Original Article

THE IMPACT OF SIBLING SPECIATION IN SIMULIUM DAMNOSUM COMPLEX ON CURRENT ONCHOCERCIASIS CONTROL EFFORTS

Nwachukwu Emmanuel Obinna

Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike, PMB 7267, Umuahia, Abia State, Nigeria

DOI: https://doi.org/10.5281/zenodo.17151292

Abstract: Human onchocerciasis is a debilitating tropical infection that can result in blindness and severe skin disease. Despite the fact that the National Onchocerciasis Control Programme (NOCP) has been highly successful, at least from a public health perspective (in terms of significant reduction in morbidity), there still remains the problem of persistent transmission in some hyper endemic areas as opposed to what is obtainable in the Americas that ran a similar programme (where interruption of transmission has been achieved). This paper, reviewed the current strategies used in the control of onchocerciasis and highlighted a cryptic factor decimating the efforts employed against the disease; culminating in an extraordinary propensity of Simulium damnosum complex to exhibit sibling speciation and the attendant poor understanding of this phenomenon in relation to disease epidemiology. The review revealed that, poor knowledge of the role of sibling species in disease transmission could be contributing directly to the persistence in transmission seen in some endemic foci despite some control interventions, as this has not been seen in the Americas where a fair knowledge of the role of different sibling species has been reported. It is recommended that any control effort should take into account the possible varied roles of different sibling species in disease transmission. This will involve proper identification of vectors at cytotaxonomic and molecular levels, since sibling species are morphologically similar but reproductively isolated, rendering morphormetrics relatively irrelevant.

Keywords: Onchocerciasis, Simulium damnosum, Control Measures, Sibling Speciation

Introduction

Human Onchocerciasis (River Blindness) is caused by infection of the parasitic filarial nematode *Onchocerca volvulus*, which can cause blindness and skin diseases. The disease is endemic to Africa, tropical America and the Yemen but 99% of cases occur in Africa, and in Africa 95% is transmitted by *Simulium damnosum sensu lato* which is responsible for 250,000 cases of blindness and the loss of nearly one million. Disability Adjusted Life

Years (DALYs) every year (WHO, 1995). As well as this direct effect on human health there is some evidence that Onchocerciasis may be associated with epilepsy, stunted growth, reduced efficacy of vaccinations, increased susceptibility to malaria and (Basanez *et al*., 2006). There is no animal reservoir of infection, and the parasite is transmitted to humans by bloodsucking black flies (Diptera Simulidae) of the *Simulium damnosum* complex. Only certain anthropophilic members of the complex transmit the parasite and the epidemiology of the *Onchocerca* shows complex interaction between vector, parasite and host factors (Post and Boakye, 1992, Basanez and Ricardez Esquinca, 2001). The parasite has recently been targeted for global elimination by the world Health Organisation using community directed treatment with the drug Ivermectin (CDTI) (WHO, 1994). Annual treatment of communities can eliminate the parasite (Awadzie *et al*., 1985, Molyneux and Davies, 1997, Mustapha, *et al*., 2005) and progress towards elimination is routinely assessed by both epidemiological and entomological parameters. WHO – recommended strategy for Onchocerciasis elimination requires the delimitation of transmission zones where parasites form a single population which is more or less homogenous within the transmission zones (Davies, 1993; Boatin *et al*., 1998; Kennedy & Basanez 2002; Kennedy *et al*., 2003). Migration of parasites can occur through human or vector migration, and the most important is thought to be vector migration, (Walsh, 1990; and Davies, 1994) "for example, Onchocerciasis transmission is continuing in the eastern part of Kogi State (Nigeria) in spite of continuing CDTI, and it is possible that this is the result of immigration of infective flies from surrounding areas".

Knowledge of the role of each vector species in transmission is important for the rational design of disease – control programmes. Unfortunately, the members of the *S. damnosum* complex are extremely difficult to differentiate morphologically, and are usually defined by specific chromosomal characters in the larvae (Vajime and Dubar 1975; Crosskey, 1987). This means that members of *S. damnosum* complex are morphologically identical but reproductively isolated. This poses a huge setback to control efforts since not all members are equally important in disease transmission. Moreover, even though they are morphologically alike they exhibit enormous differences in aspects of their behaviour and biology.

Causative Agent of Onchocerciasis

The causative agent of Onchocerciasis is a filarial worm *Onchocerca volvulus (*Nematoda; filariodea) which is transmitted from person to person by blood – feeding black flies (Diptera: Simulidae).

Onchocerca volvulus can be found mainly in West Africa, and also in central and south America. Most likely, this species was originally only in Africa, and was introduced to the Americans during the era of slave trade. There are two strains of this species, distinguishable at the DNA level by the 0 - 150 polymerase chain reaction (PCR) test. One strain is typically found in the savannah regions of West Africa and the Americas, and is responsible for the savannah type of Onchocerciasis which leads to blindness, while the other strain is commonly found in rain forest areas (Ogunrinade, *et al*., 1999; Roberts and Janovy, 2000), and is responsible for the forest type of Onchocerciasis which leads to skin diseases.

Vector Distribution and Breeding Sites

Black flies are small, stout – bodied flies that have blade-like mouth parts adapted for tearing and rasping the skin to rupture blood capillaries.

They breed in swift – running water (as contrasted with mosquitoes, which breed in still water), such as streams and rivers, where the larvae attach themselves to rocks and other objects on the bottom. Adults emerge and generally fly within a range of 12 to 18cm (some much further) looking for food and males. Females require a blood meal for egg development; the males never suck blood. Black flies occur in huge swarms, tormenting humans, wild and domestic animals. They are particularly abundant in the north temperate and subarctic zones, but many species occur in the subtropics and tropics where factors other than seasonal temperature affect their developmental and abundance patterns (Harwood, *et al*., 1979).

Original Article

However not all black flies are vectors of Onchocerciasis. In fact most species do not even bite humans. In Africa, members of the *Simulium damnosum* and *Simulium neavei* complexes are important vectors. Members of the *S. damnosum* complex are responsible for more than 90% of Onchocerciasis cases worldwide and more than 95% of cases in Africa, (Crosskey, 1996). In central and south America, vectors of Onchocerciasis include *Simulium Ochraceum* and *Simulium metallicum.* (Burnham, 1998).

Mode of Transmission of Onchocerciasis

River blindness is transmitted by infected *Simulium* black flies living near bodies of fast flowing river. In Africa infected flies carry a larval form of filarial parasitic worm, *Onchocerca volvulus* from person to person. Infected black flies spread the larvae through bites on the skin, the larvae enter the body at bite site, form subcutaneous nodule under the skin and mature into microfilaria which move throughout the body and eventually die leading to severe skin rashes and de-pigmentation (Leopard skin), itching, skin nodules, visual impairment and blindness can also occur.

Due to host immunological reactions, these group of worms often become encapsulated in the fibrous tissue mainly composed of collagen when these sites are located over bones such as a joint or skull, a conspicuous nodule called an Onchoderma appears, which is generally about 3cm in diameter. Nodule location depends on geographical area, with most infections in Africa exhibiting nodules below the waist (especially on the knees and pelvic area), and those in America exhibiting nodules above the waist (especially on the neck and head).

This relationship is based on the biting preference of the black fly vector with African flies preferring to bite above the waist. (Roberts and Janovy Jr., 2000).

Onchocerca volvulus will generally be found causing disease in savannah and rainforest areas although occasionally it can be found in arid savannah and desert areas as well (Burnham, 1998; Roberts and Janovy Jr., 2000).

Control of Onchocerciasis

The control of Onchocerciasis today is based essentially on two strategies; *Simulium* vector control and large scale chemotherapy with Ivermectin.

Targeting Vectors for Onchocerciasis control

Insecticide: This involves the reduction of the black fly population at its larval stage through aerial application of selective insecticides on infested rivers (Hougard, *et al.*, 1998) which are breeding sites for black flies. These insecticides contain DEET which is an active repellent for black flies. This method of control reduces the larval stage of the flies preventing them from reaching adults.

***Bacillius Thuringiensis var. Israeliensis* (BTI):** A number of chemical products have been applied in order to control black fly which has been very effective. However, eventual resistance and the long length of time the chemicals remain in the environment have lead to the replacement of these products with less polluting compounds. The best means of control is with the use of a biological control method which is regarded as less toxic for the environment, a biological agent *Bacillius thuringiensis var. Israeliensis* has proven the most effective as it kills the larvae and has less impact upon other macro-vertebrates from fresh water ecosystems (Charles Darwin Foundation).

Traps: the Esperanza window trap, when baited with CO_2 and Olfactory (Human smell) lures substantial members of *Simulium ochraceum S.I.*, one of the principle vectors of *O. volvulus* in Latin America. This trap has a potential for replacing humans as bait in the monitoring and surveillance of Onchocerciasis vectors. These traps have the potential to be extremely useful tools in aiding the certification of elimination of *O. Volvulus* transmission and in the post-treatment surveillance era. For gravid (egg laying) flies, the bellac trap is used.

Personal protection: Because no vaccine or chemoprevention is available to prevent Onchocerciasis, one method used to reduce the chance of infection is to take basic measures to avoid getting bitten by the black fly. These measures include;

-Avoiding the black fly habitat (i.e. fast flowing streams or rivers)
-Using insecticides like DEET (N-N-Diethyl-meta-toluamide) -Wearing long pants and sleeves as well as head nets -Sleeping under insect nettings.

These methods have been found fairly effective for travellers, but less effective for people who live in endemic areas.

Targeting Filarial Worms

Nodulectomy (Surgical removal):

This is the surgical removal of popular nodules that encapsulate adult worms. Nodulectomy serves to decrease the parasite burden by removing the source of production. Surgical removal on the skull is also credited with decreasing the risks of ocular complications of onchocerciasis.

Chemotherapy

Ivermectin (dihydroavermectin B1) is the drug of choice for the treatment of Onchocerciasis. In 1996, ivermectin (Stromectol) was registered for human use in the United States. In France, it was registered as mectizan. For animals, in the United States, it was registered previously by mercksharpe and dohme as heartgard. A widely used veterinary gastrointestinal antihelminthic and ecto-parasiticide (Coffeng, *et al.*, 2014, Evans, *et al.*, 2014).

Ivermectin is a microcyclic lactone derived from the *actinomycete streptomyces avermertilis* found in a soil sample from a Japanese Gulf course and is produced by fermentation (Burnham, 1998). Ivermectin enters the worm by the transcuticular route.

Ivermectin kills microfilariae within female worms as well as those in human tissues. Dead microfilaria within the uteri of the female worm degenerate and prevents further microfilariae productions for 6-12 months. Ivermectin not only kills microfilariae, it also functions as a microfilariae suppressant. Two to three months after ivermectin therapy, microfilariae usually disappear from the eyes, halting the advancement of ocular lesions. Patients with visual field defect, anterior segment disease and optic nerve damage benefits. Advanced disease of the posterior segment (example; Chorioretinitis) does not improve but stabilizes with treatment. (Abiose, 1998).

Antibiotics

Wolbachia organisms appear to play a critical role in the biology and metabolism of filariae worms. The use of tetracycline to kill the Wolbachia organisms appear to be lethal to adult *O. ochengi*, and recent evidence suggests it is also effective for and perhaps other filarial worms (Langworthy *et al.*, 2000).

Sibling Species Recorded In West Africa and Nigeria

In West Africa, human Onchocerciasis is transmitted exclusively by sibling species of the *S. damnosum* complex (Ikpeama *et al.*, 2006).

The nine sibling species of *S. damnosum* complex identified in West Africa include:

S. damnosum Sensu Stricto
S. sirbanum
S. dieguerense
S. sanctipauli
S. soubrense
S. squamosum
S. yahense
S. leonense
S. konkorense

The first three species are regarded as savannah flies which transmit the savannah strain of *Onchocerca volvulus* which causes blindness while the remaining six belong to the forest group and transmit the forest strain of the disease of which the pathogenicity is more of skin disease with less blinding effects. (Adeleke *et al.*, 2010). The ratio of the length of the thorax to the antenna is generally a very useful character in distinguishing savannah flies from the forest flies (Okeke *et al*., 2011).

The migrating habit noted in black fly sibling species makes cross – border identification very important, with the strong possibility of vector migration into Nigeria from the surrounding countries. In summary, the cytospecies and cytotypes reliably recorded in Nigeria, according to Post *et al.*, (2007). Include:

S. damnosum subcomplex
S. damnosum s.s Nile form
S. damnosum s.s volta form
S. sirbanum sirba form
S. sirbanum Sudanese form
S. sanctipauli SUBCOMPLEX
S. soubrense beffa form
S. squamosum SUBCOMPLEX
S. squamosum A
S. squamosum B
S. squamosum C
S. yahense (typical form)

Challenges posed by sibling speciation to Control Efforts

Efforts to eliminate Onchocerciasis have been seriously hampered by inaccurate delimitation of transmission zones and this situation in turn, has been occasioned by the extra – ordinary propensity of the members of *S. damnosum* complex to exhibit sibling speciation. For example, the very large number of sibling species in the *Simulium damnosum* complex (>55) creates a problem in proper identification of vectors – since all species appear morphologically similar but do not interbreed (Wilson & Post, 1994; Kruger, 2005). Sibling species are real species, which differ in aspects of their biology, but cannot be distinguished on the basis of morphology. Their existence has been proven and they have been described upon the basis of variation in the polytene chromosomes, mostly fixed inversion differences but also sex-linked inversions hence the sibling species are also called cytospecies. Cytotypes are chromosomally distinctive populations of unknown species – status. Some cytotypes are probably cytospecies, but others are probably geographic races. Cytospecies and cytotypes are known together as cytoforms.

The real problem in control effort is that not all members of the complex are medically important – some bite animals in preference to man. Since their behaviour and host preference differ, the knowledge of which cytospecies dominate a breeding site becomes a very useful tool in planning intervention strategies-since all species appear morphologically similar but reproductively isolated-(Wilson and Post, 1994; Kruger, 2005).

Unfortunately, although the advent of the NOCP has been a significant stimulus to studies on the transmission and control of onchocerciasis (see for example Okonkwo *et al*., 1991 Adewale *et al*., 1999; Nwoke and Dozie, 2001; Ubachukwu and Anya, 2001; Oyibo and Fagbenro- Beyioko, 2003; Ubachukwu, 2004; Idowu *et al*., 2004; Opara *et al*., 2005; Ibe *et al*., 2007 ; Oluwole *et al*., 2009; Adeleke *et al*., 2010; Adeleke *et al*., 2011; Ebido *et al* 2011; Ugwuanyi *et al*., 2016), these have largely been epidemiological or ecological works and only a few studies have been published on the cytotaxonomic status of the vectors in this region. This has led to a poor understanding of the role of each member of the complex and has negatively affected control efforts. The negative effects on control effort can easily be seen in the fact that transmission has been reported to persist in most of the endemic foci undergoing Community Directed Programme with Ivemectin CDTI in the country. The knowledge of sibling species status in a particular endemic area will help solve the mystery behind this trend. Given that other factors such as poor treatment coverage and noncompliance to WHO guidelines for CDTI are likely factors that may encourage the reported case of persistent transmission, one cannot overlook the role of poor understanding of sibling species composition in an area. For instance, any control of onchocerciasis involving larvicidal treatment of breeding sites requires reliable species identification. This is because marked differences between the individual species in the *S. damnosum* complex with respect to macro and micro geographical distributions have been documented (Crosskey, 1981). Again, since adult members of different sibling species vary in their behaviour and biology, adequate knowledge of their sibling species status will help in understanding the transmission indices e.g. their preferred season of biting, migratory habit etc.

Over twenty years after the identification of vector breeding areas in relation to Onchocerciasis foci, the cytotaxonomic and molecular identity of *Simulium damosum* complex population in Nigeria has remained insufficiently studied. The entomological identification techniques of adult black flies are known to have technical limitations and this has greatly affected the assessment of control programmes (Dang and Peterson 1980; Garms, *et al*. 1982) such that cytotaxonomy and molecular systematic have remained the most reliable means of identification.

Since sibling species are real and differ in aspects of their biology and indistinguishable on morphological basis, it then follows that morphologically similar sibling species may not necessary be playing the same role in Onchocerciasis transmission. This is one of the cardinal challenges facing the (NOCP) in controlling vectors of Onchocerciasis in Nigeria.

Summary

The greatest burdens related to human Onchocerciasis are the results of the eye and skin lesions and severe itching produced by the micro-filariae (Kale, 1998). Onchocerciasis affects the productivity, social and sexual lives of infected persons due to blindness and other debilitating effects (Nwoke, 1990) and is a major obstacle to socio-economic development (WHO, 1980). The main strategies for control of the disease centre on the reduction of vectors and chemotherapy with Ivemectin. Blackflies show sibling speciation and these can only be separated using cytotaxonomy and molecular systematic. Accurate identification of sibling species has been found to be useful in controlling the disease in the Americas.

Insufficient knowledge of the sibling species is thought to hamper the efforts made to eliminate the disease in West Africa.

Recommendation

More financial and technical supports should be given to the (NOCP) and other related agencies responsible for control of Onchocerciasis in Nigeria by the Government. This is with the view to helping them to delimit more foci through employing epidemiological and taxonomic studies. This in turn will help in proper understanding of the role of each sibling species in disease transmission and guide the design of effective control measures. The development and application of alternative control strategies in Nigeria will benefit from a better understanding of the epidemiology of the disease, and this should take into account, the differences between the vector cytospecies.

References

Abiose, A.,(1998). Onchocercal eye disease and the impact of mectiyan treatment. *Ann Trop med parasitol 92*:511.

Adeleke M. A., Olaoye I. K., & Ayanwale A. S. (2010). Socio-economic implication of *Simulium damnosum* complex infections in some rural communities in Odeda Local Government Area of Ogun State, *J. Public health Epidemiol.,* 2(5): 109-112.

Adewale, B., Mafe, M. A. & Oyerinde, J. P. O. (1999). Infectivity and transmission dynamics of Simulium damnosum s.l. around Owena Dam (Ondo state). West African Journal of Medicine, 18, 257–260.

Awadzie, K., Dadzie, K Y., Schulzkey, H., Haddock, D. R. W., Gilles, H. M. & Aziz, M. A. (1985). The chemotherapy of Onchocerciasis X. An assessment of four single dose treatments in Onchocerciasis patients. *Acta leidensis* 59 (1 x 2): 193-199.

Basanez, M. G. & Ricardez- Esquinca, J. (2001). Models for the population biology and control of human onchocerciasis. *Trends in Parasitology,* 17: 430 – 438.

Basanez, M. G., Pion, SDS., Churcher T., Breitling, Lp., Littler, MP & Boussines, M., (2006). River blindness: a success story under threat? *Plos Medicine* 3, e37.

Boatin, B. A., Hougard, J. M., Alley, E. S., Akpabova, L. K., B. Yameoge, L. Dembeke, N.

Seketeti, A. & Dadzie, K. Y. (1998). The impact of Mectizan on the transmission of Onchocerciasis. *Annals of Tropical Medicine and Parasitology*, 92C suppl. 1, 547561.

Burnham, G. (1998). Onchocerciasis *lancet,* 351: 1341-1346.

Crosskey R. W. (1987) Blackfly Species of the World. *An annotated checklist of the world blackflies* (Diptera: Simulidae) pages 425-520.

Crosskey, R. W. (1981). A review of *Simulium damnosum* s.l. and human onchocerciasis in Nigeria, with special reference to cytographical distribution and development of a Nigerian national control campaign. Tropenmedizin und Parasitologie, 32, 2–16.

Crosskey, R. W., Lane R. P. & Crosskey, R.W., (1996). Medical Insects and Arachnids. London: Chapman and Hill; 241 287.

Dang, P. T. & Petertson, B. V. (1980). Pictorial keys to the main species groups within the Simulium damnosum Theobald complex occurring in West Africa. Tropenmedizin und Parasitologie, 31, 117–120.

Davies, J. B. (1993), Description of a Computer Model of forest Onchocerciasis Transmitting and its Application to Field Scenarios of Vector Control and Chemotherapy. *Annals of Tropical medicine and parasitology*, 87. 41-63.

Davies, J. B. (1994). Sixty years of onchocerciasis control: a chronological summary with comments on eradication, reinvasion, and insecticide resistance. *Annual Review of Entomology*, **39**, 23-45.

Ebido, C.,Okeke, O.C. & Ubachukwu,P.O. (2011). Studies on the Morphometric Chracteristics of the *Simulium damnosum* Complex in Uzo-Uwani, Enugu State , Nigeria. *Animal Research International* **8**: 1337 – 1344.

Evans, D. S., Alphonsus K, Umaru J, Eigege A, Miri E, & Mafuyai H,(2014). Status of Onchocerciasis transmission after more than a decade of mass drug administration for Onchocerciasis and lymphatic Filariasis elimination in Central Nigeria: Challenges in co-ordinating the stop MDA decision. *Plos Neglected Tropical Disease.*

Garms, R., Cheke, R. A., Vajime, C. G. & Sowah, S. (1982). The occurrence and movements of different members of the Simulium damnosum complex in Togo and Benin. Zeitschrift fur Angewandte Zoologie, 69, 219–236.

Hougard, J-M., Agoua, H., Yaméogo, L, Akpoboua, K.L.B., Sékétéli, A. & Dadzie K.Y.(1998). Blackfly control: what choices after onchocerciasis? *World Health Forum*, 19: 281-284.

Ibeh, O. O., Nwoke, B. E. B. & Adegoke, J. A. (2007). Distribution and ecology of breeding sites of *Simulium damnosum* s.l. in south–eastern primary health zone of Nigeria. Nigerian Journal of Parasitology, 28, 32–38.

Idowu, E. T., Adewale, B., Mafe, M. A., Appelt, B. & Bamgbose, A. (2004). Endemicity of onchocerciasis in some local government areas of Niger state. African Journal of Medical Sciences, 33, 31–34.

Ikpeama, C. A., Nwoke, B. E. B. & Anosike, J. C. (2006). Studies on the ecology and distribution of blackflies (Diptera: Simuliidae) in Imo state, Nigeria. International Journal of Natural and Applied Sciences, 2, 412–416.

Kale, O. O. (1998) Onchocerciasis the burden of disease. *Annals of Tropical medicine and parasitology* 92: 5101-s 115.

Kennedy, S. & Basanez, M. G. (2002). The effect of different Onchocercal – *Simulium* combinations on the outcome of ivermectin-based control programmes. *British Simulids Group Bulletin*, 19, 6-9.

Kennedy, S., Basanez, M. G. & Williams, J. R. (2003). The sentivity of a Mathematical model for the transmission dynamics and control of human onchocerciasis to vectorrelated parameters. *American Journal of Tropical Medicine and Hygiene*, **69**, 229230.

Kosisochukwu, U., Eneanya, C. I., Onyido, A. E., Okonkwo, N. J., Aribodor, D. N., Nwankwo, E. N., Ukonze, C. B. & Okoye C. K. (2016). Microhabitat Assessment of the Breeding Sites of Simulium damnosum Theobald Complex in Oji River Basin, Enugu State Nigeria. *Int. J. Trop. Dis. Heal.* **16,** 1–7.

Kruger, A. (2005). Descriptions of members of the *Simulium damnosum* Complex (Diptera; Simuliidae)from southern African, Ethopia and Tanzania. *Annals of Tropical medicine and parasitology*, 99(3) 293-306.

Langworthy, N. G., Renz, A., Mackenstedt, U., Henkle-Duhrsen, K., de Bronsvoort, M.B., Tanya,V.N., M. Donnelly, J. & Trees, A.J. (2000). Macrofilaricidal activity of tetracycline against the filarial nematode *Onchocerca ochengi*: elimination of *Wolbachia* precedes worm death and suggests a dependent relationship .*Proc. R. Soc. Lond. B* 267:1063-1069.

Molyneux, D. H. & Davies, J. B. (1997). Onchocerciasis control: Moving towards the Millenium. *Parasitology Today* **13** (11): 418-425.

Mustapha, M. Kruger A, Tambala P. A. & Post, R. J. (2005). Incrimination of simulium thyolence (Diptera: Simullidae) as the anthropophilic black fly in the thyolo focus of human Onchocerciasis in Malawi. *Annals of Tropical medicine and parasitology*, 99(2):181 – 192.

Nwoke, B. E. B. & Dozie, I. N. S. (2001). Operational research and its success in onchocerciasis Control in Nigeria. Nigerian Journal of Parasitology, 22, 3–10.

Nwoke, B. E. B. (1990). The Socio-economic Aspects of Human Onchocerciasis in Africa:

The present appraisal, Hygiene, Epidemiology, Microbiology and Immunology 34: 37-44.

Ogunrinade, A., D. A., Baokye, Therriweather, A. & Unnasch, T. (1999). Distribution of the Blinding and Non-blinding strains of *Onchocerca volvulus* in Nigeria. *The journal of Infectious Disease.* 179 : 1577-1579.

Okeke, O. C., Ogochukwu, C. & Ubachukwu, P. O. (2011). Studies on the Morphometric characteristic Simulium- damnosum complex in Uzo-Uwani, Enugu State, Nigeria. *Ani. Res. Inter.* 8(1): 1337-1344.

Okonkwo, P., Akpa, A., Ihekwaba, A., Nwagbo, D., Umeh, R., Adibua, S., Ezike, V. & Ogbuokiri, J. (1991). Studies on onchocerciasis in forest–savannah mosaic areas of Nigeria. I. Investigations in Gbaragu, Oji River. Annals of Tropical Medicine and Parasitology, 85, 617–623.

Oluwole, A.S., Ekpo, U.F., Mafiana, C.F., Adeofun, C.O. & Idowu, A.O .(2009). Preliminary study on temporal variations in biting activity of *Simulium damnosum s.l*. in Abeokuta North LGA, Ogun State, Nigeria. *Parasit. and vect.* 2: 55 doi: 10.1186/1756-3305-255.

Opara, K. N. & Fagbemi, O. B. (2005). Physico– chemical indices of breeding sites of Simulium damnosum in the lower Cross River basin, Nigeria. Journal of Environmental Sciences, 17, 511–517.

Oyibo, W. A. & Fagbenro-Beyioko, A. F. (2003). Effect of repeated communitybasedivermectin treatment on the intensity of onchocerciasis in Nigeria. Rural and Remote Health, 3, 211.

Post, R. J. & Boakye, D. A. (1992). Vector taxonomy and the Control of human Onchocerciasis in West Africa. *Proc. Exper. And Appl. Entomology*., *N.E.E.V. AMSTERDAM*. 3: 105 – 109.

Post, R. J., Mustapha, M. & Kruger, A. (2007). An inventory of the cytospecies and cytotypes of the *Simulium damnosum* complex Diptera: Simulidae. *Tropical Medicine and International Health* 121342 – 1353. 1353 [pubmed].

Roberts, L. & J. Janovy, Jr. (2000). *Foundations of parasitology,* sixth edition, New York; McGraw Hill.Harwood, RF. and James MT.(1979), *Entomology in Human and Animal Health*. 7th edition. New York: Macmillan: 144 – 148.

Roberts, L. & J. Janovy, Jr. (2000). *Foundations of parasitology*, sixth edition, New York; McGraw Hill. Coffeng, L. E., Stolk W. A., Hoerauf A, Habbema D. M., Bakker, R, Hopkins A. D.(2014). Elimination of African Onchocerciasis: Modelling the Impact of Increasing the frequency of ivermectin mass treatment. *Plos One.*. 9 (12).

Ubachukwu, P. O. & Anya, A. O. (2001). 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, 22, 163– 168.

Original Article

Ubachukwu, P. O. (2004). Human onchocerciasis: epidemiological status of Uzo-Uwani local government area of Enugu state, Nigeria. Nigerian Journal of Parasitology, 25, 93–99.

Vajime, C. G. & Dunbar R. W. (1975) Chromosomal Identification of eight species of the sub genus *Edwarsellum* including *Simulium (Edwarsellum) damnosum* Theobald (Diptera:

Simulidae). *Tropenmedizin und parasitologies* 26. 11 – 138.138.

Walsh, J. F. (1990); Review of Vector Control Prior to the *Ocp. Acto Leidensia*. 59, 61-78.

Wilson, M. D. & Post, R. J. (1994). Integration of Morphometric, Cytogenetic and Molecular Techniques: a case study of *Simulium damnosum*. *The identification and characterization of pest organisms* (ed. By D. L. Hawksworth). pp 215-224, CAB International, Wallingford.

World Health Organization (1994). Onchocerciasis and its control. Report of a WHO expert committee of Onchocerciasis. WHO Technical Report 852.

World Health Organization. (1995). Ochocerciasis and its control. Report of a WHO expert committee on Onchocerciasis. Geneva, World Health Organization Technical Report 852.