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ENTOMOLOGICAL EVALUATION OF ONCHOCERCIASIS STATUS IN EASTERN OBAN HILLS COMMUNITIES OF CROSS RIVER STATE, NIGERIA (AFTER 20 YEARS OF IVERMECTIN INTERVENTION)

[1]*Dr. Cletus Inah Iboh, [2]Dr. Oliver E. Effiom and [3]Theophilus Effiong Akpe

[1,3]Department of Biological Sciences, Cross River University of Technology, PMB 1123, Cross River State, Nigeria.

***Corresponding Author: Dr. Cletus Inah Iboh**
Department of Biological Sciences, Cross River University of Technology, PMB 1123, Cross River State, Nigeria.

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ABSTRACT
Ivermectin (Mectizan) treatment programme commenced in Cross River State by the effort of United Nation International Children Emergency Fund (UNICEF) and government in 1995. Since then, no information had been provided on *Onchocerca volvulus* infection of *Simulium damnosum* s.l. and onchocerciasis status in communities of Eastern Oban Hills. This necessitated an entomologic evaluation for 12 months (January to December 2015) at Ibe, Mankor and Kwa Falls to provide information on the infection rate and the interruption of onchocerciasis transmission after 20 years of annual community-directed treatment with ivermectin. Out of 590 female *Simulium damnosum* flies collected, 3486 (97.1%) flies were dissected and only 17 (0.5%) were infected. There was significant variation F0. (11, 22) (P = 001), in the relative fly abundance and diurnal biting pattern of parous flies at the three foci. The infectivity rates of parous flies recorded were very low: 0.20%, 0.60, and 0.59% at Ibe, Mankor and Kwa Falls respectively. There was a biting nuisance with a high annual biting rate (ABR) of 5894.8, 7124.6 and 8790.4 infective larvae per person per year at Ibe stream, Mankor stream and Kwa Falls respectively. The annual transmission potentials (ATPs) of 0.54, 18.4 and 18.3 recorded at Ibe, Mankor, and Kwa Falls respectively, are lower than the tolerable values provided by World Health Organization in 1995 for interruption of onchocerciasis. There was, therefore, suppression of *Onchocerca volvulus* transmission in the three foci.

KEYWORDS: *Simulium damnosum* s.l. and onchocerciasis.

1. INTRODUCTION
Onchocerciasis also called ''river blindness'' is caused by a filarial worm *Onchocerca volvulus*, transmitted to human through the bite of *Simulium damnosum* complex.[1] The disease has a wide geographical spread and mostly found in sub-Saharan Africa.[2] The consequences of this disease does not only range from clinical manifestations to abandonment of fertile farm lands[3], but also total liability.[4] It has been revealed by epidemiological studies that 37 million people in 34 endemic countries are infected with onchocerciasis, and about 90 million at risk of infection.[5,6,7] However, 99% of the global burden is found in Africa.[2] One of the most endemic areas of onchocerciasis in the world is Nigeria with approximately 3.2 million infections.[4] The severity of the disease varies in relation to ecological zones and different cytogenetic vectors, giving rise to two types of vectors – savannah and forest vectors.[8] The Savannah vectors transmit the savannah strain of. *volvulus* which causes blindness, while the forest type transmits the forest strain of *O. volvulus* responsible for skin diseases like pruritus and leopard skin.[9,10] Researchers have observed that the prevalence of human onchocerciasis is directly related to the abundance of the vector.[8,11] Thus, the need for proper understanding of the transmission dynamics of onchocerciasis in advancing knowledge on how vector competence, behaviour and abundance is key to the level of infection and disease in the susceptible human population.[12] Successful reports of interruption of transmission of *O. volvulus* in former endemic areas of America, West and East Africa have raised the question whether the elimination of this debilitating disease is underway after long-term treatment of population at risk with ivermectin.[13] Even though mass chemotherapy with Mectizan, and Albendazole had been used formerly to interrupt *O. volvulus* transmission in most African countries, interrupting disease transmission could also be through reduction of human-fly contact.[6,14] It has been asserted that to evaluate the impact of onchocerciasis control programs and the annual transmission potential, it is customary to monitor the man-biting rate of the vector *Simulium* and the number of *O. volvulus* infective larvae in them.[15] The abundance of onchocerciasis vectors affects the epidemiology of the disease in communities of Eastern Oban hills of Cross River State.

Inhabitants of this area had been treated with annual doses of Ivermectin and albendazole for over 20 years The current onbchocerciasis incidence of 9.4/5.7 in the study area was recorded in 2005 at Kwa Falls. This study is therefore designed to obtain baseline data for the evaluation of interruption of *Onchocerca volvulus* transmission after 20 years (1995-2015) of annual community-directed treatment with ivermectin and the man-biting rate of the vector in the study area.

2. MATERIALS AND METHODS

2.1 Study Site

Communities in Eastern Oban Hills occupy a landmass of 36.9 square kilometres within the tropical rainforest belt.[16] This area is located at 488 metres/1600 feet altitude of the Oban hills,[17] and between latitude 5^0 19` 43`` North of the equator and longitude $8^0$ 35` 27`` East of the Greenwich meridian (Fig. 1). The climate of this area is characterized by tropical humid conditions with a mean annual rainfall of 273.9 mm, a mean annual temperature of 30.8^0C and a relative humidity of 85.7% at the peak of the rainy season.[18] There are two seasons in the area, the rainy season from April to October and dry season from November to March. The area is drained by numerous tributaries like Ibe stream and Mankor stream, (the study sites) and Ojuk stream. These streams originate from Oban hills, an extension of the Western Cameroon Highlands, draining the land south westwards. Ojuk and Mankor streams meanders eastward across Calabar-Aking road to meet with Ibe stream which originate from Okarara region of the Oban hills. This finally joins Kwa river southwards which empties at the Atlantic Ocean. On the south-eastern side of Oban hills is Kwa Falls (another study site) which drains southwards to meet with the Idundu River. Due to the rocky topography of this area, streams and rivers have stony substratum with rapids which enhanced fast flowing streams and rivers, therefore constitutes good breeding sites for *Simulium* flies. Agriculture is the main stay of the people.

2.2 Ethics Statement

Ethical clearance was obtained from the ethics committee of Cross River University of Technology, Calabar. There was no blood sample or tissue taken from human subjects. Although no human samples were obtained for analysis, participating vector collectors received ivermectin as part of the national programme following the annual schedule by WHO.[19]

2.3 Selection of Site

Criteria used for the selection of the study sites were based on nearness to living population as source of blood meals for *Simulium* flies, and previous survey of vectors of onchocerciasis. Kwa Falls was previously surveyed by us for morphological studies of female *S. damnosum*, and is a tourist destination in Cross River State. It is about 300 metres from Aningeje community. Ibe and Mankor streams were selected because of their proximity to Ibe and Mankor communities. These streams have also been surveyed by us for immature stages of *S. damnosum*. Communities in the selected sites have received ivermectin and albendazole treatment every year for the past 20 years (1995-2015).

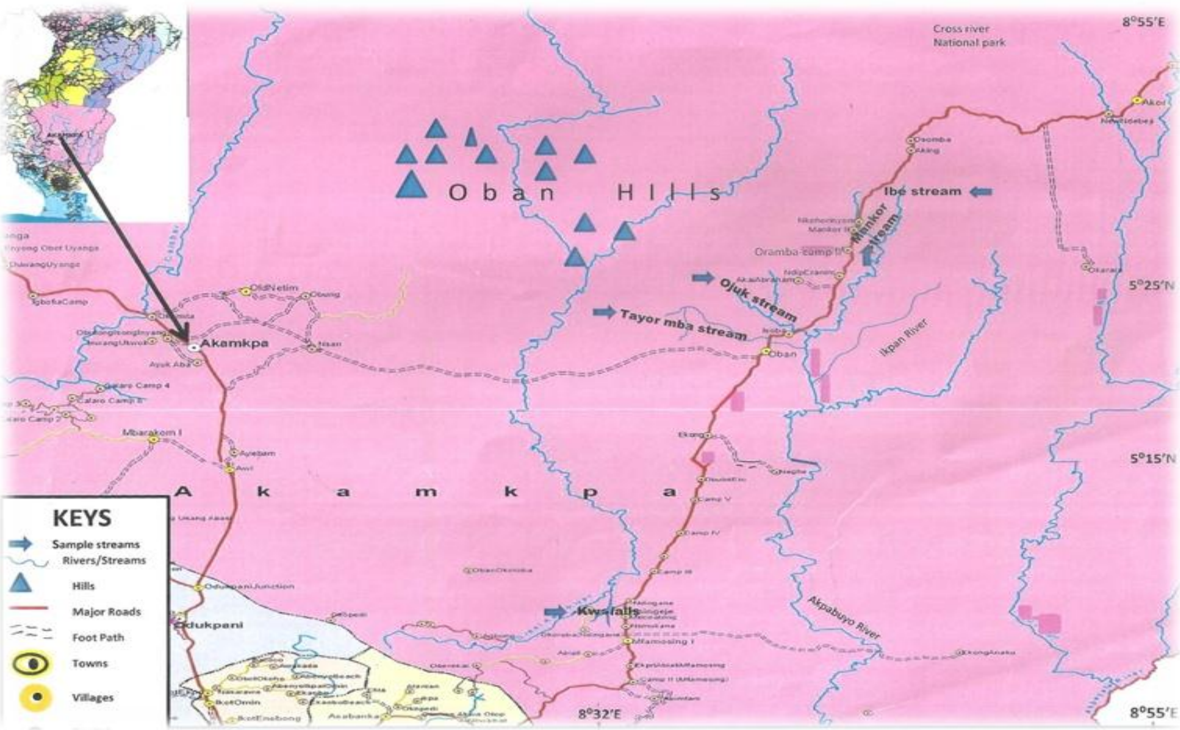

Fig. 1: Showing communities and streams in Eastern Oban Hills of Cross River State.

2.4 Method of Collecting Adult Black Flies

The human bait method was used in collecting black flies at Ibe, Mankor and Kwa Falls between January and December 2015. Each of these study sites was sampled five times in a month. Collection was conducted between 0600 hours in the morning and 0600 hours in the evening by two fly collectors who worked on a 6 hour shift daily. The fly collectors sat on wooden stool at the banks of streams and waterfall exposing their legs from the knee and hands.[1,15,20] 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. Tubes containing flies caught were labelled according to the site of capture, time and date, and transported to the laboratory in ice packed boxes to prevent further microfilariae development before dissection.[11,21]

2.5 Collection of Larvae

The method used for larvae collection was that of Srisuka et al.,[20][22] Larvae and pupae were collected from submerged rock surfaces, submerged dead wood, dead decayed leaves, and submerged weeds. Larvae and pupae were preserved in separate plastic vials (7.5 cm long and 2.1 cm wide), containing 70 per cent ethyl alcohol before transportation to Biological Science Laboratory of Cross River University of Technology, Calabar, for cytotaxonomic studies. Larvae were identified using keys provided by Crosskey (1962).[23]

2.6 Dissection of Adult Black Flies

Flies caught in every sampling week were anaesthetized with chloroform and dissected fresh with dissecting needles[1] to determine their parity (physiological age).[20] Before dissection, each fly was placed on a glass slide in normal saline solution and the head, thorax and abdomen separated using a scalpel under a dissecting microscope.[1] Flies were recorded as nulliparous indicating that they had not yet taken a blood meal and could not have a parasite larva, resulting in tightly coiled trachea systems and absence of follicular relics. However, flies were identified as parous indicating that they are blood-fed and completed at least one gonotrophic cycle, resulting in the presence of follicular relics below the maturing oocyte and loosely stretched condition of the tracheal system.[24,25,26] The parous flies were further dissected in search of filarial worms which were classified as L1 (sausage stage), L2 (pre-infective stage) and L3 (infective stage) according to the literature of Potter and Collins.[27]

2.7 Calculations of Transmission Indices

The calculations of daily biting rate (DBR), daily parous biting rate (DPBR), monthly biting rate (MBR), monthly parous biting rate (MPBR), monthly transmission potential (MTP), and annual transmission potential (ATP) were carried out using formulae provided by several researchers,[15,28,29,30,31,32,33] as follows:

DBR = (Total No. of flies caught in a month/ Number of catching days).

DPBR = (Total No. of parous flies caught in a month/ Number of catching days)

MBR = {(No. of flies caught in a month x number of days in the month)/ Number of catching days}

MTP = [{(No of days in the month x No of infective larvae)/ Number of days worked} x (No of flies caught/ No of flies dissected)]

MPBR = {(No parous flies caught in a month x No of days in the month)/ No of catching days}

ABR = Sum of 12 MBRs for the year.

ATP = Sum of 12 MTPs for the year.

2.8 Statistical Analysis

Data generated from the sampling sites were subjected to two-way analysis of variance (ANOVA) and chi-square test. The chi-square was used in testing the significant difference between morning and afternoon fly abundance as well as variation between monthly biting rate and monthly transmission potential. The analysis of variance was used to compare the difference in relative abundance of flies and diurnal biting pattern of parous flies at the three sites.

3. RESULTS

3.1 Relative Abundance of *S. damnosum* Flies

A total of 3590 *Simulium damnosum* adult female flies were caught from the three sampled sites, 974 from Ibe stream, 1171 from Mankor stream and 1445 from Kwa Fall. There was significant variation F0. (11, 31) (P = 001) in the relative fly abundance at the three sites. A total of 2272 *S. damnosum* flies were caught in the morning hours and 1318 flies in the afternoon throughout the study period. The morning and afternoon fly abundance showed a significant variation (X^2 = 197.1, P = 001) at the three sites. The monthly variation of fly abundance of *S. damnosum* is shown in Fig. 2. There was increase in fly abundance from January until April where the morning peak was observed, then declined between May and July where it started rising until September when the second peak was seen and finally declined till December.

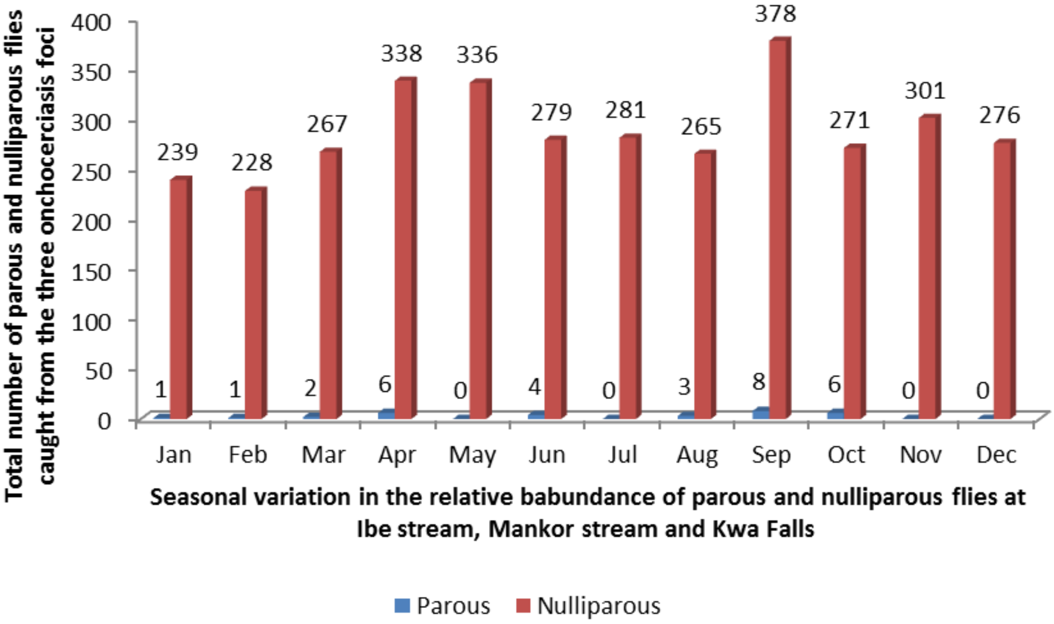

Fig. 2: Seasonal variation and abundance of flies in three streams, Nigeria 2016.

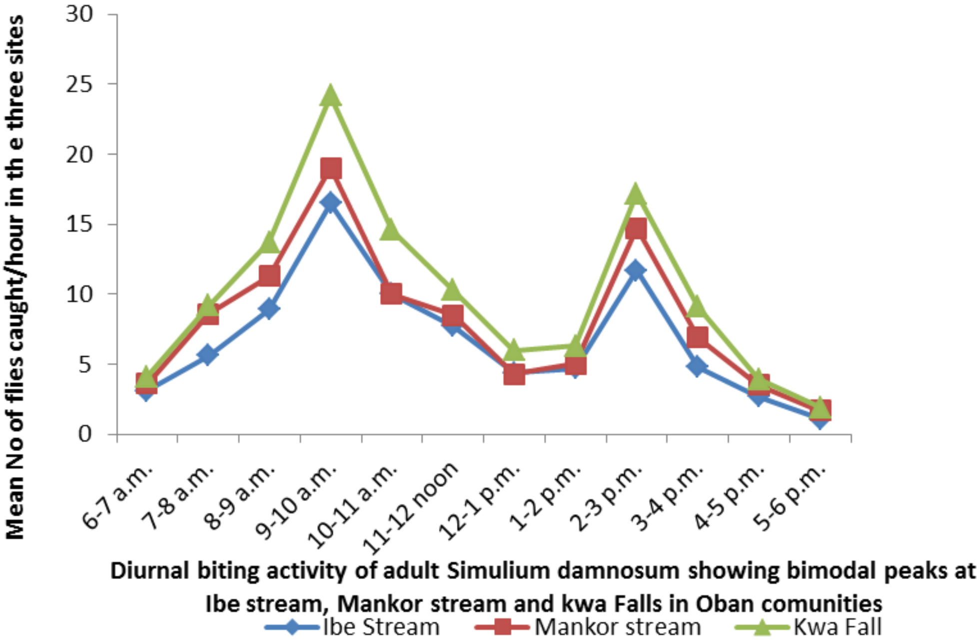

Fig. 3: Diurnal biting activity of adult Simulim damnosum, Nigeria 2016.

3.2 Diurnal Biting Pattern of *S. damnosum*

The diurnal biting pattern of *S. damnosum* flies in Ibe stream, Mankor stream and Kwa Falls showed bimodal peaks of activity, with the morning peaks being more conspicuous than the afternoon peaks (Fig. 3). The morning peaks were observed between 0900 and 1000 hours and the afternoon peaks between 0200 and 1500 hours. The diurnal biting peaks corresponded with peak farming activity in the study area. The biting cycle increased from 0600 to between 0900 and 1000 hours forming the morning peak, then declined between 1100 and 1400 hours when it increased until 1500 hours forming the afternoon peak.

3.3 Vector Transmission Indices

The monthly entomologic transmission indices at Ibe stream are shown in Table 1. A total of 974 *S. damnosum* adult female flies were collected from Ibe stream and 968 (99.4%) were dissected, of which 963 (99.5%) were nulliparous flies and only 6 (0.6%) were parous flies. A total of 2 (33.3%) parous flies were infected with L1, L2 and L3 larvae of *O. volvulus* and the 2 flies were infective, having L3 larvae. The peak monthly biting rate

(MBR) was recorded in September with 719.2 bites per person per month, and the least was observed in February with 308 bites per person per month. However, the peak monthly transmission potential (MTP) was observed in September with 0.54 L3 per person per month. An annual biting rate (ABR) of 5894.8 bites per person per year was recorded in this site. There was significant variation (X^2 = 46, P = 001) in the monthly biting rates (MBR) and monthly transmission potential (MTP) in Ibe stream. An ATP of 0.54 L3 per person per year was recorded at Ibe stream.

Similarly, the monthly entomologic transmission indices at Mankor stream are shown in Table 2. Of the 1171 *S. damnosum* flies caught 1163 (99.3%) were dissected, of which 1155 (98.9%) were nulliparous flies and 8 (0.8%) parous flies. A total of 7 (87.5%) parous flies were infected with L1, L2 and L3 *O. volvulus* larvae. The peak MBR was seen in September with 720 bites per person per month, while the least was observed in February with 464.8 bites per person per month. The ABR recorded in Mankor was 7123.6 bites per person per year. An ATP of 18.4 L3 per person per year was recorded in this site. There was a significant difference (X^2 = 148.2, P = 001) in the monthly biting rate (MBR) and MTP at Mankor stream.

Monthly entomologic transmission indices of *S. damnosum* at Kwa Falls are shown in Table 3. A total of 1445 *S. damnosum* flies were caught at Kwa Falls, and 1355 (93.8%) were dissected of which 1340 (98.9%) were nulliparous flies and 16 (1.10%) were parous flies. Of these parous flies 6 (37.5%) were infected with L1 and L2 of *O. volvulus*, of which 2 (0.15%) were infective with L3 larvae. The highest peak of MBR was recorded in September with 942 bites per person per month, while the lowest MBR was observed in February with 515.2 bites per person per month. An ABR of 8790.4 bites per person per year was recorded in Kwa Falls, while the ATP at Kwa Falls was 18.3 L3 per person per year. There was significant difference (X^2 = 185.4, P = 001) in the monthly biting rates (MBR) and MTP at this site.

A summary of population and annual transmission indices of *S. damnosum* is shown in Table 4. A total of 3590 *S. damnosum* flies were collected from the three sites and 3486 (97.1%) were dissected of which 3458 (96.3%) were nulliparous flies and 30 (0.86%) were parous flies. Of the parous flies dissected, 12 (40%) were infected and 7(23.3%) flies were infective. Annual biting rates of 5894.8, 7123.6 and 8790.4 bites per person per year were observed at Ibe stream, Mankor stream and Kwa Falls respectively. A mean ABR of 7269.6 bites per person per year and a mean ATP of 12.4 L3 per person per year were recorded for the three sites.

Table 1: Population density and transmission indices of *S. damnosum* at Ibe stream in Eastern Oban Hill, Nigeria, 2016.

Activity	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Person number of days worked	5	5	5	5	5	5	5	5	5	5	5	5	60
Total number of flies caught	50	55	74	90	88	76	83	75	116	87	93	87	969
Average daily catch per person	25.0	27.5	37.0	45.0	44.0	38.0	41.5	37.5	58.0	43.5	46.5	43.6	40.6
Number of flies dissected	50	54	74	89	88	74	83	75	116	85	93	87	968
No (%) of nulliparous flies caught	50 (100)	53 (98.1)	74 (100)	88 (98.9)	88 (100)	72 (97.3)	83 (100)	75 (100)	115 (99.1)	83 (97.6)	93 (100)	87 (100)	962 (99.4)
No (%) of parous flies caught	0 (.00)	1 (1.90)	0 (0.00)	1 (1.10)	0 (0.00)	2 (2.70)	0 (0.00)	0 (0.00)	1 (0.90)	2 (2.4)	0 (0.00)	0 (0.00)	6 (0.6)
Total No (%) of flies infected	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.10)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.20)	0 (0.00)	0 (0.00)	2 (0.20)
Flies (%) with L1 and L2	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.10)	0 (0.00)	0 (0.00	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.20)	0 (0.00)	0 (0.00)	2 (0.20)
Flies (%) with L3	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.90)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.10)
Biting density	0.2	0.2	0.25	0.3	0.29	0.25	0.28	0.25	0.39	0.29	0.31	0.29	1.35
Daily biting rate (DBR)	10	11	14.8	18.0	17.6	15.2	16.6	15.0	23.2	17.4	18.6	17.4	19.4
Daily parous biting rate (DPBR)	0 (0.00)	1 (0.20)	0 (0.00)	1 (0.20)	0 (0.00)	2 (0.4)	0 (0.00)	0 (0.00)	1 (0.20)	0 (0.00)	0 (0.00)	0 (0.00)	5 (0.6)
Monthly biting rate (MBR)	310	308	458.8	540.0	545.6	456.0	514.8	465.0	719.2	522.0	558.0	539.4	5894.8
Monthly parous biting rate (MPBR)	0 (0.00)	1(5.6)	0 (0.00)	1 (5.6)	0 (0.00)	2 12.0)	0 (0.00)	0 (0.00)	0 (0.00)	(0.00)	0 (0.00)	0 (0.00)	6 (36.5)
Monthly transmission potential (MTP)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0 (0.54)	0.0	0.0	0.0	0.54

Table 2: Population density and transmission indices of *S. damnosum* at Mankor stream in Eastern Oban Hills, Nigeria, 2016.

Activity	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Person number of days worked	5	5	5	5	5	5	5	5	5	5	5	5	60
Total number of flies caught	79	83	81	119	101	93	106	112	120	86	97	94	1171
Average daily catch per person	39.5	41.5	40.5	59.5	50.5	46.5	53.0	56.0	60.0	43	48.5	47	48.9
Number of dissected flies	78	83	81	117	101	93	106	112	117	84	97	94	1163
No (%) of nulliparous flies caught	77 (98.7)	83 (100)	81 (100)	115 (98.3)	101 (100)	93 (100)	106 (100)	112 (100)	114 (97.4)	82 (97.6)	97 (100)	94 (100)	1155 (99.3)
No (%) of parous flies caught	1(1.3)	0 (0.00)	0 (0.00)	2 (1.7)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (2.6)	2 (2.4)	0 (0.00)	0 (0.00)	8 (0.69)
Total No (%) of flies infected	0 (0.00)	0 (0.00	0 (0.00)	2 (1.7)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (2.6)	1 (1.2)	0 (0.00)	0 (0.00)	7 (0.6)
Flies (%) with L1 and L2	0 (0.00)	0 (0.00)	0 (0.00)	2 (1.7)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (1.7)	0 (0.00)	0 (0.00)	0 (0.00)	4 (0.34)
Flies (%) with L3	0 (0.00)	0 (0.00)	0 (0.00)	2 (1.7)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.85)	0 (0.00)	0 (0.00)	0 (0.00)	3 (0.26)
Biting density of flies	0.26	0.28	0.27	0.40	0.34	0.31	0.35	0.37	0.4	0.29	0.32	0.31	1.63
Daily biting rate (DBR)	15.8	16.6	16.2	23.8	20.2	18.6	21.8	22.4	24.0	17.4	19.4	18.8	19.5
Daily parous biting rate (DPBR)	0.2	0	0	0.8	0	0	0	0	0.6	20.4	0	0	2.0
Monthly biting rate (MBR)	489.8	464.8	502.2	714.0	626.2	558.0	657.2	694.4	720.0	533.2	582.8	582.8	7123
Monthly parous biting rate (MPBR)	6.2	0	0	24.0	0	0	0	0	18.0	12.4	0	0	60.6
Monthly transmission potential (MTP)	0.0	0.0	0.0	12.2	0.0	0.0	0.0	0.0	6.2	0.0	0.0	0.0	18.4

Table 3: Population density and transmission indices of *S. damnosum* at Kwa Falls in Eastern Oban Hills, Nigeria, 2016.

Activity	Jan	Fe	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Person number of days worked	5	5	5	5	5	5	5	5	5	5	5	5	60
Total number of flies caught	114	92	123	138	150	107	100	73	157	110	111	97	1445
Average daily catch per person	57	46	61.5	69	75	53.5	50	36.5	78.5	55	55.5	48.5	60.2
No (%) of flies dissected	112	92	119	138	147	105	100	73	153	108	111	95	1355
No (%) of nulliparous flies caught	112 (100)	92 (100)	104 (87.4)	135 (97.8)	147 (100)	103 (98.1)	100 (100)	70 (95.9)	149 (97.4)	106 (98.1)	111 (100)	95 (100)	1340 (98.9)
No (%) of parous flies caught	0 (0.00)	0 (0.00)	15 (12.6)	3 (2.2)	0 (0.00)	2 (1.9)	0 (0.00)	3 (4.1)	4 (2.6)	2 (1.9)	0 (0.00)	0 (0.00)	16 29 (2.14)(1.1)
Total No (%) of flies infected	0 (0.00)	0 (0,00)	1 (0.8)	2 (1.4)	0 (0.00)	0 (0.00)	0 (0.00)	2 (1.4)	3 (2.0)	0 (0.00)	0 (0.00)	0 (0.00)	8 (0.59)
Flies (%) with L1 and L2	0 (0.00	0 (0.00)	0 (0.00)	2 (1.4)	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.4)	3 (2.0)	0 (0.00)	0 (0.00)	0 (0.00)	6 (0.44)
Flies (%) with L3	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (1.3)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.15)
Biting density	0.38	0.31	0.41	0.46	0.5	0.36	0.33	0.24	0.52	0.37	0.37	0.32	2.0
Daily biting rate (DBR	22.8	18.4	24.6	27.6	30	21.4	20.0	14.6	31.4	22.0	22.2	19.4	24.1
Daily parous biting rate (DPBR)	0	0	0.4	0.6	0	0.4	0	0.6	0.8	0.4	0	0	0.2
Monthly biting rate (MBR)	706.8	570.4	762.6	828.0	930.0	642.0	620.0	452.6	942	682.0	666.0	601.4	8790.4
Monthly parous biting rate (MPBR)	0	105.4	12.4	18	0	12.0	0	18.6	24	12.4	0	0	97.3
Monthly transmission potential (MTP)	0	0	0	0	0	0	0	0	18.5	0	0	0	18.3

Table 4: Summary of population and transmission indices of *S. damnosum* at Ibe stream, Mankor stream and Kwa Falls in Eastern Oban Hills, Nigeria, 2016.

Activity	Ibe stream	Mankor stream	Kwa Fall
Persons number of days worked	60	60	60
Total number of flies caught	969	1171 (32.6)	1445 (40.3)
Average daily catch per person	40.6	48.9	60.2
Number of flies dissected	968	1163	1355
No (%) of nulliparous flies caught	962 (99.4)	1155 (89.3)	1340 (98.9)
No (%) of parous flies caught	6 (0.6)	10(0.8)	16 (1.1)
Total No (%) of flies infected	2 (0.20)	7 (0.6)	8 (0.59)
Flies (%) with L1 and L2	2 (0.20)	4 (0.34)	6 (0.44)
Flies (%) with L3	1 (0.10)	3 (0.26)	3 (0.22)
Biting density	1.35	1.63	2.0
Daily biting rate (DBR)	19.4	19.5	24.1
Monthly biting rate (MBR)	5397.4	7124.2	8790.4
Monthly biting rate (MBR)	6 (36.5)	60.6	97.3
Monthly transmission potential (MTP)	0.54	18.4	18.3

Table 5: Monthly parous infection rate of *S. damnosum* in the three onchocerciasis foci.

Month	Ibe Stream					Mankor Stream					Kwa Falls				
	Total flies dissected	Parous flies caught	Parous rate	No +ve for larval stages each month	Infection rate	Total flies dissected	Parous flies caught	Parous rate	No +ve for larval stages each month	Infection rate	Total flies dissected	Parous flies caught	Parous rate	No +ve for larval stages each month	Infection rate
Jan	50	0	0	0	0.00	78	1	1.3	0	0.00	112	0	0.00	0	0.00
Feb	54	1	1.9	0	0.00	83	0	0.0	0	0.00	92	0	0.00	0	0.00
Mar	74	0	0.0	0	0.00	81	0	0.0	0	0.00	121	2	1.65	1	0.83
Apr	89	1	1.1	1	1.10	117	2	1.7	3	2.56	138	3	2.17	2	1.45
May	88	0	0.0	0	0.00	101	0	0.0	0	0.00	147	0	0.00	0	0.00
Jun	74	2	2.7	0	0.00	93	0	0.0	0	0.00	105	2	1.90	0	0.00
Jul	83	0	0.0	0	0.00	106	0	0.0	0	0.00	100	0	0.00	0	0.00
Aug	75	0	0.0	0	0.00	112	0	0.0	0	0.00	73	3	4.10	2	2.74
Sep	116	0	0.0	1	0.86	117	3	2.6	3	2.6	153	4	2.61	3	1.96
Oct	85	2	2.4	0	0.00	84	2	2.3	1	1.2	108	2	1.85	0	0.00
Nov	93	0	0.0	0	0.00	97	0	0.0	0	0.00	111	0	0.00	0	0.00
Dec	87	0	0.0	0	0.00	94	0	0.0	0	0.00	95	0	0.00	0	0.00
Total	968	6 (0.0)	0.62	2	0.20	1163	8	0.69	7	0.60	1355	16	1.18	8	0.59

3.4 Infection Rates

Monthly parous infection rates of *S. damnosum* in Ibe stream, Mankor stream and Kwa Falls onchocerciasis foci are shown in Table 5. Parous rate of 968 flies dissected at Ibe stream was 0.62% for the year, with a monthly range of 1.1–2.7%. The infection rate (of L1, L2 and L3 larval stages) was 0.20% for the year with a monthly range of 0.86 – 1.10%, while the infective rate was 0.10 L3. The parous rate of 1163 flies dissected at Mankor stream was 0.69% with a monthly range of 1.3 – 2.6%. The infection rate was 0.60% for the year with a monthly range of 1.2 – 2.56, while the infective rate was 0.26 L3. Also the parous rate of 1355 flies dissected at Kwa Falls was 1.18% for the year, with a monthly range of 1.65 – 2.61%. The infection rate was 0.59 and a monthly range of 0.83 – 2.74% with an annual infective rate of 0.15 L3.

4. DISCUSSION

We have used a longitudinal entomologic study to investigate onchocerciasis status in three sentinel villages, Ibe, Mankor and Aningeje, located in Eastern Oban Hills. Entomologic data generated in this study provided information on the infectivity of *S. damnosum* and suppression of *O. volvulus* transmission by *S. damnosum* s. l. in Eastern Oban Hills communities, after 20 years of ivermectin treatment of the population at risk. There was significant variation in the monthly collections of *S. damnosum* s.l. at the three sites with the highest collections in the rainy season (April to October) and the lowest in the dry season (November to March). This finding is in consonance with the report of some investigators,[26,34] but in contrast with another reported work.[33] The highest number of *S. damnosum* flies was collected in Kwa Falls, compared to Ibe stream and Mankor stream. Such increased fly population could be influenced by increased provision of food and oxygen during the rainy season in the habitat, leading to emergence of new breeding sites and development of larvae into adults. This observation is consistent with earlier reported work.[8] However, the reduction in fly population in Ibe and Mankor streams may be attributed to the turbidity and flooding of These streams during the rainy season, causing disappearance of pre-existing breeding sites. This finding supports some earlier reported observations.[11] Undoubtedly, the West African vector of onchocerciasis is a rainy season breeder as earlier reported.[14,26,14]

The monthly variation in the relative abundance of *S. damnosum* flies in this study showed a high proportion of nulliparous flies as they accounted for 99.3%, 99.3% and 98.9% of the dissected flies from Ibe stream, Mankor stream and Kwa Falls respectively. This finding is in line with that of[14] who reported 53.90%, 57.86% and 59.58% of nulliparous flies from Osun Budepo, Osun Eleja and Osun Ogbere respectively. These high proportions of nulliparous flies observed in our study sites could be a reflection of non-transmission of onchocerciasis since they harbour no microfilariae, or migration from an endemic neighbouring Cameroon. This finding suggest that nulliparous flies have a wider flight range for their blood meals from human and animal population than parous flies that stay near their sites of oviposition, and hence feed and transmit the disease near the river. Similar high proportions of nulliparous flies have earlier been reported.[14,34,35]

The diurnal biting activity of parous flies observed in this study showed a bimodal biting pattern with a more conspicuous morning peak (0900 to 1000 hours) and a less conspicuous afternoon peak (1400 to 1500 hours). This finding is in agreement with the reports of several authors[1,36,37,38,39] in Cross River State, in Guatamala, in Uganda and in Enugu State respectively, but differ from the findings of[14] who observed three biting peaks in Osun River, and[40] who observed a single (unimodal) peak activity in Liberia. The cause of biting peaks lack vivid explanation, even though researchers have suggested circadian rhythms as the phenomenon behind this pattern of vector behaviour. These biting peaks are believed to be synchronized by biological rhythms,[36] especially circadian rhythms which are dependent on endogenous cues.[41] However, diurnal variations have been related to variations in temperature as reported by Leberre[42] or to the intensity of light as observed by Kaneko et al[43] These observed biting peaks in this study are synchronized by variation of daily temperature and illumination characterizing the climatic conditions in each study site. Similar findings have earlier been reported by some researchers.[14,26,35] From our results, there was significant variation in the transmission of onchocerciasis due to location. It was observed that the variation might be influenced by the longevity of the fly and its ability to allow the development of microfilariae into the infective stage and its subsequent transmission. This is in agreement with earlier reported findings.[44,45] Shelley[45] asserted that onchocerciasis transmission is influenced by the availability to flies *O. volvulus* reservoir in the human population, and the fly-to-human ratio in the endemic focus.[34]

The dissection results of parous flies in our study revealed that there was significant difference in the infection rates and infectivity of *S. damnosum* s. l. according to the three locations and month of the year. Consequently, parous rates, infection rates and infective rates (IR) were higher in the rainy season than in the dry season, especially at the beginning of the rains and the later part of rainy season, with higher infectivity rates in April and September. This finding is consistent with the reports of several authors.[14,20,26,46,47] Incidentally, these months are the peak period of farming activities, in the study area like planting of oil palms, plantain suckers and pineapple suckers, as in other rural areas in Nigeria. This finding confirmed earlier reports by.[20,34] These activities have great impact on the epidemiology of onchocerciasis in the study area. Impliedly, people who worked in their farms during these months would receive more *S. damnosum* bites. This argument is substantiated by the fact that the daily biting rates (DBR) and monthly biting rates (MBR) obtained in this study were higher in the rainy season than in the dry season, in agreement with the reported works.[48,49]

Attempts at onchocerciasis control have focussed on the use of synthetic insecticides to control the vector and thereby limit the number of infective bites per person per year. This tactic was first used successfully in Kenya where *S. neavei* the only vector species in that country, was eliminated from the area using DDT as the primary larvicide.[50] The Kenya eradication success was then used to launch a massive programme in West Africa in 1975. This programme, the Onchocerciasis Control Programme (OCP), eventually spanned 11 countries and utilized aerial treatment of waterways with temephos as a primary control approach. The OCP was successfully closed in 2002.[23] Annual mass treatment with ivermectin for 12 – 15 years in endemic communities is the control strategy adopted by the African programme for onchocerciasis control (APOC) for the control of onchocerciasis in Nigeria. Entomological results were considered satisfactory when annual biting rates were less than 1000 and the annual transmission potential (ATP) (number of *O. volvulus* L3s transmitted per person in a year) was less than 100.[4] There was variation in human onchocerciasis transmission by the vector, *S. damnosum* in the study area. In this study, black flies show serious biting nuisance as exemplified by the high ABR values obtained from the three sites (Ibe stream, Mankor stream and Kwa Falls. The ATP values obtained in the three onchocerciasis foci are lower than those given by WHO,[4] for interruption of onchocerciasis. The infectivity of parous flies was very low, suggesting a high influx of nulliparous flies from neighbouring endemic Cameroon, resulting in high ABR witnessed in this study. Although the disease relatively circulates in the study area, the incidence of the disease at Ibe was 6.2/0.62, at Mankor was 6.8/0.68 and at Kwa Falls was 20.1/2.0. The overall incidence in the study area was 11.9/1.2.

5. CONCLUSION

In conclusion, the findings in this study area have confirmed no total interruption of onchocerciasis, but suppression of *Onchocerca volvulus* transmission in Eastern Oban Hill communities. We therefore recommend that to achieve total interruption of *O. volvulus* transmission in the study area, integrated control approach should be introduced through intensified ivermectin distribution by CDTI, larviciding and continuous monitoring of onchocerciasis transmission in the area.

AUTHORS' CONTRIBUTIONS

This study was carried out by Iboh CI, Akpe TE and Effiom, OE. Dr Iboh designed the study, performed the statistical analysis, wrote the literature review and the first draft of the manuscript. Iboh, CI, Akpe, TE and Effiom, OE collected Simulium flies used in the study. The final manuscript was read and approved by all the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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