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Amaechi AATropical Disease Research Unit,
Department of Zoology, Imo
State University Owerri, Nigeria**Ukaga CN**Tropical Disease Research Unit,
Department of Zoology, Imo
State University Owerri, Nigeria**Ajero CMU**Tropical Disease Research Unit,
Department of Zoology, Imo
State University Owerri, Nigeria**Iwunze JI**Tropical Disease Research Unit,
Department of Zoology, Imo
State University Owerri, Nigeria**Uzowuru DI**Tropical Disease Research Unit,
Department of Zoology, Imo
State University Owerri, Nigeria**Iheanacho JN**National Veterinary Research
Institute Vom Jos, Plateau
State, Nigeria**Nwachukwu MO**Biology Department, Federal
University of Technology
Owerri, Nigeria**Tony-Nze CP**United African Company LTD,
Nigeria**Okpara KK**Biology Department, Federal
University of Technology
Owerri, Nigeria**Achusim CC**Environmental Health Science
Department, Federal University
of Technology Owerri, Nigeria**Corresponding Author:****Amaechi AA**Tropical Disease Research Unit,
Department of Zoology, Imo
State University Owerri, Nigeria

Onchocerciasis transmission indicators following long-term ivermectin treatment in Imo river Basin, Nigeria

Amaechi AA, Ukaga CN, Ajero CMU, Iwunze JI, Uzowuru DI, Iheanacho JN, Nwachukwu MO, Tony-Nze CP, Okpara KK and Achusim CC

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Abstract

Simulium damnosum SI, the vector of Onchocerciasis in Nigeria, breeds in fast flowing and well oxygenated rivers and streams with nutrients. To monitor the impact of repeated community directed treatment with Ivermectin on the interruption of transmission and/or elimination of Onchocerciasis, the transmission indices of female flies caught between May 2019 and March 2020 were calculated. Captured black flies at Lolo and Ibbi Rivers were morphologically identified, dissected and processed for parity, infection/ infectivity as well as transmission status using a standard protocol. Of the 889 adult females caught, rainy season months (May-September) had a greater proportion than dry season months (October-March). Nulliparous black flies density (78.97%) was significantly higher than parous (21.08%) black flies ($P < 0.05$). 0.67% of the parous flies were infected with different parasite stages, but none had infective larvae (L_3 in the head). The calculated monthly biting rate (MBR) ranged from 259 (in February) to 899 (in September) with a mean biting rate of 662.925 bites/ person/ month. The monthly parous biting rate (MPBR) ranged from 23 (in February) to 247.5 (in November) with a mean parous biting rate of 140.225 bites/person/month. The transmission potential (TP), the number of infective bites/ person/ month was zero. These findings indicated entomological evidence of reduced vector biting activities and a low risk of river blindness infection in the study area. The public health implications were discussed, especially regarding the on-going community directed treatment with ivermectin (CDTI) and elimination strategy.

Keywords: Onchocerciasis transmission, ivermectin treatment, entomologic indices, Imo River Basin

Introduction

Onchocerciasis is one of the most debilitating yet neglected tropical diseases, NTDs [1] affecting the skin and eye leading to visual impairment and blindness. It is caused by a filarial nematode worm, *Onchocerca volvulus* and is transmitted by the bite of *Simulium* species (black flies) from person to person. Since the vector breeds in fast flowing rivers and those within the area are exposed to the disease, it is called river blindness. The female black flies are haematophagous and execute vectorial capacity by alternately seeking and picking blood meals from *O. volvulus* infected and uninfected patients in endemic areas. The infective larvae (L_3) are transmitted through the repeated bites of infective black flies which give rise to adult worms. It resides in humans' subcutaneous tissues where they can survive for about 15 years under drug pressure with adult females hatching about 1600 mf [2]. The MF migrates through the skin where they can be ingested by a black fly where it transforms into L_1 and L_2 larval stages and becomes infected. The L_2 subsequently develop into the L_3 stage, which migrates to the black fly's proboscis rendering it infective and as such can infect humans when taking a blood meal³ Water bodies which support the breeding of these flies have been identified in Nigeria including the Imo River Basin [4, 5, 6]. Over 20 million people are projected to be infected, 1 million are blind and 70 million are at risk of infection worldwide [7]. Nigeria accounts for one third of these estimates. The disease is found in all states of Nigeria with varying degrees of endemicity and intensity of clinical manifestations [8, 9, 10].

Various large-scale control programs through community-directed treatment of onchocerciasis with ivermectin (CDTI) have been implemented in onchocerciasis endemic areas in Africa, to mitigate the public health and socio-economic burden of onchocerciasis [11]. Ivermectin (IVM) or stromectol (mectizan) is a drug previously used for the veterinary purpose as broad-spectrum antihelminthes.

After IVM was discovered to have microfilaricidal activity and proven to be well tolerated, it was patented for human use for mass treatment of onchocerciasis [12, 13]. IVM was adopted in Nigeria in 1992 under the primary health care (PHC) scheme. Despite its challenges, its main objective is to break the disease transmission cycle [13]. It could also assuage deterioration of existing lesions, prevent anterior and posterior segment lesions in the eyes [14] or may aggravate them [8]. Further studies on the impact assessment of ongoing CDTI and its long term effect on parasitological, clinical changes and entomologic indices need to be fully evaluated. Epidemiological studies prior to and during the large scale distribution of IVM in communities close to the vicinity of some streams and rivers have confirmed the endemicity of the disease at different levels [15, 16, 17]. It will be worthwhile to fully understand the vector activities, including biting rates and transmission potential of the disease as part of the benefits of long term annual treatment practiced in Nigeria. The breeding activities of black flies in the Imo River Basin, Nigeria have been documented [4, 6] and findings revealed the presence of immature *S. damnosum* sl in the river bodies at varied populations, which is considered as a possible reflection of fly density in the area. This study investigated the relative abundance, biting activity and transmission potentials of the black flies following repeated IVM treatment in the Imo River Basin communities in Okigwe, Nigeria.

Materials and Methods

Study Area/ Choice of Capture Sites

The study was conducted on the river banks of a section of Ibbi River and Lolo River in two rural communities (Umulolo and Amuro) of Okigwe, Nigeria. These sites: Umulolo (N 05.85794; E007.32866) and Amuro (N05.7808; E007.26703) have been previously described [18,9].

Ethical Consideration

Ethical clearance for this study was obtained from the Imo State Ministry of Health, Okigwe Local Government Area Health Unit and Heads of study communities for the conduct of the research activities. Persons acting as human attractants for black fly catches were informed about the risks and benefits of participation.

Adult Black flies Collection

The collection of black flies was done twice each month, from 7:00 am to 6:00 pm each catching day. Carter Center Trained fly catchers in the communities were adopted. They worked on alternating hourly basis [19] and caught the flies as they landed on the exposed parts of their legs for blood meal. The

black flies captured were kept in a cold box containing ice packs to stop further microfilariae development.

Morphological Identification

The morphological identification was done using [20] criteria. Adult black flies were ascribed to either Savannah or forest species on the basis of color of some anatomical parts of the fly (the wing tuft, arculus, fore coxa and basal segment of the anterior) as either pale or dark for savannah and forest species respectively.

Dissection and Assessment for Parity, Infection and Infectivity Rates

Standard protocol for the dissection of black flies was adopted [21]. Black flies were inactivated individually with chloroform and then placed on a clean glass slides containing a drop of physiological saline. Parous black flies were further examined for stages of microfilariae and larvae in different anatomical sites (head, thorax and abdomen). Onchocerciasis transmission parameters (biting rates, biting parous rates and transmission potentials) were calculated following standard methods [19].

Data Analysis

The monthly relative abundance of *S. damnosum* was subjected to two-way analysis of variance (ANOVA) and the difference in infection/ infectivity rates evaluated by Chi-Square test. Prevalence was calculated as simple percentages. Probability level <0.05 was considered significant.

Results

The presence of black flies in Imo River Basin was recorded all year round. However, more black flies were caught in the rainy season months than dry season months (Figure 1). Nulliparous black flies were significantly higher in proportion than the parous black flies (Figure 2). The biting density and infection / infective rates are shown in Table 1. Overall, 187 (21.08%) of the 889 black flies were parous and varied with months of collection ($P < 0.05$). Six (6) black flies among the flies caught in May, November and March accounted for 0.6% infection but none was infective (L_3 in the head). Monthly entomological parameters pertaining to transmission of onchocerciasis are shown in Table 2. The monthly biting rate (MBR) ranged from 259 in February to 899 in September with a mean biting rate of 662.925 bites/ person/ month. Similarly, the monthly parous biting rate (MPBR) values ranged from 28 in February to 247.5 in November with a mean parous biting rate of 140.225 bites/ person/ month. In all the months, the value for the transmission potential was zero.

Table 1: Density and infection/ infectivity rates of black flies around Rivers Lolo and Ibbi.

Months	Total Catch/Dissected	No (%) Parous	No (%) Nulliparous	No Infected	%Infected	No Infective	% Infective
May	98	20 (20.4)	78 (79.6)	1	(1.0)	0	0.0
June	105	26 (24.8)	79 (75.2)	0	(0.0)	0	0.0
July	90	14 (4.4)	76 (84.4)	0	(0.0)	0	0.0
August	57	09 (15.8)	48 (84.2)	0	(0.0)	0	0.0
September	133	27 (20.3)	106 (79.7)	0	(0.0)	0	0.0
October	116	24 (20.7)	92 (79.3)	0	(0.0)	0	0.0
November	93	23 (24.7)	70 (75.3)	2	(2.2)	0	0.0
January	53	20 (37.7)	33 (62.3)	0	(0.0)	0	0.0
February	37	04 (10.8)	33 (89.2)	0	(0.0)	0	0.0
March	107	20 (8.7)	87 (81.3)	3	(2.8)	0	0.0
Total	889	187 (21.03)	702 (78.97)	6	(0.67)	0	0.0

Table 2: Monthly biting rate and Transmission Potentials of black flies around Rivers Lolo and Ibbi

Transmission Indices	May	June	July	August	September	October	November	January	February	March	Total
Total black flies caught/dissected	98	105	90	87	133	116	93	53	37	107	889
Total Parous	20	26	14	09	27	24	23	20	04	20	187
Total infected	1	0	0	0	0	0	2	0	0	3	6
Total infective	0	0	0	0	0	0	0	0	0	0	0
Total L ₃ larvae	0	0	0	0	0	0	0	0	0	0	0
Calculated MBR	759.5	785.5	697.5	44.75	817.25	899	697.5	410.75	259	829.25	6,629.25
Calculated MPBR	155	195	108.5	69.75	20.25	186	247.5	155	28	155	1,402.25
Calculated MTP	0	0	0	0	0	0	0	0	0	0	0

MBR Monthly biting rate
 MPBR Monthly parous biting rate
 MTP Monthly transmission potentials

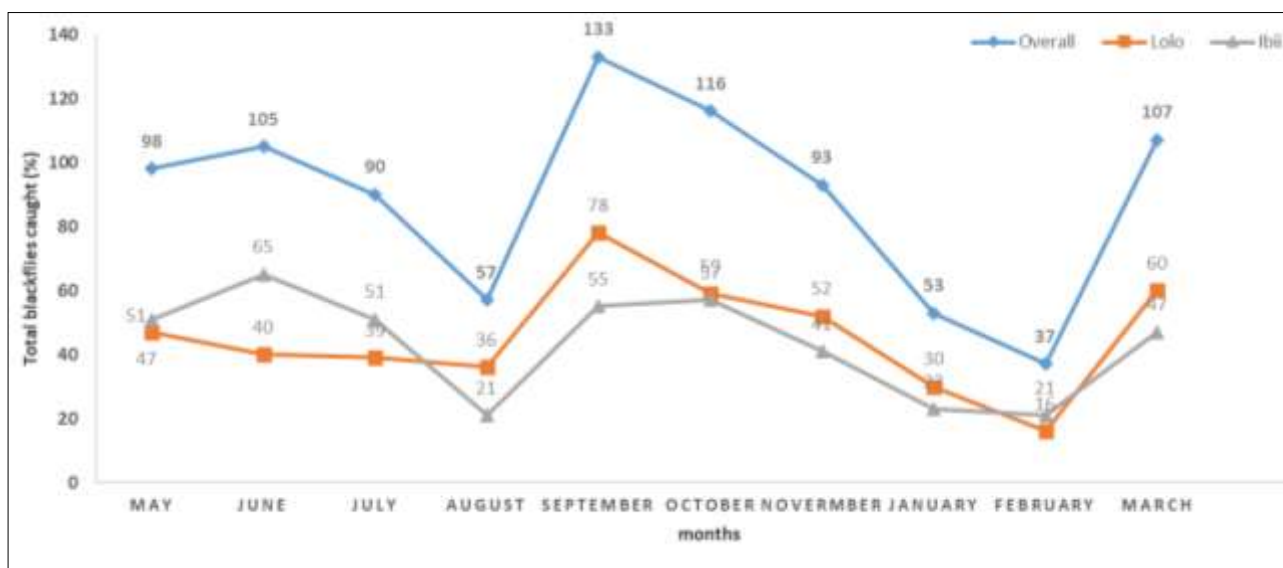


Fig 1: Monthly catches of biting black flies around Lolo and Ibi Rivers

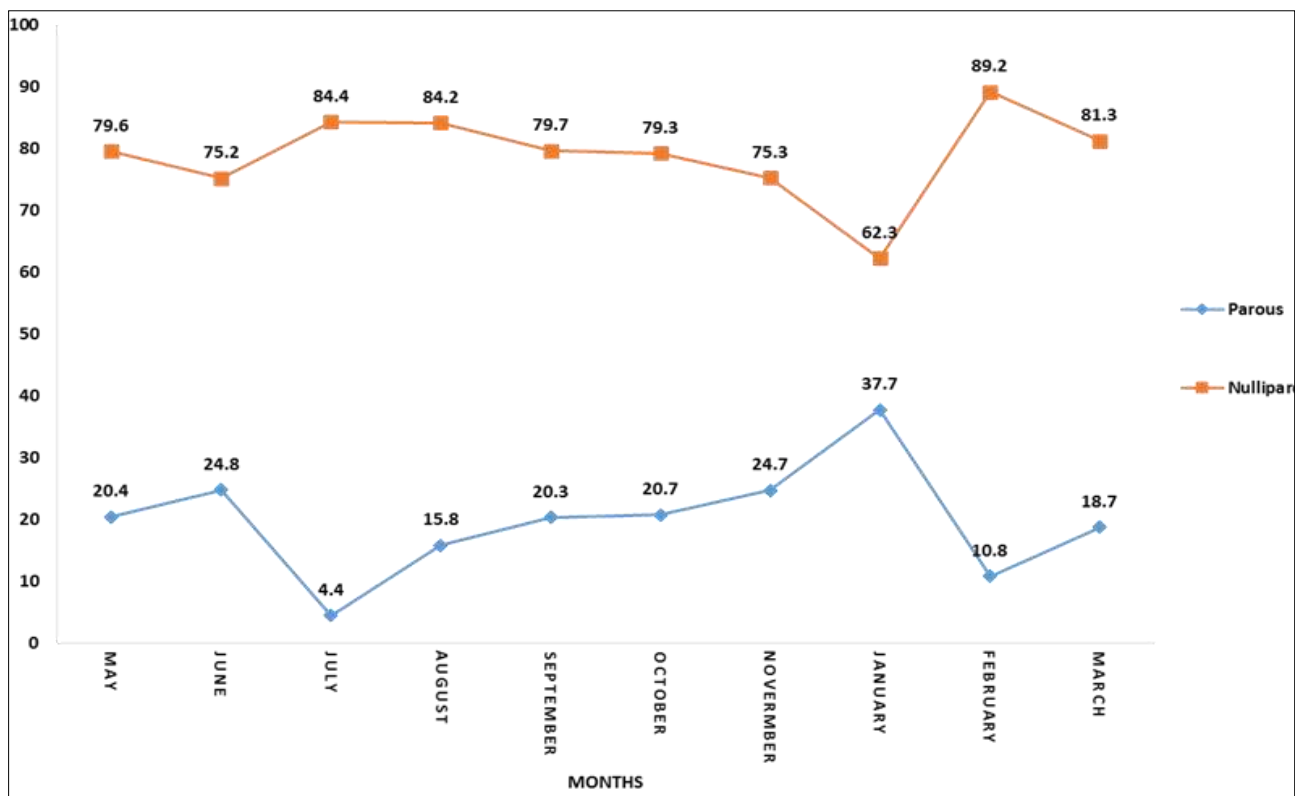


Fig 2: Physiological/Parity status of biting black flies around the River sites.

Discussion

In central Nigeria (Plateau and Nasarawa States) [22] accounted for interruption of transmission of human onchocerciasis and first stop MDA for onchocerciasis in Nigeria. This study was a follow up to the halt LF MDA in these states [23, 24]. Current study was initiated by the fundamental lack of information as to whether or not MDA should be stopped in Imo River Basin communities, part of south east Nigeria that have been under MDA with ivermectin for the past two decades. The study accounted for dataset of biting density and transmission from two sites in onchocerciasis-endemic Imo River Basin. We observed monthly activity of *S. damnosum* around Lolo and Ibi Rivers all year round which showed that rainy season months accounted for majority of the black flies. This is in agreement with previous reports where populations of black flies were at peak in the wet season [15, 25]. In previous studies on population dynamics of immature forms of *S. damnosum sl* in Imo River Basin [4, 6] both larvae and pupae of *Simulium* flies were found in both seasons attributable to the occurrence to biotic and abiotic factors favorable to the development of the aquatic stages of the flies to adult. Carter Center/ Global 2000, a nongovernmental organization has carried out a lot of intervention research on tropical diseases in south eastern Nigeria including Imo River Basin. They train locals and volunteers on ownership of projects and participation. Thus, the use of Carter Center trained black flies catchers in these areas contributed equally to the success of abundant flies recorded in the study as some of the flies that did not probe on landing before flying off were minimized. Few of the flies that probed and settled to feed suggested the presence of non anthropophilic members of *S. damnosum* in the area which may be *S. squamosum* as documented by [4]. These species have been found to show zoophilic behavior by feeding on non-human blood meal [26]. Blood meal analysis and speciation of the black flies beyond morphological status will be needed to determine their roles in the study.

Entomological evaluations as highly specific indicators of changes in community microfilarial load are known to correlate well with ivermectin coverage [27]. Proportion of infected black flies (0.67%) recovered points to parasite present in the human and population. Probably parasite resistance to ivermectin due to intensive and widespread use [28]. Differential resistance by subjects with different blood group phenotypes to onchocerciasis filarial infection is needed to clarify this.

Thus these findings suggested that ivermectin distribution should continue since DNA from mf or L₂ stages is used to evaluate the presence of the parasite in humans and cattle [29]. Since assessment of infectivity rate was the standard for ascertaining the level of transmission, our result probably indicate possible interruption of transmission within the communities around the study areas. We observed a break of the transmission cycle on the sites as transmission seems to be halted in the foci due to ivermectin intervention. The treatment intervention in these foci has passed the estimated life expectancy of the worm (10-15 years) almost by factor two. Parity status revealed lower parous rate than nulliparous rates among dissected black flies. The observed low parous rate could be indicative of low vector-human contact or absence of practices within the body of the host which the black flies could pick during blood meals. The observed low infection rate is consistent with low parous rate and may

indicate success with drug coverage or adherence. Poor ivermectin drug adherence and its contribution to persistence levels of meso endemicity have been reported [30] contrary to our finding.

Interestingly, the monthly biting and infection rates were low with absence of infectivity rate and monthly transmission potentials. The result could indicate that transmission has been halted considering that the presence of onchocerciasis parasite in black fly bodies does not necessarily imply current transmission which usually need the presence of L₃ in the head [29]. In the light of the on-going CDTI in the country, the epidemiological significance of these findings is that onchocerciasis patients within the villages carry low skin microfilariae (skin mf) which is resultant from the long term ivermectin intervention [31, 32, 17]. It also revealed low transmission of onchocerciasis within the communities around the study sites. Low values of MBR and MPBR points to sporadic or low disease transmission as the values especially annual transmission potential is <1,000 which is tolerable transmission value stipulated by [7].

Ivermectin and efficacy at suppressing the uptake of skin mf by black flies for up to 6 months after treatment was reported by Cupp *et al.* (1986). The current study was conducted approximately 5 months after CDTI round and the possibility of diminished effect of ivermectin on black flies cannot be ruled out. A study in a community in south eastern Nigeria [32] has shown skin mf reduction after years of ivermectin therapy. This could account for low infection and zero infectivity rates in the black flies since they could pick only few or no mf in their blood meals. Similarly, in a study on diethylcarbamazine treatment against *Wuchereria bancrofti* transmission, it was found that the proportion of *Culex quinquefasciatus* infected and the number of the parasite larvae per infected vector were proportional to the mf density at the time of feeding [34]. WHO/APOC entomologic criteria for onchocerciasis stop MDA decision requires infection rate in vector < 0.05%. Obstacle to this in our study remains our inability to capture up to 6,000 black flies which cannot be ignored due to logistic capacity. Also, from epidemiological perspective infection status <1.0% will be insufficient to maintain the basic reproductive cycle rate/ population and eventually it will lead to the extinction of the parasite. Based on the observations made in this study, it is predicted that onchocerciasis in communities around Imo River Basin will be sporadic and of low prevalence preparatory to elimination. Notwithstanding, the entomologic assessment of all neighboring communities is needed before taking decision on annual MDA to avoid recrudescence of the disease as was the case in Burkina Faso [35]. The use of OV16 serology test to detect exposure of the

O. volvulus parasite in children < 10 years cannot be overlooked [36]. An understanding of the local vectors, bionomics and transmission parameters are very vital for successful control of onchocerciasis and any strategy aiming at control will have to account for this heterogeneity.

Conclusively, the density of black flies and sporadic nature of infection suggested the scaling up of both biannual CDTI which started in 2018 and biannual CDTI supplemented with vector control. These methods have recently been effective in interrupting transmission in foci in Uganda and Tanzania [37, 38] and should be considered elsewhere in the River Basin to facilitate quick elimination of the black flies and mf from the skin.

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