

Entomological Evaluation by Dissection of Adult *Simulium damnosum* Complex for Larvae of *Onchocerca volvulus*, Following CDTI in Amagu Agba Community, Ishielu L.G.A-Ebonyi State, Nigeria

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Abstract The study was carried out to verify reports of persistent transmission of onchocerciasis, despite long-term Community Directed Treatment with Ivermectin, (CDTI). Blackflies were collected along Asu River using human baits. A total of 91 female *Simulium damnosum* complex adults were caught and dissected- using a dissecting microscope- for microfilariae of *O. volvulus*. 58 flies representing (63.70%) of the total number of captured flies were caught in the month of June while 33 (36.20%) were caught in July. Despite ivermectin treatment, evidence of *O. volvulus* transmission was documented in Amagu Agba. A total of 27 larvae were recovered from the three body segments of the dissected flies, of this number, 17 larvae were recovered from the head region and a total of 10 were recovered from the thorax and abdomen representing 62.96 % and 37.04 % respectively of the total number of larvae recovered. The proportion of infected flies recorded was 8 (8.8 %) while the proportion of infective flies recorded was 17 (18.7%) of the total number of infected and infective flies. The high proportion of infective flies is indicative of active transmission in and around the study area. This should not be so as CDTI is currently going on in the area. Some militating factors such as: patterns of treatment coverage and compliance, parasite ivermectin susceptibility, parasite immigration in flies or people, may be responsible for the current unsuccessful ivermectin treatment strategy. There is therefore need for consistent and effective ivermectin distribution pattern and its resultant onchocerciasis prevalence, detected with blackfly dissection techniques and or more reliably detected in both blackfly population and human subjects through a combination of some molecular-based detection techniques and biocontrol approaches. Adopting these measures together with community-ownership participation concept, will successfully interrupt transmission in Amagu Agba community and other endemic areas.

Keywords: Amagu Agba, CDTI, Ivermectin, *Onchocerca volvulus*, *Onchocerciasis*, *Simulium damnosum* complex

Cite This Article: Nnadozie R.I.A., Onyenwe E., Ibediugha B.N., and Okorie A.G., “Entomological Evaluation by Dissection of Adult *Simulium damnosum* Complex for Larvae of *Onchocerca volvulus*, Following CDTI in Amagu Agba Community, Ishielu L.G.A-Ebonyi State, Nigeria.” *American Journal of Public Health Research*, vol. 6, no. 2 (2018): 26-30. doi: 10.12691/ajphr-6-2-1.

1. Introduction

Onchocerciasis, also known as river blindness, caused by the filarial nematode *Onchocerca volvulus*, is a chronic parasitic infection with public health and socioeconomic consequences of considerable magnitude in many sub-Saharan African countries [1]. It is an insect-borne disease transmitted by adult blackflies. It is the world’s second leading infectious cause of blindness. In most endemic countries, it constitutes a public health problem and a serious obstacle to socio-economic development [2].

According to [3], the disease affected about 17 to 18 million people in 37 countries of the world with approximately 123 million being at risk of infection. In West Africa, the predominant species is *Simulium damnosum*

complex, which coexists with other species such as *S. sirbanum*, *S. sanctipauli* and *S. soubrense* [4]. In Nigeria, onchocerciasis is widespread and a cause of blindness in most rural communities. Of all the countries of the world, Nigeria has the largest number of persons with onchocerciasis accounting for about a third of the global prevalence with about 40 million at risk of infection [6]. Cytotaxonomic studies of the *S. damnosum* complex from different parts of Nigeria have revealed the presence of five cytospecies, these include: *S. damnosum sensu stricto*, *S. sirbanum*, *S. squamosum*, *S. yahense* and *S. soubrense* [4]. An understanding of the transmission dynamics of onchocerciasis as in other forms of filariasis is important in advancing knowledge of how vector capacity, vector abundance, survival rate, feeding habit and behaviour influence the level of infection and disease in susceptible human population. The knowledge of vectorial capacity

would be of immense value in formulating the most appropriate control strategies in a given locality [7].

The adverse socio-economic importance of onchocerciasis led to the launching of Onchocerciasis Control Programme (OCP) by the World Health Organization (WHO) in 1974 [8,9]. The OCP was initially mandated to effectively control onchocerciasis by eradicating the black fly vector in seven endemic countries of West Africa including Nigeria by spraying dichlorodiphenyl-trichloroethane (DDT) along their fast flowing river breeding sites. In spite of the significant successes achieved, the use of this insecticide was abandoned because of the high toxicity to humans and the environment. Similarly, the relative therapeutic successes achieved in the attempts to control the scourge of onchocerciasis using diethyl carbomazine [10] and Suramin [11] had to be abandoned due to their high toxicity to the human patients being treated [12,13]. In 1987, a pharmaceutical firm (Merck) developed and introduced oral formulation of ivermectin (Mectizan) as the most effective, free and safest larvicide for the treatment of onchocerciasis [14]. Nigeria initiated the mass ivermectin (Mectizan) treatment of onchocerciasis in 1991 intending to effectively control the disease in 32 endemic States and the Federal Capital Territory (FCT) [15]. This implied that ivermectin clearance of *O. volvulus* microfilaria in the host will disrupt the transmission chain of onchocerciasis. This also implied that the infective larval load in the black fly vector will also be gradually cleared, since microfilaria will increasingly be unavailable in human host for the fly vectors to pick during blood meals [16].

Till date, the current strategy for the elimination of onchocerciasis still relies on mass treatment with ivermectin. A variety of treatment regimens, including quarterly, semi-annual and annual treatment have been adopted and they have proven effective in focally interrupting transmission and eliminating the parasite in isolated foci in Africa over different time frames [17,18,19]. High coverage (> 85% of eligible persons) community-wide treatment of residents for a minimum of 15 years is believed to be sufficient to reduce the load of microfilariae in human hosts below the threshold that can sustain transmission by blackfly vectors, thus locally eliminating the infection [20].

The elimination guidelines set forth by the World Health Organization (WHO) uses the prevalence of the infective stage of *O. volvulus* larvae in the blackfly vectors as a major metric for determining whether or not transmission has been successfully interrupted in an endemic community [21]. The threshold used for declaring elimination is less than one *O. volvulus* per 2000 female blackflies per endemic community. This means that the elimination of the causative agent of the disease holds the key to onchocerciasis control. The idea behind this is that if transmission can be effectively suppressed for a suitable period of time, the reproductive rate of the parasite will be brought below the minimum replacement rate, and the infection will die out. If one is to use a strategy of suppressing transmission using chemotherapy to eliminate human onchocerciasis, monitoring the level of transmission is necessary [22]. This is required to demonstrate that the control strategy being applied is effective and to ensure that once control activities are in place, transmission has been brought to the point

where the parasite population can no longer sustain itself. *O. volvulus* transmission has historically been measured through dissection of vector blackflies [23,24]. This an efficient method of monitoring transmission in areas not subject to control, because the prevalence of infection in the vector population is usually high [25,26].

Though Ebonyi state participates in NOCP, head dissection of female flies is still required to resolve conflicting published and unpublished epidemiological reports emanating from the study area even as CDTI continues. Despite these epidemiological studies [27], there are no other known works on entomological evaluation of vectors in the study area, hence the present study was aimed at establishing the transmission status in the study area using head dissection of female blackflies for largely L3 larvae or infective larvae.

2. Materials and Methods

2.1. The Study Area

Amagu Agba is a rural community in Ishielu Local Government Area of Ebonyi State, Nigeria. The village lies between longitude 54° east and latitude 6° north. It also lies in the tropical rainforest zone of Nigeria and is located at about 140 Kilometres North East of Abakaliki the state capital. There is an estimated population of 3200 people in Amagu Agba according to 2006 National Population Census. Amagu Agba has two different climate seasons; the rainy season from March to October and the dry season from November to February. The annual rainfall is approximately 230mm and atmospheric temperature range from 28 ± 30°C. The village is transversed by the Asu River. The river experiences lots of anthropogenic activities including sand mining, farming and irrigation, washing and bathing respectively. The river is characterized with sand dunes amidst the water body. Other tributaries abode healthy vegetation with *Elaeis guineensis* dominating.

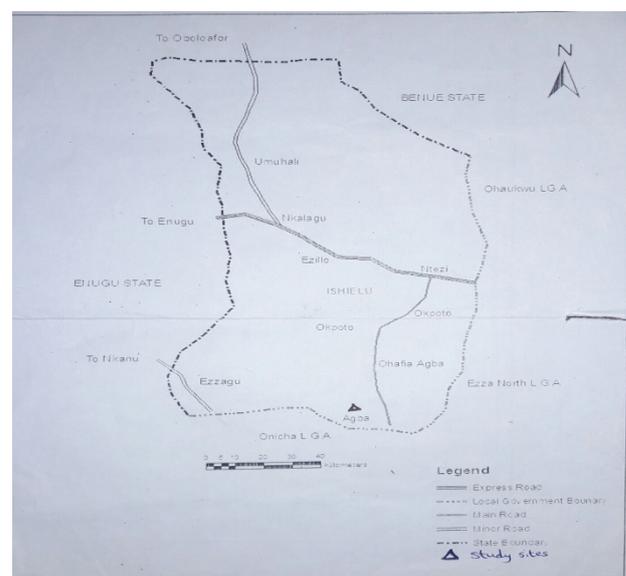


Figure 1. Map of Ishielu L. G. A. and environs showing study sites. (Source: Department of Geology and Geophysics, Ebonyi State University)

The people have non-functional electricity, distantly located pipe-borne water and two built local schools. The people depend majorly on rain water, streams and rivers as alternative sources of drinking water. The economy of these communities is mostly agrarian which includes sales of primary farm produce such as Cassava, Yam, Vegetables and Vegetable fruits, processed agricultural products like garri, rice and livestock such as hens, sheep, goats and fishes. Commerce also forms part of the economy in terms of petty trading and sand mining.

2.1.1. Ethical Statement

Ethical clearance was obtained from the community leaders prior to the research. They provided a respondent who helped in the study. They also provided human baits for the purpose of the study.

2.1.2. Blackfly Collection

Human baits (HB) were used for catching the female adult flies [1,7,28,29]. The site was sampled for two months between June and July 2016. The flies were usually collected between 7.00am and 6.00pm (11 hours) by two fly collectors sitting far apart [1,4,7]. The human baits were given doses of Ivermectin as preventive measures. The fly collectors were dressed in knickers exposing the lower legs. The collectors sat at convenient places with the feet and the legs below the knees exposed. Any fly perching on the exposed parts was collected before it started feeding by inverting a small glass tube over it and replacing the cap immediately [1,4,24]. The glass tubes containing the captured flies were wrapped tightly with poly bags bound with rubber bands and placed in a cold box containing ice packs to prevent moisture from getting into the glass tubes and to stop further development of microfilariae in the flies. The samples were then transported to the Applied Biology Laboratory, Ebonyi State University for dissection.

2.1.3. Dissection of the Blackflies

The flies were removed from the cold box where they were preserved and placed soaked for one hour in distilled water. They were then placed in small drops of water on a slide (3 flies each in 3 drops of water on a single slide). The head and abdomen were separated from the thorax and each was broken open without separating the pieces

(this was to allow stain to penetrate). A drop of diluted lactopropionic orcein stain was then added to the drop of water and allowed to stain and clear for 30 minutes. The preparation was then observed under a dissecting microscope. Tissues were seen to be transparent and stained light pink while filarial larvae were more densely stained. The head was teased further open to look for the 3rd stage larvae while the abdomen was opened to examine for microfilariae. The bundles of thoracic muscles were equally teased apart to examine for darker stained larvae.

3. Results

3.1. Result of Dissection

A total number of 91 adult female *S. damnosum* were caught and examined for larval stages of *O. volvulus* during the two months study. A total of 27 larvae were recovered from the three body segments of these flies throughout the sampling period. Of these, 17 larvae were recovered from the head segment representing 18.7 % of the total number of larvae recovered (Table 1). These are also called L3 larvae or infective larvae. 10 filariae worms were recovered from the thorax and abdomen of eight flies. Two of these eight flies harboured one larva in the thorax and one in the abdomen, making a total of 4 larvae, while the remaining six harboured 1 larva each in the abdomen only. The total number of L1 and L2 larvae recovered (10) represents 10.9 % of the total number of larvae recovered.

3.1.1. Proportion of Infected and Infective Flies

The proportion recorded as infective flies, that is, flies having the L3 larvae capable of causing the disease, was 17 and this represented 18.7% of the total number of infected and infective flies, while the proportion recorded as infected, that is, flies having the L1 & L2 larvae was 8 representing 8.8% of the total number of infected and infective flies. Information on the proportion of infected and infective flies is shown in Table 2. The overall % number of both infected and infective flies was recorded as 25 representing 27.5% of the total number of flies captured and examined. The figures are also shown in Table 2.

Table 1. Distribution of *O. volvulus* larvae in the head, thorax and abdomen of *S. damnosum* in Amagu Agba community

Body segment of <i>S. damnosum</i> isolated	Number of <i>S. damnosum</i> examined	Number of L1 and L2 larvae recorded (%)	Number of L3 larvae recorded (%)	Total number of <i>O. volvulus</i> larvae recorded (%)
Head	91	-	17	17
Thorax	91	2	-	2
Abdomen	91	8	-	8
Total	91*	10 (10.9)	17 (18.7)	27 (29.7)

*Not Additive.

Table 2. Proportion of infected and infective flies

Collection Site	Number of flies examined	Number of infected flies (%)	Number of infective flies (%)	% number of infected and infective flies
Amagu Agba	91	08 (8.8)	17 (18.7)	25 (27.5)

3.1.2. Monthly Relative Abundance of Flies

The total number of flies captured for the month of June was 58 while the July figure was 33. These represent 63.7% and 36.2% respectively of the total number of flies captured in the two months. Monthly relative abundance of flies is shown in Table 3 and Figure 2 respectively.

Table 3. Relative abundance of *S. damnosum* complex in Amagu Agba from June 2016 to July 2016

Collection site	June (%)	July (%)	Total number of flies caught
Amagu Agba	58 (63.7)	33 (36.2)	91

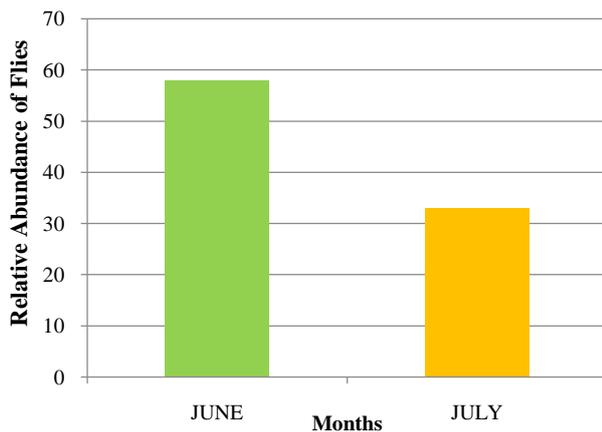


Figure 2. Monthly relative abundance of *S. damnosum* from June 2016 to July 2016

4. Discussion

The study was carried out close to the peak of the rainy season, June and July. This accounts for the relative small number of flies caught in the two months. This agrees with [29] but contradicts the findings of [30] and [4] who reported larger number of flies in the rainy season. The catching of 91 female *S. damnosum* complex during this study period might indicate that Amagu Agba is moderately infested with these blood-sucking flies as earlier reported by [31]. The results also revealed that there were a lot of activities along the river since it was the rainy season and all the farms around were cultivated [32]. The increase in anthropogenic activities provides sources of blood meal for the blackflies [16]. The study revealed that *Simulium damnosum* complex along Asu River were heavily infected. This is an indication that the hosts (humans) around the river might harbour microfilariae despite ivermectin treatment [32].

The results gleaned from this study also showed an enormous number of infective larvae of *Onchocerca volvulus* in the study area. This corroborates with the findings from epidemiological studies of the study area by [27]. The presence of parasites in the head region of dissected flies suggests the risk of infection to the inhabitants of Amagu Agba and its environs. This is because the infective larvae (L3) found in the head region of infected flies enter the human host through bite wounds when the female blackflies take blood meals [33].

The results are far from expectations given that the area is currently receiving CDTI through the NOCP. It therefore shows that some factors are interfering with the smooth

running of the programme [34]. The likely causes are not yet known but may not be far from the following factors:

- Patterns of treatment coverage and compliance
- Parasite ivermectin susceptibility
- Duration and effectiveness of former vector control if any
- Seasonality of transmission in relation to ivermectin distribution
- Parasite immigration in flies or people
- Vector species mix and their associated vectorial capacity and competence for *O. volvulus* [34].

5. Conclusion and Recommendations

The isolation of *O. volvulus* in the dissected head, thorax and abdomen suggests that these flies are actively engaged in the transmission of these parasites. It is also an indication of the availability of human sources of blood meals, some of which probably are reservoir hosts of these filarial parasites. Clear interpretation of the results is difficult since the period of study was a short one. Therefore, prolonged entomological studies should be carried out in Amagu Agba to really ascertain the biting patterns and transmission potentials of *S. damnosum* in the area.

However, there is a strong call for introducing more frequent ivermectin treatments or other strategies if elimination is to be attained in Amagu Agba village.

CDTI should be stepped up with efficient ivermectin distribution pattern, to be able to successfully interrupt transmission in these areas.

We recommend that a comprehensive strategic work plan be drawn up by the Ebonyi State Ministry of Health and other stakeholders to assess the infestation profile of *S. damnosum* along the Asu River for the institution of effective control measures.

Furthermore, river channeling improvement to clean and deepen Asu River with the removal of stones, logs and other obstructions, that cause ripples attractive to blackfly larvae should be encouraged.

A More eco-friendly-biocontrol approach by means of entomopathogenic spore-forming bacteria species, such as *Bacillus thuringiensis var. Israeliensis (Bti)*, as successfully accomplished in Florida State, in United State of America [35], Can also be applied in Asu River and other potentials sites.

Finally, with the ongoing CDTI programmes in the study area and in other areas, this survey advocates, that all the concerned communities with detectable onchocerciasis, should adopt some of the newly established molecular based detection techniques such as [Loop Mediated Isothermal Amplification Assay, LAMP [36], Restriction Fragment Length Polymorphism RFLP analysis [37], and Real-Time Polymerase Chain Reaction, [RT-PCR], q PCR analysis [38], for accurate and specific detection of *onchocerca volvulus* parasite in both blackfly population and human subjects, in combination with biocontrol [biopesticides] applications, and the blackfly dissection technique, as described in this study, to effectively control or eliminate onchocerciasis, in order to achieve the most sustainable environmental and ecological benefits.

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