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Detection of *Onchocerca volvulus* in Blackflies Collected from Some Communities in Madakiya, Zangon Kataf Local Government Area of Kaduna State

Dangana, H. F[1]., Dadah, A. J[2]. and Omole, V. M[3].

[1]Department of Microbiology Faculty of Science Kaduna State University, Kaduna.

*Corresponding Author:danganahannatu00@gmail.com; *Phone: +2348034525220*

Abstract

Onchocerca volvulus is a parasitic filarial worm of public health concern in Nigeria known as a causative agent of Onchocerciasis transmitted by female blackflies. The research is aimed at detecting Onchocerca volvulus in blackflies collected from some communities in Madakiya, Zangon Kataf Local Government Area of Kaduna State. Research was carried out in five Villages of Madakiya community. Blackflies were caught and stored in 70% alcohol, then transported to the Nigeria Institute for Trypanosomiasis Research, Kaduna for entomological studies and dissected after which the blackflies were transported to the Nigerian Institute for Medical Research for morphological identification and molecular detection of Onchocerca parasite. The highest catches of blackflies was recorded in the month of November with 54 total number of catches respectively. Morphological identification revealed the forest species of parasite present in the study area. Onchocerca volvulus was not detected in any of the blackflies by microscopic and molecular methods with a target gene of 200bp. There is need for increased public health enlightenment and health education on the negative effects of blackflies on the cause of Onchocerciasis.

Keywords: Detection, O. volvulus, Blackflies, Kaduna, Molecular and Onchocerciasis.

INTRODUCTION

Blackflies are important biting pests and vectors of several pathogens of humans and other warm blooded animals (Adler *et al.*, 2004). The common name for these flies vary with blackflies being used in some countries or sand-flies in others countries, here in Nigeria, it is known as shakwibi in Hausa; makurgi in Nupe; mukwai in Fulani and abunbu in Tiv (Davies, 2006). A blackfly (sometimes called a buffalo gnat, turkey gnat, or white socks) is any member of the family *Simuliidae* of the *Culicomorphainfra* order (Crosskey, 1990). Blackflies are divided into two subfamilies: Parasimuliinae which contains only one genus and four species; and Simuliinae which contains all the other genera. Over 1,800 species belong to the genus *Simulium* (Adler *et al.*, 2017). There are four genera among over 2,200 species that are of principal importance to humans, *Simulium*, *Prosimulium*, *Austrosimulium* and *Cnephia*. However *Simulium* is the most important that acts as vectors for pathogens that causes diseases, in Africa the main concern is with *Simulium damnosum* complex and *Simulium neavei* group, while central and South America have *S. ochraceum*, *S. metallicum* and *S. exiguum* complexes that transmit nematodes that cause Onchocerciasis (Service, 2008).

Blackflies are generally regarded as the second most medically important group of insects (Adler *et al.*, 2004). Blackflies are carriers of several pathogens which causes diseases like river blindness in Africa and the Americas (Adler *et al.*, 2017). Female blackflies feed on the blood of mammals, including humans for the food and ovary maturation with males feeding mainly on nectar. They are usually small, black or grey, with short legs, antennae and can be a common nuisance for humans ((CDC, 2018). Blackflies bite in daytime and out of doors, some species prefer to feed only on certain parts of the body like the legs or the upper part of the body (Davies, 2006; WHO, 2017). When a female blackflies bites an infected person during a blood meal, it also ingests microfilariae which develops further in the blackfly and are then transmitted to the next human host during subsequent bites (WHO, 2017). The adult worms can live in the nodules for up to 15 years. Some nodules may contain numerous male and female worms (CDC, 2018). The disease causal pathogen (*Onchocerca volvulus*) transmitted by blackflies is prevalent in 35 countries of the world of which 28 are in Africa and Nigeria accounts for one quarter of the global infection rate (CDC, 2010).

Onchocerca volvulus is among the eight parasitic nematode (roundworm) species that account for most cases of filariasis in humans.

Onchocerca volvulus is one of the three species responsible for most of the morbidity attributable to filariasis (the other two being *Wuchereria bancrofti* and *Brugia malayi*, which cause lymphatic filariasis). Onchocerciasis occurs mainly in Africa, with additional foci in Latin America and the Middle East (CDC, 2018). Onchocerciasis- or "river blindness" is a disease caused by the filarial worm *Onchocerca volvulus* transmitted by repeated bites of infected blackflies (*Simulium* sp.). These blackflies breed along fast-flowing rivers and streams, close to remote villages located near fertile land where people rely on agriculture (WHO, 2017). It is one of the Neglected Tropical Diseases (NTD) and has been estimated that approximately 85 million people are at risk of this infection while approximately 20 million people are infected with *Onchocerca volvulus*. In view of this, the research sought to detect the presence of *Onchocerca volvulus* in the female blackflies among the rural dwellers in Madakiya community, Zangon Kataf Local Government Area of Kaduna State, Kaduna Nigeria.

MATERIALS AND METHODS

Study Area

Madakiya is a rural and farming community with houses made of concrete buildings and traditional wooden and mud structures. The main occupation in the community is farming. The community has a flowing stream through it that cascades into the popular Matsirga waterfalls which is located in Matsirga, an area in Madakiya. The Matsirga waterfalls located in Madakiya is about 227 kilometers, south of Kaduna in Zangon Kataf Local Government Area and closely situated to Kafanchan. The water at Matsirga waterfalls drop 30 meters into a gorge that has been supported by beautiful rocks. The waterfalls take it source from springs on the Kagoro Hills cascading from four different natural funnels off the sheer rock cliff from about 25 meters to form a large pool at the bottom (10 beautiful places in Kaduna State May 2017). The cool mist from the water is often rainbow – coloured and there is plenty of shade so one can easily relax beside the waterfalls and take in the beauty and serenity of the area (Waterfalls of Nigeria-February 2015). The environs of this amazing work of nature provide very suitable breeding sites for the matured and immature stages of the blackflies. Also, a lot of human activities take place in and around the stream even as it flows down from upstream into the waterfalls and at the bottom of the falls where the water current and force has reduced. The locals patronize the stream for their drinking water, washing, bathing, agricultural activities (dry season irrigation farming and rainy season farming), block making, sand digging, etc

Collection and Sorting of Blackflies

Flies were caught using human baits and scoop nets. The blackflies were gently caught using inverted plastic tubes as they perch to take blood meal. The caps of the tubes were replaced immediately to prevent the flies escaping. This was done even before the fly feeds. Each fly was caught in a different tube and these were later pooled together (Otabil *et al.*, 2020). According to Otabil *et al.* (2020), this human landing collection is a gold standard for blackfly collection in Onchocerciasis surveys. For the scoop nets, the nets were swiped across the vegetation along the riverbank. The nets containing the flies were soaked in water to immobilize the flies. They were hand-picked into sample containers containing 10% formal saline for further analysis (Otabil *et al.*, 2020).

Using morphological characteristics profile, the blackflies harvested were sorted out and separated from other insects. The blackflies were then separated further into male and female flies using morphological features the female flies were counted (Maikaje *et al.*, 2015). Morphological identification of the blackflies was done and calculated according to the method described by Davies and Crosskey, (1992).

$$\text{Monthly Biting Rate} = \frac{\text{No. of Flies Caught X No. of Days in a Month}}{\text{No. of Catching Days}}$$

(Davies and Crosskey, 1992).

Microscopic Detection of *O. volvulus* Larvae in Adult Blackflies

The head, thorax and abdomen of each adult female blackfly were dissected under the dissection microscope on a clean glass slide in saline solution. This was done in search of the larval stages of the parasite, *O. volvulus*. The flies were stored in 70% alcohol which were then transported to the Nigerian Institute for Medical Research (NIMR), Yaba- Lagos for molecular for investigations.

Molecular Detection of *Onchocerca volvulus*

DNA Extraction

DNA extraction was carried out by pooling the head of black fly using the Zymo Quick-DNA Tissue/Insect Miniprep Kit according to manufacturer's instructions.

PCR Amplification of the ITS (Internal Transcribed Spacer) Gene

Polymerase chain reaction was carried out on the extracted DNA using the primer pair O-150-

1632 (5'GATTYTTCCGRCGAANARCGC3') and O-150-1633 (5' GCNRTRTAAATNYGNAATTC3C) previously described by Laurent *et al.* (1998). The PCR reaction was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. Polymerase chain reaction (PCR) was performed in 20 µl of a reaction mixture, and the reaction concentration was brought down from 5x concentration to 1X concentration containing 1X Blend Master mix buffer (Solis Biodyne), 1.5 mM $MgCl_2$, 200µM of each deoxynucleoside triphosphates (dNTP)(Solis Biodyne), 20pMol of each primer, 2 unit of Hot FIREPol *DNA* polymerase (Solis Biodyne), Proofreading Enzyme, 5µl of the extracted DNA, and sterile distilled water was used to make up the reaction mixture (Huang *et al.*, 2017).

Thermal cycling was conducted in a PTC 200 Gradient Thermal Cycler for an initial denaturation of 95˚C for 15 minutes followed by 5 cycles of 95˚C for 1 minute, 37˚C for 2 minutes and 72˚C for 30 seconds, then another 29 cycles of 95˚C for 30 seconds, 37˚C for 1 minute and 72˚C for 30 seconds. The reaction was completed with a final extension of 72˚C for 10 minutes. The PCR products were separated in a 2% agarose gel electrophoresis followed by addition of Ethidium bromide to the gel. The gel was ran at 100 V for 60 minutes and visualized with an ultraviolet trans illuminator.

Data analyses

Data obtained were analysed using descriptive statistics such as frequency and percentages and presented on Tables and figures.

RESULTS

A total of 216 flies were caught with the highest caught within November 54 (25%). The month of January, had the least number of flies caught 11(5.09%). Other months as August 36(16%), September 29(13%), October 37(17%) and December 26(12.04%) had the moderate number of flies as the time of the research (Table 1).

On the biting rates, November had the highest; 540 (bites/person/month) while January had the least; 113 (bites/person/month) (Table 2).

Morphological, microscopic and molecular screening of dissected head, thorax and abdomen of blackflies revealed that larvae of *Onchocerca* were not present as shown in Table 3.

Molecular detection revealed negative at the targeted gene of the screened blackflies as shown in figure 1.

Table 1: Rate of Distribution of Flies Caught from the Month of July to January, 2021.

Months	Frequency	Percentage (%)
July	23	10.65
August	36	16.67
September	29	13.43
October	37	17.13
November	54	25.00
December	26	12.04
January	11	5.09
Total	**216**	**100.00**

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Table 2: Detection of *O. volvulus* from Female Blackflies Caught in the Collection Months.

Characteristics	July	August	Sept.	Oct.	Nov.	Dec.	Jan.
Number of Days Worked	3	3	3	3	3	3	p3
Total Flies Caught	23	36	29	37	54	26	11
Average Daily Catch/Day	8	12	10	12	18	9	4
Biting Rates(B/P/M)	237	372	290	357	540	268	113
No: of Flies Dissected (%)	23(100)	36(100)	29(100)	37(100)	54(100)	26(100)	11(100)

Biting Rates=NC X NDM/NCD: **NC**=Number of flies caught, **NDM**=Number of days in a month, **NCD**=Number of catching days.

Table 3: Microscopic and Molecular Screening of *Onchocerca* Larvae in Blackflies

Morphologic Features	Microscopic Screening	Molecular Screening
Head,	0(0)	0(0)
Thorax	0(0)	0(0)
Abdomen	0(0)	0(0)

Key: M=Ladder, **-ve**=Negative control, **+ve**= Positive control **bp**= base pair
Figure 1: Gel electrophoregram of PCR Amplicons of Genomic DNA of Onchocercal parasites targeting the O-150 repeat sequence

DISCUSSION

A total of 216 flies were caught. The highest numbers of flies caught were in November, August and October. From the month of July, the flies caught showed an undulating increase features with decline in the number of catches towards the dry season. The highest number of blackflies catches recorded were within the months November despite the onset of the dry season which is in line with the work reported by Takaoka (2015; Adler *et al.*, 2017) where most of the flies are easily found flying within the period of the season of October and November. This may be due to the fact that the rainy season just ended with the gradual onset of the dry season but there was still the presence of luxurious vegetation all around which is a good breeding and resting place for the blackflies and increased human activities. From the number of flies caught monthly in the study area, the place can be said that the rate of population of flies varies from season to season. Hence, making it difficult to estimate and control their rate of distribution. It is usually difficult to estimate the population of adult blackflies due to their peculiar migration abilities WHO, (2017), but this is needed for any meaningful vector control studies (Davies, 2006).

As the highest number of flies are recorded within November, October and August, the biting rates substantially increases within the period of November with the highest; 540 (bites/person/month) while January had the least; 11 (bites/person/month). The morphological and microscopic examination of the Onchocercal parasite in the head, thorax and abdomen of the blackflies (by dissection) was zero as reported by Takaoka (2015); Adler *et al.*, (2017) where the highest bite was recorded in October and November, this may be due to the variation of season as the rainy season ended affect the distribution of blackflies with decrease in biting rate despite an increased in human activities. This finding is consistent to the work of Takaoka (2015) that blackflies caught varies from season to season; this may be as a result of the dry season that had fully set in and the reduction of human activities around the stream. The result of morphological identification showed that most flies harvested in the study area were of the forest group, characterized by dark colour of the hairs on the 9th abdominal tergite setae, antennae, the fore-coxae, scutella setae, wing arculus and wing tufts, a lot of the flies had dark wing tufts while some were observed to bear pale wing tufts, in some cases, there were mixture (Wilson *et al.*, 1993; 1994). The dark coloration is very significant for the identification of the Forest strain of the blackflies. Microscopic examination further revealed the absence of *Onchocerca volvulus* parasite in the examined female flies, this may be due to the fact that the biting rate of flies that result to Onchocercal infection as at the time of the research may had been minimal due to the reduction in human activities in the area. This is consistent to Service, (2008) that Onchorcecal infection reduces as the biting rate reduces with decreases in human activities.

Molecular analysis on the detection of *Onchocerca volvulus* parasite in female blackflies in the study area further revealed the absence of the parasite since there were no gel

bands in the electrophoregram. This may be due to the methods adapted from microscopic examination to molecular technique. It could be due to the absence of parasite in the blackflies as at the time of the research. The importance of the polymerase chain reaction during post-control in insects has proven to be sensitive (Huang *et al.*, 2017), but time consuming.

CONCLUSION

The highest catches of blackflies were recorded in the month of November with a total of 54 numbers of catches respectively. Morphological identification revealed the forest species of parasite present in the study area. *Onchocerca volvulus* was not detected in any of the blackflies by microscopic and molecular methods with a target gene of 200bp.

REFERENCES

Adler, P.H.,Crosskey, M. and Roger, W. (2017).World Blackflies (Diptera: Simuliidae): A Comprehensive Revision of the Taxonomic and Geographical Inventory 44(2):. 11-22.

Adler, P.H., Currie, D.C. and Wood, D.M. (2004).The Black Flies (Simuliidae) of North America.xv+941 pp., Cornell University Press, Ithaca, New York, U.S.A.

CDC. (2013). Onchocerciasis-Prevention and Control. June, 2021.

Crosskey, R. W. and Howard T. M. (2004). A Revised Taxonomic and Geographical Inventory of World Blackflies (Diptera: Simuliidae). The Natural History Museum, London. http://www.nhm.ac.uk/research-curation/projects/blackflies/ .

Crosskey, R. W. (1979). An Appraisal of Current Knowledge of Simulium damnosums in the Federal Republic of Nigeria in Relation to the Development of an Onchocerciasis Control Campaign. 1-11.

Davies, J. B. (2006). Blackfly Vernacular Names; Based on the List Published in the StudiaDipterologia Supplement, 44:129-135.

Huang, H. I., Weng, K. F. and Shih, S. R. (2017). Viral and Host Factors that Contribute to Pathogenicity of Enterovirus 71. Future Microbiology, 22(7): 467-479. **[Crossref]**

Laurent, Toe.,Boakye, A., Boatin, A.A., Back, C., Anthony, M. and Thomas, U. (1998). Detection of Onchocerca volvulus Infection by O-150 PCR Analysis of Skin Scratches. The Journal of Infectious Diseases 21 (11): 89-93.

Maikaje, D.B., Dibal, D.M., Umar, Y.A. and Egbe, N.E. (2015).Investigations on the Transmission Potentials of Simulium damnosum and the Risk of Human Onchocerciasis in Kaduna Metropolis, Kaduna State, Nigeria. International Journal of Modern Biological Research. IJMBR 3(12):1-5.

Olajide, J. A. (2019). Biting Preferences of Blackfly in two Endemic Communities of Ose Local Government Area, Ondo State, Nigeria. New Nigerian Journal of Clinical Research 8(14):64-67. **[Crossref]**

Osue, H.O., Inabo, H.I., Yakubu, S.E., Audu, P.A., Galadima, M.,Odama, L.E., Musa, D., Ado, S.A. and Mohammed, M. (2013). Impact of Eighteen Year Varied Compliance to Onchocerciasis Treatment with Ivermectin in Sentinel Savannah Agrarian Communities in Kaduna State of Nigeria. Parasitology 55 (33): 189-196. **[Crossref]**

Otabil, K.B., Samuel, F.G., Henk, D. and Schallig, H. (2020).Biting Rates and Relative Abundance of Simulium Flies under Different Climatic Conditions in an Onchocerciasis Endemic Community in Ghana. Parasites & Vectors, 13(4): 229. **[Crossref]**

Service, M. W. (2008). Medical Entomology for Students, Cambridge University Press, pp 81-92 and 289

Takaoka, H. (2015). Review of the Biology and Ecology of Adult Blackflies in Relation to the Transmission of Onchocerciasis in Guatemala.Tropical Medicine and Health. 43:71-81. **[Crossref]**

WHO. (2017). Onchocerciasis -Fact sheet, updated September, 2020.

Wilson, M.D., Cheke, R.A., Flasse, S.P.J., Grist, S., Osei-Ateweneboana, M.Y., *et al.* (2002). Deforestation and the Spatio-Temporal Distribution of Savannah and Forest Members of the Simulium damnosum Complex in Southern Ghana and South Western Togo. Transactions of the Royal Society of Tropical Medicine and Hygiene. 96:632-639. **[Crossref]**

Wilson, M.D., Post, R.J., Gomulski, L.M. (1993). Multivariate Morphotaxonomy in the Identification of Adult Female Simulium damnosum Theobald Complex (Diptera: Simuliidae) in the Onchocerciasis Control Programme area of West Africa. Ann. Trop. Med. Parasitol. 87(6): 65-82. **[Crossref]**

Wilson, M.D., Mafuyai, H.B. and Post, R. J. (1994). Morphological Identification of Sibling Species of the Simuliumdamnosum (Diptera: Simuliidae Complex from Nigeria, Cameroon and Bioko. Proc. Exper. and Appl. Entomol; N.E.V. Amsterdam. 5(23):181-185.