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Assessments of Onchocerca Volvulus Infection in Humans and Black flies and Onchocerciasis Response to Ivermectin in among Villagers around Gurara Dam, Kaduna State, Nigeria

Ozovehe, L. O.

Research officer, Nigeria Institute for Trypanosomiasis and Onchocerciasis Research, Nigeria

Abdulkadir, M. K.

Research officer, Nigeria Institute for Trypanosomiasis and Onchocerciasis Research, Nigeria

Rabiu, A. I.

Research officer, Nigeria Institute for Trypanosomiasis and Onchocerciasis Research, Nigeria

Abstract:

Purpose: A study on the assessments of onchocerciasis rates in humans and black flies and its response to ivermectin therapy among villagers around Gurara dam, Kaduna state, Nigeria was conducted between June to November 2016.

Principal methodologies: A total of 158 skin snips were collected and 4994 black flies were caught. The skin snips and female black flies were analysed by means of microscopy to detect Onchocerca volvulus infection. An amplified Polymerase Chain Reaction (PCR) detected O. volvulus DNA from skin snip of some individuals.

Key finding: From the skin snips analysed, 4(2.6%) were positive for Onchocerca volvulus infection. The highest number of flies caught (1274) and highest number of parous flies (1089) were recorded in June. The highest number of infected flies, 14(38%) was obtained in the month of July. The drug Ivermectin, administered in the study population was effective in humans, with 75% complete resolution of symptoms. The highest incidence of Onchocerca volvulus was found among the age group of 50 years and above (75%). The prevalence was higher among the farmers (75%) with equal distribution among females (50%) and males (50%), followed by artisans (25%). The risk factor implicated was regular visit to the dam. The signs and symptoms of onchocerciasis observed were; white patches, severe body itch, nodules and hanging groins.

Conclusion: This study revealed that infective black flies are still hovering around Gurara dam, thus the persistence of onchocerciasis among the inhabitants, especially farmers, of the sampled villages. Hence the call for an effective vector control and a well supervised and prolonged ivermectin distribution

Keywords: Assessment, onchocerca volvulus, ivermectin

1. Introduction

Onchocerciasis or “river blindness” is a parasitic disease caused by the filarial nematode *Onchocerca volvulus* and transmitted by repeated bites of infected blackflies of the *Simulium* species⁽¹⁶⁾.

The microfilariae are the main cause of the clinical manifestations of the disease⁽¹⁵⁾. Onchocerciasis is characterized by chronic skin disease, severe itching, and eye lesions that can progress to complete blindness⁽¹⁶⁾. It is endemic in Nigeria⁽⁵⁾. It is the second leading cause of blindness (after trachoma) in the world⁽⁴⁾. The current strategy of controlling the disease in Africa relies mostly on the annual chemotherapeutic treatment of the endemic communities through mass distribution of Ivermectin which has substantially reduced the disease burden in many affected communities^{(13), (17)}.

In the face of a successful control program, the prevalence of infection in the vector populations is drastically reduced. This is true both for programs relying on vector control⁽¹⁴⁾ and for those using mass Ivermectin distribution⁽²⁾.

2. Materials and Methods

2.1. Study Area

The study area consisted of Kurmi Bango and Ungwan Jaba villages around Gurara dam in Kagarko LGA, Kaduna state.

2.2. Ethical Clearance

Clearance was obtained from Kagarko Local Government Authority.

2.3. Sample Collection

Skin snips were taken from consenting individuals aged 5yrs and above, using sterilized sclerocorneal punches. A total of 158 persons were skin snipped. The 96 wells of the microtitration trails were filled with 50µl of saline solution before adding skin snip samples. Similarly, the biting adult females of *Simulium damnosum* were collected using two humans as bait. Blood meal searching female *Simulium* flies were naturally attracted to the exposed legs of fly catchers in an attempt to take a blood meal, as soon as they landed. The fly catching was organized alternately between 07:00 to 17: 00 hours every sampling day for three (3) consecutive days monthly through six (6) months of the year. Flies were dissected on the field while skin snips were examined, stored in a cooler containing ice packs, then transported to and refrigerated at Onchocerciasis Research Department laboratory of the Nigerian Institute for Trypanosomiasis Research (NITR) at the end of each week's activity.

Structured questionnaires were administered to the study subjects to capture the socio-economic, demographic, risk factors and symptoms associated with onchocerciasis.

2.4. Dissection of the Flies

Each of the flies was dissected, the ovaries of the dissected flies were stretched and classified as parous or nulliparous after observing other characters such as absence or presence of fat bodies and the colour of malpighian tubules. The parous flies were dissected further to determine the presence of *Onchocercavolvulus*, while the nulliparous flies were discarded.

2.5. Microscopic Examination of Skin Snip Biopsies

The skin snip samples in microtitration trails containing normal saline were examined after 24 hours to observe the presence or absence of microfilariae with the aid of a microscope.

2.6. Extraction of *Onchocerca volvulus* DNA

Positive skin snip samples gotten from microscopy with other negative samples were subjected to PCR.

2.7. PCR amplification

Polymerase chain reaction (PCR) was performed using a S4 forward primer (5' AATAACTGATCCTATGACC3') and a biotinylated S3 reverse primer (5' ATCAATTTTGCAAAATGCG3') at a concentration of 20 pmol per 50 µl each.

3. Results

- The prevalence of *O. volvulus* obtained from skin snip samples of some subjects in Kurmi Bango and Ungwan Jaba villages around Gurara Dam, Kaduna state was 4(2.5%) with a significant P-value of 0.002. The prevalence was higher from Ungwan Jaba 3(1.9%) than those from Kurmi Bango 1(0.6%).
- The monthly distribution of *O. volvulus* from *S. damnosum* harvested around Gurara Dam, is presented as follows; The highest number caught (1274) and parous flies (1089) were in June. The lowest number caught (895) and parous flies (706) were obtained in November. The highest number of infected flies (14) was obtained in July and the least (2) in October.
- Polymerase Chain Reaction (PCR) to detect *Onchocerca volvulus* DNA from skin snips was performed. A total of one hundred and fifty-eight skin snip samples were subjected to PCR (including four positives gotten from parasitological method), five turned out positive. These five samples were picked and later re-run on a single gel for a simplified presentation. There were amplifications at 150bp in lane 1, 2, 3, 4 and 5. Lanes 1 and 2 were from Kurmi Bango while lanes 3, 4 and 5 were from Ungwan Jaba. Three out of the four positive samples from parasitology result were confirmed positive by PCR.

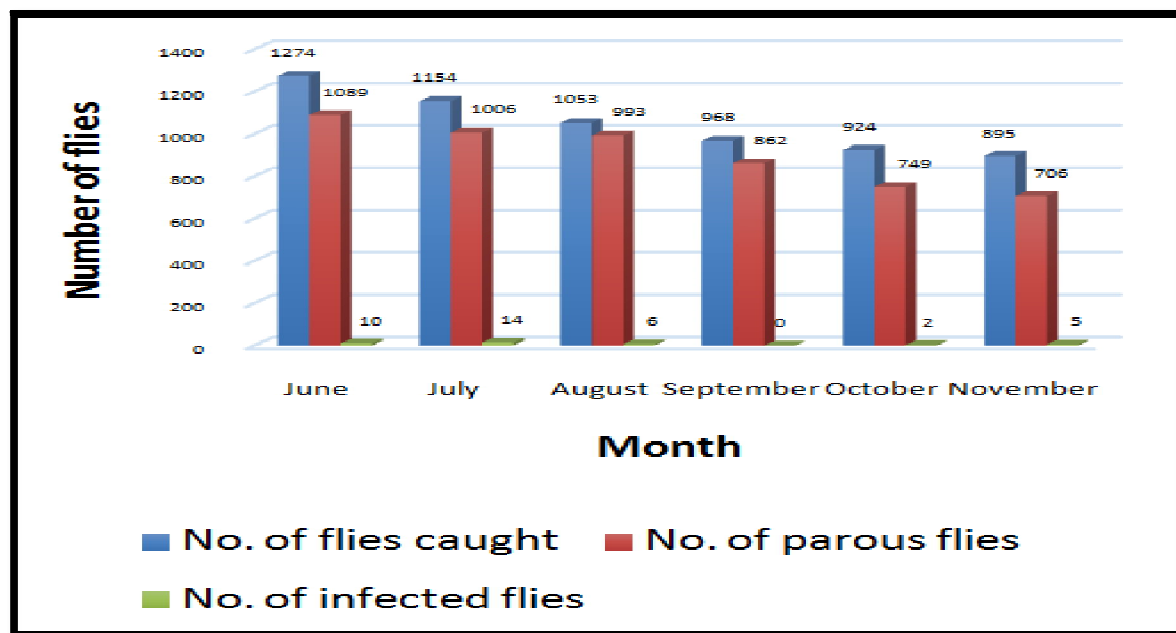


Figure 1: Monthly Distribution of Onchocerciasis in Simulium Damnosum Detection of Onchocercavolvulus

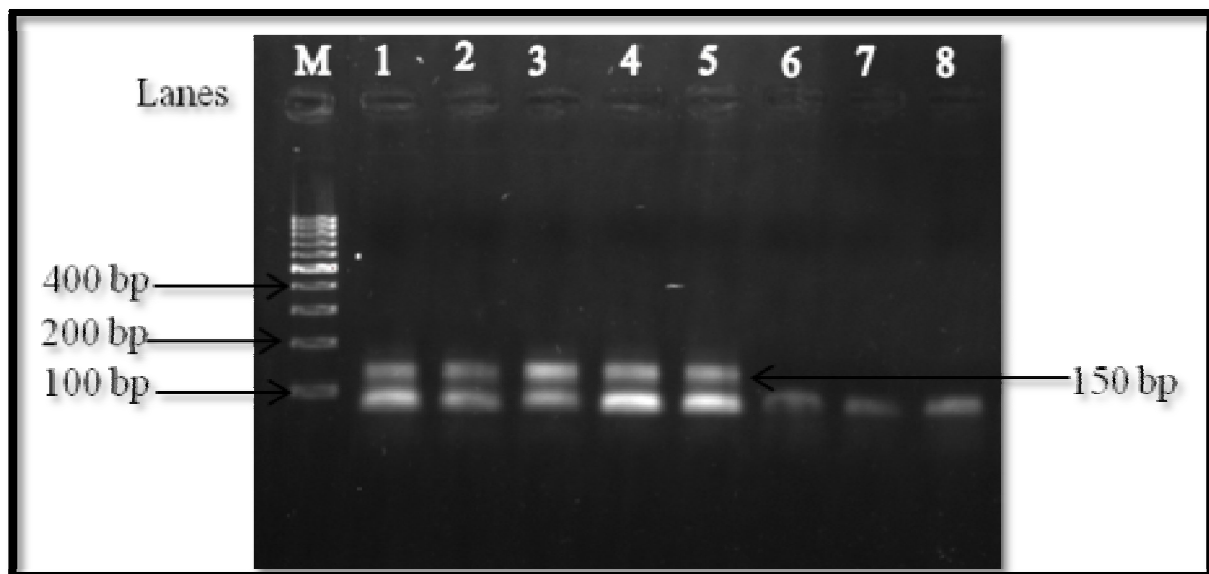


Figure 2: Harvested around Gurara Dam in Kaduna State
Dna From Skin Snip Samples

- The rate of access and usage of the drug Ivermectin varied in the population. A total of forty-nine individuals used the drug once since the exercise began, sixteen used it twice, twenty-nine used it thrice, and twenty-two used the drug more than three times. Onchocerciasis was not detected in subjects who either used the drug thrice or more times, however, those who used the drug either once or twice had most of the infection.
- The outcome of the issued questionnaire among the residents of the two communities studied revealed Ivermectin treatment of onchocerciasis results as follows: a total of thirty-six subjects claimed complete resolution of symptoms due to the use of the drug for over three years, thirteen subjects had incomplete resolutions, forty subjects did not use the drug at all, while sixty-nine did not respond to the question.
- The demographic factors of the subjects were analyzed for their relationship with the occurrence of onchocerciasis; Subjects within the age ≥ 50 years were mostly infected (75.0%), those within the age group of 15 to 19 years (25.0%), other age brackets were negative. This association was statistically significant ($p= 0.019$) between onchocerciasis and age. There was equal occurrence of onchocerciasis between the male and female subjects 50.0% each, but the association was not significant ($p=1.000$) between onchocerciasis and gender. The highest prevalence for onchocerciasis was recorded among some civil servants who also engage in part-time farming (50.0%). Same prevalence was recorded among the farmers and artisans (25.0%) each. In other words,

highest prevalence was recorded among the farmers (75.0%). There was a statistically significant association ($p=0.035$) between onchocerciasis and occupation.

- Assessment of the epidemiological factors predisposing to onchocerciasis is as follows: All the subjects had knowledge of black fly and can identify these vectors. Likewise, they all use water from the dam. Seventy-five percent of those diagnosed with onchocerciasis said they visited the dam on daily basis while the other twenty-five only visited the dam about once in a week. The relationship between visit to the dam and onchocerciasis prevalence was found to be statistically significant ($p=0.004$).
- The signs and symptoms of onchocerciasis as observed in this study are presented as follows: All the symptoms except blindness and impaired vision showed statistical significant association with *O. volvulus*. White patches ($p=0.002$), body itch ($p=0.016$), nodules ($p=0.002$) and hanging groin ($p=0.001$).

4. Discussion

- The overall prevalence of *O. volvulus* infection in humans obtained in this study was 2.5%. The standard parasitological assessment (microscopy) was carried out on all the one hundred and fifty-eight skin snip samples collected from the two sampled villages. This low prevalence is attributed to the mass distribution of Ivermectin carried out in the area over the years. Similar observations have been reported^{(3),(9),(8)}.
- Villagers who regularly visited the dam site for activities as farming, swimming, fetching of water, washing of clothes constituted an all year-round providence for black flies to thrive. The high number of parous flies observed in June was also reported in Mobi, Adamawa state⁽⁷⁾.
- Amplified PCR products were observed on lane 1-5 using gel photos in form of bands at 150 bp. This confirmed the presence of *O. volvulus* DNA in the subjects. It agrees with the result of the microscopy previously carried out. A total number of one hundred and fifty-eight samples (this included the four positive samples obtained through microscopy with the one hundred and fifty-four negative samples) were processed for the molecular assay. DNA of *O. volvulus* from skin snip was isolated by a modified proteinase K digestion protocol, followed by PCR using forward and reverse primers to target the mitochondrial encoded *O. volvulus* *cox1* gene. All the one hundred and fifty-eight samples were run on gel photos and finally, the positive samples were picked and re-run on a single gel photo for a clearer view. Five positive samples were obtained; this included three out of four positive samples obtained through microscopy and other two samples. This positive from PCR is greater than the result obtained from microscopy, thereby confirming that molecular detection is more sensitive than microscopy. Several studies using polymerase chain reactions (PCR)-based methods for the detection of *O. volvulus* DNA in the skin or in the vectors have been reported^{(18),(17)}.
- About 74% had access to the drug ivermectin and the relationship between onchocerciasis and symptom resolution from treatment with Ivermectin was found to be statistically significant ($p=0.001$). This indicated that the drug, Ivermectin, used for the treatment of onchocerciasis in this area has been very effective.
- The relationship between the prevalence of onchocerciasis and the demographic factors showed the factor age ($p=0.019$) and occupation (0.035) to be statistically significant.
- There was no statistical association of onchocerciasis prevalence and gender in this study.
- There was equal distribution of the infection among the males and females in this study. A 38.7% male, 23.1% females in Ebonyi Central and 32.0% males, 25.0% females in Ebonyi North positive for *Onchocerca volvulus* infection was previously reported⁽¹⁰⁾.
- Age was shown to be associated with the infection with more infection in the elderly probably because the infection takes a long time of about 18 to 24 months to be established and could persist for as long as 9 to 11 years⁽¹¹⁾. A similar observation was made in Ibarapa L.G.A. of Oyo State Nigeria⁽¹⁾.
- The occupation of the villagers especially farming exposes them to black fly bites leading to infection as implicated in this study ($p=0.035$). There has been a report on the association of Onchocerciasis with occupation⁽⁶⁾, and another report on farmers being the most *Onchocerca volvulus* affected group⁽¹⁰⁾.
- Regular visit to dam was found to be a statistically significant risk factor in the acquisition of onchocerciasis ($p=0.004$). Since the dam has served as a point of contact. Other studies reported a similar finding⁽¹¹⁾.
- Although all the study population claimed to have awareness of black flies, they were yet infected. This implies that the knowledge of black flies or the ability to identify them is not enough to curb the infection.
- These are some of the indices considered to show the presence of the disease; white patches on the skin, painful and severe body itching, nodules, presence of impaired vision, hanging groin and blindness.
- All the symptoms studied with the exception of blindness and impaired vision, were found to be related to onchocerciasis thereby confirming them to be the symptoms of onchocerciasis as observed in this study. This finding agrees with previous ones⁽¹²⁾.

5. Conclusions

- The prevalence of 2.5% (0.6% from Kurmi bango and 1.9% from Ungwan jaba) of *O. volvulus* was obtained.

- The highest number of parous flies (20%) was obtained in June while the highest number of infected flies (38%) was obtained in July.
- The Agarose gel amplified PCR products of *O. volvulus* DNA from skin snips of some persons in the selected villages showed amplifications at 150bp.
- The association of *O. volvulus* and some demographic factors such as age (p-value 0.019) and occupation (p-value 0.035) was established.
- The association of *O. volvulus* and symptoms of onchocerciasis such as white patches (p-value 0.002), painful and severe body itches (p-value 0.016), nodules (p-value 0.002) and hanging groins (p-value 0.001) was established.
- The association of *O. volvulus* and the risk factor "frequent visit to the dam" (p-value 0.004) was obtained.
- The relationship between *Onchocercavolvulus* and symptoms resolution from treatment with ivermectin was established (p-value 0.001).

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