



Seasonal biting rate and transmission potentials of host-seeking black flies (Diptera: Simuliidae) and its implications for human onchocerciasis control in parts of Imo River Basin, Nigeria

Amaechi Austin^{1*}, Iwunze John¹, Alisi Goodlick¹, Uzoagba Deborah²

¹Parasitology/Entomology Unit, Zoology Department, Imo State University, Owerri, Nigeria, ²Biology/Microbiology Department, Federal Polytechnic Nekede Owerri, Nigeria

ABSTRACT

Biting and parity status of black flies were evaluated between the transmission seasons (rainy and dry) after long-term Ivermectin treatments in Imo River Basin, Nigeria. Captured black flies were morphologically identified, dissected and assessed by standard protocol. Although more black flies were caught in the rainy season (54.3%) than dry season (45.3%), there was insignificant difference in the transmission parameters ($P > 0.05$). Infection rate (0.2% versus 1.2%), parous rate (19.9% versus 22.4%), Infectivity rate (0.0% versus 0.0%), monthly biting rate (3,533.75 versus 3,095.5) bites/person/month, monthly parous biting rate (703.75 versus 771.5) bites and monthly transmission potential (0.0% versus 0.0%) were comparable for the seasons respectively. The data showed the existence of residual infection at the level of transmission likely to be insignificant to maintain parasite population.

KEYWORDS: Biting and parity rates, Transmission potentials, Ivermectin treatment, Onchocerciasis, Rainy and dry seasons, Imo River Basin

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*Corresponding author:

Amaechi Austin
Email: amaechiaustin@gmail.com

INTRODUCTION

Onchocerciasis is a skin and eye infection caused by the filarial nematode *Onchocerca volvulus* through the bites of *Simulium* species (black flies). It is estimated that 18-20 million people in the world are infected, 1-2 million people are visually impaired and 270,000 people are rendered completely blind (Okwa *et al.*, 2009). Nigeria is believed to have more persons infected by the disease than other country in the world. Thus, accounting for over one-third of global cases with the disability-adjusted years (DALYs) estimated at 1.49 million (Uttah *et al.*, 2004). The manifestations of the disease include itching, severe skin disease, partial or total blindness, scrotal elephantiasis, lizard skin etc. These symptoms make it difficult for affected individuals to concentrate, work and interact socially (Nwoke, 1990).

Mass drug administration (MDA) with Ivermectin/mectizan is the World Health Organisation recommended strategy for the control of onchocerciasis (WHO, 2006). Merits of the

adoption of ivermectin as the national strategy had been variously enumerated (Foucault *et al.*, 2006; Gutman *et al.*, 2010). In spite of the adoption and merits of ivermectin, the issues of coverage, manpower, concerns about possible evolution of ivermectin-resistance in *Onchocerca* species and the long duration of 10-15 years for which an individual must repeatedly take the drug to achieve control become important factors. In addition to these was compliance due to considerable geographical and religious disparities. Several years (>20 years) of ivermectin intervention, evaluation conducted in some community-directed treatment with ivermectin (CDTI) projects in the Savannah regions have reported prevalence rates and microfilarial loads as well as transmission indices below the thresholds for elimination (Traore *et al.*, 2012; Wanji *et al.*, 2015). However, there is a conspicuous lack of information from rainforest regions where conditions are not the same for black fly transmission. The presence of perennial fast flowing rivers favour the breeding and development of black flies that contribute to transmission in these places. Also in these forest

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areas, CDTI can be compromised due to *Loa loa* which triggers severe adverse reactions (SARs) in high microfilaremic patients following ivermectin treatment (Wanji *et al.*, 2002).

In Imo State, rapid epidemiological mapping of onchocerciasis (REMO) had placed the study areas in the hyperendemic zone (Nwoke *et al.*, 1994) and treatment with ivermectin has been on-going. Entomological evaluation has therefore become imperative to complement parasitological efforts in the certification of elimination of human onchocerciasis. In this study an attempt was made to ascertain the biting and parity status and its implications for the control of onchocerciasis in the middle Imo River Basin, Nigeria.

MATERIALS AND METHODS

Study Sites and Selection

The study was conducted on the riverbanks of a section of the Ibii River and the Lolo River in two rural communities (Umulolo & Amuro), Okigwe Local Government Area, Imo State from May 2022 to March 2023. These sites and their communities; Umulolo (Lat 05.8794°N, Long. 007.32566°E) and Amuro (Lat 05.78808°N, Long. 007.26703°E) are drained by the Imo River, a known breeding site of the black fly vector. These communities falls outside the middle Imo River Basin and had earlier been placed as hyperendemic (Nwoke *et al.*, 1994). The inhabitants had received yearly doses of ivermectin for 25 years counting (including bi annual CTDI which started in 2018. The study area has been described in detail elsewhere (Ukpai & Ezeji, 2003; Dozie *et al.*, 2006). In brief it lies in the tropical rainforest belt of southern Nigeria characterised by two distinct seasons: rainy season (April to November) and dry season (December to March) with rainfall and temperature ranging between 1,700-2,200 mm and 20 °C-30 °C (mean 29 °C) respectively. The communities are mainly Christians engaged in occupational activities such as farming, fishing, hunting, palm wine tapping and petty trading.

Ethical Approval

The work was performed under the project titled Assessment for Onchocerciasis Elimination/Stop MDA in the Imo River Basin - A Pilot study approved by the Scientific Advisory Committee and Institutional Ethical Committee of Zoology Board of Post Graduate Studies Imo State University, Owerri. Imo State Ministry of Health and Okigwe Local Government Area Health Unit and heads of study communities gave informed consent. People acting as human attractants for black flies catches were informed about the risks and benefits of participation.

Collection of Adult Black Flies

The human-bait catch method was adopted for catching adult black flies (Barbiero & Trpis, 1984) along the bank of Lolo River and Ibii River from Amuro and Umulolo respectively. Each station was sampled four times a month and fly catches

were between 7:00 am to 6:00 pm by two collectors working alternatively (Walsh *et al.*, 1978). The collection lasted from May 2019 to March 2020. May - September represented the rainy season and October-March represented the dry season.

Speciation and Parity Status Assessment

In the field each fly was carefully packed in a cold box containing ice packs to stop further microfilariae development before they were transferred individually with chloroform and then placed on a clean glass slide containing a drop of physiological saline. They were identified morphologically on the basis of the color of some anatomical parts. Determination of parity followed observations made on the ovariole characteristics (Nwoke, 1993).

Infection/Infectivity Status and Entomological Parameters Assessment.

Standard protocol for dissection of black flies was adopted (Mokry, 1980). Each black fly was dissected by teasing the head, thorax and abdomen separately in a drop of normal saline on a clean glass slide. The preparations were assessed for the larvae of *O. volvulus*. The number of L₁, L₂ and L₃ stages of development at these sites was recorded. The black fly density and level of transmission of onchocerciasis were quantified by two entomologic parameters (the monthly biting rates, MBR and transmission potentials, TP). The evaluation of MBR and TP followed established methods (Walsh *et al.*, 1978).

Data Analysis

Data was analysed based on percentages and Chi-Square. The seasonal density of black flies was subjected to two-way analysis of variance (ANOVA) and the difference in infection/infectivity rates evaluated by Chi-Square test. Probability level <0.05 was considered significant.

RESULTS

Of the 483 black flies caught during the rainy season, 96 (19.9%) were parous while 80.1% were nulliparous. The overall infection rate for the rainy season was 0.2% with an MBR of 3533.33 bites/person/month. The average monthly parous biting rate (MPBR) of 703.75 bites was recorded at the two sites. There were no monthly transmission potentials, MTP (Table 1). During the dry season an average MPBR of 771.5 bites was observed in the sites (Table 2). The highest MPBR were recorded in October for Ibii 116.25 and in January and March for Lolo Rivers 100.75; no MTP was observed. Altogether 150 persons (73 males and 77 females) were interviewed to ascertain factors which could influence community compliance to mectizan treatment, showed that 6 leading factors were identified. These were adverse effect (95%), free cost (93%), time of distribution (92%), availability of drugs (90%), methods of distribution (87%), and distributors attitudes (66%). The most preferred time for distribution by the respondents was community festival (96%), evening times (79%) and dry season (94%), Table 2.

Table 1: Summary of the biting, parity and transmission potentials for Raining Season (May-September 2022)

	May		June		July		August		September		Total
	Ibii	Lolo	Ibii	Lolo	Ibii	Lolo	Ibii	Lolo	Ibii	Lolo	
Person per day	4	4	4	4	4	4	4	4	4	4	8
Total flies caught	51	47	65	40	51	39	21	36	55	78	483
Average daily catch per person	24		26		23		14		33		121
(%) flies dissected	(20.3)		(21.7)		(18.6)		(11.8)		(27.5)		483
No (%) parous	07 (13.7)	13 (27.7)	11 (16.9)	15 (37.5)	09 (17.6)	05 (12.8)	06 (28.6)	03 (8.3)	11 (20.0)	16 (20.5)	96 (19.9)
No (%) Nulliparous	44 (86.3)	34 (72.3)	54 (83.1)	25 (62.5)	42 (82.4)	34 (87.2)	15 (71.4)	33 (91.7)	44 (80.0)	62 (79.5)	387 (80.1)
No (%) with L1 and L2 (Infection)	0 (0.0)	01 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	01 (0.2)
No (%) with L3 (Infective)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Monthly biting rate (MBR)	395.25	364.25	487.5	300	395.25	302.25	162.75	279	412.5	585	3533.75
Monthly Parous Biting Rate (MBPR)	54.25	100.75	82.5	112.5	69.75	38.75	46.5	23.25	82.5	120	703.75
Monthly Transmission Potential (MTP)	0	0	0	0	0	0	0	0	0	0	0

Table 2: Summary of the biting, parity and transmission potentials for Dry Season (October2022- March 2023)

	October		November		January		February		March		Total
	Ibii	Lolo	Ibii	Lolo	Ibii	Lolo	Ibii	Lolo	Ibii	Lolo	
Person per day	4	4	4	4	4	4	4	4	4	4	8
Total flies caught	57	59	41	52	23	30	21	16	47	60	406
Average daily catch per person	29		23		13		9		27		102
(%) flies dissected	(28.6)		(22.9)		(13.1)		(9.1)		(26.4)		406
No (%) parous	15 (26.3)	09 (15.3)	12 (29.3)	11 (21.2)	07 (30.4)	13 (43.3)	01 (4.8)	03 (18.8)	07 (14.9)	13 (21.7)	91 (22.4)
No (%) Nulliparous	42 (73.7)	50 (84.7)	29 (70.7)	41 (78.8)	16 (69.6)	17 (56.7)	20 (95.2)	13 (81.3)	40 (85.1)	47 (78.3)	315 (77.6)
No (%) with L1 and L2 (Infection)	0 (0.0)	0 (0.0)	0 (0.0)	02 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	03 (6.4)	0 (0.0)	05 (1.2)
No (%) with L3 (Infective)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Monthly biting rate (MBR)	441.75	457.25	307.5	309	178.25	232.5	147	112	364.25	465	3095.5
Monthly Parous Biting Rate (MBPR)	116.25	69.75	90	82.5	54.25	100.75	07	21	54.25	100.75	771.5
Monthly Transmission Potential (MTP)	0		0		0		0		0		0

DISCUSSION

The possible effect of ivermectin intervention was explored on onchocerciasis vectors biting, parity and transmission indices to assist with certification of the WHO/APOC stop MDA decision. As African countries look forward to onchocerciasis elimination goals, criteria for the certification of elimination including guidelines for entomological evaluation of the impact of interventions like ivermectin distribution are required. These guidelines include determination of biting and infection rates by the vectors which reflect human exposure to and vector density during transmission seasons, maximize the detection of infection/infective flies.

Onchocerciasis elimination with ivermectin in the rain forest regions with optimal breeding conditions for the vector (due to the perennial nature of breeding sites) is yet to be demonstrated.

In these areas hilly nature contributes to the creation and maintenance of the river flow rate for the establishment of breeding sites. This could explain why *Simulium* flies forage on both seasons and all year round. Despite the observed significant difference in black flies densities, between the two seasons the bites of these flies are proven discomfort to inhabitants of Umulolo and Amuro around the riversides. This could be traceable to their residential proximity and occupational activities which are indicative of a lot of anthropogenic activities that increases the sources of blood meal for the black flies (Maikaje *et al.*, 2015).

Entomological transmission indices which include infectivity and biting rates are known to vary from year to year because of differences in rainfall and other climate phenomena (Kovats *et al.*, 2001). Data from the study indicated the absence of seasonal difference in black flies entomologic indices; parous,

infection/infectivity rates. Three factors could explain these 1) comparable human activities leading to vector exposure rates 2) ivermectin coverage rates and 3) migrant workers activities. In addition to the above-named, factors such as vector abundance, more efficient vectorial capacity, presence of breeding sites, limitation phenomenon etc all probably contributed to the establishment of non-seasonal transmission variation. Thus, unlike in savannah regions more time would be needed to interrupt transmission by CDTI in the rainforest region. For whatever reason(s) these factors may point to a refusal to follow directives and a high population of people visiting the rivers unprotected. This would be addressed by aggressive orientation (behavioural change and health education). Lolo and Ibbi Rivers in Amuro and Umulolo are known breeding sites for *Simulium*. Indeed a challenge to maintaining the Imo River Basin free of onchocerciasis is its proximity to the border with Ebonyi and Abia States with reports of onchocerciasis transmission. There has been conjecture that these foci were linked through migrant farmers moving between these states and acquiring infection. This group of people was always absent for ivermectin treatment in their native areas which are still endemic in Ebonyi State (Nnadozie et al., 2018) and Abia State (Ezigbo et al., 2013). The likelihood of these people being able to maintain onchocerciasis transmission in the Imo River Basin is yet to be reported as was done for migrants elsewhere (Lindblade et al., 2009). Therefore collaboration between the states to harmonize the intervention strategy is needed in order to reach the goal of elimination. Preliminary mathematical models prediction had revealed that annual ivermectin treatment would not eradicate the parasite from the endemic area within 25 years (Habbema et al., 1992). The infection status of black flies as observed in the study areas for both seasons support this prediction. There is an indication that the hosts (human) around the rivers harbor skin microfilariae, mf (residual infection) despite ivermectin treatment (Wealtheral, 1995). However, the low/reduced and comparable entomologic indices for both seasons were a vivid reflection of the success story of ivermectin intervention onchocerciasis control. Our findings/results are remarkable and should be used in support of MDA the decision for Imo River Basin.

Monthly difference in parous rate (PR) may be attributed to natural annual variation, sample sizes and or changes over time with species composition and vector control. The values for MBR fall below the tolerable levels of 1,000 bites/person/year for both seasons. MPBR is one of the most important epidemiological measures in entomological monitoring which encompasses both biting density and parous status. Comparable PRs and resulting MPBR also reflected comparable MBRs observed for both seasons. PR and MPBR which used to be higher during the rainy seasons (Atting et al., 2005) were reduced drastically. Also, MTP, which usually oscillate between low and high (Cheke et al., 1997; Atting et al., 2005) and are known to be higher for forest vectors than Savannah types (Duke, 1968) were zero. Generally, transmission is expected to be higher during the rainy season with some sites drying out in the dry season. Our results showed comparable MPBR for the seasons indicative of perennial breeding sites of the rivers. In Imo River Basin, the zero value for MTP compared to high number of adult female

black flies caught is suggestive of biting nuisance. Ivermectin probably affected the parasite-vector relationship by significantly reducing the number of mf available for ingestion by the flies, and production of infective larvae. Further to this, the mf that survives in the treated people might migrate deep inside the dermis than normal making them inaccessible to the black flies (Jurgens & Schulz-Key, 1990). From an epidemiological prism for transmission to be significant, it must occur at a level that is sufficient to maintain the basic reproductive rate at a value that is >1%. Transmission below this level will be insufficient to maintain the population and eventually lead to parasite's extinction. While the annual transmission potential (ATP) to maintain reproduction at a level sufficient to sustain the population is not precisely known, previous modeling studies using data from West Africa have posited that the annual transmission (AT) necessary to maintain a reproductive rate of 1.0 may be between 12-29 larvae/person/year and in the absence of any perturbations (Basáñez et al., 2007).

Conclusively, the data confirmed mf existence and acquisition by the vectors in the study sites. The *O. volvulus* population of 0.2% and 1.2% recorded is likely to be on its way to extinction. Additional entomological and parasitological surveys will need to be conducted to strengthen the facts contained herein.

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