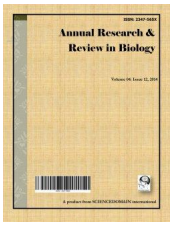

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Seasonal Variations in Biting Density and Infectivity of *Simulium damnosum* Complex in Ezeagu and Oji-River Local Government Areas of Enugu State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FMC, NRU, KNO and EKE designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors FMC and OKN managed the analysis of the study. Authors FMC, NRU, KNO and EKE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background and Objectives: Onchocerciasis is a parasitic filarial disease of the tropics, having serious effects on global public health and the economy of endemic countries. Nigeria is among the most endemic countries, necessitating much research and control program interests. This study determined the rate of transmission of onchocerciasis in Oji-River and Ezeagu Local Government Areas of Enugu State, Nigeria.
Methodology: Human landing sampling method was used to collect black flies. Parity assessment was conducted to determine the age of fly populations. Parous flies were further dissected to detect the presence or absence of *Onchocerca* larvae. Biting rates and transmission potentials were calculated using standard methods. The principal vector groups in the study area were identified morphologically.

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Results: A total of 352 adult female flies were collected during the study period. These were identified as members of the forest species of the *S. damnosum* complex. There were significant differences between monthly abundances of black flies caught during the entire study period ($P < 0.04$). Comparison of the monthly biting rates (MBRs) showed a significant difference in ($P < 0.02$). The MBR was lowest in February but highest in October. Generally, parity rate of over 50 % was observed throughout the period except for November. None of the flies dissected was infective. Diurnal biting activities of black flies between the months were significantly different from each other ($P < 0.01$).

Conclusions: Onchocerciasis transmission is low in the studied area, and is largely by means of the forest black flies as these were the only observed group. None of the dissected flies was found to be infective. The findings of this study will be of tremendous benefit to policy makers in the National Onchocerciasis Elimination Programme. It calls for a continuous monitoring of onchocerciasis disease conditions in the study area.

Keywords: Elimination; disease; ivermectin; Simulium damnosum.

1. INTRODUCTION

Onchocerciasis was regarded as one of the world's major health problems [1], a status it is gradually losing due to successes in control and elimination efforts. This parasitic filarial disease of the tropics has serious consequences on public health and the economy of endemic countries. Onchocerciasis is deemed responsible for the annual loss of approximately 1 million disability adjusted life years (DALYs), — healthy life years lost due to disability and mortality [2]. It is a disease of both the eyes and the skin and is spread by infected black flies of the *Simulium damnosum* complex in West Africa where distinct savanna and forest species occur. Among other symptoms, onchocerciasis results in total loss of vision, partial visual impairment, skin lesions, hanging groin, and hernia [3]. Over 90 million people are at risk of contracting the disease in Africa, where Nigeria contributes very significantly to this at-risk-population [4-7]. Concerted effort to control and eliminate onchocerciasis is ongoing throughout all endemic countries, with positive result.

In Nigeria and West Africa, the *S. damnosum* complex are solely responsible for onchocerciasis transmission [8-11]. The occurrence of *S. damnosum* s.s, *S. sirbanum, S. sudanense*, *S. squamosum* Volta form [12], *S. squamosum* Enderlein, *S. yahense*, *S. sanctipauli*, *S. soubrense* and the Beffa form of *S. soubrense* [13] have been reported from different parts of the country. However, the main vectors species in Nigeria have been found to be *S. damnosum* s.s, *S. sirbanum, S. sanctipauli, S. soubrense, S. squamosum* and *S. yahense* [14]. Analysis of larval samples from 23 sites across 4 bioclimatic zones in Nigeria by [15] revealed the presence of 5 cytospecies namely: S. *damnosum s.str., S. sirbanum, S. squamosum, S. yahense* and *S. soubrense* (including the Beffa form); excluding *S. sanctipauli* as one of the species found.

Capture and dissection of adult flies and/or molecular screening of flies for infection is an advantageous and non-insidious means for assessing the progress of various control measures [16] as well as disease elimination. It does therefore serve the purpose of monitoring levels and magnitudes of parasite transmission [17]. Evaluations and re-evaluations of transmission and epidemiological disease parameters continue as integral part of the control and elimination drives. Fly infectivity rates vary with species and location. The forest black fly species are known to vector the non-blinding, mild forest strain of *O. volvulus* while the savanna species carry the blinding, severe savanna strain of the same filarial parasite [18,19].

The World Health Organization (WHO) together with the Nigerian Federal Ministry of Health through the National Onchocerciasis Elimination Programme (NOEP) remain focused in their effort to eliminate onchocerciasis in Nigeria. Assessment of onchocerciasis burden and transmission frequency have continued. Entomological, parasitological and epidemiological evaluations are carried out from time to time in line with the elimination mandate. Chemotherapy with ivermectin to endemic communities also continues. These and other independent research activities on the disease have remained a significant stimulus to onchocerciasis elimination [17,20-26].

Following a recent study in one of the endemic communities [27] in Oji-River Local Government

Area of Enugu State, Nigeria which found no infective flies in the area, the need arose that some other neighbouring endemic communities be studied to provide comprehensive data of communities with on-going onchocerciasis transmission in the State, and the nation at large. This is necessary as it serves for monitoring the effectiveness of control and elimination efforts and successes. This study therefore was designed to determine the seasonal transmission indices of onchocerciasis by *S. damnosum* s.l. in Gbaragu, Amaetiti, Inyi, Ezeagu and Umunkpa communities and to identify the principal vector group in the areas.

2. MATERIALS AND METHODS

2.1 Study Area

An entomological study was conducted in Gbaragu (Oji-River, 0.006º 12.809′ N, 007º 19.353′ E), Akwu Inyi (Mmamu Rivers, 06º 06.092′ N, 007º 15.634′ E) communities in Oji-River Local Government Area (LGA) known to be onchocerciasis meso-endemic [28] and Umunkpa community (Du River, 06º 30.038′ N, 007º 07.113′ E) in Ezeagu LGA of Enugu State, Nigeria. These communities are populated with mainly farmers and petty business men and women, few students and public servants. Each of the communities has functional health facilities which include at least a Primary Health Care facility. The communities also have ivermectin distributors. Most community members had knowledge of ivermectin popularly known as Mectizan®. However, majority have not taken this drug in the past three years.

There are two seasons in the community namely: wet season (April to November) and dry season (December to March). The annual mean rainfall ranges between 1520-2030 mm and the mean monthly temperature varies between 22.4ºC and 30.8ºC [29]. The Oji, Mmamu and Du Rivers are relatively large rivers, covered in most of their areas by dense forest and supplied with very minimal sunlight in most of its expanse. The study areas varied from semi-urban to rural settlements. Most of the adult fly samples collected during this study were from Gbaragu. Very few flies were caught in Inyi and Umunkpa communities. Identification and dissection of flies was done in a small field laboratory. The State Ministry of Health, the Head of Department (HOD) for Health of each LGA and local authorities were duely involved in other to make the impact of the study more sustainable. The study was carried out during the dry months of January-March and late rains of September-November 2015.

2.2 Adult Black Flies Collection

Sampling for adult flies were at designated catching points in each community using two consented and trained fly collectors. Fly collection was conducted between 6:00 and 18:00 hours GMT following the procedure of [30]. The sampling period covered the period of diurnal activities of the flies. The collected vectors were identified and transmission parameters were determined following standard procedures [30,31].

2.3 Morphological Identification

Adult female black flies were identified morphologically in line with the published keys of [31] In this study, morphological identification was done only to separate savanna fly species from those of the forest origin based on 6 qualitative morphological characters, namely: the colour of the 9th abdominal tergite setae, antennae, fore-coxae, scutella setae, wing arculi, and wing tufts. Pale wing tufts, antennae, 9th abdominal tergite setae, scutella hair and fore-coxae are characteristics of savanna species of black flies and flies with these features were considered as such. On the other hand, those with pale or dark wing tuft; dark 9th abdominal tergite setae, antennae, fore-coxae, and scutella setae were considered as forest flies.

2.4 Dissection of Adult Flies

Dissection to determine fly parity rates and infection levels were carried out according to [30]. Parity determination was carried out first before further dissection for infections was made. Flies were recorded as parous or nulliparous. All nulliparous flies were discarded while all parous flies were further dissected minutely to detect the presence of *O. volvulus* larvae. The number and developmental stages of any *Onchocerca* larva was documented.

2.5 Entomological Indices

Fly densities and level of transmission of onchocerciasis were quantified using two entomological indices, the monthly biting rates and transmission potentials. The monthly biting rates (MBR) were measured as the theoretical number of black fly bites received by a person

stationed at a catching site during the twelve hours of the daylight for one complete month in any given catching point. The monthly transmission potential (MTP) was established as the total number of infective larvae (third stage larvae found in these black flies) that would be received in one month by an individual stationed at a catching point for 12 hours of the daylight. These parameters were analyzed using the two-way ANOVA.

3. RESULTS

All flies collected were of the forest origin, characterized by dark colour of the 9th abdominal tergite setae, antennae, fore-coxae, scutella setae, wing arculus, and wing tufts among others. Some flies were observed to bear pale wing tufts while the rest of the features were dark. In some cases, there was a mixture of dark and pale tufts.

Fig. 1 presents the monthly biting rates (MBRs) of black flies in the study communities. Throughout this period, the month of October recorded the highest MBR of 782.75 bites/person/month while the lowest MBR of 178.25 bites/person/month was recorded in February. A comparison of the monthly biting rates showed that there was a significant difference in MBRs ($P < 0.02$).

Hourly diurnal biting activities of black flies showed a characteristic bimodal pattern, with morning peak at about 10.00-12.00 and evening peak at about 15.00-16.00. However, there were differences in the peak periods, in one case just a single peak was observed while in some others, more than two peaks were observed. The diurnal biting activities of *S. damnosum* in the study communities is presented in Fig. 2.

The month of January had just one peak at about 11.00 am to 12.00 GMT. Fly biting activities for February showed a little peak at 11.00 and 16.00 GMT. The month of March had a little peak at about 10.00 and 16.00 GMT. In September, peak of activities were observed at 11.00 and 15.00 hours while for October, it was between 10.00 to 12.00 and 15.00 GMT; November however showed multiple peaks. The lowest number of flies was collected during the hour of 6.00 to 8.00 and 17.00 to 18.00 while the highest was collected during the hours of 9.00 and 12.00 GMT. The diurnal biting activities of black flies between the months were significantly different from each other ($P < 0.01$).

A total of 352 adult female flies were collected during the study period. The highest number of flies caught was 105 during the month of October while February recorded the lowest number of 23 flies. There were significant differences between monthly abundances of black flies caught during

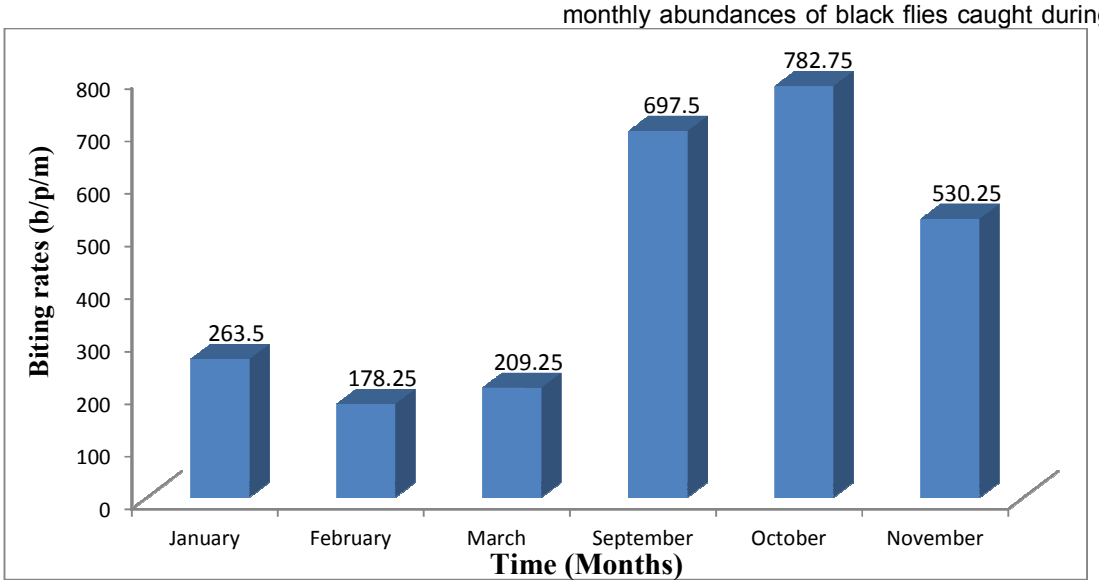

Fig. 1. The monthly biting rates of black flies in the study community

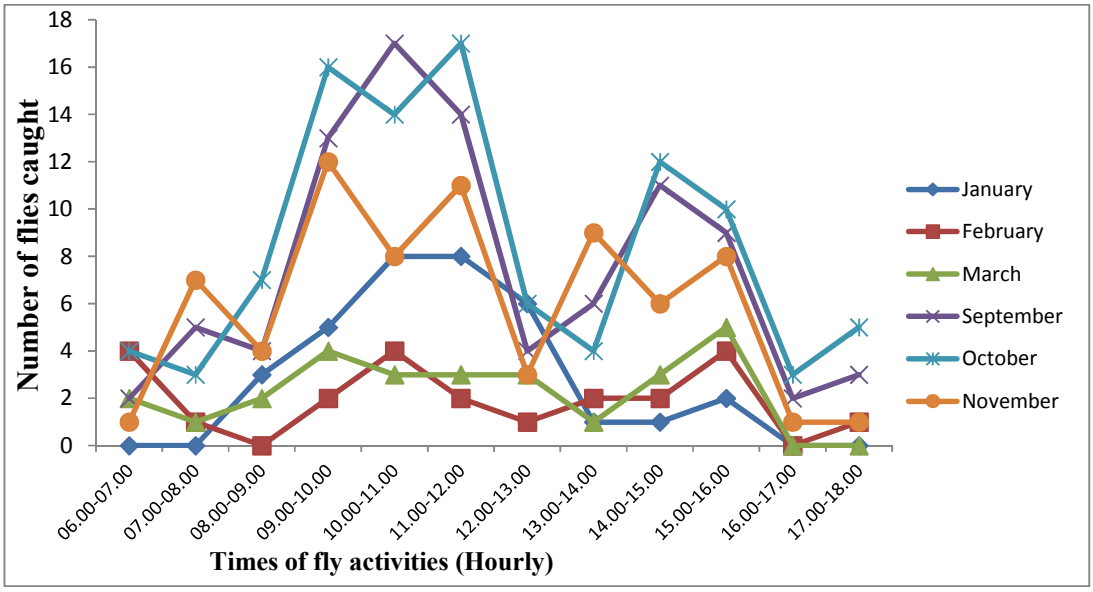

Fig. 2. The diurnal biting activities of *S. damnosum* in the study community

the entire study period (P < 0.04). Table 1 presents the summary of transmission indices of *S. damnosum* and *O. volvulus* for the study period. In dry months, the percentage monthly black fly parity rates ranged from 63.0% to 69.6%. The rainy months maintained parity of over 50% except November which recorded 47.9%. However the parity was lower in the rainy than dry season.

Table 1 further revealed that none of the dissected flies were infected nor infective. Since no fly carried any of the developmental larval stages of *Onchocerca*, the monthly transmission potentials were zero for the period of this study as shown in Table 1.

4. DISCUSSION

The key changes for shifting from control to elimination are the scale-up of Community Directed Treatment with Ivermectin (CDTI) to hypo-endemic areas and the implementation of regular epidemiological and entomological surveys along with ongoing surveillance [32]. Nigeria is presently working towards the stage of onchocercaisis elimination and entomological evaluation is one of the key tools for elimination. It provides information on transmission-related indices, vector composition and abundance. These information are obtained from adult female black flies landing and biting activities on human baits [33]. Human landing catches therefore remains key to monitoring disease transmission which in this instance is majorly by the forest species of black fly.

The presence of dark ninth abdominal tergite setae in all samples examined and the presence of flies with pale wing tuft may be pointing towards *S. yahense* and *S. squamosum* since the characteristics of the breeding sites here is not quite different from that known already for this species [15,29]. Members of the forest species of black flies were the only group identified in this study. Recognizing strain differences in *O. volvulus* transmitted by *S. damnosum* in West Africa, forest onchocerciasis, characterized by onchocercal skin diseases is expected to dominate in the area; mass drug administration with ivermectin has however significantly reduced disease transmission.

Biting rates and water levels of breeding sites in the study areas were found to be directly related. This type of relationship had been reported by [34]. In savanna areas, at the beginning of the rainy season, savanna flies that may have migrated due to unfavorable conditions may migrate back to such former sites or others to repopulate rivers, streams and other breeding sites that had become unfavorable during the preceding dry season. This phenomenon called “re-invasion” when first discovered in controlled areas of the WHO Onchocerciasis Control Programme (OCP) in the Volta river basin [35], was not observed in the studied areas as the rivers were perennial and never dried up

Table 1. Summary of transmission indices of *S. damnosum* in the four collection months

Characteristics	January	February	March	September	October	November	Totals
Person' days worked	4	4	4	4	4	4	24
Total flies caught (%)	34 (9.7)	23 (6.5)	27 (7.7)	92 (26.1)	105 (29.8)	71 (20.2)	352
Average daily catch per person	8.5	5.8	6.8	23.0	26.3	17.8	88
No. of flies dissected (%)	34 (100)	23 (100)	27 (100)	92 (100)	105 (100)	71 (100)	352
No. (%) of parous flies	23 (67.6)	16 (69.6)	17 (63.0)	50 (54.3)	57 (54.3)	34 (47.9)	197
No. (%) of nulliparous flies	11(32.4)	7 (30.4)	10 (37.0)	43 (45.7)	47 (44.8)	37 (52.1)	155
Total no. of flies infected (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0
Flies (%) with L_1 and L_2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0
Flies (%) with L_3	0 (0)	0(0)	0 (0)	0(0)	0 (0)	0(0)	0

completely. The works of [6] and [36] reported that the presence of migratory flies could account for high parity rates. There was a significant decline in fly numbers during the dry season. The age composition of flies is closely linked to variations in total biting density, with highest parous rates being observed when biting rates were highest. In this study, we observed that parity rate always exceeded 60 % during the dry months which happened to cover the generally cold harmattan period of the dry season, and therefore in agreement with the findings of [37] who observed that during the hot dry season, parous proportions were low. The remarkably high parity rate of black flies found in this study may be suggestive of the fact that vector populations had access to blood meal, the source of which however was not determined, and that they live long enough to reproduce.

Diurnal fly biting activities observed in this study did not show a regular pattern. Fly activities peaked differentially with months. Diurnal fly activities corresponded to periods of high human activities and working habits of the people in the communities who are predominantly farmers, usually working close to the rivers. This finding aligns with the earlier findings by [27,37,38]. The flies appear to have carefully adapted their activities to suit those of their host, thereby posing high risks of fly bites and potential infection with *O. volvulus*. It has been reported by [17,27] that human activities around breeding sites/water bodies such as farming, fishing and hunting among others, are known to be predisposing factors to onchocerciasis infection. In this study, such activities are actively going on around the breeding sites. In fact, sand mining, mostly carried out by the youths and able-bodied men, is one of the most lucrative sources of livelihood in the areas studied, and as a result many people spend the whole day in the rivers and other related sites excavating sand and are therefore exposed to regular fly bites. Due to the high energy requirement of this type of job and the harsh environmental conditions of the area, those engaged in them were found with bare bodies, further exposing them to high risks of infection. Change in some of the anthropogenic factors that expose inhabitants to fly bites could help sustain the gains already experienced in control and elimination of onchocerciasis.

In the work of [37], the highest fly densities were observed from 16.00 to 17.00 hours during the rainy season. The present study however found that late morning and early evening hours were the most significant in terms of biting of black flies and transmission of onchocerciasis due to increase in fly densities at these time. This observation supported that of [36]. Avoiding outdoor activities, especially around breeding sites during this time would reduce exposure to infection. The observed differences between these two reports may be attributed to environmental, time and location factors.

The monthly catches clearly showed that more parous than nulliparous flies were caught except in the month of November when nulliparous flies slightly outnumbered parous flies. This observation supported that of [36]. The differences in behavior and daily biting activities of parous and nulliparous flies are likely due to some inherent parameters [39,18]. Daily rhythms were found to be affecting nulliparous flies more than older, parous flies [37]. Low temperatures also limited the activities of blood-searching adult flies in line with [36].

The absence of infections in the flies is likely to be as a result of the relatively fewer number of flies caught or the actual absence of infection within the black fly populations. Interestingly, a previous study in a nearby site by [27] revealed absence of infective flies. Still of interest is the finding from a few inhabitants interviewed who indicated that ivermectin distribution had not taken place in the study communities for over three years to the point of the study. It further suggests possible transmission interruption in the areas.

The results of this study showed some seasonal variation in monthly biting rates. The minimum monthly MBR of (178.25 bites/person/month) and the maximum of (782.75 bites/person/month) black flies were observed in this study. Seasonal transmission potential was zero. There was absence of infective larvae in the head of local black fly vectors. Assessment of fly infectivity rates was used as a tool for determining transmission levels. It is reassuring and noteworthy that no transmission was observed as no infective fly was found during the study period. This finding is consistent with the report of [39]. This could be suggestive of a possible interruption of onchocerciasis transmission due to long-term ivermectin distribution which has drastically reduced the parasite density making it difficult for flies to pick them up while feeding. However, it further presents a need for sustained mass Ivermectin distribution in these areas to achieve local elimination of onchocerciasis in

view of the reported presence of subcutaneous nodules and lack of treatments for some years now. There is need for more concerted research efforts to further monitor fly infectivity in the area, especially because the traditional dissection method lacks the sensitivity to detect low infectivity.

The WHO mass drug administration with Ivermectin is a boost to onchocerciasis control in Africa and the South and Central Americas [40] and more recently, its ongoing elimination. For over a decade, the communities studied has benefited immensely from this initiative through the Community Directed Treatment with Ivermectin (CDTI) [41]. The achievements of onchocerciasis elimination efforts should therefore be sustained.

5. CONCLUSION

Onchocerciasis transmission is low in the studied area, and is largely by means of the forest black flies as these were the only observed group. This study serves to provide updated information on the status of onchocerciasis transmission in the area as well as the biting activities of the vectors. The findings of this study will be of tremendous benefit to policy makers in the National Onchocerciasis Elimination Programme. It calls for a continuous monitoring of onchocerciasis disease conditions in the study area.

COMPETING INTERESTS

The authors hereby declare that they do not have any competing interest associated with this paper.

REFERENCES

1. Murdoch ME, Hay RJ, Mackenzie CD, et al. A clinical classification and grading system for the cutaneous changes in onchocerciasis. Br J Dermatol. 1993;129:260-269.
2. Remme JHF. The Global Burden of Onchocerciasis in 1990. Geneva: WHO; 2004.
3. WHO. Onchocerciasis (River blindness), WHO information fact sheets; 2002.
4. Basanez M-G, Pion SDS, Churcher TS. River blindness: a success story under threat? PLoS Medical Journal. 2006;3:1454–60.
5. Post RJ, Onyenwe E, Somiari SAE, Mafuyai HB, Crainey JL, Ubachukwu PO. A guide to the *Simulium damnosum* complex (Diptera: *Simuliidae*) in Nigeria, with a cytotaxonomic key for the identification of the sibling species. Annals of Trop Med Parasitol. 2011;105(4):277–297.
6. WHO. Report of a WHO Expert Committee on Onchocerciasis Control. WHO Technical Report Series WHO, Geneva 1995;852.
7. WHO. Epidemiology of onchocerciasis. Report of WHO Expert Committee. WHO Technical Report Series, No 752 Geneva 1987;1.
8. Dunbar RW, Vajime CG. The *Simulium* (*Edwardsellum*) *damnosum* complex. A report of cytotaxonomic studies to April 1972. WHO mimeographed document WHO/ONCHO /72.100. 1972;10-23.
9. Vajime CG, Dunbar RW. Chromosomal identification of eight species of the subgenus Edwardsellum near and including *Simulium* (Edwardsellum) *damnosum* Theobald (Diptera: Simuliidae). Tropenmedizin and Parasitologie. 1975;26:11-18.
10. Boakye DA. A pictorial guide to the chromosomal identification of members of the *Simulium damnosum* Theobald complex in West Africa with particular reference to the Onchocerciasis Control Programme area during the decade of 1984-93, following intensive larviciding since 1974. Medical and Veterinary Entomology. 1993;12:345-358.
11. Boakye DA, Post RJ, Mosha FW, Surtees DP, Baker RHA. Cytotaxonomic revision of the *Simulium sanctipauli* subcomplex including the description of two new species within the *Simulium damnosum* complex in Guinea and surrounding countries**.** Bulletin of Entomological Research. 1993;83:171–186.
12. Vajime CG, Gregory WG. Onchocerciasis: Species complex of vectors and epidemiology. Acta Leidensia. 1990;59:235-252.
13. Meredith SEO, Cheke RA, Garms R. Variation and distribution of forms of *Simulium soubrense* and *S. sanctipauli* in West Africa. Annals of Tropical Medicine and Parasitology. 1983;77:627–640.
14. Nwoke BEB, Shiwaku K, Takahashi H. Nigeria onchocerciasis: Epidemiology perspective. The Nigerian Journal of Parasitology. 1991;22(1):3-10.
15. Mafuyai HB, Post RJ, Vajime CG, Molyneux DH. Cytotaxonomic identification

of the *Simulium damnosum* complex (Diptera: Simuliidae) from Nigeria. Trop Med Int Health. 1996;1:779–785.
16. Ottesen EA, Ramachandran CP. Lymphatic filariasis infection and disease: control strategies. Parasitol Today. 1995;11:129–131.
17. Opara KN, Fagbemi BO, Ekwe A, Okenu DMN. Status of forest onchocerciasis in the lower Cross River basin, Nigeria. Entomologic profile after five years of ivermectin intervention. Am J Trop Med Hyg. 2005;73(2):371–376.
18. Philippon B. Etude de la Transmission d'*Onchocerca volvulus* (Leuckart, 1893) (Nematoda, Onchocercidae) oar *Simulium damnosum* Theobald (1903) (Diptera-Simulidae) Afrique Tropicale. Paris: L'Office de la Recherche Scientifiqueet Technologiqued'Outre Mer. *Travaux et Documents deL'ORSTOM.* 1977;62-64.
19. Leveque C, Hougard JM, Resh V, Statzner B. Freshwater ecology and biodiversity in the tropics: what did we learn from 30 years of onchocerciasis control and the associated biomonitoring of west African rivers. Hydrobiologia. 2003;500:23–49.
20. Okonkwo P, Akpa A, Ihekwaba A, Nwagbo D, Umeh R, Adibua S, et al. Studies on onchocerciasis in forest – Savannah Mosaic areas of Nigeria. Investigation in Gbaragu Oji River. Ann Trop Med Parasitol. 1991;38:153–167.
21. Adewale B, Mafe MA, Oyerinde JO. Infectivity and transmission dynamics of *Simulium damnosum* s.l. around Owena Dam (Ondo State). West Afri J Med.1999;18(4):257-260.
22. Nwoke BEB, Dozie INS. Operational research and its success in onchocerciasis control in Nigeria. Nigeria Journal of Parasitology. 2001;22:3-10.
23. Ubachukwu PO, Anya AO. Studies on the diurnal biting activity pattern of *Simulium damnosum* complex (Dipteran: Simuliidae) in Uzo-Uwani local government area of Enugu state, Nigeria. Nigerian Journal of Parasitology. 2001;22163–22168.
24. Oyibo WA, Fagbenro-Beyioko AF. Effect of repeated community-based ivermectin treatment on the intensity of onchocerciasis in Nigeria. Rural and Remote Health. 2003;3211-3216.
25. Idowu ET, Adewale B, Mafe MA, Appelt B, Bamgbose A. Endemicity of onchocerciasis in some local government areas of Niger state. African Journal of Medical Sciences. 2004;3331–3334.
26. Ubachukwu PO. Human onchocerciasis: epidemiological status of Uzo-Uwani local government area of Enugu state, Nigeria. Nigerian Journal of Parasitology. 2004;2593–2599.
27. Chikezie FM, Ezihe EK, Uzoigwe NR, Opara KN, Igbe MA, Okonkwo NJ, Nwankwo EN, Nwoke BEB. A concise entomological evaluation of onchocerciasis transmission in Ahani-Achi community in Oji-River LGA of Enugu State. Intl J Cur Res Rev. 2015;7(5):56–62.
28. State Ministry of Health Enugu. Demographic Profile of Enugu State Nigeria; 2000.
29. WHO. Vector control series. *Simulium*. Advanced Level Training and Information Guide. 1991;WHO/VBC/92.992.1.
30. Wilson MD, Post RJ, Gomulski LM. Multivirate morphotaxonomy in the identification of adult females of the *Simulium damnosum* Theobald complex (Diptera: Simuliidae) in the Onchocerciasis Control Programme area of West Africa. Annals of Tropical Medicine and Parasitology. 1993;87:65–82.
31. Kim YE, Remme JHF, Steinmann P, Stolk WA, Roungou J-B, Tediosi F. Control, Elimination, and Eradication of River Blindness: Scenarios, Timelines, and Ivermectin Treatment Needs in Africa. PLoS Negl Trop Dis. 2015;9(4):e0003664. DOI:10.1371/journal.pntd. 0003664.
32. Le Berre R. Contribution à l' étude biologique et écologique de *Simulium damnosum* Theobald, 1903 (Diptera: Simulidae) Memoires. O.R.S.T.O.M Paris. 1966;1.
33. Crosskey RW. An appraisal of current knowledge of *Simulium damnosum* s.l. in the Federal Republic of Nigeria in relation to the development of an Onchocerciasis Control Campaign. 1979;1–11.
34. Garms R, Walsh JF, Davies JB. Studies on the re-invasion of the Onchocerciasis control programme in the Volta River basin by *Simulium damnosum* s.l. with emphasis on the Southwestern areas. Tropenmed. Parasitol. 1979;30:345-362.
35. Henry MC, Meredith SEO. The onchocerciasis focus at Kinsuka/Kinshasa Republic of Zaire in 1985. Entomological aspects. Ann Trop Med Parasitol. 1990;84:369–379.

36. Renz A. Studies on the dynamics of transmission of onchocerciasis in a Sudan savanna area of North Cameroon III. Infection rate of the *simulium* vectors and *Onchocerca volvulus* transmission potentials. Ann Trop Med Parasitol. 1987;81:239–252.
37. Umeh RE, Chijioke CP, Okonkwo PO. Eye disease in an onchocerciasis-endemic area of the forest-savanna mosaic region of Nigeria. Bulletin of the World Health Organization. 1996;74(1):95–100.
38. Opara KN, Usip LP, Akpabio EE. Transmission dynamics of onchocerciasixs in rural communities of Akwa Ibom State, Nigeria. Journal of Vector Borne Disease. 2008;45:225–230.
39. Hausermann W. On the biology of *Simulium damnosum* Theobald, 1903, the main vector of onchocerciasis in the Mahenge Mountains, Ulange, Tanzania. Acta Tropica. 1969;26:29-69.
40. WHO. Working to overcome the global impact of neglected tropical diseases: first WHO report on neglected tropical diseases. World Health Organization. Geneva; 2010. Available:http://www.who.int/neglected_diseases/2010report/NTD_2010report_embargoed.pdf
41. Onwujekwe EN, Shu EN, Nwagbo D, Akpala CO, Okonkwo PO. Willingness to pay for community-based ivermectin distribution: A study of three onchocerciasis endemic communities in Nigeria. Tropical Medicine and International Health. 1998;3(10):802-808.

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