms and microfilariae in the host. The male:female sex ratio of infection from serological data was close to that of questionnaire. While in the serological data more *O. volvulus* +ve males than female resulted, in questionnaire more female than male were reported. Results from the questionnaire showed that more female than male had all the symptoms and asymptomatic manifestation of *O. volvulus* infection. This could be because the research areas is predominantly agrarian area and more females than male goes to the farms and are exposed to bites. This finding is inline with Chikezie et al. (2023), who noted that peak transmissions and biting season of *S. damnosum* was October. It is known that October is harvest period in Nigeria when most farmers visits their farms more often. The higher infestation rate of female than male is also in line with Nmorsi & Kio, (1994) who noted that females frequent water bodies to fetch water for domestic uses. The research area is agrarian and known for rice production. The vegetation are usually cleared negating the fact that biting is as result of vegetation (Smith et al. 2019). Evidently the visit to streams and rivers exposes the female population to bites. The manifestations of the clinical signs highlights the developmental stages of the parasite in the individuals Murdoch et al (2017). The adult worms usually aggregate in the body subcutaneous nodules, (onchocercomata), and aggravation of the infection leads to itching, skin rashes, thin skin and loss of skin elasticity and eventually leopard skin. 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In the research area at Awgu farmers and villagers had the highest number of *O. volvulus* DNA +ve traits. The results of this experiment is in line with Onojafe et al. (2015), who found that in Delta state of Nigeria, farmers had twice the prevalent rate of all infected. The results in this experiment are also supportive of position of Iroha et al (2010) who noted that field workers were predisposed to be infected with *O. volvulus.* Moreover the results here aligns strongly with Nworie et al. (2014), who noted that farmers had highest prevalent rate among other exposed to *O. volvulus* infection. The results in this experiment also support the position of Surakat et al. (2018), who noted that farmers were vulnerable to the infection with *O. volvulus.* This shows the exposure to bites is associated with occupation (Fig 1). The zero prevalence for students could be because the students population in this research were nursing students of Enugu State school of Nursing. Most of those student nurses were not indigenous of Awgu and came from different parts of the state and Nigeria. Its is possible that if the research was done with student of secondary school the results would be different for students. However the results here tallies with previous findings of Nworie et al. (2014), who noted that students followed by civil servants and traders population were the least infected in the survey at Ebonyi state Nigeria. In this research traders were more infected than civil servant and students. This could be due to their life style and exposure to bites of female. There was higher prevalence of body itching among the females population than male in the study area. This is in line with Akinbo and Okaka (2010) who noted similar trend in Ovia Edo State Nigeria. The prevalence of itching for the female population was 57.25 ± 0.32% while it was 42.75 ± 0.15% for the male.

The sex ratio for the prevalence of male and female body itching was 0.74. The sex ratio for prevalence of nodules in the male and female population was 1. Seemingly Nodules were reported among the respondents and the prevalence of nodules among female population was 28.48 ± 0.01%. The higher prevalence of body nodules among the female than males shows that females harbor more parasites than the males. Onchocerciasis is an

occupational disease with higher incidence in female than the male population. This could be because Awgu is an agrarian local government meant for production of rice and many food items. More women than men engage in all sorts of agricultural produce like rice, vegetables, cassava. The high prevalence of rashes, itching and leopard skin and discoloration points to high proliferation of skin microfilaremia (Fig 2). This suggest a resurgence of infection in the area and suggests also that the research area should be subjected to O. volvulus infection interruption program like mass drug administration (MDA).

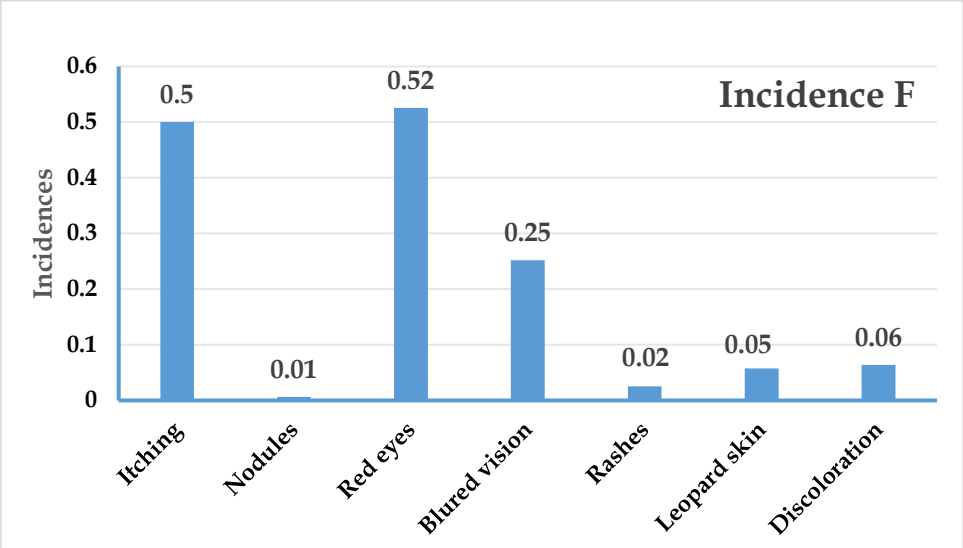

Fig. 2. The incidence of itching, nodules, red eye, blurred vision, rashes, leopard skin and skin discoloration among female population of Awgu in Enugu State Nigeria that visit Fat flowing rivers and streams

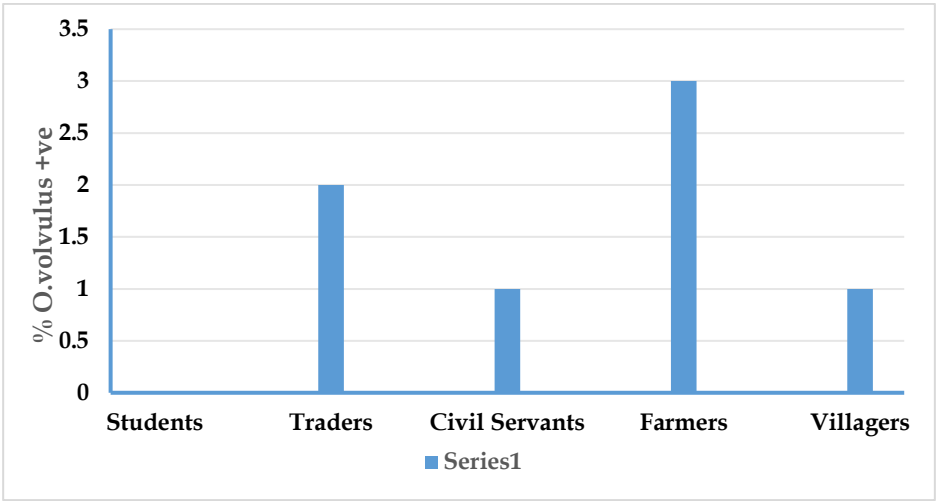

Fig. 3. The rate of *O. volvulus* +ve traits among the students, traders, civil servants, farmers and villagers of Awgu in Awgu local government area of Enugu State

The prevalence of the microfilaremia and clinical signs in the research area was noted to be highly influenced by the visitation of the people to fast flowing rivers and streams n the area. The number of the people that got infected were far higher for those that visit the rivers and streams than those that did not (Fig. 3). This can be explained by the biting exposure. The black flies are associated with fast flowing rivers and streams where the larval stages are aquatic. The prevalence of body itching was found in over 150 people that visit the water bodies while it was found in only 33 people that do not visit the water bodies (Fig. 4). The results suggests that visitation to the stream expose the subjects to more bites which increases mean intensity and therefore the abundance and clinical manifestation of the parasites (Figs. 3 and 4). The lack of skin nodules among people

that do not visit the water bodies is indicative of the fact that the parasite is not incubated in them. Moreover the number of people found to be with leopard skin among those that do not visit the water bodies was less than 5 compared to almost 20 of those that visit the water bodies. The result is novel and shows that prevalence of the parasites and the clinical signs can be linked to visitation of streams and the rivers. More a quick comparison at the prevalence rate of infection among those that visit the streams and rivers and those that don't shows a very striking difference (Fig. 5). There is predominantly higher cases of infection and manifestation of clinical signs among those that visit streams and rivers contrary to those that don't. This finding supports the position of Nmorsi & Kio, (1994) who noted that higher rate of O. volvulus infection among females, is because females frequent water bodies to fetch water for domestic uses. The cases of blurred vision is clearly more in those that visit the stream than those that do not. The cases of leopard skin and skin discoloration like wise testify the same.

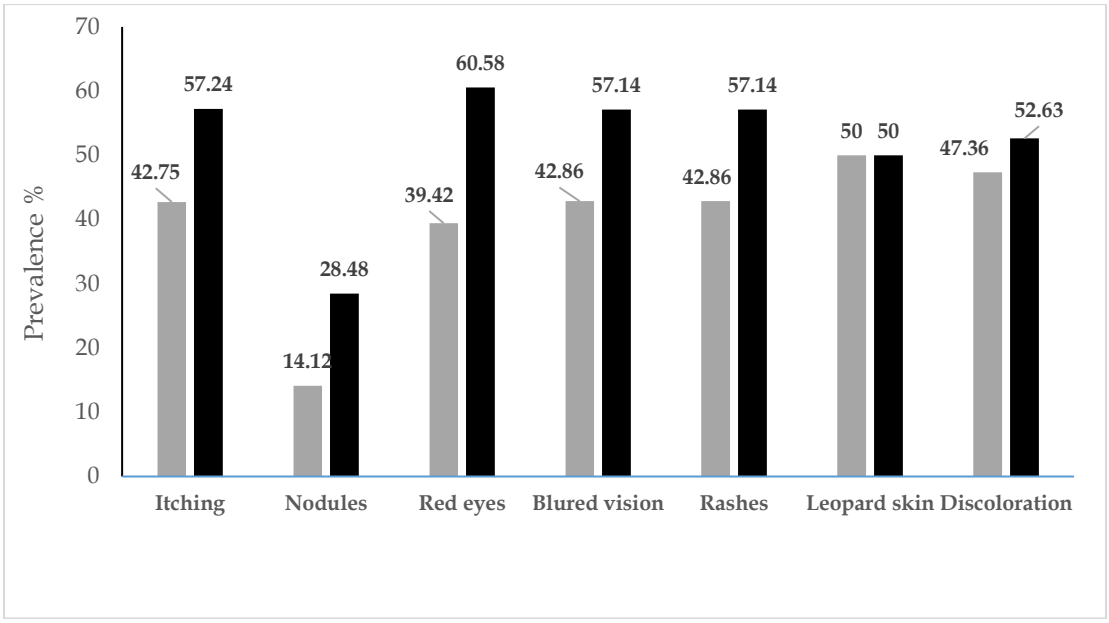

Fig. 4. The prevalence of *O. volvulus* clinical signs on residents, male (ash color) and female (black color) of Awgu in Enugu State that frequent fast flowing streams and rivers

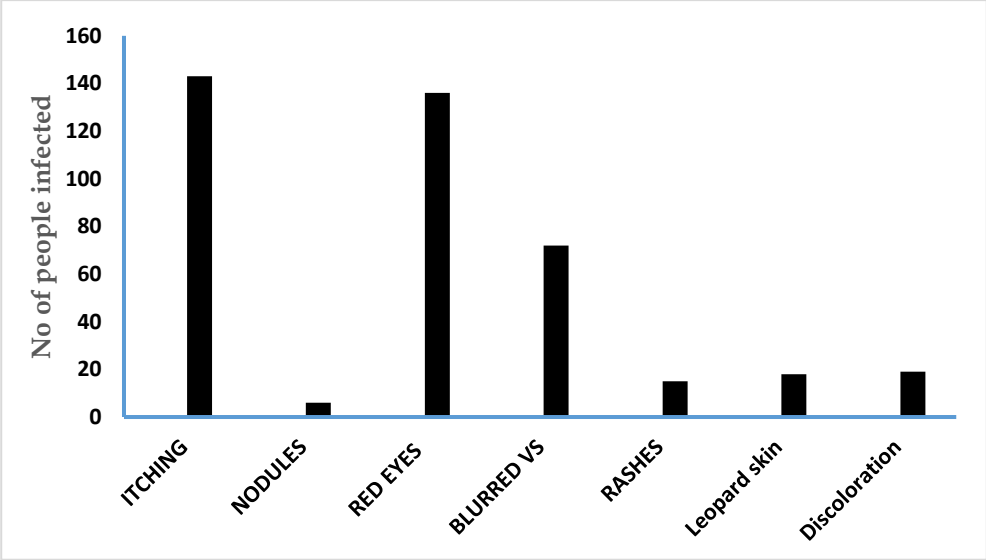

Fig. 5. The demography of people that frequent fast flowing streams at Awgu, and the observation of Clinical signs and symptoms of those infected with *Onchocerca volvolus*

Table 6. The prevalence, abundance and mean intensity of red eyes, blurred vision noddles and body itching, clinical signs and symptoms of *O. volvulus* infection among residents of Awgu in Awgu LGA Enugu state

Blindness	Itching	Red Eye	Blur Vision	Nodules
Prevalence	82.83 ± 0.08[a]	46.26 ± 0.04[b]	2.69 ± 0.09[c]	2.70 ± 0.12[c]
Abundance	25.71 ± 0.03[a]	14.35 ± 0.05[b]	0.84 ± 0.01[c]	0.83 ± 0.03[c]
Mean intensity	0.85 ± 0.08[a]	0.47 ± 0.01[b]	0.03 ± 0.11[c]	0.02 ± 0.05[d]

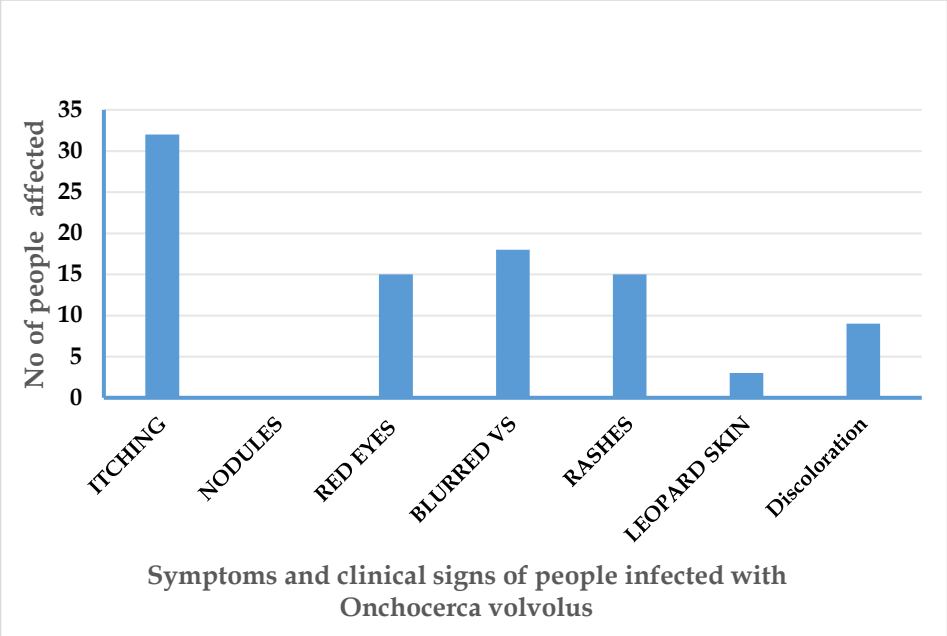

Fig. 6. The demography of manifestation of clinical sings of the infection of *O. volvulus* among inhabitants of Awgu in Enugu State that never visit fast flowing streams and rivers

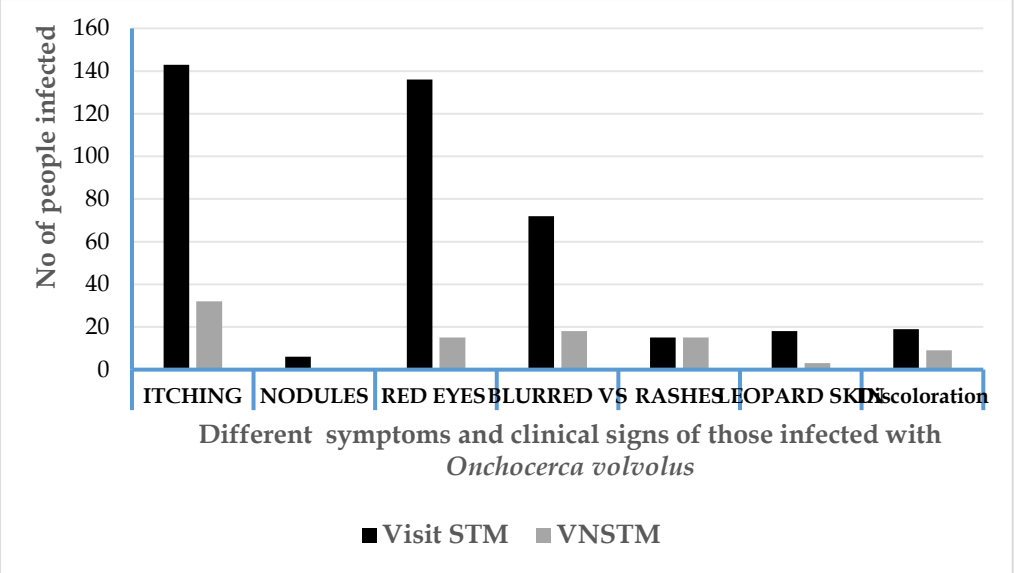

Fig. 7. The comparative demography of manifestation of clinical sings of the infection of *O. volvulus* among inhabitants of Awgu in Enugu State that visit fast flowing streams and rivers (In black color) and those that do not (in ash color)

4. Conclusion

There is prevalence and incidence of onchocerciasis in Awgu Enugu state. There are infestation and incidence rate for the female that male population. The use molecular biology technique is useful in early detection of the infection instead of traditional detection at the point of microfilariae emergence from the skin. There was much higher clinical and asymptomatic manifestation among respondents that visits streams and rivers than those that doesn't. Based on the onchocerca control program, the prevalence rate in the research area can be said to be sporadic and hypertensive.

Consent

As per international standards or university standards, respondents' written consent has been collected and preserved by the author(s).

Ethical Approval

This experiment was performed according to all ethics and animal rights (Desert Research Center). As much as this work had considering all rules and regulations in conformity with the European Union directive for the protection of experimental animals (2010/63/EU).

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Competing Interests

Authors have declared that no competing interests exist.

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