

**ASSESSMENTS OF *ONCHOCERCA VOLVULUS* INFECTION IN HUMANS AND
BLACKFLIES AND ONCHOCERCIASIS RESPONSE TO IVERMECTIN AMONG
VILLAGERS AROUND GURARA DAM, KADUNA STATE, NIGERIA**

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FACULTY OF LIFE SCIENCES,
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NIGERIA.**

MAY, 2017.

TITLE PAGE

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**A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,
AHMADU BELLO UNIVERSITY, ZARIA**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD
OF A
MASTER OF SCIENCE DEGREE IN MICROBIOLOGY**

**DEPARTMENT OF MICROBIOLOGY,
FACULTY OF LIFE SCIENCES
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA**

MAY, 2017.

DECLARATION

I declare that the work in this dissertation entitled, “**ASSESSMENTS OF *ONCHOCERCA VOLVULUS* INFECTION IN HUMANS AND BLACKFLIES AND ONCHOCERCIASIS RESPONSE TO IVERMECTIN AMONG VILLAGERS AROUND GURARA DAM, KADUNA STATE, NIGERIA**” was carried out by me. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

OZOVEHE Lydia Onyeche

Name of Student

Signature

Date

CERTIFICATION

This dissertation entitled “**ASSESSMENTS OF *ONCHOCERCA VOLVULUS* INFECTION IN HUMANS AND BLACKFLIES AND ONCHOCERCIASIS RESPONSE TO IVERMECTIN AMONG VILLAGERS AROUND GURARA DAM, KADUNA STATE, NIGERIA**” by OZOVEHE Lydia Onyeche meets the regulations governing the award of the degree of Master of Science in Microbiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to the triumphant arrival of my lovely Son, King David, Ariel, Omeiza. May you spend your days in plenty, your years in abundance and your entire life passionately serving the Lord.

ACKNOWLEDGEMENT

To the only one who has no beginning nor ending, the Source and Supplier of all Knowledge, the Omnipotent, my Sufficiency, the Father and Lord of all creation; with a grateful heart, I return all glory to you for the successful completion of this programme.

My immense gratitude goes to my supervisors, Prof. J. B. Ameh and Prof. O. S. Olonitola for their tremendous support in guidance during the course of the entire thesis.

I am most grateful to Prof. I. O. Abdullahi, Head, Department of Microbiology, Ahmadu Bello University and the entire teaching and non-teaching staff of the Department for their various contributions to the successful completion of this work.

My sincere thanks go to Prof. M. Muhammad, Director General/CEO, Dr A. C. Igweh, Head, Onchocerciasis Research Department and the entire staff of Onchocerciasis Research Department (especially Mr J. Gazama who played a pivotal role in the field work and Mrs M. Benjamin), Nigerian Institute for Trypanosomiasis and onchocerciasis Research, (NITOR) for their support and constructive contributions in diverse ways.

To all volunteers who consented and bore the pain involved in sample collection during this work (and the fly catchers), I am sincerely grateful.

To my wonderful parents, Chief and Mrs Michael Ozovehe: thank you for this indelible legacy, also for your encouragement and tremendous support throughout the course of this programme, I am forever grateful. Also, my siblings Mrs Mercy Abraham, Mrs Christiana Adams, Mr Samuel Ozovehe (of blessed memory), Mrs Grace Oluwasegun and Mrs Helen Asun: thank you for your patience, understanding, prayers and solid support towards this study, God bless you all.

I appreciate my dear husband Dr David Yakubu for his patience and utmost contributions towards the success of this work, thank you.

To my son and sweetheart, King David Omeiza, thank you for your forbearance.

Reverend and Mrs. E. J Ayeni, thank you for your hospitality and goodwill.

To my good friends and colleagues: Henry Bishop, Emmanuel Atai, Katherine Zira, Mercy Otori, Maryam Vandu, Rebecca Bitiyong, David Balogun, James Ochigbo, Helen Chaman La'ah, Steven Ade, Lami Danladi; your contributions in diverse ways made this work a success, thank you very much.

TABLE OF CONTENT

Title Page..... ii

Declaration..... iii

Certification..... iv

Dedication..... v

Acknowledgement..... vi

Table of Content..... vii

List of Tables..... xi

List of Figures..... xii

List of Plates..... xiii

List of Appendices..... xiv

Abstract..... xv

CHAPTER ONE.....1

1.0 INTRODUCTION.....1

1.1 Background of the study.....1

1.2 Statement of Problem4

1.3 Justification4

1.4 Aim6

1.5 Objectives6

| | |
|--|-----------|
| CHAPTER TWO..... | 7 |
| 2.0 LITERATURE REVIEW..... | 7 |
| 2.1 Onchocerciasis (River blindness)..... | 7 |
| 2.1.1 Etiology and life cycle of <i>Onchocerca volvulus</i> | 8 |
| 2.1.2 Epidemiology of Onchocerciasis | 11 |
| 2.1.3 Geographic Distribution of <i>O. volvulus</i> | 15 |
| 2.2 Clinical presentation of onchocerciasis..... | 15 |
| 2.2.1 Signs and Symptoms of onchocerciasis..... | 19 |
| 2.3 Risk Factors of Onchocerciasis..... | 20 |
| 2.4 Pathophysiology of Onchocerciasis..... | 20 |
| 2.5 Diagnosis of onchocerciasis..... | 21 |
| 2.5.1 Physical examination for onchocerciasis..... | 21 |
| 2.5.2 Clinical Diagnosis of onchocerciasis..... | 22 |
| 2.5.3 Differential diagnosis of onchocerciasis..... | 24 |
| 2.6 Prophylactic and therapy control of onchocerciasis..... | 24 |
| 2.6.1 Prognosis of onchocerciasis therapy..... | 25 |
| | |
| CHAPTER THREE..... | 27 |
| 3.0 MATERIALS AND METHODS..... | 27 |
| 3.1 Study area..... | 27 |
| 3.2 Study design/population..... | 27 |

| | | |
|--------------------------|--|-----------|
| 3.3 | Inclusion Criteria..... | 27 |
| 3.4 | Exclusion Criteria..... | 27 |
| 3.5 | Ethical approval..... | 27 |
| 3.6 | Data Collection..... | 30 |
| 3.7 | Collection of Samples..... | 30 |
| 3.7.1 | Collection of skin snips..... | 30 |
| 3.7.2 | Collection of black flies..... | 33 |
| 3.7.3 | Collection of control programme indices..... | 34 |
| 3.8 | Laboratory analyses of sample..... | 34 |
| 3.8.1 | Dissection of the Flies..... | 34 |
| 3.8.2 | Microscopic examination of skin snip biopsies at 30 mins and 24hrs..... | 34 |
| 3.8.3 | Extraction of DNA | 35 |
| 3.8.4 | PCR Amplification..... | 36 |
| 3.8.5 | Data Analyses..... | 36 |
| CHAPTER FOUR..... | | 37 |
| 4.0 | RESULTS..... | 37 |
| 4.1 | Prevalence of onchocerca volvulus among rural dwellersin Kurmi bango and Ungwan jaba, Kaduna..... | 37 |
| 4.2. | Monthly Distribution of <i>Onchocerca volvulus</i> in <i>Simulium damnosum</i> | 37 |
| 4.3. | PCR detection and amplification of <i>Onchocerca volvulus</i> DNAfrom | |

| | |
|---|-----------|
| Skin Snips collected from Kurmi bango and Ungwan jaba villages..... | 40 |
| 4.4 Assessment of the Therapeutic response of Onchocerciasis to | |
| Ivermectin..... | 40 |
| 4.5 Ivermectin therapeutic response..... | 43 |
| 4.6. The Relationship between Onchocerciasis and some Demographic | |
| Factors..... | 43 |
| 4.7. The Relationship between <i>Onchocerca volvulus</i> and Epidemiological | |
| Risk Factors..... | 46 |
| 4.8. The Relationship between <i>Onchocerca volvulus</i> load and | |
| Symptoms of Onchocerciasis..... | 46 |
| | |
| CHAPTER FIVE..... | 49 |
| 5.0. DISCUSSION | 49 |
| | |
| CHAPTER SIX..... | 55 |
| 6.0 CONCLUSION AND RECOMMENDATION..... | 55 |
| 6.1 Conclusion..... | 55 |
| 6.2 Recommendation..... | 57 |
| Contribution to knowledge..... | 58 |
| References..... | 59 |
| Appendices..... | 69 |

LIST OF TABLES

| Table | Title | Page |
|-------|--|------|
| 1. | Prevalence of <i>Onchocerca volvulus</i> among rural dwellers in Kurmi Bango and Ungwan Jaba, Kaduna | 38 |
| 2. | The relationship between <i>Onchocercavolvulus</i> load and frequency of treatment with Ivermectin | 42 |
| 3. | The relationship between <i>Onchocercavolvulus</i> infection and onchocerciasis symptom resolution rates due to ivermectin treatment regime..... | 44 |
| 4. | The relationship between onchocerciasis and Demographic factors | 45 |
| 5. | The relationship between <i>Onchocerca volvulus</i> and epidemiological risk factors..... | 47 |
| 6. | The relationship between <i>Onchocerca volvulus</i> load and Symptoms of Onchocerciasis | 48 |

LIST OF FIGURES

| Figure | Title | Page |
|---------------|--|-------------|
| 1: | Life cycle of <i>Onchocerca volvulus</i> | 9 |
| 2a: | Map of Kagarko LGA | 28 |
| 2b: | Google earth map showing the major villages and roads around Gurara Dam | 29 |
| 3: | The Setup of the facilities and organization of movement for skin sniping..... | 32 |
| 4. | Monthly distribution of <i>Onchocerca volvulus</i> infection in <i>Simulium damnosum</i> harvested around Gurara Dam in Kaduna State..... | 39 |

LIST OF PLATES

| Plate | Title | Page |
|-------|--|------|
| I. | Onchocerciasis dermatitis (leopard skin)..... | 16 |
| II. | Subcutaneous nodules caused by onchocerciasis..... | 17 |
| III. | Blindness resulting from onchocerciasis..... | 18 |
| IV. | Detection of <i>O. volvulus</i> DNA from skin snip samples. | 41 |

LIST OF APPENDICES

| Appendix | Title | Page |
|----------|---|------|
| I: | Ethical clearance..... | 69 |
| II: | Structured questionnaire..... | 70 |
| III: | Analysis of monthly flies caught | 72 |
| IV a: | Picture showing part of Gurara dam..... | 81 |
| IV b: | Picture showing the three (3) segments of a blackfly viewed under a dissection microscope..... | 82 |
| IV c: | Picture showing the internal organs of the blackflies abdomen..... | 83 |
| V a: | Picture showing the skin snipping process..... | 84 |
| V b: | The skin snipping process..... | 85 |
| V c: | Fixing of skin snip samples with formalin..... | 86 |
| VI a: | Skin snip samples in microtitre wells with normal saline..... | 87 |
| VI b: | Skin snip samples on microtitre plates from ungowan-jaba village..... | 88 |
| VI c: | Skin snip samples on microtitre plates from kurmi-bango village..... | 89 |

ABSTRACT

A study on the assessment of *Onchocercavolvulus* infection in humans and blackflies and the onchocerciasis response to ivermectin therapy among some villagers around Gurara dam was conducted between June to November 2015. 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*. 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 amplified Polymerase Chain Reaction (PCR) detected *O. volvulus* DNA from skin snip of some individuals. The drug of choice, Ivermectin, administered in the study population was observed to be effective in humans, with 75% complete resolution of symptoms such as dermatitis and reduction in nodules. 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 in this study were; white patches, severe body itch, nodules and hanging groins. This study revealed that infective blackflies are still hovering around Gurara dam and vicinity, 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 is necessary.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

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 (WHO, 2013).

The adult filarial worms are usually found in subcutaneous nodules and have an average longevity of around 9-11 years. The adult female worm produces millions of microfilariae who migrate to the skin of the host (Marroquin, 1981).

The microfilariae are the main cause of the clinical manifestations of the disease (Shibuya, 2006). Onchocerciasis is characterized by chronic skin disease, severe itching, and eye lesions that can progress to complete blindness (CDC, 2013). It is endemic in Nigeria (Evans *et al.*, 2011). Onchocerciasis is the second leading cause of blindness (after trachoma) in the world (Dent and Kazura, 2011).

The disease was highly endemic in many parts of the world before the commencement of control activities, affecting millions of people in Africa, South and Central America, and Yemen (Babalola, 2011). Approximately 123 million persons are at risk of contracting onchocerciasis in 38 endemic countries with at least 25.7 million infected, and about 1 million reported blinded or exhibiting severe visual impairment. Nigeria accounts for one-third of these estimates (CDC, 2013).

Onchocerciasis is basically a rural disease affecting communities sited along fast-flowing rivers with symptoms particularly irritating and disabling, often associated with long-term exposure to infection and this affects the social and economic activities of the inhabitants concerned (Okonkwo *et al.*, 2010).

Both the savannah type that is associated with severe eye disorders and blindness and the forest type which causes more skin damage are present and responsible for the divergent clinic-epidemiologic picture. One of the major reasons northern Nigeria is reported to have higher blindness rates than the southern part is owing to the widespread distribution of savannah species of *O. volvulus*. In the south, the forest species that cause mostly skin diseases abound (Okonkwo *etal.*, 1991).

In Nigeria, most of the activities of the individuals in affected communities include farming, fishing, handicraft and trading. Rural dwellers flee from this plague and migrate to urban cities disrupting the socioeconomic development in their communities (Alonso *et al.*, 2009).

According to Ubachukwu (2006), one of the social consequences associated with onchocerciasis is the disruption of family life and relationships as disabled persons desert rural villages to urban cities for better quality of life. However in the city, they are unable to access and secure employment, and thus experience extreme poverty and become beggars on the streets, they end up settling in shanty towns and resort to living in slums.

In addition, the burden of the disease impacts women and children, it affects the age of marriage in young women, limiting their choice of partners such as older men and divorcees. It also prevents infected mothers from practicing exclusive breastfeeding, reducing bonding between new born and mother. The educational impact of the disease is especially on children as they drop out from school to care for and lead blind family members (Ubachukwu, 2006).

The vector (black fly) breeds in fast flowing rivers and those within the area are exposed to the disease. In West Africa, members of *Simuliumdamnosum* complex are the only known vectors of human onchocerciasis (Post and Crampton, 1988).

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 been proved to have microfilaricidal properties and substantially reduced the disease burden in many affected communities (Post *etal.*, 1987; Yameogo *etal.*, 1999).

Humans acquire infections after being bitten by black flies of the *Simuliumdamnosum* strains carrying infective larvae of *Onchocercavolvulus* (Yameogo *etal.*, 1999). Therefore, surveillance on infectivity status of the *Simulium* vectors biting along the river systems is a vital component in monitoring the transmission level of onchocerciasis which thus gives a non-invasive method of measuring the success of the control measures (Opara *etal.*, 2005).

1.2 Statement of the problem

Nigeria bears the highest burden and diversity of neglected tropical diseases (NTDs) in sub-Saharan Africa (Okorie *etal.*, 2014). Onchocerciasis is one of the five most prevalent neglected tropical diseases in the world (Keenan *etal.*, 2013). It is ranked among the four major preventable causes of blindness in the world, after trachoma, cataract, and glaucoma and the leading cause of blindness in sub-Saharan Africa (WHO, 1987). About 33 million Nigerians are at risk of acquiring onchocerciasis.

Mass treatment with Ivermectin controls onchocerciasis as a public health problem, but it was not known if it could also interrupt transmission and eliminate the parasite in endemic foci in Africa where vectors are highly efficient (Traore *etal.*, 2012). Thus this is a surveillance study to assess if the mass treatment with Ivermectin also interrupts transmission and is able to eliminate the parasite.

1.3 Justification

Humans acquire onchocerciasis infections after being bitten by *Simuliumdamnosum* carrying infective larvae of *Onchocercavolvulus* (Yameogo *etal.*, 1999). Therefore, surveillance on parasite carrying status of the *Simulium* vectors biting along the river systems is a vital component in monitoring the transmission level of onchocerciasis which thus gives the most direct and non-invasive method of measuring the success of the control/ elimination measures (Opara *etal.*, 2005).

The presence of *Onchocercavolvulus* infective larvae in the vector population is a direct demonstration that transmission is occurring, thus providing early warning that new infections may be developing. This is important since the incubation period for the infection in humans is eighteen months to two years (WHO, 1987).

Measurements of transmission can thus be used to rapidly identify potential areas of recrudescence of infection. *Onchocercavolvulus* transmission has historically been measured through dissection of wild-caught vector black flies. This method is efficient in monitoring transmission in areas in which onchocerciasis is hyperendemic or mesoendemic because the prevalence of infection in the vector population is usually high (Cheke, 1992).

However, 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 (Philippon, 1990) and for those using mass Ivermectin distribution (Boussinesq *et al.*, 1997).

1.4 Aim

To employ parasitological and molecular techniques to assess the prevalence of onchocerciasis in Kurmi bango and Ungwan jaba villages around Gurara Dam, Kagarko LGA, Kaduna State.

1.5 Specific Objectives

The specific objectives of this research were to:

1. Assess *Onchocercavolvulus* infection rates among residents of the selected villages around the dam using parasitological methods;
2. Determine *Onchocercavolvulus* infection rates in harvested *S. damnosum* around the dam using parasitological method;
3. Molecularly detect *Onchocercavolvulus* DNA from skin snips using polymerase chain reaction (PCR), and;
4. Determine the associating demographic and risk factors, signs and symptoms of onchocerciasis using questionnaires.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Onchocerciasis (River blindness)

Onchocerciasis (“African River Blindness”) is a terminally blinding infectious disease caused by the filarial nematode *Onchocerca volvulus*. It is one of the nine filarial nematodes in which humans are the definitive hosts. Of the three syndromes associated with filarial infection (lymphatic, subcutaneous, and visceral), onchocerciasis falls into the subcutaneous category, as one of its main typical features is subcutaneous nodules. These nodules are followed in turn by ocular manifestations which if left untreated, can lead to blindness (Diemert, 2011).

Onchocerciasis is a chronic infection transmitted exclusively to humans through the bites of black flies. The parasite impact on human health is mainly manifested by the infection on the skin and eyes. *Onchocerca volvulus* embryonic forms (microfilariae) migrate through the skin and cause severe itching, disfiguring skin and ocular lesions. Visual impairment and blindness may occur as a result of heavy parasite loads in the human host over time (WER, 2013).

Human onchocerciasis is characterized by initially painless skin manifestations such as itching of the body and pruritis or papular onchodermatitis, followed by fibrosis, atrophy, depigmentation and nodular swellings containing adult filarial worms (Tekle *et al.*, 2014).

2.1.1 Etiology and life cycle of *Onchocerca volvulus*

Onchocerca volvulus develops exclusively in black flies found only in certain endemic areas near fast-flowing rivers and streams. The life cycle of *Onchocerca volvulus* (as shown in figure 1) starts from a black fly biting an infected human host and ingesting microfilariae from the skin or blood. These microfilariae migrate from the gut to the thoracic flight muscles of the fly, where they develop from the initial larval phase into the infective microfilariae after one week (Stages L1 - L2). They then migrate to the salivary glands of the fly and are ready to be transmitted again (Stage L3), (Fox, 2009; Diemert, 2011; Dent and Kazura, 2011).

The third stage larvae are then introduced into the skin of the new human host via a fly bite, within a period of 6-12 months, they develop into mature adult parasites. The larger female nematode migrates to the subcutaneous or deeper fascial tissues and becomes encapsulated by a fibrous shell. The males migrate into these capsules in order to fertilize the females. It is these capsules that are the characteristic subcutaneous nodules of onchocerciasis.

Fertilized adult females in these nodules produce millions of microfilariae, which are responsible for the systemic and ocular signs and symptoms of onchocerciasis (Fox, 2009).

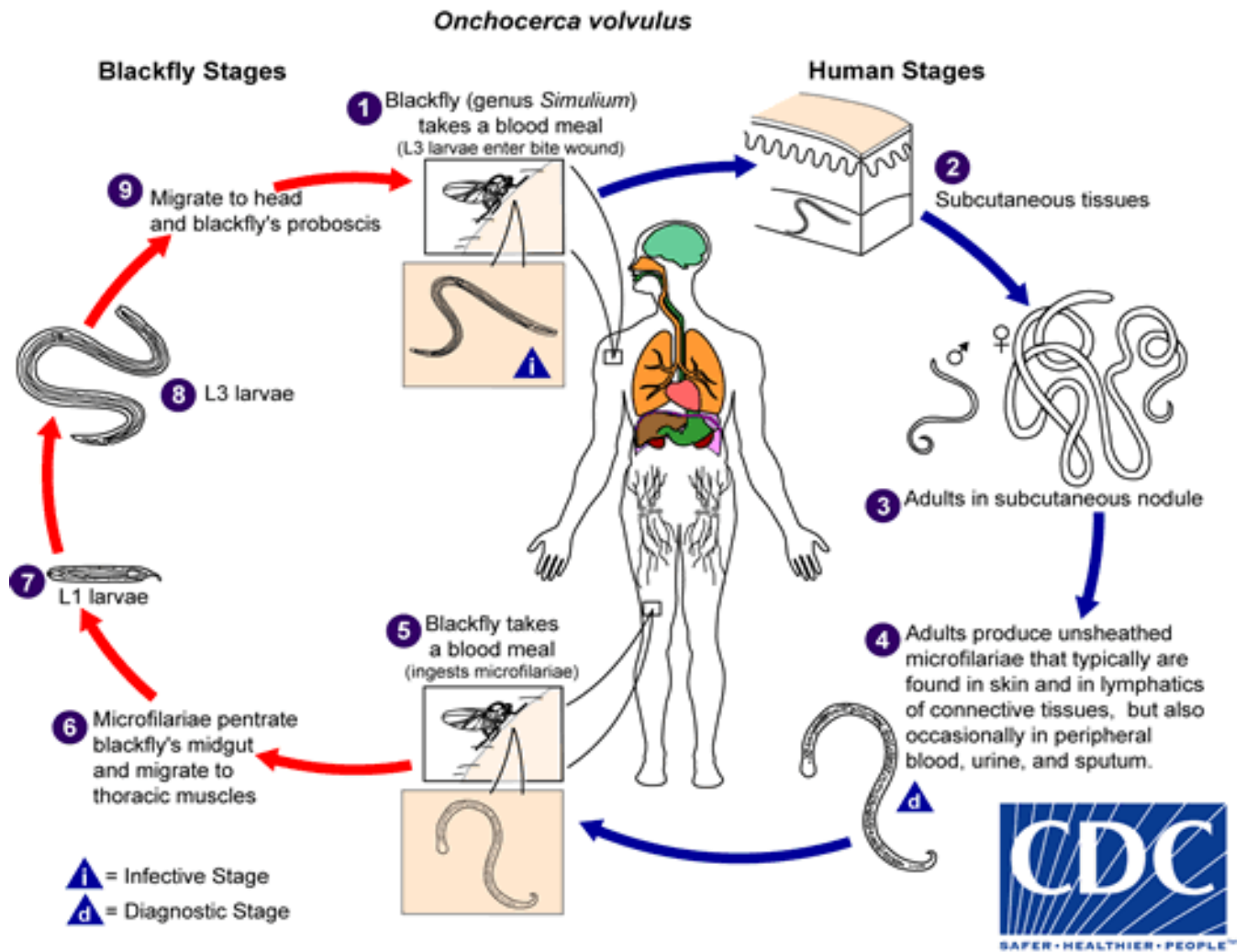


Figure 1: Life cycle of *Onchocerca volvulus*

Source: CDC, 2013.

During a blood meal, an infected blackfly (genus *Simulium*) introduces third-stage filarial larvae onto the skin of the human host, from where they penetrate into the bite wound. In subcutaneous tissues the larvae develop into adult filariae, which commonly reside in nodules in subcutaneous connective tissues.

The adults can live in the nodules for approximately 15 years. Some nodules may contain numerous male and female worms. The females measure 33 to 50 cm in length and 270 to 400 μm in diameter, while the males measure 19 to 42 mm by 130 to 210 μm . In the subcutaneous nodules, the female worms are capable of producing microfilariae for approximately 9 years. The microfilariae, measuring 220 to 360 μm by 5 to 9 μm and unsheathed, have a life span that may reach 2 years. They are occasionally found in peripheral blood, urine, and sputum but are typically found in the skin and in the lymphatics of connective tissues.

A blackfly ingests the microfilariae during a blood meal. After ingestion, the microfilariae migrate from the blackfly's midgut through the hemocoel to the thoracic muscles. There the microfilariae develop into first-stage larvae and subsequently into third-stage infective larvae. The third-stage infective larvae migrate to the blackfly's proboscis and can infect another human when the fly takes a blood meal (CDC, 2013).

2.1.2 Epidemiology of onchocerciasis

Onchocerciasis is the second leading cause of blindness globally (after trachoma) due to infection (Dent & Kazura, 2011). It is estimated that between 18 -37 million people are infected with up to 1-2 million people with various degrees of visual impairment and 270,000 people rendered completely blind (WHO, 1995 and Basañez *et al.*, 2006; Mcleod, 2008; Richards *et al.*, 2001; Diemert, 2011;).

Of the 37 endemic countries, 30 are in Africa and 6 are in Latin America (Diemert, 2011; Mcleod, 2008; Richards *et al.*, 2001; Cupp *et al.*, 2011). It should be noted, however, that public health efforts by public/private partnerships involving organizations such as the Carter Center have resulted in significant progress, especially in Latin America.

As of November 2011, the cycle of transmission of *O. volvulus* has officially been broken in Colombia, Ecuador, Mexico, and Guatemala. These countries (especially Mexico and Guatemala, the newest countries to have declared that they had stopped onchocerciasis transmission) are undergoing a three-year period of post-treatment surveillance by the World Health Organization (CCRB, 2012).

However, it remains that more than 90 million people are estimated to be at risk in Africa, with Nigeria and Zaire being the most affected countries (WHO, 1995). In these highly endemic areas, infection rates can be as high as 80-100% among people of 20 years of age, with clinical manifestations usually peaking at 40-50 years of age (Duerr *et al.*, 2011).

These infections often result in major socioeconomic liabilities, as working-aged adults are often the ones debilitated, leaving the young to care for the adults as well as to provide for the family (Diemert, 2011).

Nigeria carries the highest burden and diversity of neglected tropical diseases (NTDs) in sub-Saharan Africa between 1995 through 2015 (Okorie *et al.*, 2014). Onchocerciasis is one of the five most prevalent neglected tropical diseases in the world, and each is frequently treated with mass drug administration (Keenan *et al.*, 2013).

Ivermectin has been used to control onchocerciasis as a public health problem in Africa (Pion *et al.*, 2013). Since 1995, the African Programme for Onchocerciasis Control (APOC) has coordinated annual mass treatment with ivermectin (Coffeng *et al.*, 2013). APOC has been working with ultimate goal of reducing the public health and socioeconomic problems associated with onchocerciasis within a period of 12-15 years (Weldegebreal *et al.*, 2014). Mass treatment with ivermectin controls onchocerciasis as a public health problem, but it was not known if it could also interrupt transmission and eliminate the parasite in endemic foci in Africa where vectors are highly efficient (Traore *et al.*, 2012).

It has been proposed that switching from annual to biannual (twice yearly) mass community directed treatment with ivermectin (CDTI) might improve the chances of onchocerciasis elimination in some African foci (Turner *et al.*, 2013; Turner *et al.*, 2014).

Biannual treatment yields only small additional health gains, its benefit is pronounced in the context of the elimination goals, shortening the time frames for and increasing the feasibility of reaching the proposed operational thresholds for stopping treatment (Turner, *et al.*, 2014). It may not always be feasible to implement biannual treatment, particularly in hard-to-reach populations (Turner *et al.*, 2014).

According to Higazi *et al.*, (2013), long-term community-directed treatment with ivermectin (CDTI) alone can interrupt transmission of onchocerciasis.

Periodic, communitywide mass drug administration (MDA) with ivermectin (Mectizan, Merck) prevents eye and skin disease and might interrupt transmission of the infection, depending on the coverage, duration, and frequency of MDA (CDC, 2013). While there might be some level of success in fighting onchocerciasis, there is the need to worry about the issue of drug resistance.

Drug resistance was thought to be a major limitation of mass drug administration for all five neglected tropical diseases (Keenan *et al.*, 2013). Over the years the control of onchocerciasis has relied on the mass drug administration to the at-risk communities (Convit *et al.*, 2013). In the words of Molyneux, *et al.*, (2014), there are some communities in which ivermectin cannot be safely administered due to the risk of serious adverse reactions and therefore makes pertinent the need for an alternative strategy to combat onchocerciasis.

The drugs that were used before ivermectin were found to be toxic and unsuitable for mass distribution, in particular, they precipitated optic nerve disease (Babalola, 2011).

The fact that the producers of ivermectin are willing to distribute the drug for as long as needed is no reason why alternatives cannot be explored. The problem with ivermectin is that it is a monotherapy microfilaricide which has limited effect on the adult worm, and thus will need to be continued for the life span of the adult worm, which may last up to 15 years (Babalola, 2011).

Although mass drug administration of ivermectin has had a profound effect on the control of the disease, additional tools are critically needed (Hess *et al.*, 2014).

According to Awadzi *et al.* (2014), new tools are needed to achieve elimination of onchocerciasis infection. This is why alternatives should be sought to the use of drugs.

The vector of onchocerciasis thrives on fast flowing water; an alternative source of water for communities other than fast flowing streams may help in breaking the chain of transmission.

The suggestions of Coffeng *et al.* (2013) that APOC has had a remarkable impact on population health in Africa between 1995 and 2010 and this health impact is likely to double through to 2015. This notwithstanding, there is a need for development of novel prevention and treatment modalities, such as next-generation small molecule drugs and vaccines (Barry *et al.*, 2013).

Empowering people with adequate health information is a good means of preventing onchocerciasis where prevalence is high (Okanlawon and Osanyintolu, 2011). According to Traore *et al.* (2012) onchocerciasis elimination with ivermectin treatment is feasible in at least some endemic foci in Africa.

The need to attain the set target of eliminating onchocerciasis by 2025 has led to a shift in onchocerciasis control policy, changing from prevention of high morbidity toward elimination of infection (Turner *et al.*, 2014).

2.1.3 Geographic distribution of *Onchocerca volvulus*

The agent of river blindness, *Onchocerca volvulus*, occurs mainly in Africa, with additional foci in Latin America and the Middle East (CDC, 2013).

2.2 Clinical presentation of onchocerciasis

Onchocerciasis can cause pruritus, dermatitis, onchocercomata (subcutaneous nodules), and lymphadenopathies. The most serious manifestation consists of ocular lesions that can progress to blindness (CDC, 2013). Some of the clinical presentations of onchocerciasis are as seen in plate 1a-1c.



Plate I: Onchocerciasis dermatitis (leopard skin) resulting from *O.volvulus* infection

Source: Nigerian Institute for Trypanosomiasis Research quarterly report, 2012.



Plate II: Subcutaneous nodules caused by onchocerciasis.

Source: Nigerian Institute for Trypanosomiasis Research quarterly report, 2012.



Plate III: Blindness resulting from onchocerciasis.

Source : Nigerian Institute for Trypanosomiasis Research quarterly report, 2012.

2.2.1 Signs and symptoms of onchocerciasis

Typically, dermatologic manifestations are the initial presenting symptoms. Ophthalmic signs often present several years afterwards. The severity of these ocular symptoms depends on the duration of infection and the microfilarial load, as well as the strain of microfilaria that is infecting the host (Meredith *etal.*, 1991).

O. volvulus involves all ocular tissues. Initially, it can involve the eyelid and conjunctiva, leading to eyelid nodules and edema, chronic conjunctivitis, chemosis, and phlyctenule-like kerato-conjunctival lesions. Then, by direct invasion, microfilariae infect the cornea and sclera. corneal manifestations include fine interpalpebral sub-epithelial punctate lesions. These “snowflake opacities” can lead to a chronic sclerosing keratitis and discrete nummular scars with stromal edema, corneal infiltration, and neovascularization. It is the sclerosing keratitis that contributes to this nematode's permanent blinding effect.

The microfilariae invades the iris and the ciliary body, leading to iridocyclitis that can be severe. This can result in correctopia, iris atrophy and extensive synechiae that can result in secondary glaucoma, as well as early cataract formation (McLeod, 2008; Diemert, 2011; ; Murdoch, 2012; Freedman, 2015). Posterior chamber involvement can include chorioretinal lesions. Peripapillary chorioretinitis can result in optic nerve dysfunction secondary to optic nerve edema and optic neuritis, eventually leading to optic atrophy (Egbert *etal.*, 2005).

Typically, patient presents with cutaneous symptoms including itching and dermatologic changes years before the onset of ocular symptoms. These symptoms can be very severe, leading to excoriation, bleeding, and even suicide (Diemert, 2011; Fox, 2009). In terms of ocular disease, patients will classically complain of an insidious onset of decreased vision, eye redness, eye pain and photophobia related to iridocyclitis, and perceived corneal changes related to the sclerosing keratitis. These changes often manifest in the patients' 4th to 5th decade (Freedman, 2005; Mcleod, 2008; Fox, 2009).

2.3 Risk factors of onchocerciasis

The people most at risk for acquiring onchocerciasis are those who live near fast flowing streams or rivers where *Simulium* (blackflies) breeds. Most of the areas where the blackflies are found are rural agricultural areas in sub-Saharan Africa. Usually, many bites are needed before being infected, so people who travel for short periods of time (generally less than 3 months) to areas where the parasite is found have a low chance of becoming infected with *O. volvulus*. Those travelers to areas where they are most likely to become infected are long-term missionaries, Peace Corps and other long-term volunteers, and field researchers (CDC, 2013).

2.4 Pathophysiology of onchocerciasis

The multiple sequelae result from direct invasion of the nematodes, which leads to induction of inflammation of the eyes in the infected host.

Eventually, these inflammatory changes can lead to blindness following corneal sclerosing keratitis, secondary glaucoma, cataracts, chorioretinitis, and optic atrophy (Diemert, 2011).

While still alive, *O. volvulus* causes very little in terms of pathology, the adult worms are protected by the fibrous skin nodules in which they reside, and the microfilariae are non-immunogenic. It is when they die that they cause an immune response. Antigens that are released by dead or dying organisms cause a T-helper cell (TH2) response, which leads to the release of interleukins, resulting in the influx of neutrophils and eosinophils, and the production of antibodies by plasma cells. These inflammatory responses lead to corneal opacification (Mcleod *etal.*, 2008; Diemert, 2011).

Specifically, it is thought that the sclerosing keratitis is an effect of modification of ICAM-1 expression and production of IL-4 and IL-14 (Berger *etal.*, 2002).

2.5 Diagnosis of onchocerciasis

Diagnosis is made clinically with confirmation by superficial skin snip biopsies. Newer adjuvant tests include PCR amplification and serology (Mcleod *etal.*, 2008; Diemert, 2011).

2.5.1 Physical examination for onchocerciasis

Slit lamp examination can be used to visualize the ocular changes associated with Onchocerciasis.

Occasionally, the microfilaria in the anterior chamber or cornea can be visualized, aiding in diagnosis. The nematodes can be seen using retro-illumination as S or C-shaped, fine motile elements.

To increase the sensitivity of this test, patients are asked to sit with their head between their knees for at least 2 minutes in order to mobilize the microfilariae that are suspended in the anterior chamber (McLeod, 2008; Murdoch, 2012; Freedman, 2015).

These ocular findings are most often accompanied by cutaneous findings, warranting a full dermatologic examination. These can include freely mobile subcutaneous nodules measuring 0.5 – 3.0 cm in diameter, which are most often palpable over bony prominences (especially over the hips and lower limbs or head). These nodules present concurrently with the development of diffuse papular dermatitis, orange-peel textured skin, lichenified dermatitis, atrophy, fine wrinkles, lymphadenitis ("hanging groin"), and eventually depigmentation ("leopard skin") (Diemert, 2011).

2.5.2 Laboratory tests for onchocerciasis

Confirmation of suspected onchocerciasis involves a bloodless skin snips taken from areas of apparently involved skin, as well as from the scapula, over each iliac crest and each calf. These specimens are then incubated for up to 24 hours in normal saline and stain is applied in order to identify the species of the motile elements seen in the specimens. *O. volvulus* has no sheath or nuclei in their tails, differentiating them from other nematodes.

This test is very specific but not sensitive for those with lower worm burdens and early in their infection. It is more valuable at 18+ months post-infection for the worms to be abundant, large and mature enough to be detected (Murdoch, 2012). Serology can also be used to detect exposure to *O. volvulus* with high sensitivity and specificity.

Newer ELISA and Western Blot techniques have been used to quantitatively detect antibodies to *O. volvulus* antigens in the skin, tears, and urine. Other assays detect levels of antibody subclass IgG4 for diagnosis (Freedman, 2005; Murdoch, 2012).

Polymerase Chain Reaction, ultrasound of the nodules and sclerocorneal biopsy have also been suggested as other tests to help confirm the diagnosis (Fox, 2009).

Proposed tests that are currently being investigated include the diethylcarbamazine (DEC) skin patch test, the antibody card test, and the antigen detection test (Weil, *etal.*, 2000; Richards *etal.*, 2001). However, these tests are not currently available for clinical use and are not commonly available in endemic areas.

More antiquated methods include using the Mazzotti test, in which DEC (diethylcarbamazine) is given to individuals who are suspected to be infected, leading to systemic reactions including rashes, fevers, generalized body pains, uveitis, and possible anaphylaxis. The presence of these findings were highly suggestive of onchocerciasis (as well as other filarial infections), and the killed microfilariae could sometimes be found in the blood or urine. However, due to the severity of the reactions, it is no longer used clinically (Fox, 2009; Diemert, 2011; Murdoch, 2012).

2.5.3 Differential diagnosis of onchocerciasis

The differential diagnosis of the dermatology, in isolation from the ophthalmic findings, is extensive. The diffuse papular dermatitis may be due to food allergies, syphilis, leprosy, vitamin A deficiency and yaws (Fox, 2009; Diemert, 2011; Murdoch, 2012).

The differential diagnosis using subcutaneous nodules is also extensive, and can include multiple types of tumors (mesenchymal, metastatic, etc.), skin appendage lesions, inflammatory or infectious lesions, or other tumor-like lesions or cysts (Beaman, *et al.*, 2007).

In the context of the characteristic ocular findings, the differential includes infection with other microfilaria (including *Marsonellaperstans*, *Loa loa*, *Onchocercagutturosa*, or *Dracunculusmedinensis*), inflammatory lesions involving iridocyclitis including systemic inflammatory etiologies such as sarcoidosis, and other corneal degenerative/sclerotic diseases (McLeod, 2008; Udall, 2007).

2.6. Prophylactic and therapeutic control of onchocerciasis

Global preventative efforts are currently concentrated in population-based prophylactic treatment through programs such as the African Programme for Onchocerciasis Control (APOC) (Richards *et al.*, 2001). Community-wide ivermectin administration (Mectizan®, donated by Merck) and vector (black fly) control have been the mainstay of these programs, which have been markedly successful.

These measures have resulted in a situation where an estimated 35 million people are no longer at risk of infection. Similarly, two million infected people who have been appropriately treated, and over 200,000 people in whom blindness has been prevented (Cupp *et al.*, 2011; Greene, 1992; Duerr *et al.*, 2011). However, in some highly endemic areas, more than three decades of annual treatment are still needed in order to effectively control this preventable disease (Cupp *et al.*, 2011).

Individual prevention can include protective measures to avoid contact with the black flies in endemic areas, including avoiding areas, using insect repellent and wearing appropriate clothing (McKechnie *et al.*, 2002; Fox, 2009; Diemert *et al.*, 2011).

2.6.1 Prognosis of onchocerciasis therapy

Prognosis depends on the stage at which the infection is treated. If treated early during the course of the disease, the corneal opacities may be minimal and may recede with appropriate treatment length of time. However, as the disease progresses, the visual outcomes can progress towards blindness and may be irreversible. In a global sense, studies have found a direct relationship between *O. volvulus* microfilarial load and early host mortality (Little *et al.*, 2004). Other studies have shown significant socioeconomic and public health effects of the disease.

Overall, this is a devastating disease with a multitude of ramifications that manifests a poor prognosis among the poor villagers.

However, it should be noted that major strides have been made through the combined efforts of public and private sector partnerships, involving such organizations as the Carter Center, Hellen Keller International, Lions Clubs International, Onchocerciasis Control Programme (OCP), and African Programme for Onchocerciasis Control (APOC), in conjunction with local health ministries.

As a result of this work, it was recently announced that parts of Uganda and Sudan have broken the cycle of transmission of onchocerciasis (Uganda's Success Against River Blindness, 2012; Abu, 2012). The progress made in these previously endemic areas serves as an inspiration for countries in which the burden of disease is great and challenges the preconception that this is a disease that will forever afflict these disadvantaged persons (Abu, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study areas were Kurmi Bango and Ungwan Jaba villages around Gurara dam in Kagarko LGA, Kaduna state, as shown in Figures 3.1 and 3.2.

Kaduna State is located in the North-West Geo-political Zone of Nigeria. It is the successor to the old Northern Region of Nigeria with 23 Local Government Areas including, Kagarko.

3.2 Study design/population

This is a longitudinal study where fly collection was carried out for a period of six consecutive months (June to November) and skin snips were collected in three days. The study population comprised of male and female of 5 years and above resident in the two selected villages.

3.3 Inclusion criteria

Male and female volunteers aged 5 years and above in the two selected villages.

3.4 Exclusion criteria

All children below 5 years, including those above 5 years that did not give consent.

3.5 Ethical approval

Approval was sought for and granted from Kagarko Local Government Authority.

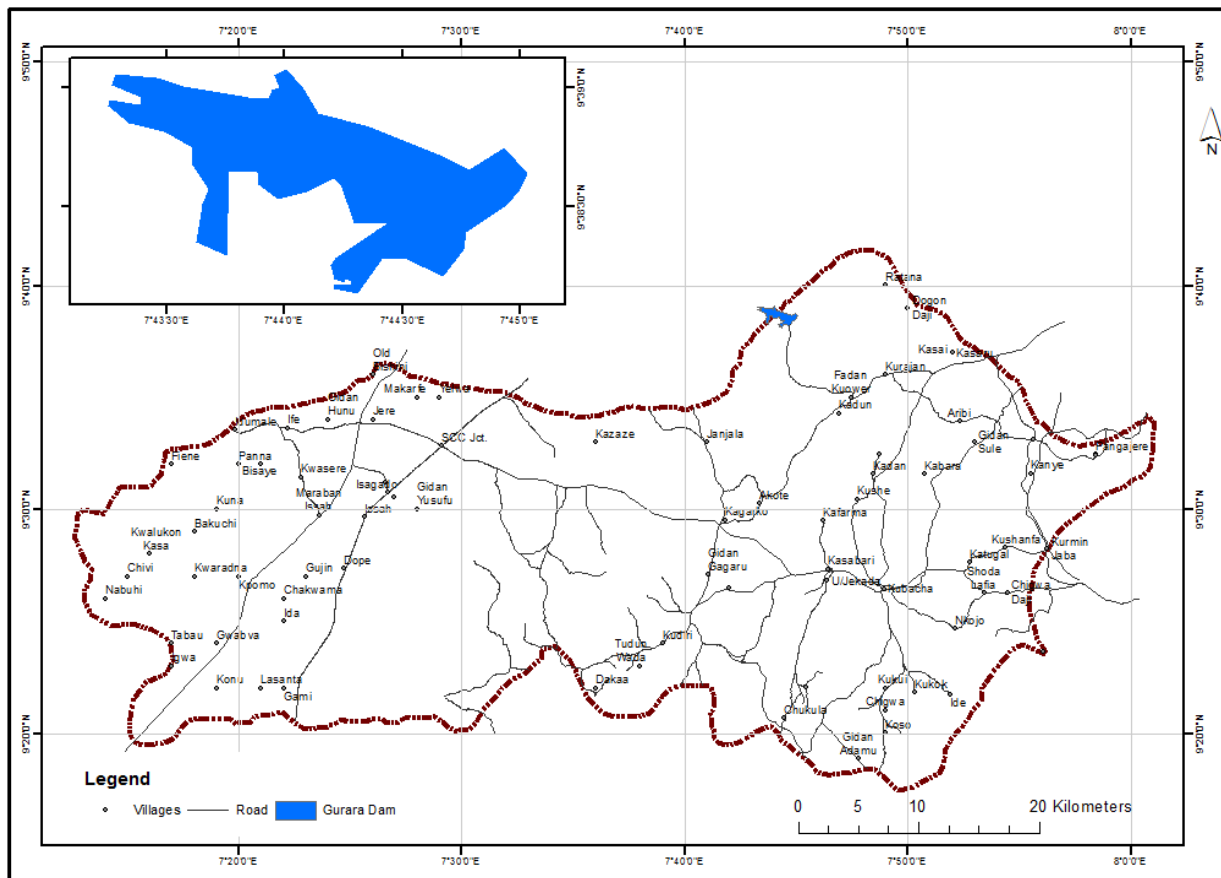


Fig 2a: Map of Kagarko LGA showing the major villages, roads and Gurara Dam.

Source: Adopted from the Administrative map of Kaduna State, 2014

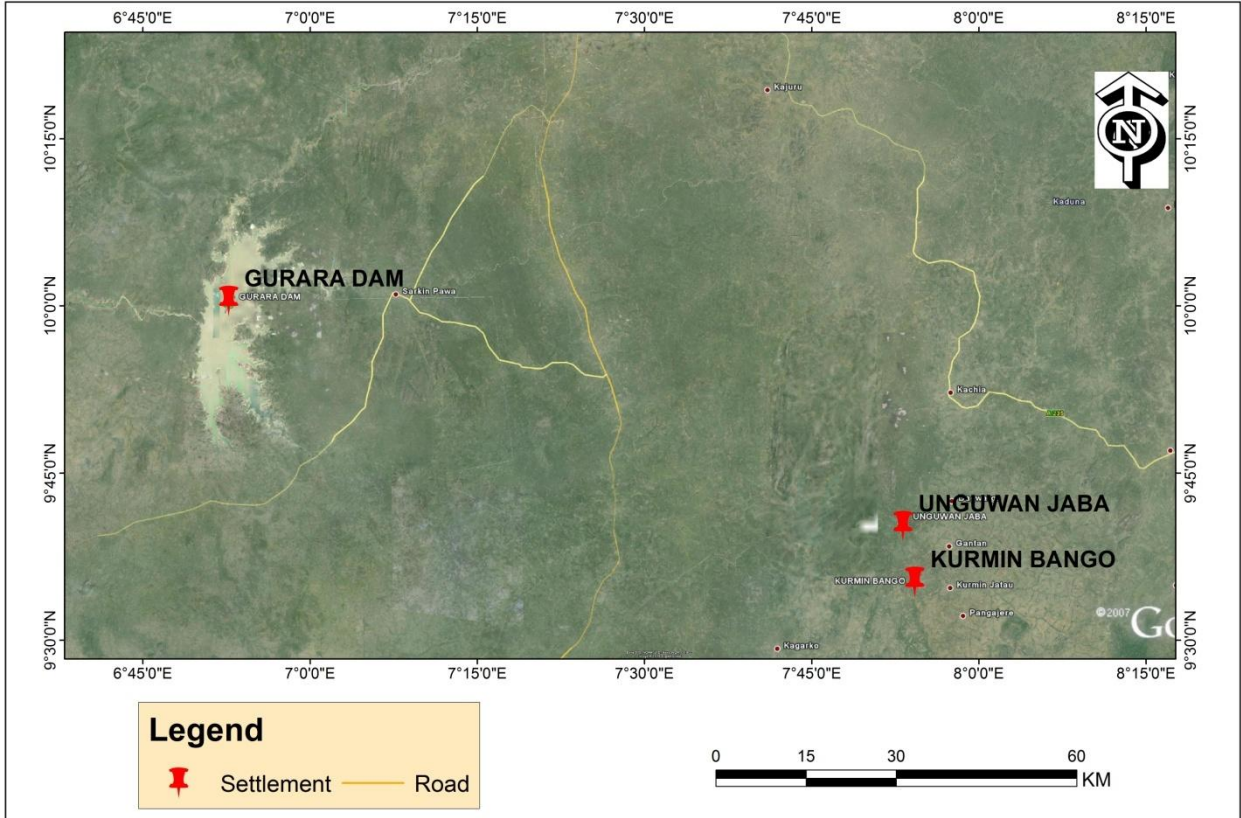


Fig 2b: Google earth map showing the major villages, roads and Gurara Dam.

Source: Adapted from Google Earth

3.6 Data collection

Questionnaire forms were administered to collect to volunteers prior to sample collection to gather information regarding the bio-data of individuals, contributing environmental factors and symptoms related to *Onchocerca volvulus* infection.

3.7 Collection of Samples

3.7.1 Collection of skin snips

A two day pre-survey visit was embarked upon to the two selected villages, a day to each village for sensitization and awareness creation. This was done a week earlier before the actual skin snipping exercise was performed. The main survey took another three days visit to the two villages where data and skin snips were collected. Skin snips were taken from consenting individuals aged 5yrs and above in the selected villages. A total of 158 persons were skin snipped. The setup of conduct of this exercise is shown in Figure 3.3.

Using a hypodermic syringe or pipette, the 96 wells of the microtitration trails were filled with 50µl of saline solution. Consent forms were signed by the study subjects prior to sample collection and identification numbers allotted. Each examinee was led into an isolated tent where left and right crest iliacs were disinfected with an alcohol-soaked swab and allowed to dry for few seconds, then a snip was taken using a sclerocorneal punch, and placed in appropriate labeled well.

After sampling each subject, the sclerocorneal punch was sterilized by intense boiling using a pressure cooker for a minimum of 5mins. After snipping 48 persons, the cells of the first microtitration trail became full and the trail was sealed with adhesive tape and labelled appropriately.

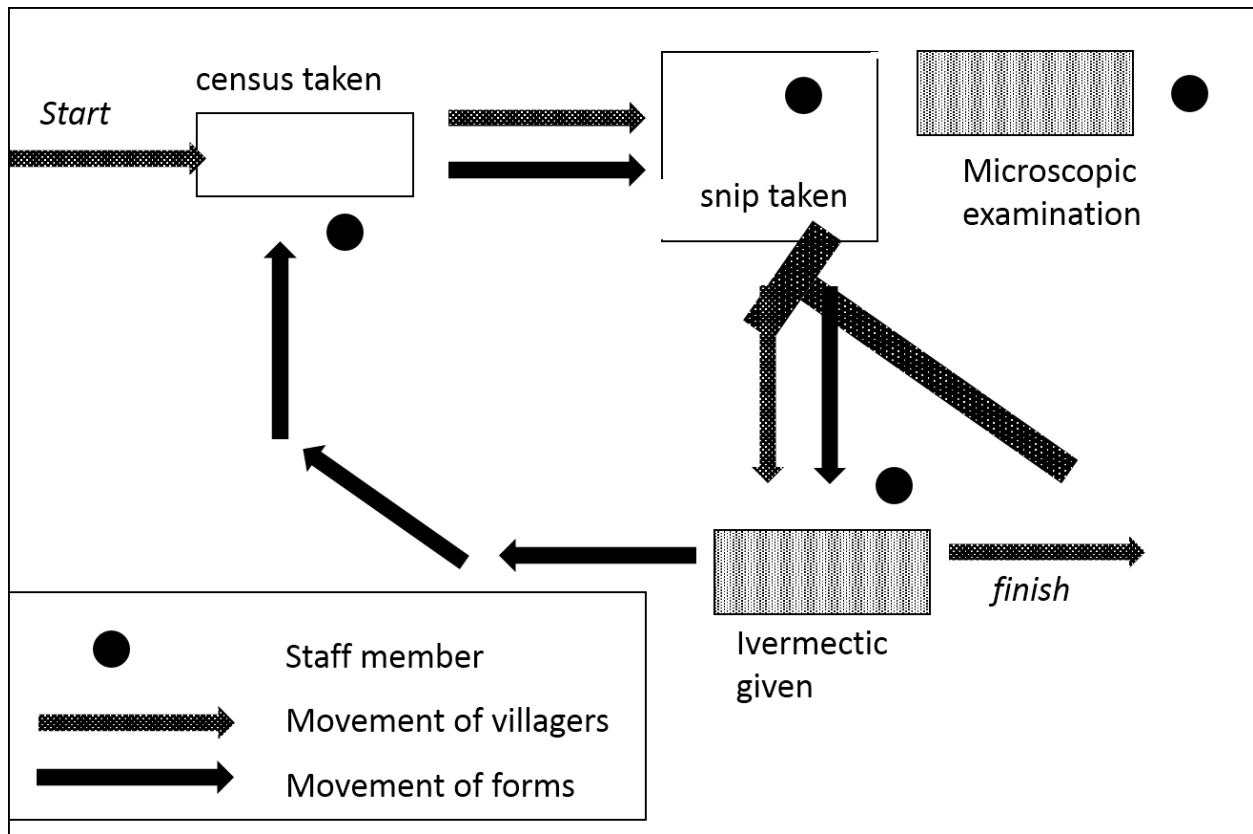


Figure 3: The Set up of the facilities and organizing of movement for skin sniping

On arrival of volunteers to the arena, their consents were first sought, then questionnaire forms were administered prior to sample collection to gather information regarding the bio-data of individuals, contributing environmental factors and symptoms related to *Onchocerca volvulus* infection. Skin snip samples were collected and examined and where volunteer indicated to have been on ivermectin treatment or shows visible signs of onchocerciasis, ivermectin was administered

3.7.2 Collection of black flies

The biting adult females of *Simulium damnosum* were collected using two trained adult fly catchers who were positioned near the bank of the river during the sampling period. They were given Ivermectin as prophylaxis. 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, they were trapped by sucking tubes before starting to probe, this was followed by screw-capping.

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. This allowed for determination of hourly and monthlyinfection transmission. The entrapped flies were kept in coolers containing ice packs and transported to Onchocerciasis Research Department laboratory of the Nigerian Institute for Trypanosomiasis Research (NITR) at the end of daily catch for dissection.

3.7.3 Collection of control programme indices using structured questionnaires

Structured questionnaires (as shown in Appendix 1) were administered to the study subjects to capture the socio-economic, demographic, risk factors and symptoms associated with onchocerciasis.

3.8 Laboratory Analyses of Samples

3.8.1 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.

3.8.2 Microscopic examination of skin snip biopsies

The skin snip samples in microtitration trails containing normal saline were examined at 30 minutes and after 24 hours to observe the presence or absence of microfilariae with the aid of a microscope.

A microscope was cleaned and the lens focused, microtitration trails were arranged to correspond with the serial numbers on the consent form, each well was viewed for emerging *Onchocerca volvulus* microfilariae.

Then, both the positive and negative samples were stored at refrigeration temperature for polymerase chain reaction (PCR).

3.8.3 Extraction of *Onchocerca volvulus* DNA

All the four positive skin snip samples gotten from microscopy with some other twenty-four negative samples were randomly picked and subjected to PCR. DNA from the parasite was isolated by a modified proteinase K digestion protocol as previously reported by Zimmerman *etal.* (1994). From a solution of normal saline and skin snip samples, 200µl was drawn using a pipette and added to 400 µl TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 7.6) with 5 µl of 10 mg/ml Proteinase K (Sigma Chemical Co., St. Louis, MO) and 5 µl of 10% sodium dodecyl sulfate (SDS, Sigma).

After overnight incubation at 56°C, 10 µl of 1M dithiothreitol was added and the samples were heated in a boiling water bath for 30 min and then treated with 3 cycles of freezing and thawing. The Deoxyribonucleic acid (DNA) was precipitated by the addition of 1 ml of 100% ethanol and 4N sodium acetate. After 1 hr incubation at 70°C and centrifugation at 14,000 xg for 15 min, the samples were dried and resuspended in 200 µl of TE buffer.

3.8.4 PCR amplification

Polymerase chain reaction (PCR) was performed using a S4 forward primer (5'AATAACTGATCCTATGACC3') and a biotinylated S3 reverse primer (5'ATCAATTTTGCAAATGCG3') at a concentration of 20 pmol per 50µl each (Fischer *et al.*, 1996).

Using 7 µl of DNA, 3.5 µl of water, 1µl each of forward and reverse primers was added to 12.5 µl of the mastermix, amplification was performed in aGeneAmp PCR system 9700 (ABI) using 35 cycles at 94°C denaturation, 55°C annealing and 72°C extension of 30s each, then final extension of 72°C for 7min.

An activation step of 12 min at 94 °C preceded the cycling programme. For control, 5µl of the PCR products was checked by agarose gel electrophoresis.

3.8.5 Data Analyses

The percentage prevalence of onchocerciasis from the male and female subjects in the two sampled villages was recorded. The diurnal data of the parous flies were pooled for hourly and monthly variations. Data obtained from this study were analyzed statistically using Chi Square and Fisher's Exact Test; Results were recorded and presented as tables and charts as appropriate.

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence of *Onchocerca volvulus* Among Rural Dwellers in Kurmi Bango and Ungwan Jaba, Kaduna

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 is presented in Table 1. The overall prevalence recorded 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%).

4.2. Monthly Distribution of *Onchocerca volvulus* in *Simulium damnosum*

The monthly distribution of *O. volvulus* from *S. damnosum* harvested around Gurara Dam, Kaduna State is presented in Figure 4, showing the number of flies caught, number of parous flies and the number of infected flies. The parous flies connote the female adult flies that have the capacity to harbor the infection. 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.

Table 1: Prevalence of Onchocerciasis Among Rural Dwellers in Kurmi Bango and Ungwan Jaba, Kaduna

| Village | Number of persons Examined | Number of positive (%) | P-value (Fisher's Exact test) |
|----------------|-----------------------------------|-------------------------------|--------------------------------------|
| Kurmin Bango | 128 | 1 (0.6) | |
| Ungwan Jaba | 30 | 3 (1.9) | 0.002* |
| Total | 158 | 4 (2.5) | |

* Significant at $P < 0.05$

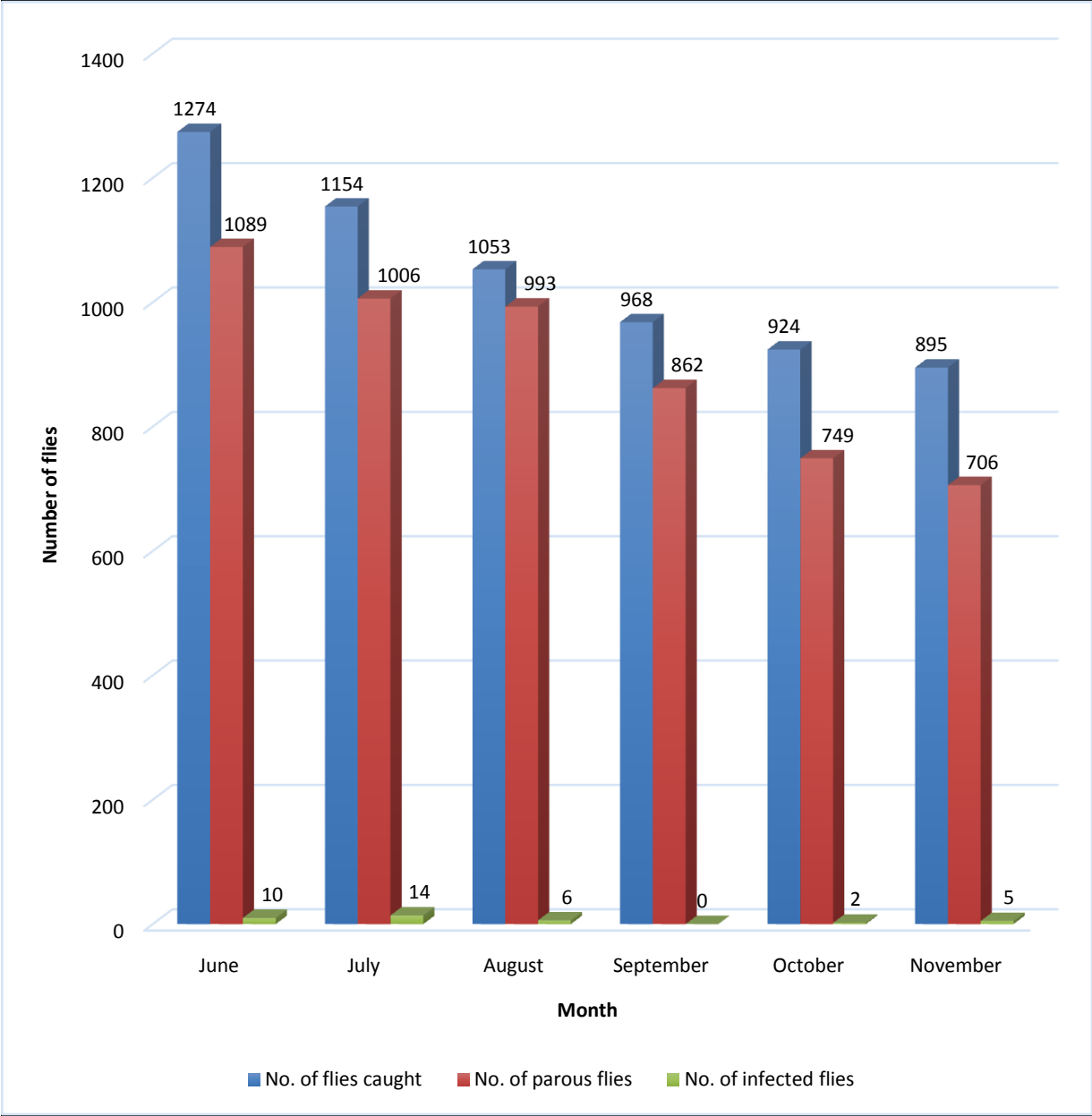


Figure 4: Monthly Distribution of Onchocerciasis in *Simulium damnosum* Harvested Around Gurara Dam in Kaduna State.

4.3. Detection and Amplification of *Onchocerca volvulus* DNA from Volunteers Skin Snips in Kurmi Bango and Ungwan Jaba villages

Polymerase Chain Reaction (PCR) to detect *Onchocerca. volvulus* DNA from skin snips is shown in Plate 2. Out of the twenty-eight total skin snip samples 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.

4.4 Assessment of the Yearly Treatment and Therapeutic Response of Onchocerciasis to Ivermectin

The rate of access and usage of the drug Ivermectin varied in the population. This is presented in Table 2. 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.

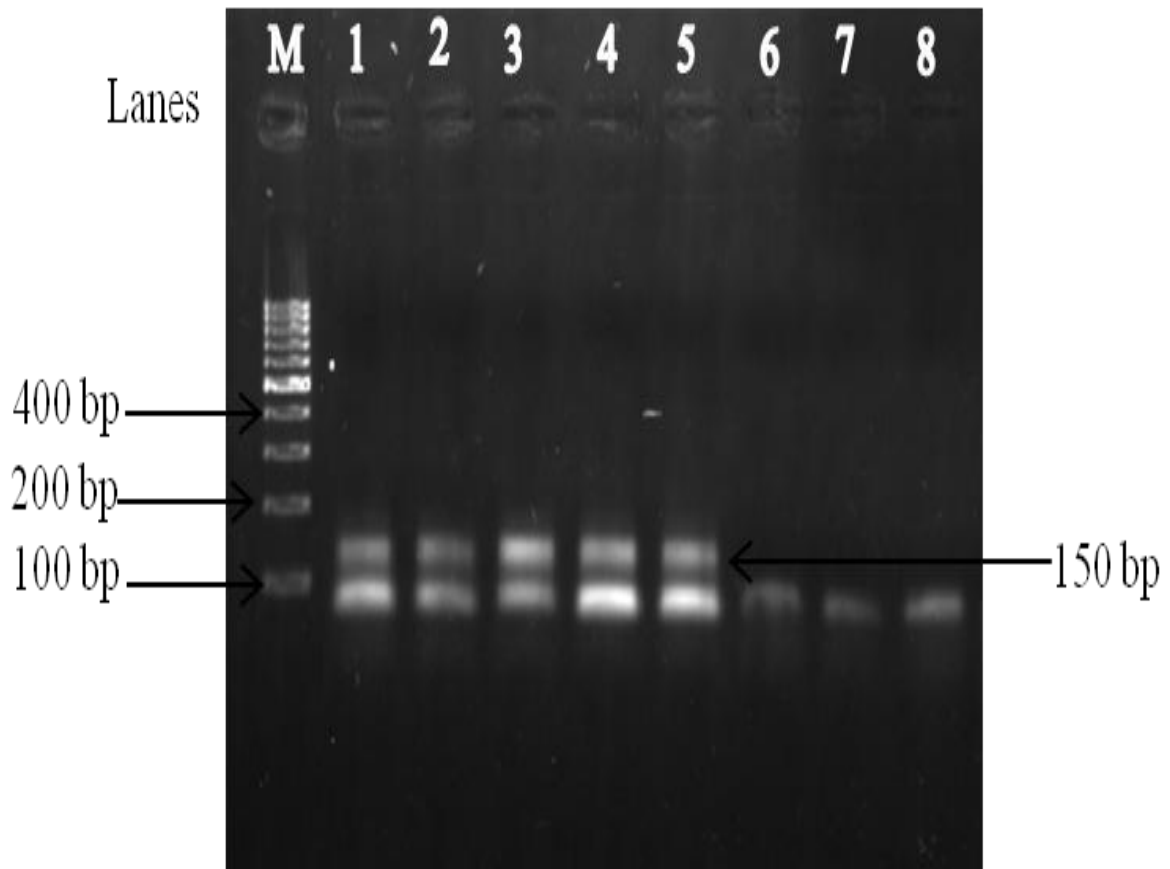


Plate IV: Detection of *Onchocerca volvulus* DNA from Skin Snip Samples.

Table 2. The Relationship between *Onchocerca volvulus* Recovery and Frequency of Treatment with Ivermectin Since Onset of Exercise.

| Treatment regime | Number of persons examined | Number positive for onchocerciasis (%) | Number negative for onchocerciasis (%) | Chi-square value | p-value |
|-------------------------|-----------------------------------|---|---|-------------------------|----------------|
| Once | 49 | 2 (50) | 47 (30.5) | 9.336 | 0.053 |
| Twice | 16 | 2 (50) | 14 (9.1) | | |
| Thrice | 29 | 0 (0.0) | 29 (18.8) | | |
| Many times | 22 | 0 (0.0) | 22 (14.3) | | |
| Not applicable | 42 | 0 (0.0) | 42 (27.3) | | |
| Total | 158 | 4 | 154 | | |

4.5 Ivermectin Therapeutic Response

The outcome of the issued questionnaire among the residents of the two communities studied revealed Ivermectin treatment of onchocerciasis results as presented in Table 3: 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.

4.6 The Relationship between Onchocerciasis and Some Demographic Factors

The demographic factors of the subjects were analyzed for their relationship with the occurrence of onchocerciasis; this is presented in Table 4:

Subjects within the age ≥ 50 years were mostly infected (75.0%), followed by 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.

Table 3. The Relationship between *Onchocercavolvulus* Infection and Onchocerciasis Symptom Resolution Rates due to Ivermectin Treatment Regime

| Ivermectin treatment status | Number of persons on treatment | Regime of treatment (years) | No. with onchocerciasis positive symptoms (%) | No. with onchocerciasis negative symptoms (%) | Chi-square value | p-value |
|------------------------------------|---------------------------------------|------------------------------------|--|--|-------------------------|----------------|
| Complete | 69 | ≥3 | 0 (0.0) | 69 (43.5) | 23.074 | 0.001* |
| Incomplete | 13 | <3 | 3 (75) | 10 (7.1) | | |
| No response | 36 | 0 | 1 (25) | 35 (22.7) | | |
| Not applicable | 40 | 0 | 0 (0.0) | 40 (25.3) | | |
| Total | 158 | | 4 | 154 | | |

* P-value significant at ≤ 0.05

Table 4. The Relationship between Onchocerciasis and Some Demographic Factors to which the studied subjects were exposed

| Demographic factors | Number of persons examined | No. with onchocerciasis positive symptoms (%) | No. with onchocerciasis negative symptoms (%) | Chi-square value | p-value (Chi-square) | p-value (Fisher's Exact test) |
|----------------------------|-----------------------------------|--|--|-------------------------|-----------------------------|--------------------------------------|
| Age (years) | | | | | | |
| 5-9 | 23 | 0 (0.0) | 23 (14.9) | 13.516 | 0.019* | |
| 10-14 | 38 | 0 (0.0) | 38 (24.7) | | | |
| 15-19 | 23 | 1 (25.0) | 22 (14.3) | | | |
| 20-29 | 20 | 0 (0.0) | 20 (13.0) | | | |
| 30-49 | 31 | 0 (0.0) | 31 (20.1) | | | |
| 50 and above | 23 | 3 (75.0) | 20 (13.0) | | | |
| Gender | | | | | | |
| Male | 77 | 2(50.0) | 75 (48.7) | 0.003 | 0.959 | 1.000 |
| Female | 81 | 2(50.0) | 79 (51.3) | | | |
| Occupation | | | | | | |
| Farming | 87 | 1 (25.0) | 86 (55.8) | 10.546 | 0.035* | |
| Civil servants & Farming | 16 | 2 (50.0) | 14 (9.1) | | | |
| Artisans | 10 | 1 (25.0) | 9 (5.8) | | | |
| Others | 45 | 0 (0.0) | 45 (29.2) | | | |
| | | | | | | |

* P-value significant at ≤ 0.05

4.7. The Relationship between Onchocerciasis and Epidemiological Risk Factors

Assessment of the epidemiological factors predisposing to onchocerciasis is presented in Table 5. 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 ($p=0.004$).

4.8. The Relationship between *Onchocerca volvulus* load and Symptoms of Onchocerciasis

The signs and symptoms of onchocerciasis as observed in this study are presented in Table 6. 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 ($p=0.001$).

Table 5: The Relationship between Onchocerciasis and Epidemiological Risk Factors to which the Studied Subjects Were Exposed

| Risk factors | Number of persons Examined | Number positive for onchocerciasis (%) | Number negative for onchocerciasis (%) | Chi-square value | p-value | p-value (Fisher's exact test) |
|--------------------------------------|-----------------------------------|---|---|-------------------------|----------------|--------------------------------------|
| Knowledge of black fly | | | | | | |
| Yes | 158 | 4 (100) | 154 (100) | - | - | - |
| No | 0 | 0 (0.0) | 0 (0.0) | | | |
| Ability to identify black fly | | | | | | |
| Yes | 123 | 4 (100) | 119 (77.3) | 1.168 | 0.280 | 0.576 |
| No | 35 | 0 (0.0) | 35 (22.7) | | | |
| Use of water from the dam | | | | | | |
| Yes | 158 | 4 (100) | 154 (100) | - | - | - |
| No | 0 | 0 (0.0) | 0 (0.0) | | | |
| Regular visit to dam | | | | | | |
| Daily | 25 | 3 (75.0) | 22 (14.3) | | | - |
| Weekly | 50 | 1 (25.0) | 49 (31.8) | 11.296 | 0.004* | |
| Not often | 83 | 0 (0.0) | 83 (53.9) | | | |

* P-value significant at ≤ 0.05

Table 6: The Relationship between *Onchocerca volvulus* Infection and Symptoms of Onchocerciasis

| Symptoms | Number of persons with symptoms | Number positive for onchocerciasis (%) | Number negative for onchocerciasis (%) | p-value (Fisher exact test) |
|-------------------------|---------------------------------|--|--|-----------------------------|
| White patches | | | | |
| Yes | 13 | 3 (75.0) | 10 (6.5) | 0.002* |
| No | 145 | 1 (25.0) | 144 (93.5) | |
| Severe body itch | | | | |
| Yes | 27 | 3 (75.0) | 24 (15.6) | 0.016* |
| No | 131 | 1 (25.0) | 130 (84.4) | |
| Nodules | | | | |
| Yes | 14 | 3 (75.0) | 11 (7.2) | 0.002* |
| No | 143 | 1 (25.0) | 142 (92.8) | |
| Impaired vision | | | | |
| Yes | 44 | 3 (75.0) | 41 (26.6) | 0.066 |
| No | 114 | 1 (25.0) | 132 (73.4) | |
| Hanging groin | | | | |
| Yes | 3 | 2 (50.0) | 1 (0.6) | 0.001* |
| No | 155 | 2 (50.0) | 153 (99.4) | |
| Blindness | | | | |
| Yes | 1 | 0 (0.0) | 1 (0.6) | 1.000 |
| No | 157 | 4 (100) | 153 (99.4) | |

* P-value significant at ≤ 0.05

CHAPTER FIVE

5.0 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 could be as a result of the several intervention exercises carried out in the area over the years. There has been mass distribution of Ivermectin, which is the drug of choice for intervention in onchocerciasis. Similar observations have been reported by Cuppet *et al.* (2011); Njepuomeet *et al.* (2014) and Manyiet *et al.* (2014). However, Ezeigbo *et al.* (2013) reported a lower prevalence of 0.55% in Abia State, Nigeria.

The regular availability of humans around the dam whose blood serves as meal to blackflies, that is, villagers who regularly visited the dam site for activities as farming, swimming, fetching of water, washing of clothes constituted an all year round providence, hence a suitable breeding and resting conditions must have resulted in the persistence of blackflies in these areas. Since all the villagers use water from the dam as gathered from questionnaires, also June to July constituted the peak breeding season for this species of black flies and consequently the period of highest *O. volvulus* transmission in the study area. The high number of parous flies observed in June was also reported in Mobi, Adamawa state by Maikaje *et al.* (2008).

The month of June and July are characterized by more suitable breeding conditions such as suitable temperature, turbulent and highly oxygenated waters; giving the flies the opportunity to breed. The high percentage of female black flies found to harbour *O. volvulus* larvae at different stages of development is an indication of the presence and persistence of microfilaria in human hosts in the area.

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. These subjects could be a source of infection to the general population if measures to curb the spread are not taken.

Of the one-hundred and fifty-eight skin snip samples obtained, a total number of twenty-eight samples (this included the four positive samples obtained through microscopy with other twenty-four randomly selected samples) were processed for the molecular assay putting into consideration the different age brackets examined. 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 twenty-eight samples were run on gel photos and finally, the positive samples were picked and 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; also the unprocessed samples could probably give some more positives if subjected to PCR analysis.

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 (Meredith *et al.*, 1991; Zimmerman *et al.*, 1994; Fischer *et al.*, 1996; Yameogo *et al.*, 1999). All these assays are based on the amplification and detection of the tandem and repeated DNA sequence 0-150 which is present in about 4000 copies per haploid genome of *O. volvulus*.

It was shown that parasite DNA can be sensitively detected in the skin by Zimmerman *et al.* (1994). It has also been reported that PCR is more sensitive than standard parasitological assessment of emerging microfilaria through microscopy. However, in individuals with low microfilaria densities, whose skin samples may be devoid of whole or partial microfilaria, the PCR might be negative because free parasite DNA is not stable in the tissue (Fischer *et al.*, 1996). Therefore, the PCR on tissue samples is a sensitive, direct method to prove the presence of intact, disintegrating microfilaria or remnants of them.

Structured questionnaires were administered to assess the associating demographic and risk factors, signs and symptoms to *Onchocerca volvulus*;

The impact of repeated mass treatment with Ivermectin on the prevalence of onchocerciasis in the study area was assessed. About 74% had access to the drug and the relationship between onchocerciasis and symptom resolution from treatment with Ivermectin was found to be statistically significant ($p=0.001$) as shown in table 2. This indicated that the drug, Ivermectin, used for the treatment of onchocerciasis in this area has been very effective.

The observed significant reduction in the prevalence of onchocerciasis after the intervention with ivermectin was an indication of the efficacy of the drug in curbing the spread of onchocerciasis in the sampled communities.

One of the most important and striking characteristics of ivermectin is that despite its short half-life, a single dose does not only eliminate skin microfilariae but also provides long lasting suppression of microfilariderma (Awadzi *et al.*,1986).

Ivermectin kills 99% of microfilariae with a single treatment (APOC/WHO, 2005).

Significant reduction in nodule prevalence as a result of treatment with ivermectin had been reported earlier (Emukahet *al.*,2004). Also, other authors have reported the efficacy of this drug in curbing the spread of onchocerciasis in communities. Ezeigbo *et al.* (2013) observed a remarkable reduction in the prevalence and intensity of onchocerciasis in their study in Abia State. Kogi and Bulus (2008) reported a post-ivermectin reduction in symptoms and complete elimination of microfilaria in their study in Kaduna State. Tekle *et al.*(2012) asserted that ivermectin treatment alone can eliminate onchocerciasis infection and probably disease transmission in endemic foci as Africa.

The problem with ivermectin is that it is a monotherapy microfilaricide which has limited effect on the adult worm, and thus will need to be continued for the life span of the adult worm, which may last up to 15 years (Babalola, 2011). Alternatively, development of novel prevention and treatment modalities, such as next-generation small molecule drugs and vaccines should be considered (Barry *etal.*, 2013).

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.

Nworie *et al.*(2014) reported 38.7% males, 23.1% females in Ebonyi Central and 32.0% males, 25.0% females in Ebonyi North positive for *Onchocerca volvulus* infection. 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 24months to be established and could persist for as long as 9 to 11years (Okonkwo *et al.*, 2010). A similar observation was made in Ibarapa L.G.A. of Oyo State Nigeria (Akinboye *et al.*, 2010).

The occupation of the villagers especially farming exposes them to black fly bites leading to infection as implicated in this study ($p=0.035$). Kogi and Bulus (2008) reported an association of Onchocerciasis with occupation. Nworie *et al.*(2014) reported farmers to be the most *Onchocerca volvulus*affected group.

Regular visit to dam was found to be a statistically significant risk factor in the acquisition of onchocerciasis ($p=0.004$). This shows that visit to the dam has served as a point of contact between the villagers and black flies resulting in a bite and the subsequent transmission of the *O. volvulus*infection. Other studies reported a similar finding (Okonkwo *et al.*, 2010).

Although all the study population claimed to have awareness of blackflies, they were yet infected. This implies that the knowledge of blackflies or the ability to identify them is not enough to curb the infection.

The relationship between *Onchocercavolvulus* and symptoms of onchocerciasis was studied. 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. As such the presence of these symptoms in an individual should arouse a suspicion for the possibility of onchocerciasis. These findings agree with Okoye and Onwuliri (2007).

Okonkwo *et al.* (2010) reported in their study several onchocerciasis manifestations in terms of nodules, blindness, leopard skin, puritus and other dermatitis, hanging groin and elephantiasis. Similarly, Nworie *et al.*(2014) also reported onchocercal lesions such as nodules, leopard skin, hanging groin and blindness.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Onchocerciasis was highly endemic in many parts of Africa before control activities; it is a disease affecting millions of people in Africa, South and Central America, and Yemen (Babalola, 2011). It is endemic in Nigeria, the vector (black fly) breeds in fast flowing rivers and those within the area are exposed to the disease (Evans *et al.*, 2011). A low prevalence (2.5%) of *O. volvulus* infection in humans was recorded in this study from the two sampled villages using standard parasitological assessment (microscopy). This success is largely as a result of the several intervention exercises carried out in the area over the years. There has been mass distribution of Ivermectin, which is the drug of choice for intervention in onchocerciasis. Similar observations have been reported by Cuppet *al.* (2011); Njepuomeet *al.* (2014) and Manyiet *al.* (2014).

June to July constituted the peak breeding season for this species of black flies and consequently the period of highest *O. volvulus* transmission in the study site (gurara dam). The regular availability of humans around the dam whose blood serves as meal to blackflies constituted an all year round providence, hence a suitable breeding and resting conditions. The high number of parous flies observed in June was also reported in Mobi, Adamawa state by Maikaje *etal.*(2008).

Molecular assay was used to confirm the presence of *O. volvulus* DNA in human skin snip samples. The positive result from PCR is greater than the result obtained from microscopy, confirming the fact of molecular detection to be more sensitive than microscopy. Similar findings have been reported by Zimmerman *et al.* (1994).

It has been established that the overall prevalence of *O. volvulus* obtained in this study is 2.5%.

The highest collection of parasitic black flies was in the month of June.

Amplified PCR products were observed on the gel photo in form of bands at 150 bp confirming the presence of *O. volvulus* DNA in the skin snip of these subjects

The use of the drug of choice, Ivermectin against onchocerciasis in the study area has been effective.

There is a relationship between the prevalence of *O. volvulus* and age and occupations of the participants. Epidemiological risk factor implicated was the visit to dam.

The symptoms that showed indication of onchocerciasis were white patches, severe body itch, nodules and hanging groin.

6.2 Recommendations

In view of the above findings it is recommended that:

1. Thorough surveillance for the intervention against the endemic situation of *O. volvulus* be extended to other neighboring communities.
2. Effective control strategy of the *Simulium* vectors should be implemented and carried out routinely around dams: surveillance on parasite carrying status of the *Simulium* vectors biting along the river systems is a vital component in monitoring the transmission level of onchocerciasis which thus gives the most direct and non-invasive method of measuring the success of the control/ elimination measures. Hence, those who visit the dam must be adequately protected.
3. Other sensitive diagnostic procedures such as rapid test kits and test strips methods should be adopted to ensure ease and effectiveness in diagnosis of the infection,
4. Better sources of water should be established for the villagers to have alternatives to dams, thereby avoiding regular visit to the place of high prevalence of black fly
5. To facilitate effective control of these infections, policy makers should ensure that treatments of these infections are provided to the various communities as at when due.

CONTRIBUTION TO KNOWLEDGE

- ❖ 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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APPENDICES

Appendix I: Ethical clearance

**OFFICE OF THE EXECUTIVE CHAIRMAN
KAGARKO LOCAL GOVERNMENT
KAGARKO, KADUNA STATE**

Our Ref: KGKLG/SWT/AD/166/VOL.I

Your Ref: _____



LOCAL GOVERNMENT SECRETARIAT
KAGARKO.
Date: 15th July, 2015


Head,
Department of Microbiology,
Ahmadu Bello University,
Zaria.

REPORT ON COMPLETION OF RESEARCH.

I am directed to write and inform you that ~~OZOVHE~~ **LYDIA ONYECHE** has successfully completed her research work on 'Isolation and Molecular Characterization of Onchocerca volvulus from vector and human hosts', in Kurmin-Bango and Ungwan Doya around Gurara Dam, in Kagarko Local Government, Kaduna State. She is a hard working, punctual and dedicated student that is ready to accept responsibilities.

Thank you for your usual cooperation please.

I


Yakubu Umar
Staff Welfare and Training
For: Chairman

Home of Cassava & Ginger

Appendix II: Structured questionnaire

Dear volunteer, my name is Mrs. Lydia Ozovehe, a master student of Ahmadu Bello University, Zaria. Your consent is appreciated!

Research topic: Isolation and molecular detection of *Onchocercavolvulus* from vector and human hosts around Gurara dam, Kaduna state, Nigeria.

Instruction: please fill in the blank spaces or tick as appropriate in the spaces provided below:

SECTION A: BIODATA/ DEMOGRAPHY

Name:

Gender: Female () Male ()

Village name/code:

Age group: 05-09 () 10-14 () 15-19 () 20-29 () 30-49 () 50+ ()

Occupation:

Sample Number_____

Evaluation Date_____

SECTION B: EPIDEMIOLOGICAL RISK FACTORS

Have you heard of black flies? Yes () No ()

Can you identify a black fly? Yes () No ()

Do you use water from the dam for domestic activities? Yes () No ()

How often do you visit the dam? Daily () Weekly () Not often ()

SECTION C: SYMPTOMS OF ONCHOCERCIASIS

Which of these are you currently suffering from?

a. white patches on the legs (leopard skin) and/or hard skin (lizard skin)

b. constant and severe body itching

c. painless swellings (nodules)

d. impaired vision

e. hanging groin

f. blindness

SECTION D: TREATMENT RECEIVED AND ITS OUTCOME

- What treatment(s) have you received?
 - a. mectizan
 - b. others (specify)

- If mectizan, how many times have you taken this drug?
 - a. once
 - b. twice
 - c. thrice
 - d. many times

- If any, what are the difficulties faced in getting these drugs? (specify)

- What was the outcome of treatment?
 - a. completely resolved
 - b. incompletely resolved

APPENDIX III

Monthly analysis of blackflies caught

Flies caught in June.

Day 1

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 13/06/2015 | 7-8 | 64 | | |
| 13/06/2015 | 8-9 | 56 | | |
| 13/06/2015 | 9-10 | 53 | | |
| 13/06/2015 | 10-11 | 48 | | |
| 13/06/2015 | 11-12 | 56 | | |
| 13/06/2015 | 12-1 | 37 | | |
| 13/06/2015 | 1-2 | 18 | | |
| 13/06/2015 | 2-3 | 00 | | |
| 13/06/2015 | 3-4 | 24 | | |
| 13/06/2015 | 4-5 | 39 | | |
| Total | | 395 | | |

Day 2

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 14/06/2015 | 7-8 | 62 | | |
| 14/06/2015 | 8-9 | 60 | | |
| 14/06/2015 | 9-10 | 49 | | |
| 14/06/2015 | 10-11 | 56 | | |
| 14/06/2015 | 11-12 | 53 | | |
| 14/06/2015 | 12-1 | 42 | | |
| 14/06/2015 | 1-2 | 39 | | |
| 14/06/2015 | 2-3 | 14 | | |
| 14/06/2015 | 3-4 | 33 | | |
| 14/06/2015 | 4-5 | 20 | | |
| Total | | 428 | | |

Day 3

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 15/06/2015 | 7-8 | 78 | | |
| 15/06/2015 | 8-9 | 57 | | |
| 15/06/2015 | 9-10 | 65 | | |
| 15/06/2015 | 10-11 | 62 | | |
| 15/06/2015 | 11-12 | 59 | | |
| 15/06/2015 | 12-1 | 34 | | |
| 15/06/2015 | 1-2 | 07 | | |
| 15/06/2015 | 2-3 | 40 | | |
| 15/06/2015 | 3-4 | 18 | | |
| 15/06/2015 | 4-5 | 31 | | |
| Total | | 451 | | |

Total no. of flies caught in June = 1,274 flies

Flies caught in July. Day 1

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 13/07/2015 | 7-8 | 100 | | |
| 13/07/2015 | 8-9 | 82 | | |
| 13/07/2015 | 9-10 | 50 | | |
| 13/07/2015 | 10-11 | 43 | | |
| 13/07/2015 | 11-12 | 20 | | |
| 13/07/2015 | 12-1 | 45 | | |
| 13/07/2015 | 1-2 | 30 | | |
| 13/07/2015 | 2-3 | 44 | | |
| 13/07/2015 | 3-4 | 30 | | |
| 13/07/2015 | 4-5 | 33 | | |
| Total | | 477 | | |

Day 2

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 14/07/2015 | 7-8 | 40 | | |
| 14/07/2015 | 8-9 | 24 | | |
| 14/07/2015 | 9-10 | 66 | | |
| 14/07/2015 | 10-11 | 20 | | |
| 14/07/2015 | 11-12 | 25 | | |
| 14/07/2015 | 12-1 | 35 | | |
| 14/07/2015 | 1-2 | 42 | | |
| 14/07/2015 | 2-3 | 30 | | |
| 14/07/2015 | 3-4 | 40 | | |
| 14/07/2015 | 4-5 | 45 | | |
| Total | | 367 | | |

Day 3

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 15/07/2015 | 7-8 | 20 | | |
| 15/07/2015 | 8-9 | 37 | | |
| 15/07/2015 | 9-10 | 33 | | |
| 15/07/2015 | 10-11 | 37 | | |
| 15/07/2015 | 11-12 | 23 | | |
| 15/07/2015 | 12-1 | 30 | | |
| 15/07/2015 | 1-2 | 45 | | |
| 15/07/2015 | 2-3 | 20 | | |
| 15/07/2015 | 3-4 | 25 | | |
| 15/07/2015 | 4-5 | 40 | | |
| Total | | | | |

Total no. of flies caught in July = 1,154 flies

Flies caught in August. Day 1

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 13/08/2015 | 7-8 | 30 | | |
| 13/08/2015 | 8-9 | 35 | | |
| 13/08/2015 | 9-10 | 45 | | |
| 13/08/2015 | 10-11 | 37 | | |
| 13/08/2015 | 11-12 | 25 | | |
| 13/08/2015 | 12-1 | 20 | | |
| 13/08/2015 | 1-2 | 38 | | |
| 13/08/2015 | 2-3 | 42 | | |
| 13/08/2015 | 3-4 | 33 | | |
| 13/08/2015 | 4-5 | 28 | | |
| Total | | 333 | | |

Day 2

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 14/08/2015 | 7-8 | 40 | | |
| 14/08/2015 | 8-9 | 38 | | |
| 14/08/2015 | 9-10 | 34 | | |
| 14/08/2015 | 10-11 | 46 | | |
| 14/08/2015 | 11-12 | 54 | | |
| 14/08/2015 | 12-1 | 36 | | |
| 14/08/2015 | 1-2 | 30 | | |
| 14/08/2015 | 2-3 | 37 | | |
| 14/08/2015 | 3-4 | 39 | | |
| 14/08/2015 | 4-5 | 26 | | |
| Total | | 380 | | |

Day 3

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 15/08/2015 | 7-8 | 40 | | |
| 15/08/2015 | 8-9 | 34 | | |
| 15/08/2015 | 9-10 | 26 | | |
| 15/08/2015 | 10-11 | 33 | | |
| 15/08/2015 | 11-12 | 35 | | |
| 15/08/2015 | 12-1 | 31 | | |
| 15/08/2015 | 1-2 | 29 | | |
| 15/08/2015 | 2-3 | 32 | | |
| 15/08/2015 | 3-4 | 43 | | |
| 15/08/2015 | 4-5 | 37 | | |
| Total | | 1053 | | |

Total no. of flies caught in August = 1,053 flies

Flies caught in September. Day 1

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 13/09/2015 | 7-8 | 40 | | |
| 13/09/2015 | 8-9 | 36 | | |
| 13/09/2015 | 9-10 | 30 | | |
| 13/09/2015 | 10-11 | 32 | | |
| 13/09/2015 | 11-12 | 38 | | |
| 13/09/2015 | 12-1 | 34 | | |
| 13/09/2015 | 1-2 | 31 | | |
| 13/09/2015 | 2-3 | 41 | | |
| 13/09/2015 | 3-4 | 34 | | |
| 13/09/2015 | 4-5 | 25 | | |
| Total | | 341 | | |

Day 2

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 14/09/2015 | 7-8 | 32 | | |
| 14/09/2015 | 8-9 | 28 | | |
| 14/09/2015 | 9-10 | 29 | | |
| 14/09/2015 | 10-11 | 22 | | |
| 14/09/2015 | 11-12 | 31 | | |
| 14/09/2015 | 12-1 | 28 | | |
| 14/09/2015 | 1-2 | 21 | | |
| 14/09/2015 | 2-3 | 23 | | |
| 14/09/2015 | 3-4 | 20 | | |
| 14/09/2015 | 4-5 | 24 | | |
| Total | | 258 | | |

Day 3

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 15/09/2015 | 7-8 | 56 | | |
| 15/09/2015 | 8-9 | 43 | | |
| 15/09/2015 | 9-10 | 41 | | |
| 15/09/2015 | 10-11 | 36 | | |
| 15/09/2015 | 11-12 | 29 | | |
| 15/09/2015 | 12-1 | 20 | | |
| 15/09/2015 | 1-2 | 31 | | |
| 15/09/2015 | 2-3 | 35 | | |
| 15/09/2015 | 3-4 | 38 | | |
| 15/09/2015 | 4-5 | 40 | | |
| Total | | 369 | | |

Total no. of flies caught in September = 968 flies

Flies caught in October. Day 1

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 13/10/2015 | 7-8 | 46 | | |
| 13/10/2015 | 8-9 | 51 | | |
| 13/10/2015 | 9-10 | 39 | | |
| 13/10/2015 | 10-11 | 27 | | |
| 13/10/2015 | 11-12 | 18 | | |
| 13/10/2015 | 12-1 | 12 | | |
| 13/10/2015 | 1-2 | 17 | | |
| 13/10/2015 | 2-3 | 33 | | |
| 13/10/2015 | 3-4 | 38 | | |
| 13/10/2015 | 4-5 | 41 | | |
| Total | | 322 | | |

Day 2

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 14/10/2015 | 7-8 | 39 | | |
| 14/10/2015 | 8-9 | 47 | | |
| 14/10/2015 | 9-10 | 44 | | |
| 14/10/2015 | 10-11 | 36 | | |
| 14/10/2015 | 11-12 | 24 | | |
| 14/10/2015 | 12-1 | 13 | | |
| 14/10/2015 | 1-2 | 24 | | |
| 14/10/2015 | 2-3 | 18 | | |
| 14/10/2015 | 3-4 | 24 | | |
| 14/10/2015 | 4-5 | 39 | | |
| Total | | 308 | | |

Day 3

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 15/10/2015 | 7-8 | 42 | | |
| 15/10/2015 | 8-9 | 39 | | |
| 15/10/2015 | 9-10 | 33 | | |
| 15/10/2015 | 10-11 | 25 | | |
| 15/10/2015 | 11-12 | 26 | | |
| 15/10/2015 | 12-1 | 10 | | |
| 15/10/2015 | 1-2 | 26 | | |
| 15/10/2015 | 2-3 | 29 | | |
| 15/10/2015 | 3-4 | 36 | | |
| 15/10/2015 | 4-5 | 28 | | |
| Total | | 294 | | |

Total no. of flies caught in October = 924 flies

Flies caught in November. Day 1

| Date | Hourly catch | No of flies | Initials | Remarks |
|------------|--------------|-------------|----------|---------|
| 13/11/2015 | 7-8 | 41 | | |
| 13/11/2015 | 8-9 | 37 | | |
| 13/11/2015 | 9-10 | 42 | | |
| 13/11/2015 | 10-11 | 31 | | |
| 13/11/2015 | 11-12 | 27 | | |
| 13/11/2015 | 12-1 | 13 | | |
| 13/11/2015 | 1-2 | 23 | | |
| 13/11/2015 | 2-3 | 22 | | |
| 13/11/2015 | 3-4 | 28 | | |
| 13/11/2015 | 4-5 | 34 | | |
| Total | | 298 | | |

Day 2

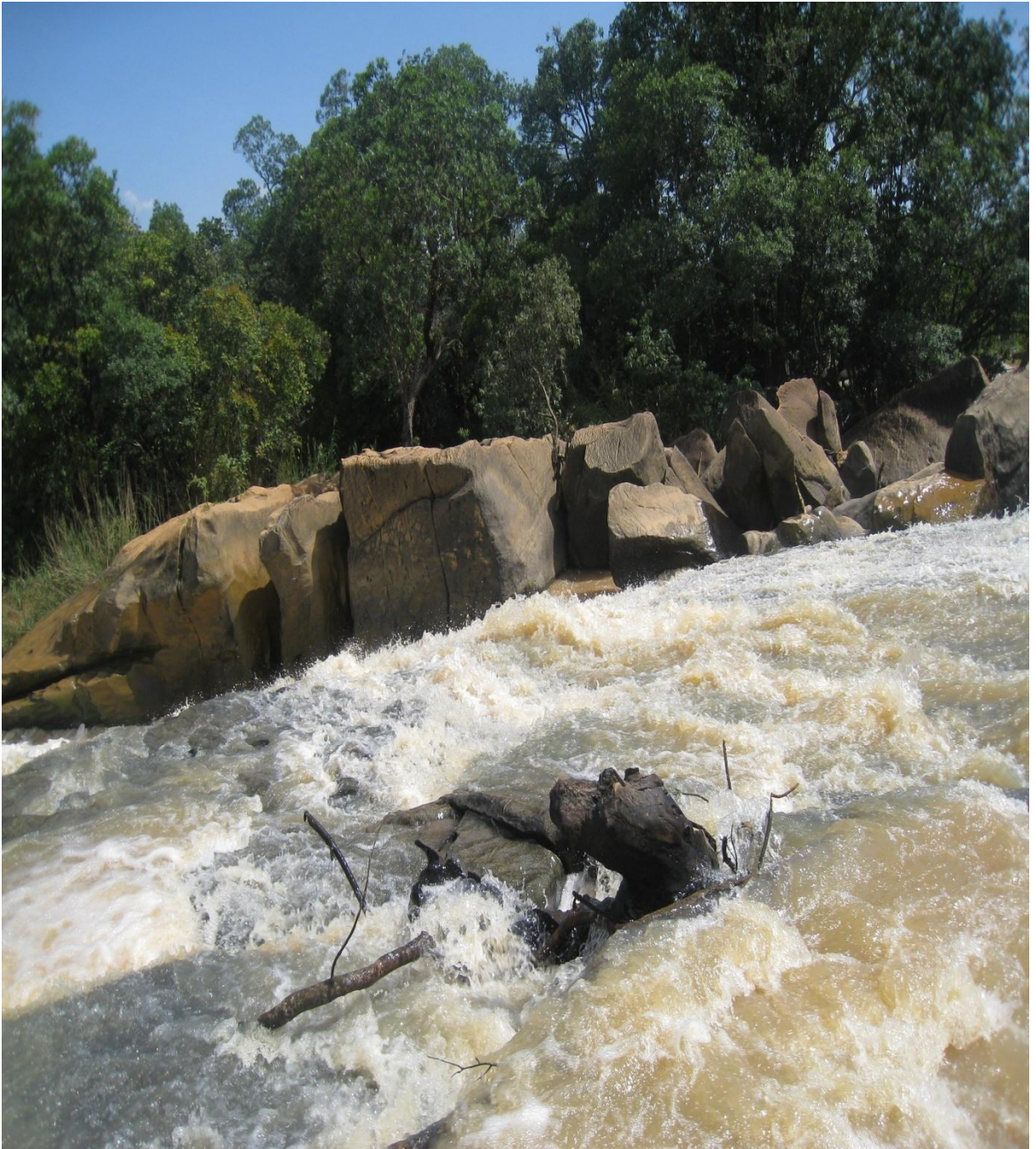
| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 14/11/2015 | 7-8 | 32 | | |
| 14/11/2015 | 8-9 | 35 | | |
| 14/11/2015 | 9-10 | 36 | | |
| 14/11/2015 | 10-11 | 26 | | |
| 14/11/2015 | 11-12 | 28 | | |
| 14/11/2015 | 12-1 | 09 | | |
| 14/11/2015 | 1-2 | 20 | | |
| 14/11/2015 | 2-3 | 31 | | |
| 14/11/2015 | 3-4 | 33 | | |
| 14/11/2015 | 4-5 | 40 | | |
| Total | | 290 | | |

Day 3

| Date | Hourly catch | No. of flies | Initials | Remarks |
|------------|--------------|--------------|----------|---------|
| 15/11/2015 | 7-8 | 30 | | |
| 15/11/2015 | 8-9 | 43 | | |
| 15/11/2015 | 9-10 | 30 | | |
| 15/11/2015 | 10-11 | 29 | | |
| 15/11/2015 | 11-12 | 20 | | |
| 15/11/2015 | 12-1 | 13 | | |
| 15/11/2015 | 1-2 | 14 | | |
| 15/11/2015 | 2-3 | 31 | | |
| 15/11/2015 | 3-4 | 30 | | |
| 15/11/2015 | 4-5 | 37 | | |
| Total | | 277 | | |

Total no. of flies caught in November = 895 flies

Overall no. of flies caught = 4,994 flies



Appendix IV a: Picture showing part of Gurara dam



Appendix IV b: Picture showing the three (3) segments of a blackfly viewed under a dissection microscope



Appendix IV c: Picture showing the internal organ of the blackfly's abdomen



Appendix V a: Picture showing the skin snipping process



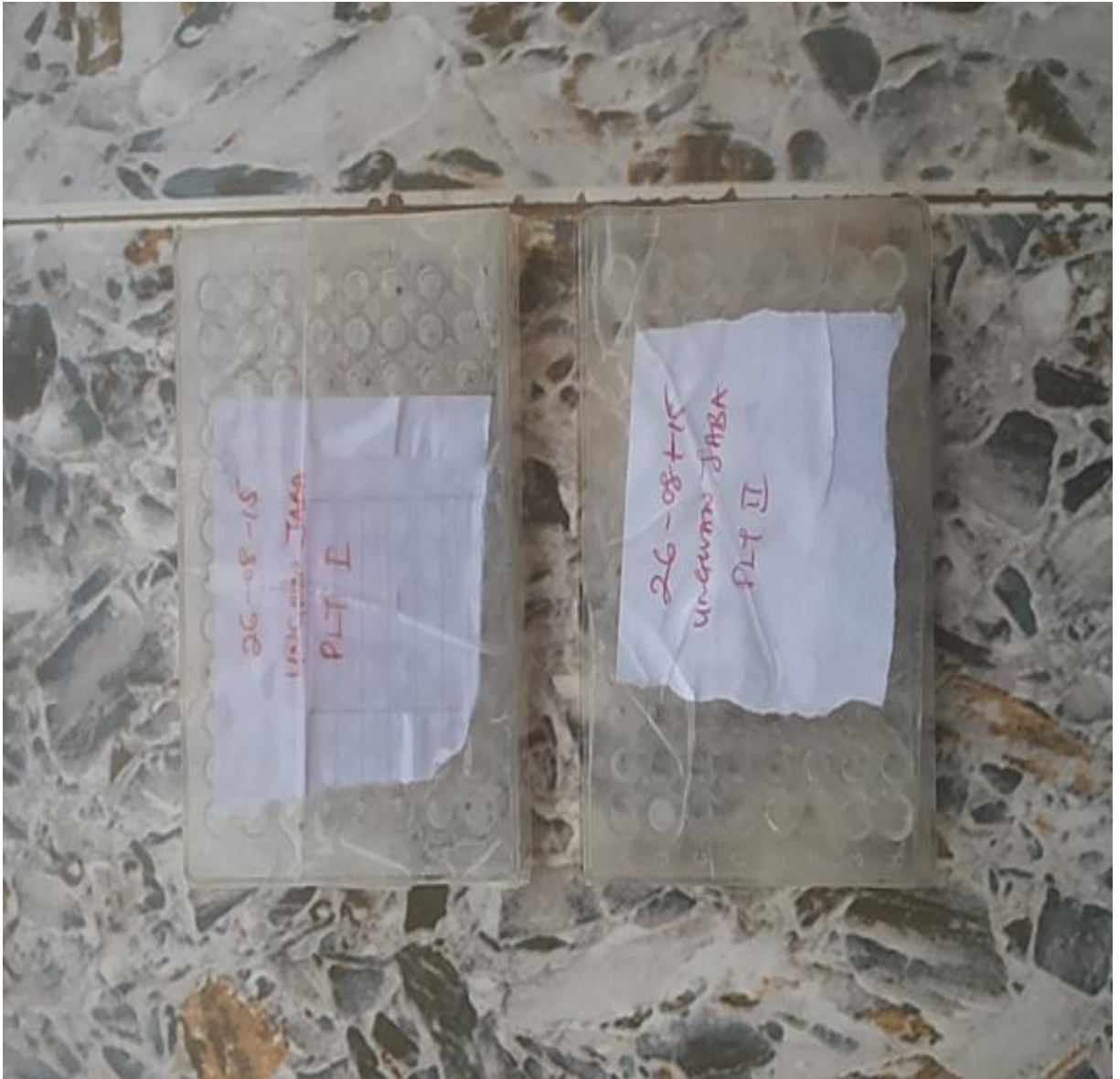
Appendix V b: The skin snipping process



Appendix V c: Fixing of skin snip samples with formalin



Appendix VI a: Skin snip samples in microtitre wells with normal saline



Appendix VI b: Skin snip samples on microtitre plates from ungwan-jaba village



Appendix VI c: Skin snip samples on microtitre plates from kurmi-bango village