DETERMINATION OF *ONCHOCERCA VOLVULUS* STRAINS

PREVALENT IN THE NKWANTA NORTH DISTRICT OF GHANA

BY

ROWLAND ADUKPO

(10508507)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL
FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL
MICROBIOLOGY DEGREE

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES

FEBRUARY, 2019
DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree in any other institution. I have duly acknowledged references made to other authors’ work in the reference section of this thesis.

Signature……………………………… Date………………………………

ROWLAND ADUKPO
(STUDENT)

Signature……………………………… Date………………………………

DR. SIMON KWAKU ATTAH
(SUPERVISOR)

Signature……………………………… Date………………………………

DR. PATIENCE B. TETTEH-QUARCOO
(SUPERVISOR)
DEDICATION

To Hetty and Gabriella
ACKNOWLEDGEMENTS

My sincere thanks and appreciation go to my supervisors, Dr. Simon K. Attah and Dr. Patience B. Tetteh-Quarcoo for their patience, invaluable support and guidance throughout my course work and during the preparation of this thesis. Special appreciation also goes to Prof. Yaw Afrane and Prof. Kwamena W. C. Sagoe for their encouragement.

I wish to also express my profound gratitude to Dr. Michael Osei-Atweneboana, Head of the Biomedical and Public Health Research Unit of the Council for Scientific and Industrial Research (CSIR) for his mentorship and direction and also for allowing us to use the laboratory for the bench work. Special thanks also go to the staff of the Biomedical and Public Health Research Unit and the Molecular Biology Laboratory (CSIR) especially Dr. Samuel Armoo, Edward Jenner Tettevi, Queenstar Naa Dedei Quashie for their support. Special mention is to be made of Mr. Isaac Owusu Frimpong for whom I am eternally grateful to for his technical support and guidance during the molecular work. God bless you Ike for your sacrifice.

My appreciation also goes to Dr. Laud Boateng and his team at the Nkwanta North District Health Directorate of the Ghana Health Service for facilitating the community entry and data collection. I want to thank especially Mr Amatus Nambagyira and Dominic Nanga both of Nkwanta North District Health Directorate and Mr. Reuben Tettey Martey and Michael Dicko of the Pentecost Health Centre, Kpassa, for their support on the field. I also wish to thank all the chiefs, elders and community leaders in the study communities.
Additionally, I am eternally indebted to my wife Ms. Henrietta Appiah who is always available to tell me in that sweet assuring voice “Rowland, you are capable, go for it”. To my siblings and family, I say thank you for your love and support. Also to my very good friends; Francis Dzidefo Krampa of West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), Israel Mensah-Attipoe of the Department of Medical Microbiology, School of Biomedical and Allied Health Sciences and Richard Kutame of the National Public Health and Reference Laboratory, Korle-Bu for the diverse roles they played in making this work a success.

And finally, to God be the glory and great things He has done.
# TABLE OF CONTENTS

DECLARATION .............................................................................................................................. i

DEDICATION .............................................................................................................................. ii

ACKNOWLEDGEMENTS ........................................................................................................ iii

LIST OF FIGURES ....................................................................................................................... ix

LIST OF ABBREVIATIONS ........................................................................................................ x

ABSTRACT .................................................................................................................................. xi

CHAPTER ONE ............................................................................................................................. 1

1.0 INTRODUCTION .................................................................................................................... 1

1.1 General introduction ............................................................................................................... 1

1.2 Research problem .................................................................................................................. 3

1.3 Justification ............................................................................................................................ 5

1.4 Aim of the study .................................................................................................................... 5

1.5 Specific objectives ................................................................................................................ 5

CHAPTER TWO ........................................................................................................................... 6

2.0 LITERATURE REVIEW ......................................................................................................... 6

2.1 Onchocerca volvulus .............................................................................................................. 6

2.2 The genome of *Onchocerca volvulus* .................................................................................. 8

2.2.1 The coding sequence ....................................................................................................... 8

2.2.2 The non-coding sequences ........................................................................................... 9

2.3 Life cycle of *Onchocerca volvulus* .................................................................................... 10
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4</td>
<td>Epidemiology and socioeconomic significance of <em>Onchocerca volvulus</em></td>
<td>12</td>
</tr>
<tr>
<td>2.5</td>
<td>Clinical manifestations and pathogenesis of onchocerciasis</td>
<td>15</td>
</tr>
<tr>
<td>2.5.1</td>
<td>Ocular onchocerciasis</td>
<td>15</td>
</tr>
<tr>
<td>2.6</td>
<td>Parasite, vector and host dynamics of onchocerciasis</td>
<td>21</td>
</tr>
<tr>
<td>2.7</td>
<td>Laboratory diagnosis of <em>Onchocerca volvulus</em></td>
<td>24</td>
</tr>
<tr>
<td>2.7.1</td>
<td>Skin snip microscopy</td>
<td>25</td>
</tr>
<tr>
<td>2.7.2</td>
<td>Mazzotti test</td>
<td>25</td>
</tr>
<tr>
<td>2.7.3</td>
<td>Immunological tests</td>
<td>26</td>
</tr>
<tr>
<td>2.7.4</td>
<td>Molecular techniques</td>
<td>26</td>
</tr>
<tr>
<td>2.8</td>
<td>Onchocerciasis control</td>
<td>27</td>
</tr>
</tbody>
</table>

CHAPTER THREE                                                                 | 31   |
| 3.0     | MATERIALS AND METHODS                                                | 31   |
| 3.1     | Study area and population                                            | 31   |
| 3.2     | Sample size calculation                                              | 33   |
| 3.3     | Sampling techniques                                                  | 33   |
| 3.3.1   | Community selection and inclusion criteria                           | 33   |
| 3.3.2   | Participants selection                                               | 34   |
| 3.3.4   | DNA extraction of *O. volvulus* from skin snips                       | 35   |
| 3.3.5   | *Onchocerca volvulus* DNA amplification using diagnostic primer      | 35   |

CHAPTER FOUR                                                                 | 38   |
| 4.0     | RESULTS                                                               | 38   |
4.1 Analysis of skin snip microscopy and *O. volvulus* DNA PCR results ........................................... 38

4.1.2 Analysis of skin microscopy and DNA PCR results by occupation ........................................... 39

4.2 Analysis of results of Clinical manifestations of onchocerciasis ..................................................... 39

4.2.1 Analysis of subjects manifesting onchocercal lesions by sex ..................................................... 39

4.2.2 Analysis of subjects manifesting onchocercal lesions by age groups .......................................... 40

4.2.3 Analysis of subjects manifesting onchocercal lesions by occupation .......................................... 40

4.3 DNA results analysis ....................................................................................................................... 41

4.3.1 Detection of *O. volvulus* using Diagnostic primers ................................................................. 41

4.3.2 Analysis of PCR test results for determination of *O. volvulus* strain using forest strain specific primers ......................................................................................................................... 43

CHAPTER FIVE ........................................................................................................................................ 45

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS .................................................................. 45

5.1 Discussion ........................................................................................................................................ 45

5.2 Conclusion ...................................................................................................................................... 49

5.3 Limitations ..................................................................................................................................... 50

5.4 Recommendations .......................................................................................................................... 50

REFERENCES ....................................................................................................................................... 51

APPENDICES ......................................................................................................................................... 75

APPENDIX 1 ........................................................................................................................................... 75

PARTICIPANT INFORMATION FORM ................................................................................................... 75

APPENDIX 2 ........................................................................................................................................... 77
LIST OF FIGURES

Figure 1. Adult female worms of *Onchocerca volvulus* ................................................................. 7
Figure 2. Microfilaria of *Onchocerca volvulus* .................................................................................. 7
Figure 3. Life cycle of *Onchocerca volvulus* ...................................................................................... 11
Figure 4. Distribution of onchocerciasis worldwide, 2014 ................................................................. 14
Figure 5. Sclerosing keratitis in onchocerciasis. © Ian Murdoch & Allen Foster, 2001 ................. 17
Figure 6. Lichenified onchodermatitis in a young male ................................................................. 20
Figure 7. Chronic onchodermatitis with Leopard spotting over lower legs ................................. 20
Figure 8. Chronic onchodermatitis producing a Lizard skin appearance in a young patient ..... 20
Figure 9. District map of Nkwaso North .......................................................................................... 32
Figure 10. Agarose gel electrophoresis pattern for amplification products of samples from Kabonwule (KA) in lanes 3, 4, 7, 8 and 9 by OV Diagnostic primers ........................................... 42
Figure 11. Agarose gel electrophoresis pattern for amplification products of samples from Lemina (LM) in lanes 3, 4, and Controls from Agborlekame ABI, AB2 AB3 in lanes 6, 7, 8 and Asubende ASU 1, ASU 2, ASU3 IN lanes 9, 13, and 14 and forest controls in lane FSP 1, FSP 2, FSP 3 and FSP 4 in lanes 5, 10, 11, 12, by OV diagnostic primer .................................................. 42
Figure 12. Agarose gel electrophoresis pattern of savannah controls in lanes 2, 3, 4, 6, 7, 8 and 9 showing no amplification with nested PCR primers. Lanes 5, 10, 11 and 12 amplified with the nested PCR producing amplicon size of 153 bp. ................................................................. 44
LIST OF ABBREVIATIONS

APOCH – African Programme for Onchocerciasis Control

CDC – Centres for Disease Control and Prevention

CDTI – Community-Directed Treatment with Ivermectin

DALYs- Disability-Adjusted Life Years

DNA-Deoxyribonucleic acid

IVM – Ivermectin

LAMP- Loop Mediated Isothermal Amplification

L3- Infective stage larvae of *O. volvulus*

MDA – Mass Drug Administration

MF – Microfilariae

NTD - Neglected Tropical Disease

NTDCP – Neglected Tropical Disease Control Programme

OCP – Onchocerciasis Control Programme in West Africa

OEPA – Onchocerciasis Elimination Programme for the Americas

OSD- Onchocercal Skin Diseases

REMO- Rapid Epidemiology Mapping of Onchocerciasis

WHO – World Health Organization
ABSTRACT

Background

*Onchocerca volvulus* is a filarial parasite that causes onchocerciasis or ‘river blindness’. Two strains of the parasite exist in West Africa namely, savannah and forest strains. They differ significantly in epidemiology, disease severity and are specific to different vectors. The savannah strain found in West Africa is associated with blindness while the forest strain, on the other hand, causes less severe ocular diseases even in individuals with high parasite load. Information obtained from some workers of the Onchocerciasis Chemotherapy Research Centre who carried out some investigations in the Nkwanta North district suggested that the MF of the parasite appear morphologically longer, a character that is associated with the savannah strain. However, the preponderance of the ocular manifestations in patients that are usually associated with the savannah strain was absent in the patients. The lack of empirical data to address this issue calls for further investigation and research in this area. Therefore, this study was aimed at characterizing the strain types of *O. volvulus* present in these communities and evaluating clients for clinical lesions of onchocerciasis.

Methodology

Subjects who consented to participate in the study were physically examined for clinical signs of onchocerciasis, particularly; skin rashes, depigmentation (leopard skin), visible and palpable nodules as well as visual acuity assessment using the Snellen chart. Skin snips were collected and examined microscopically for *O. volvulus* MF. The residual skin snips were analyzed for *O. volvulus* DNA using conventional PCR. A nested-PCR was performed on positive samples with a forest strain specific primer to further characterize the strain type.
Results

A total of 218 participants were enrolled. The most predominant clinical manifestations among the participants was rashes/itches 15.1% (33/218) followed by visual impairment (low vision, severe low vision and profound low vision) 8.3% (18/218). Palpable nodules were found in only 0.5% (1/218) of the study participants while lizard and leopard skin presentations were absent. About 9.2% (20/218) participants were positive for *O. volvulus* DNA PCR as compared with 3.7% (8/218) by microscopy (p< 0.05). All the 20 *O. volvulus* samples were classified as savannah strains by the nested PCR analysis.

Conclusion

The results from this study suggest that the Nkwanta North district is endemic for savannah strains of *O. volvulus*. The prevalence of the savannah strains in these communities may indicate a changing trend in the vector population as a consequence of deforestation and climate change.

The prevalent clinical manifestations found among the study subjects were predominantly skin rashes/itches and ocular lesions with blindness in just 0.5% of the participants. The generally low prevalence of clinical manifestations and MF in skin snip microscopy is an indication of success of several years of control activities in these communities in spite of evidence of disease transmission in the area.
CHAPTER ONE

1.0 INTRODUCTION

1.1 General introduction

Onchocerciasis or ‘river blindness’ is one of the neglected tropical diseases (NTDs) that causes both health and socio-economic problems in the affected communities (Crump et al., 2012). It is a chronic disease caused by the filarial nematode parasite, *Onchocerca volvulus*. Onchocerciasis is endemic in 30 countries in Africa which accounts for over 99% of people infected worldwide. It is also present in small foci in 6 Latin American countries as well as Yemen (WHO, 1995, 2010). It has been estimated that about 123 million people globally were at risk of contracting the infection with 18 million actually infected of whom 500,000 were severely visually impaired. In addition, 270,000 were completely blind due to the disease (WHO, 1995). However, a more recent estimates indicated that about 37 million people are infected with at risk population in Africa standing at 90 million (Basáñez et al., 2006). In Ghana, the disease is endemic in nine (9) out of the ten (10) regions with an estimated 3.2 million people in 3,204 communities in 66 districts at risk of the infection (Taylor et al., 2009).

Infection with *O. volvulus* results in relentless itching and debilitating skin lesions as well as visual impairment and ultimately blindness. Onchocerciasis has serious socio-economic consequences, which includes depopulation of arable lands along river valleys. It also leads to reduction in productivity of affected persons (Murdoch et al., 2002). The disease is second to trachoma as the leading cause of blindness due to infection in the developing world (Thylefors
Onchocerciasis is least prevalent in individuals aged between 0 and 10 years, but highest in those aged between 20 and 30 years (Anosike & Onwuliri, 1995; Michael, et al., 1996; Little, et al., 2004). The reason for the low prevalence in the 0 to 10 year old group who are of school going age is largely due to reduced bites from the blackflies whose biting activity is greatest in the mornings. The disease is generally more prevalent in males than in females (Anosike & Onwuliri, 1995; Hailu et al., 2002; Wogu & Okaka, 2008). This is due to increased exposure to blackfly bites in men as they go about their daily tasks that include fishing, farming and hunting (Little et al., 2004; Wogu & Okaka, 2008).

Onchocerciasis exhibits a wide range of clinical spectrum from an asymptomatic infection or generalized onchocerciasis to severe conditions such as blindness and chronic skin diseases (Hoerauf et al., 2005). The host’s immune response to the dead or dying microfilariae (MF) is responsible for the eye damage and skin manifestations in onchocerciasis (Tamarozzi et al., 2011). It has been proposed that an rickettsia-like endosymbiont bacterium of *O. volvulus*, *Wolbachia* rather than the parasite itself is the driver of the immunopathology associated with the disease (Andre et al., 2002; Gillette-Ferguson et al., 2006).

There is no protective vaccine or chemoprophylactic drug against *O. volvulus*; therefore the control and elimination programmes being carried out currently depend on ivermectin (IVM) as the only safe and effective drug available for mass drug administration (Webster et al., 2014; WHO, 2017). Ivermectin, as a single dose of 150 µg/kg body weight, is a highly microfilaricidal agent which clears MF from the skin for many months and also inhibits uterine release of MF by adult female worms (Schulz-Key, 1990; Basáñez et al., 2006; Lustigman & McCarter, 2007).
Simulium flies or blackflies are the obligate intermediate hosts of *O. volvulus* (Hall & Pearlman, 1999), and many species of these flies have been involved in the transmission of the parasite (Crosskey, 1990.). The relative vectorial roles of the flies have contributed to shape the different transmission patterns across the endemic areas (Basáñez *et al.*, 2006). *Simulium damnosum* sensu lato (s.l.) (species complex), consisting of about 60 cytoforms, is the vector responsible for more than 95 percent of onchocerciasis cases in Africa (Crosskey, 1990). The main vectors of *O. volvulus* in Latin America are *S. ochraceum* s.l. (the principal vector), *S. metallicum* s.l., *S. guianense* s.l. and *S. exiguum* s.l. (Boakye *et al.*, 1998).

The blackflies breed in fast-flowing aerated rivers and the infective stage larvae (L3) of the parasites are released from infected blackflies when they take blood meal. In the human host, surviving infective stage larvae undergo two moults to develop into adult male and female worms which live inside thick fibrous nodules (Basáñez & Boussinesq, 1999).

### 1.2 Research problem

Prior to the implementation of the Onchocerciasis Control Programme (OCP), the risk of onchocercal blindness was very high in the West African savannah areas. In some communities, blindness reportedly affected up to 50% of adults and consequently for the fear of contracting the disease, people abandoned the fertile lands along river valleys. In the 1970s, a whopping US$30 million was estimated as economic losses due to onchocerciasis, making it a major obstacle to socioeconomic development (WHO, 2016).
Despite almost four decades of onchocerciasis control in Ghana, the disease is still endemic in all regions of Ghana except the Greater Accra region with the at risk population of infection estimated at 3.2 million in 3,204 communities in 66 districts (Taylor et al., 2009).

There is evidence that in West Africa, at least two strains of *O. volvulus* exist (Cianchi et al., 1985; Dadzie et al., 1989; Remme et al., 1989). These strains differ significantly in their transmission by *Simulium* vectors, their general epidemiology and the severity of clinical manifestation (Duke, 1976). The savannah strain found in West Africa is associated with blindness in large proportions of individuals it infects while the forest strain, on the other hand, has been found to be less likely to cause blindness, even in individuals with high parasite load (Dadzie et al., 1989; Remme et al., 1989). Some reports indicated that the blindness rate among infected people in the savannah is up to a maximum of 15% which is higher than that in the forest where the rate is usually about 2%. In the forest areas severe eye problems such as sclerosing keratitis is mostly not common but, rather, skin manifestations predominate (Duke, 1981).

Information obtained from some workers of the Onchocerciasis Chemotherapy Research Centre (OCRC) who carried out some investigations in the Nkwanta North district suggested that the MF of the parasite appear morphologically longer, a character that is associated with the savannah strain. However, the prevalence of the ocular pathology that are usually associated with infection of the savannah strains is absent in these communities. This observation is casual and not based on any empirical data, warranting further research work. The present study therefore sought to identify the strain type present in these communities.
1.3 Justification

There is little evidence from available literature on the strain type present in the Nkwanta North district of Ghana. The study, if carried out, will add on to existing knowledge by providing information on the type(s) of strain present in that community. This information will be useful for disease mapping and treatment schedules and epidemiological investigation in the area. This information will also be useful in the search for chemotherapy and vaccine development since some drugs and vaccines could be strain specific. It will also be useful in monitoring drug resistance should this also be associated with a particular strain.

1.4 Aim of the study

The main objective of the study was to characterize the *Onchocerca volvulus* strains prevalent in the Nkwanta North District.

1.5 Specific objectives

The specific objectives of the study are to determine the:

1. *Onchocerca volvulus* strains prevalent in the Nkwanta North district;
2. predominant clinical manifestation of onchocerciasis among the population in the district.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Onchocerca volvulus*

The genus *Onchocerca* consists of 28 parasite species of large hoofed animals (Anderson, 2000) except the dog parasite *O. lupi* (Eberhard *et al.*, 2000; Egyed *et al.*, 2002) and the human parasite *O. volvulus* (Hall & Pearlman, 1999). The matured adult male of *O. volvulus* measures up to 5 cm in length and diameter of 0.02 mm while the much larger females measure between 30 cm and 80 cm in length and diameter of 0.04 mm (Fig 2.1) (Forgione, 2002). The adult worms are normally found in subcutaneous nodules or onchocercomata which are most easily seen on bony prominences (Okulicz *et al.*, 2004), where they live for up to 15 years (Ranganathan, 2012). The adult female worms generally remain in the nodules while the itinerant adult male worms move between nodules inseminating the females (Brattig, 2004). The much migratory unsheathed MF (Fig 2.2) which are usually associated with disease manifestations measure between 220 µm and 360 µm in length and diameter between 5 µm and 9 µm. The MF have a life span of up to 2 years (CDC, 2016).
Figure 2.1. Adult female worms of *Onchocerca volvulus*


Figure 2.2. Microfilaria of *Onchocerca volvulus*

*Source*: [https://web.stanford.edu/group/parasites/ParaSites2006/Onchocerciais/parasite.htm](https://web.stanford.edu/group/parasites/ParaSites2006/Onchocerciais/parasite.htm)
2.2. The genome of *Onchocerca volvulus*

2.2.1 The coding sequence

The nuclear genome of *O. volvulus* has been estimated to be approximately $1.5 \times 10^8$ bp consisting of three pairs of autosomes and a pair of dimorphic sex chromosomes (Donelson *et al.*, 1988; Post, 2005). Based on the analysis of RNAseg data from eight stages of *O. volvulus* life cycle the total number of protein-coding genes was predicted to be 12,143. Approximately 91% of these genes were shared with other nematodes with just 9% being specific for *O. volvulus* which shares little or no homology with other human nematodes (Cotton *et al.*, 2016). Structurally, these genes are compact averaging approximately 5 kbp in size and interrupted repeatedly by several small introns measuring between 100 and 300 bp. Similarly, the exons are small with median length of 124 bp (Unnasch & Williams, 2000).

There is a high level of variation observed in gene density, GC content and repeat density of *O. volvulus* genome which is relatively AT-rich with an overall AT content of 68% but with slight variation between the intron sequences (73%) and exons (61%) (Unnasch & Williams, 2000). The intron-exon boundaries of these genes generally follow the GU-AG rule which is characteristic of the splice donor and acceptors of other vertebrate organisms except that there are some observed variations in the conserved GU and AG sequences at the 5´ and 3´ ends of the introns. It has also been observed from the genes examined so far that the most conserved positions in the intron are five nucleotides from each end. This conclusion was drawn from the finding that at the 5´ end of the intron, a purine is found at position +5 in 83% of the introns and at the 3´ end, a pyrimidine is found at position -5 in 88% of the introns (Aroian *et al.*, 1993).
The mRNAs of *O. volvulus* contain 22-nucleotide spliced leader (SL) at their 5’ ends with the genes encoding the SL RNA encoded in the intragenic region of the spacer of the 5S rRNA gene cluster (Zeng *et al.*, 1990).

### 2.2.2 The non-coding sequences

The best characterized non-coding sequence of *O. volvulus* is that of the O-150 family. It is a distinct, variable and tandemly repeated sequence with a unit length of approximately 150 bp which is organized into large tandem arrays (Erttmann *et al.*, 1987; Meredith *et al.*, 1989). Cross hybridization and PCR experiments using degenerate primers have shown that these sequences are found only in the genus *Onchocerca* but not in any other nematodes or the vectors. Thus, probes and primers can be used to identify specific sequences within the O-150 family to distinguish *O. volvulus* from other *Onchocerca* species as well as characterize the strains of *O. volvulus* (Erttmann *et al.*, 1987 and 1990; Ogunrinade *et al.*, 1999; Adewale *et al.*, 2005).

### 2.2.3 The mitochondrial genome

The mitochondrial genome of *O. volvulus* is the smallest among the metazoan mitochondrial genomes described to date, only 13,747 bp in size (Keddie *et al.*, 1998). In keeping with the compact nature of the genome, four gene pairs overlap, eight contain no intergenic regions and the remaining gene pairs are separated by small intergenic regions with sizes ranging from 1 to 46 bp. The genome contains two ribosomal RNA genes, genes for 12 mitochondrial proteins and genes for 22 transfer RNAs (Keddie *et al.*,1998). The protein-coding genes of the *O. volvulus* mitochondrial genome exhibit extreme codon bias, where for 15 out of the 20 amino acids, a single member of the codon is used more than 70% of the time. There is limited
intraspecific variation in both the nuclear and mitochondrial genomes of *O. volvulus* (Unnasch & Williams, 2000).

### 2.3 Life cycle of *Onchocerca volvulus*

The life cycle of *O. volvulus* begins when the adult blackfly during a blood meal ingests the MF. After ingestion, the MF which survive the peritropic membrane that forms around the blood meal penetrate the midgut and migrate to the thoracic muscles of the blackfly and differentiate into L1 larvae. By 96 hours the L1 undergo the first moult to form L2 larvae which after a second moult by day 7 differentiate into the third-stage infective larvae (L3) (Burnham, 1998). The L3 now move to the mouth parts of the blackfly. The cycle of transmission continues when during a blood meal, an infected blackfly introduces infective filarial larvae onto the skin of the human host, where they penetrate into the bite wound (Burnham, 1998; CDC, 2016). Once in the subcutaneous tissues the larvae undergo moulting to L4 stage to reach adult stage in about one year (Bari & Rahman, 2007) and become encapsulated in the nodules (Burnham, 1998). The female worm produces numerous oocytes which when not fertilized degenerate within the uterus (WHO, 1995). When the female is fertilized, MF develop in 3-12 weeks and are released from the uterus. MF move freely through the skin and connective tissue and ultimately reach the eye. They can be found also in the blood, cerebrospinal fluid, urine and internal organs (Duke, 1993).
Figure 2.3. Life cycle of *Onchocerca volvulus*

https://www.cdc.gov/dpdx/onchocerciasis/index.html
2.4 Epidemiology and socioeconomic significance of *Onchocerca volvulus*

Onchocerciasis is endemic in 37 countries, of which 30 are in sub-Saharan Africa. The endemic area starts from Senegal in the west to Ethiopia in the east and extends to the south of the equator from Angola in the west to Tanzania in the east. Pockets of onchocerciasis exist in Sudan and Yemen (WHO, 2008). The disease is also endemic in small foci in 6 Latin American countries. The disease burden of *O. volvulus* has been largely underestimated in earlier literature with just 18 million people reportedly infected with onchocerciasis (WHO, 1995). However, since then, the true extent of the disease burden has been determined by the Rapid Epidemiology Mapping of Onchocerciasis (REMO), which uses the prevalence of palpable nodules as proxy for infection. Thus, by the end of 2005, using REMO, over 22,000 additional villages outside the OCP region have been surveyed leading to the discovery of a lot more endemic areas. As a result, the current prevalence of onchocerciasis globally is estimated at 37 million with about 90 million people in Africa at risk of infection (Basáñez et al., 2006).

Onchocerciasis is a clinical condition which is generally characterized by skin, eye, lymphatic and sometimes systemic manifestations with the most severe lesion being blindness (Nwoke, 1992). It has been estimated that approximately 270,000 people are blind and 500,000 suffer from visual impairment globally as a direct result of onchocerciasis (WHO, 1995). A further 40,000 new blind cases are added to these figures each year (Alonso et al., 2009).

In addition to the debilitating health problems, onchocerciasis have caused serious social and economic problems to individuals and entire communities. For example, Remme (1989) quoting from an unpublished document of Rolland and Balay (1986), stated that over 41,000 km$^2$ of fertile land in river valleys in Burkina Faso was uninhabited as a result of onchocerciasis.
It has been reported that onchocerciasis contributed to the loss of an estimated 1 million DALYs annually globally, with severe itching accounting for 60% and visual impairment and blindness making up the remaining 40% (Remme, 2004). It has been documented that the presence of onchocercal skin lesions affected the ability of the patients to interact with peers thereby reducing their marriage prospects. Onchocercal skin disease (OSD) has also been blamed for poor school performance and a high dropout rates in affected communities. In addition, productivity of these individuals is affected as a result of time spent out of work and health related costs. Furthermore, victims of onchocerciasis suffer embarrassment, sleeplessness, and reduced concentration (Wagbatsoma & Okojie, 2004; Wogu & Okaka, 2008).
Figure 2.4. Distribution of onchocerciasis worldwide, 2014. (Source: WHO, 2015)
2.5. Clinical manifestations and pathogenesis of onchocerciasis

Previously, the view was held that filarial products were the major causes of the underlying inflammatory reactions, however, there is now a growing evidence to show that an obligatory endosymbiotic rickettsia-like bacteria, *Wolbachia* have been incriminated in the clinical manifestations of the disease and in adverse reactions after treatment (Hoerauf *et al.*, 2003). The main clinical manifestation of the disease are mainly observed in the eyes and skin with troublesome itching being the most common early clinical symptom (Alonso *et al.*, 2009). Although the mechanisms are not fully understood, musculo-skeletal pains and reduced body mass index are the systemic conditions associated with onchocerciasis (Kale, 1998). In addition, there is evidence from available literature which links onchocerciasis to epilepsy (Marin *et al.*, 2006; Pion *et al.*, 2009).

2.5.1 Ocular onchocerciasis

It is now understood that the migratory MF enter the cornea from the skin and the conjunctiva leading to ocular pathology which is generally classified into anterior and posterior eye diseases (Abiose, 1998).

The posterior ocular onchocerciasis presents with atrophy of the retinal pigment epithelium which later becomes widespread. Evidence from experimental studies showed that autoimmune responses are involved in posterior ocular onchocerciasis. This was based on the observation that patients show persistent, low level, progressive pathologic changes of the retina and pigment epithelium even after treatment (Semba *et al.*, 1990). The role of reactive antigens in posterior ocular onchocerciasis was demonstrated by McKechnie and colleagues (1997), who injected rats with 39 kDa *O. volvulus* protein and found that the antibodies cross-react with a 44
kDa, human retinal protein. They observed that the animals developed many pathological changes in the posterior region but none in the anterior region of the eye (McKechnie et al., 1997).

The anterior segment onchocerciasis mostly affects the cornea even though other parts of the anterior segment can be affected. This is initiated by inflammatory reactions to dead or dying MF and presents as completely separate areas of corneal opacification (punctate keratitis). As a result of heavy infection or continued exposure to the parasites, these opacities coalesce and sometimes become hyper pigmented (sclerosing keratitis) leading to visual impairment and ultimately to blindness. Blindness resulting from onchocerciasis is mainly due to this condition (Pearlman & Hall, 2000).
Figure 5. Sclerosing keratitis in onchocerciasis. © Ian Murdoch & Allen Foster, 2001

Although onchocerciasis is associated with skin and eye lesions, the disease pattern varies considerably between geographical zones with ocular pathology being more common in hyperendemic localities within the savannah bioclimes while the forest communities are characterized by dermal manifestations of the disease (Dadzie et al., 1989; Murdoch et al., 2002). This difference in disease presentation has been attributed to many factors but evidence from clinical, epidemiological, and genetic studies have all shown that *O. volvulus* exists as two strains in West Africa. Some researchers have attempted to link the differences in pathogenesis between the two strains of *O. volvulus* to the differences in their *Wolbachia* load. The evidence in support of this observation was provided by the quantitative measurement of the amount of *Wolbachia* DNA per nuclear genome of adult *O. volvulus* and it was found to be significantly higher in savannah strains than in forest strains (Higazi et al., 2005). This correlation between *Wolbachia* DNA copy number and blindness was disputed by Armoo et al., (2017). Much as Armoo and colleagues also found significant heterogeneity in the *Wolbachia* DNA ratio between savannah and forest strains, they found the linkage problematic. They argued that because Higazi and colleagues used whole nodule for the analysis which means that there could be an unknown mix of parasites it is difficult to understand what the *Wolbachia* density actually means in the light of the findings of Higazi and colleagues. Armoo and colleagues held the opinion that histological data from studies conducted on *Brugia malayi* by Fischer, et al, (2011) suggest less variation in *Wolbachia* density in MF and therefore concluded that since the immunopathology is caused by the MF and not adult worms the *Wolbachia* density may not be the cause of the difference in pathology by the parasites in the two ecotypes.
2.5.1 Onchocercal skin disease

The skin is the main organ affected by onchocerciasis with a variable spectrum of skin lesions (Murdoch et al., 1993). The initial manifestations of cutaneous onchocerciasis which can occur anywhere include itching, scratching and alterations in skin pigmentation (Bari & Rahman, 2007). The mildest form of cutaneous onchocerciasis presents as itching with localized maculopapular rash which may disappear completely without any treatment or may progress to chronic papular dermatitis. In some cases, bleeding, ulceration and secondary infection may occur as a result of excessive scratching (Burnham, 1998). The pathology of onchocercal skin disease may be associated with generalized lichenified skin condition known as “leopard skin” (Greene et al., 1983). With prolonged exposure to active infections, degenerative skin changes usually set in with the destruction of elastic fibres which leaves the skin very thin and wrinkled. The atrophied skin begins to sag, resulting in the so-called “hanging groin” in extreme cases (Greene et al., 1983; Brattig et al., 1994).

A less common and localized chronic papular dermatitis called Sowda is often confined to one extremity and is most commonly found in certain geographical regions such as Sudan and Yemen. This condition is associated with local lymphadenopathy as a result of exceptionally strong IgG response (Cabrera et al., 1988; Murdoch et al., 1993).
Figure 6. Lichenified onchodermatitis in a young male


Figure 7. Chronic onchodermatitis with Leopard spotting over lower legs


Figure 8. Chronic onchodermatitis producing a Lizard skin appearance in a young patient

2.6 Parasite, vector and host dynamics of onchocerciasis

The clinical pattern of onchocerciasis with regards to the preponderance of blindness and skin lesions, varies considerably between geographical zones and even between different ecotypes within a single region (Remme et al., 1989). The most striking of these differences is mostly in the prevalence of more blindness due to onchocerciasis in the savannah than forest regions of West Africa. Among the savannah populations, blindness is present in hyper endemic communities with little or no blindness found in forested communities with a comparable level of endemicity (Duke, 1981; Dadzie et al., 1989; Remme et al., 1989). These observations of greater severity and high preponderance of blindness in the savannah regions were the reasons why the Onchocerciasis Control Programme (OCP) was originally limited to the savannah regions of West Africa (WHO, 1987).

A number of hypotheses have been put forward to explain this difference in clinical manifestation of onchocerciasis in the savannah and forest regions but the most widely accepted hypothesis is that intrinsic differences exist among the strains of parasites occurring in the forest and savannah zones (Duke, 1981). Initial evidence to support this “strain difference” hypothesis was provided by vector switch experiment conducted in Cameroon (Duke, 1966; Duke et al., 1966). They concluded from the findings that separate strains of *O. volvulus* exist in the forest zones of Cameroon and that of the Sudan savannah each of which is adapted for transmission by a different form(s) of *S. damnosum* s.l.

Also, this savannah-forest strain hypothesis was experimentally demonstrated by subconjunctival injection of forest and savannah strains into rabbits by Duke and colleagues (1972). They observed that MF of the savannah strain induced a more severe inflammatory
response in the cornea compared to those of the rain forest strain (Duke & Anderson, 1972; Garner et al., 1973). Another evidence in support of the strain difference hypothesis came from isoenzyme studies on adult *O. volvulus* obtained from representative bioclimatic zones in Zaire, Ivory Coast and Mali (Cianchi et al., 1985). Even though they used a limited number of samples, Cianchi and colleagues concluded that there are genetic differences between the savannah and forest strains. Subsequent studies using oligonucleotide DNA probes led to the identification of DNA sequences specific for the two strains of the parasite (Erttmann et al., 1987 and 1990) and classification of these strains based on sequence showed that strains from the forest zones are different from those from the savannah regions (Meredith et al., 1989; Zimmerman et al., 1993).

However, this “two strain” hypothesis does not seem to apply to parasites from other regions of Africa. For example, using the forest strain specific probes, Fischer et al. (1996) observed a pattern of hybridization which does not fit the classical savannah-forest categorization i.e. neither the forest nor savannah probes hybridized with the *S. neivei*-transmitted *O. volvulus* in Uganda. Prior to this finding, Kron and Ali (1993), reported that the DNA sequence of O-150 family from *O. volvulus* isolates obtained from northern Sudan were different from those obtained from West Africa. This goes to support the preliminary hypothesis that there are differences in the parasites present in the western and eastern foci of onchocerciasis in Africa (Kron & Ali, 1993). In another study conducted in Sudan by Higazi et al. (2001) in which O-150 repeat analysis performed on DNA of parasites obtained from the three onchocerciasis hotspots in Sudan and other parts of Africa were compared, they found that sequences from isolates obtained from eastern Sudan and Yemen are genetically indistinguishable from those obtained from West Africa. However, they observed that clinical and epidemiological picture of
the disease seen in these foci in Sudan and Yemen do not resemble the pattern observed in West Africa.

A complication of the forest savannah dichotomy is the rampant deforestation in the West African sub-region which has allowed invasion by savannah flies in these areas which were previously not ecologically suitable for them (Baker et al., 1990). High levels of blindness (5.5%) was reported in a deforested region of Sierra Leone (Zimmerman et al., 1992; Wilson et al., 2002) This observation was attributed to the fact that the savannah species, *S. damnosum* s.s and *S. sirbanum* are able to migrate over a distance of 500 km with the attendant possibility of reintroduction of *O. volvulus* into areas previously brought under control (Wilson et al., 2002).

*Simulium damnosum* Theobald complex are currently the only known vectors of human onchocerciasis of which nine species have been identified in the areas covered by the OCP. These are *S. damnosum* s.s., *S. squamosum*, *S. sanctipauli*, *S. leonense*, *S. soubrense*, *S. yahense*, *S. sirbanum*, *S. konkourense* and *S. dieguerense*. Using chromosomal studies some variant forms have been identified within these species (Boakye, 1993).

According to the *Onchocerca-Simulium* complexes concept which involves savannah and forest strains of the parasite, the vectors also differed in these bioclimatic regions (Duke et al., 1966). Evidence now abounds that forest vectors such as *S. yahense* and members of the *S. sanctipauli* sub-complex have higher average parasite loads than in savannah vectors such as *S. damnosum* and *S. sirbanum*. Furthermore, the number of infective larvae transmitted by *S. damnosum* and *S. sirbanum* are generally lower than those transmitted by *S. yahense* and by most members of the *S. sanctipauli* sub-complex (Cheke & Garms, 2013). The two strain hypothesis has been used to explain the observations that the savannah vectors transmit blinding
form of the parasite but Garms and Cheke (2013) countered with arguments that there were reports of blindness in forest areas where the vector was *S. yahense*. All these data put together led to Fischer and Buttner (2002) to suggest that there could be a spectrum of different strains of *O. volvulus* and they stated that “It appears reasonable to conclude that several different strains of *O. volvulus* occur throughout its large distribution area, but strain differences are not sufficient to explain all the geographic variation of the disease. The human host, biting habit of the vector or environmental factors may also influence the clinical picture of onchocerciasis.”

The competence of a member of a particular vector species complex to transmit parasite strain was demonstrated through a number of cross-infection experiments in which flies were fed on MF of the same and distant localities. These localities were as diverse as within West Africa, between West Africa and Guatemala (De Leon & Duke, 1966), West Africa and northern Venezuela, Guatemala and northern Venezuela (Takaoka *et al.*, 1986) and then between the northern and Amazonian foci within Venezuela (Basáñez *et al.*, 2000). The conclusion drawn from the results of these experiments was that there is a strong local adaptation between the parasites and vectors within well-established endemic areas. Thus, regardless of location, these vectors have their own unique parasite transmission characteristics in that even if the “forest” and “savannah” flies are living together, they will transmit only their respective parasites (Cheke & Garms, 2013).

**2.7 Laboratory diagnosis of Onchocerca volvulus**

The tools for diagnosis of onchocerciasis in the laboratory include examination of skin snips by microscopy for emergent MF, the Mazzotti test, detection of antibodies to onchocercal antigens
or use of highly sensitive polymerase chain reaction-based (PCR) techniques for detection of MF DNA in skin snips (Udall, 2007; Winthrop et al., 2011).

### 2.7.1 Skin snip microscopy

Microscopic examination of skin snips is the most widely used standardized technique for onchocerciasis in many endemic regions. Samples are usually collected from the scapula, over the iliac crest or calf (Murdoch, 2012). It is prone to low sensitivity in light infection when MF tend to be more aggregated in host skin. Studies have shown that sensitivity of skin snip microscopy depends on the number of snips taken, the anatomic site from which samples are taken, the composition of the medium and the duration of snip incubation (Collins et al., 1980; Taylor et al., 1987). Skin snip microscopy however, is very specific but is becoming gradually unacceptable for many people because of its invasiveness (Boatin et al., 1998).

### 2.7.2 Mazzotti test

This is an indirect method to demonstrate the presence of MF in the skin by the administration of diethylcarbamazine (DEC). The diethylcarbamazine inhibits neuromuscular transmission in nematodes leading to the death of *O. volvulus* to produce such reactions as itching, rash and sometimes lymphadenitis (often referred to as Mazzotti reactions) which demonstrates the presence of MF in the skin (Toè et al., 2000). In the initial method, one 50 mg oral dose of DEC was used. This test is sensitive but yields false negative and false positive results. The false positive results according to Awadzi et al. (2015) may be due to the presence of other skin dwelling MF, for example *Mansonella streptocerca* which are sensitive to DEC. The oral test is seldom used because of the potential adverse reactions such as vomiting, hypotension and, in rare cases, sudden death (Bari & Rahman, 2007). To avoid the systemic issues associated with
oral administration of DEC, a major modification of the test has been made where it is applied topically as a “patch” which produces a local reaction to the dying MF at the patch site (Bari & Rahman, 2007).

### 2.7.3 Immunological tests

These tests cannot differentiate between previous and current infections but have found utility in control programmes as surveillance tool. Initial protocols suffered from cross reactivity with other nematodes but following the use of specific recombinant *O. volvulus* antigen, Ov16 towards the IgG4 subclass of antibodies, sensitivity and specificity of these tests have improved significantly. Many antibody tests have been identified as candidate tests for diagnosis of human infection and as surveillance tools for control programmes but the major drawback is the need for laboratory infrastructure to support performance of Enzyme Linked Immunosorbent Assay (ELISA) tests (Weil *et al*., 2000). However, a rapid format immunochromatographic test which is a point of care test to detect antibodies to Ov16, a recombinant *O. volvulus* antigen has led to significant improvement in performance of these antibody tests (Chandrashekar *et al*., 1996).

### 2.7.4 Molecular techniques

The direct skin snip microscopy for *O. volvulus* MF remains the gold standard but as has been discussed elsewhere are relatively not sensitive when MF densities are low (Taylor *et al*., 1987). Amplification of the parasite DNA in skin snips by Polymerase Chain Reaction (PCR) techniques targeted at the O-150 repeat sequence provides high sensitivity for diagnosis of onchocerciasis (Boatin *et al*., 2002). The PCR technique can be conventional or quantitative. In the conventional method, PCR products are separated on 2% agarose gel and the results
determined as positive or negative based on the presence of a specific visible band sizes using UV light. In the quantitative or the Real Time PCR the results are determined using automated measurements of fluorescence and so are less prone to contamination (Lloyd et al., 2015).

Another molecular technique which is currently being used in the detection of *O. volvulus* is the isothermal amplification method which in contrast to the PCR test does not require temperature cycles. The loop mediated isothermal amplification (LAMP), is one of the most commonly used isothermal amplification technologies currently in use. The principle is based on use of two primer sets that recognized six different sites on the DNA of interest and an optional third set of primers, often referred to as loop primers to accelerate the reaction (Notomi et al., 2015). The loop mediated isothermal amplification technology offers many advantages over other molecular diagnostic techniques because it is rapid, simple and very specific. Using LAMP on skin biopsies collected from endemic areas in Ghana, Lagatie et al. (2016) found the sensitivity of LAMP to be 88.2% and specificity of 99.2% compared with qPCR. Molecular techniques now provide the most sensitive tool for monitoring success of mass drug administration (MDA) using pool screening of blackflies (Lagatie et al., 2016).

### 2.8 Onchocerciasis control

In response to the rampant blindness in the savannah regions of West Africa, the World Health Organization (WHO) in collaboration with other United Nations agencies launched the Onchocerciasis Control Programme (OCP) in 1974 with the objective of eliminating onchocerciasis as a public health problem. This programme was initially carried out in 7 countries but was later expanded to cover 4 additional countries bringing the total number of countries covered by the OCP to 11. This was a vector control programme using weekly aerial
spraying of breeding sites of the blackflies with insecticides. This programme was phenomenally successful in reducing the transmission, incidence and blindness in these countries (Levine, 2007). In 1988, the OCP supplemented the aerial spraying with mass distribution of ivermectin (Molyneux et al., 1995; Boatin, 2008). By the end of the programme in 2002, it was estimated that 600,000 cases of blindness was averted with about 18 million children born in regions free from the risk of blindness. Also, about 25 million hectares of land have been reclaimed and safe for resettlement (Hopkins, 2005).

The success of OCP notwithstanding, the disease still remains uncontrolled in other countries endemic for onchocerciasis especially in the forest regions of West, Central and Eastern Africa where aerial spraying was not considered to be cost-effective or technically feasible (Levine, 2007). As a result, a second and much expanded control programme called the African Programme for Onchocerciasis Control (APOC) was established in 1995 to extend treatment to the remaining 19 endemic countries in Africa based on annual or biannual mass administration of ivermectin in the affected communities (WHO, 2011). In 1992, the Onchocerciasis Elimination Programme for the Americas (OEPA) was launched with the target to eliminate transmission and morbidity by 2012 through biannual large-scale treatment with ivermectin. This programme has been largely successful in that Colombia (2013), Ecuador (2014), and Mexico (2015) and Guatemala (2016) have all been certified by WHO as having successfully eliminated onchocerciasis (Carter Center, 2016).

Ghana was one of the initial countries that benefited from the OCP from its inception. The main strategy of this programme was to interrupt transmission of parasites by adopting vector control method for a period in excess of the maximum lifespan of adult O. volvulus in the human host
(Remme et al., 1989). Following the licensing of ivermectin for use in humans in 1987, Ghana became one of the countries to start mass drug administration (MDA) (Basáñez et al., 2008). Onchocerciasis control is now being implemented under the Neglected Tropical Disease Control Programme (NTDCP) whose control activities officially started in 5 regions in Ghana on pilot basis in 2007 (Taylor et al., 2009).

Despite all these years of onchocerciasis control activities in Ghana, the disease is still endemic in the country (Taylor et al., 2009). The persistence of onchocerciasis in these communities despite many years of control efforts has been attributed to poor response of the adult worms to ivermectin (Osei-Atweneboana et al., 2011). However, other researchers attributed the continued prevalence of onchocerciasis to poor ivermectin distribution coverage leading to residual transmission (Cupp et al., 2007; Mackenzie, 2007). In a recent epidemiological studies (2014) in 56 onchocerciasis sentinel villages along the Black Volta, Tano, Pru, Tain, Asukawkaw, Oti, Daka, Bia, Densu, Birim and Densu river basins, the standard prevalence ranges from 0% to 17.2% with 14 out of the 56 villages having prevalence above 1%. Also, one significant observation from this study was a general reduction in the MF loads. It was concluded therefore that these low counts offer some hope for elimination of onchocerciasis. However, there are still communities with high prevalence of onchocerciasis despite control efforts with ivermectin use over the years which needs to be given a closer attention (GHS, 2015).

In order to curb the socioeconomic consequences and public health problems associated with onchocerciasis, the Neglected Tropical Disease Control Programme of the Ghana Health
Service (GHS) initiated biannual ivermectin distribution in hyper endemic communities and annual distribution in meso and hypo endemic areas (Turner et al., 2013).

In its five-year strategic plan, 2013-2017, the NTDCP of the GHS, reaffirmed its commitment to a national goal of using community directed treatment with ivermectin (CDTI) and other effective interventions for elimination of onchocerciasis (GHS, 2012).
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area and population

The study was conducted in four (4) communities located in the Nkwanta North District of Ghana. These communities were Kabonwole, Kofinyi, Lemina and Kanjo. The district which has a total population of 64,553 is one of the twenty five (25) districts in the Volta Region. It lies between latitudes 7°30’N and 8°45’N and longitudes 0°45’E and 0°10’W. The Nkwanta North district shares boundaries with the Nkwanta South district to the south, the Nanumba South district to the north, the Kpandai district to the west and the Republic of Togo to the east. The district Capital, Kpassa is located 270 km to the north of Ho (the regional Capital) (GSS, 2014).

The Nkwanta North district is located in the tropical climatic zone, and experiences double maxima of rainfall (i.e. between April and July; August and September) and experiences both the wet and dry seasons with the dry season occurring between November and March. The district lies in the transitional vegetation zone and is covered by savannah woodland and grassland. Pockets and remnants of semi-deciduous forest also exist. The district is endowed with a number of rivers and streams, the most important of which are River Oti and River Kpassa. The streams and rivers exhibit a dendritic pattern, which forms the Oti basin and so provide favourable breeding grounds for the vectors of O. volvulus (GSS, 2014).
Figure 3.1 Map of the Nkwanta North District (Source: GSS, 2014)
3.2 Sample size calculation

The highest prevalence among the onchocerciasis sentinel communities in the Nkwanta North district was 6.9% (Unpublished data from NTDCP, 2012). The minimum sample size was determined to be 98 using the formula,

\[ N = \frac{z^2 p(1-p)}{e^2} \]

where

N= minimum sample size required;

Z= confidence level at 95% (standard value of 1.96);

P= estimated prevalence of onchocerciasis;

e= 5 % margin of error; (Sullivan, 2016).

Based on this sample size, a total of 218 samples were collected to increase the power of the study.

3.3 Sampling techniques

3.3.1 Community selection and inclusion criteria

Information about study population, IVM treatment history and prevalence was obtained from the District Disease Control Officer of the Nkwanta North District Health Directorate of the Ghana Health Service (GHS). The four (4) communities for this study were selected based on disease prevalence and community accessibility.
3.3.2 Participants selection

Selected communities were informed through their Chiefs. The study participants in each community were mobilized by the district health information officer and the purpose of the study explained to them. Those who consented were all recruited for the study. Each individual was given a unique identification number and clinical assessment by an experienced Physician Assistant was conducted on all subjects as follows: the study subjects were examined physically for clinical signs of onchocerciasis such as skin rashes, depigmentation (leopard skin), visible and palpable nodules. The visual acuity of participants at far and near distances was determined using the Snellen chart and the ability to count figures at distance up to 6 m was also determined.

In addition, a structured questionnaire was administered to each participant to collect their personal data, clinical history and to determine their treatment status.

3.3.3 Sample collection and storage

All participants who consented from each community were skin snipped. Briefly, one skin snip from each iliac crest was collected using sterilized Walzer type corneo-scleral punch from each patient. Each snip was placed separately into a well of a microtitre plate containing 200 µl of physiological saline and incubated for 12-24 hours (overnight) at room temperature. The microtitre plates were sent to the laboratory and examined under an inverted microscope. The emergent MF were identified and enumerated. The MF from the positive wells were transferred into Eppendorf tubes containing 10% formalin and stored at room temperature. The residual skin snips were also put into Eppendorf tubes containing 80% ethanol and stored for further works.
3.3.4 DNA extraction of *O. volvulus* from skin snips

The DNA extraction was done using Quick-gDNA™ MiniPrep (Zymo Research Corporation, USA) according to the manufacturer’s instructions with slight modifications. Briefly, skin snips were cut into smaller pieces on a clean glass slide using a sterile scalpel. With the aid of sterile forceps, the macerated skin snips were transferred into appropriately labeled 1.5 ml microcentrifuge tubes. Genomic Lysis Buffer, 200 µl and 10 µl of proteinase K were added to the sample and vortexed for 30 seconds to ensure mixing of the lysing reagent with the skin snips. The mixture was incubated at 60°C for 1 hour with intermittent vortexing at 30 minute-intervals. The homogenate was pipetted into Zymo-Spin Column with 2 ml collection tube and spun at 10,000 xg for 60 seconds and the flow-through discarded. The Zymo-spin column was transferred into another 2 ml collection tube and 200µl of DNA Pre-Wash Buffer added, and centrifuged at 10,000 xg for 60 seconds. The Zymo-spin column was again transferred to another new 2 ml collection tube and 500 µl of g-DNA Wash Buffer added, and centrifuged at 10,000 xg for 60 seconds. The flow-through was discarded and the silica membrane was further dried by repeating the centrifugation at 10,000 xg for 60 seconds. The Zymo-Spin Column was placed in a sterile 1.5 ml microcentrifuge tube and 30µl DNA Elution Buffer was pipetted directly onto the Zymo-Spin Column membrane, incubated at room temperature for 10 to 15 mins, and then centrifuged at 8,000 rpm for 60 seconds to elute the DNA. For maximum yield of the DNA, the elution was repeated as described above into another tube as second elution.

3.3.5 Onchocerca volvulus DNA amplification using diagnostic primer

Polymerase chain reaction amplification for *O. volvulus* identification was carried out in Quanta Green SYBR buffer in a volume containing 7.5 µl of B-R One-Step SYBR Green (Quanta Biosciences, USA), 0.2 µl of each primer, 2.1 µl of nuclease free water and 5 µl of control and
test sample genomic DNA. The thermocycling programme started with 3 minutes denaturation at 94°C followed by 45 cycles of denaturation at 94°C for 30 seconds, 48°C annealing for 30 seconds and 72°C extension of 40 seconds with final extension for 5 minutes (PTC-200, BioRad, USA). The sequences of the primers used are **OV_sd_diag-F1**: 5’-GTCTTATAGGAGTTTCTGT-3’ and **OV_sd_diag-R1**: 5’-ACCCATCAACTTATCAAAAC-3’ (Atweneboana and colleagues (CSIR, 2016), unpublished).

### 3.3.6 Gel electrophoresis

The obtained PCR products were run on 2 % w/v agarose gel. Briefly, 2 g of agarose powder was added to 100 ml 1X TE buffer and dissolved by boiling in a microwave oven. It was allowed to cool to 60°C and 3 µl of ethidium bromide was added, swirled to mix and dispensed gently into the casting mold with combs and allowed to set.

Finally, 8 µl of the PCR products were checked by agarose gel electrophoresis with appropriate molecular weight markers (1 kb in multiples of 100 bp) inserted to determine the expected size relative to the marker. The marker, positive (forest and savannah) and negative controls as well as samples were loaded. Electrophoresis was run at 100V for 45 minutes. Gel was observed under a UV Trans-illuminator (BioDoc-it Imaging System, Cambridge, UK) and the molecular weights analyzed.

### 3.3.7 Nested PCR for *Onchocerca volvulus* strain identification.

Samples that were identified as *O. volvulus* with the diagnostic primers were selected for nested PCR for identification of strain type according to the protocol and primers used by Fischer et al, (1996) with slight modifications. Also, known forest and savannah strain samples were used as controls. Nest 1 was performed in a 15 µl volume containing 7.5 µl of B-R One-Step SYBR
Green (Quanta Biosciences, USA) mix, 0.2µl of each primer, 2.1 µl nuclease free water and 5 µl of genomic DNA. The thermocycling programme (PTC-200, BioRad, USA) started with 3 minutes denaturation at 98°C followed by 40 cycles of denaturation at 98°C for 30 seconds, 58°C annealing for 30 seconds and 72°C extension of 30 seconds and final extension for 5 minutes. The primers used are S3 5'-ATCATTTTGCAAAATGCG-3' and S4 5'-AATAACTGATGACCTATGACC-3'.

The product of the first PCR (nest 1) was diluted 1:20 and 5 µl was used for strain specific amplification in a 15 µl volume containing 7.5 µl of B-R One-Step SYBR mix (Quanta Biosciences, USA), 0.2 µl of each primer and 2.1 µl of nuclease free water using the following cycling conditions: initial 3 minutes denaturation at 98°C followed by 45 cycles of denaturation at 98°C for 30 seconds 58°C annealing for 30 seconds and 72°C extension of 30 seconds with extended extension for 5 minutes (96 Universal Gradient PeQSTAR, UK). The following are the primers used: FA: 5' GCGGCATAAATCTGCAAATTC-3' and FB: 5'GATTTTTCCGACGAACAGGC3'

3.4 Statistical Analysis

The data obtained were entered into Excel and validated. The analysis was performed using R statistical software. Test of statistical significance was determined using the Chi-square test.
CHAPTER FOUR

4.0 RESULTS

4.1 Analysis of skin snip microscopy and *O. volvulus* DNA PCR results

Skin snips from a total of 218 participants were examined for the presence of *O. volvulus* MF by inverted microscopy and conventional PCR for *O. volvulus* DNA on residual skin snips. Of the 218 samples examined 3.7% (8/218) were positive for MF by skin snip microscopy and 9.2% (20/218) were positive for *O. volvulus* DNA by PCR on residual skin snips. Of the 8 microscopy positive samples, DNA from 7 residual skin snip samples showed amplification with *O. volvulus* DNA and one (1) did not show any amplification. The difference in performance of skin snip microscopy and PCR was statistically significant (p<0.05).

4.1.1 Analysis of *Onchocerca volvulus* positive results for skin snip microscopy and DNA PCR by age and sex

Males constitute 54.1% (118/218) and females 45.9% (100/218) of the study participants surveyed. About 60% (12/20) of those positive for *O. volvulus* by DNA PCR are females and 40% (8/20) being males while by microscopy, males were the majority with 62.5% (5/8) with females trailing with 37.5% (3/8). The age group with the highest prevalence by PCR was 11-20 year group with 35% (7/20) and the least being 51-60 year with 0%. However, among the 11-20 year group, only 12.5% (1/8) was positive by microscopy with the age groups having highest prevalence rates being 21-30 and 31-40 groups, both with 37.5% (3/8). Again the 51-60 age group had the lowest prevalence by microscopy with 0.0%.
4.1.2 Analysis of skin microscopy and DNA PCR results by occupation

In the Nkwanta North district, farming is the predominant occupation with 70.2% (153/218) of the respondents engaged in it. Majority of those positive for *O. volvulus*, 60% (12/20) by PCR and 75% (6/8) by microscopy are farmers. The civil/public servants had the least with positive rate for both PCR and microscopy of 0.0%.

4.2 Analysis of results of Clinical manifestations of onchocerciasis

Clinical manifestations identified in this study are skin rashes/itches, visual impairment and nodules. The most predominant clinical manifestation among the 218 participants screened was skin rashes/itches with a prevalence of 15.1% (33/218) followed by visual impairment with a prevalence of 8.3% (18/218). Of the 18 participants who had visual impairment, 55.6% (10/18) had low vision (≤6/60 ≤ 6/18), 38.9% (7/18) had severe low vision (≤3/60-6/60) and 5.6% (1/18) had profound low vision (3/60-NPL). Thus, overall, only, 0.5 % (1/218) (WHO criteria of acuity of <3/60) of the participants was suffering from blindness. Palpable nodules was found in only one person (1/218). Lizard skin and leopard skin lesions were not found among the participants examined.

4.2.1 Analysis of subjects manifesting onchocercal lesions by sex

Of the 33 participants who had rashes/itches 66.7% (22/33) were males and 33.3% (11/33) were females. Among those with visual impairment 72.2% (13/18) were males and 27.8% (5/18) were females. Also, of the 18 participants who were classified as having visual impairment, 10 had low vision of which 80% (8/10) were males and 20% (2/10) females. Severe low vision was found among 57.1% (4/7) males and 42.9% (3/7) female with the profound visual impairment. The only participant with blindness is a male.
4.2.2 Analysis of subjects manifesting onchocercal lesions by age groups

This section describes the distribution of onchocercal lesions in the four communities of Nkwanta North district by age. Of the 33 participants who had skin rashes/itches, the age group with most predominant lesion is 31-40 years, 27.3% (9/33) followed closely by 10-20 and 21-30 year groups both with 18.2% (6/33). As expected, visual impairment is commonest among the elderly group, 33.3% (6/18) and the least being among the 11-20 and 21-30 year groups with 5.6% (1/18) each.

4.2.3 Analysis of subjects manifesting onchocercal lesions by occupation

Of the 33 participants who had rashes/itches, 45.5% (15/33) were farmers. Students/pupils and traders both had 18.2% (6/33). Only one person in the public/civil servant category had rashes/itches. When the proportion of farmers with skin rashes/itches is compared with traders, fishermen and Civil/Public servants, the difference is significant (p<0.05).

A significant proportion, as high as 83.3% (15/18) of participants with visual impairment were farmers (p<0.05) including all the severe forms of low vision with only one participant each from student/pupil, public/civil servant and fisherman categories.

4.2.4 Analysis of demographic and treatment records of the study participants

The total number of participants recruited for this study was 218 made of 54.1% (118/218) males and females 45.9% (100/218) with the median ages of 35 and 30 respectively. The average length of stay of participants in the communities sampled were approximately 20 years for males and 18 years for females. The communities sampled were rural settings with majority,
69.3% (151/218) with no formal education and just 0.92% (2/218) with a tertiary education. The main occupation is farming 70.2% (153/218).

About 95% (207/218) of the participants have ever taken ivermectin treatment, as part of the community directed treatment with ivermectin programme, with the most recent being about one year prior to the sample collection.

4.3 DNA results analysis

4.3.1 Detection of *O. volvulus* using Diagnostic primers

The PCR assay used in this study amplified DNA from microfilariae in the skin snip using *O. volvulus* mitochondrial DNA primers **OV_sd_diag-F1**: 5'-GTCTTTATAGGAGTTTCTGT-3' and **OV_sd_diag-R1**: 5'-ACCCATCAACTTATCAAAAC-3'. Amplification of DNA from skin snips from Nkwanta north district and the control samples with the diagnostic primers confirms the PCR products as belonging to *O. volvulus*.

Figure 4.1 shows successful amplification from skin snips from one of the communities in the Nkwanta north district, Kabonwole (KA in lanes 3, 4, 7, 8 and 9) as depicted by visible bands on the agarose gel and figure 4.2 shows successful amplification from another community in the study area, Lemina (LM in lanes 3 and 4) and forest controls (FSP in lanes 5, 10, 11 and 12) and savannah controls (AB1, AB2, AB3, ASU 1, ASU 2 and ASU 3 in lanes 6, 7, 8, 9, 13 and 14 respectively.
Figure 4.1. Agarose gel electrophoresis pattern for amplification products of samples from Kabonwule (KA) in lanes 3, 4, 7, 8 and 9 by OV Diagnostic primers

Figure 4.2. Agarose gel electrophoresis pattern for amplification products of samples from Lemina (LM) in lanes 3, 4, and Controls from Agborlekame ABI, AB2 AB3 in lanes 6, 7, 8 and
Asubende ASU 1, ASU 2, ASU 3 in lanes 9, 13, and 14 and forest controls FSP 1, FSP 2, FSP 3 and FSP 4 in lanes 5, 10, 11, 12 respectively by OV diagnostic primer.

.  

4.3.2 Analysis of PCR test results for determination of *O. volvulus* strain using forest strain specific primers  
Differentiation of the isolates was done using a 107 bp long fragment of forest strain-specific DNA sequence on *O. volvulus* positive samples from the four (4) communities in the Nkwanta North district and controls from Agborlekame (AB) and Asubende (ASU) (all savannah) and samples from Cameroun (FSP) (forest samples). The test samples and the savannah control samples did not amplify with the forest specific primers whereas controls from the forest regions show amplification (Fig. 4.3), suggestive of the samples from the Nkwanta North district belonging to savannah strains.
Figure 4.3. Agarose gel electrophoresis pattern of two isolates from Nkwanta North district (KA 47 and LM 52) in lanes 3 and 4 and savannah controls in lanes 5, 6, 7, 8 and 9 showing no amplification with nested PCR primers. Lanes 2, 10, 11, 12 and 13 amplified with the forest-strain specific primer producing amplicon size of 153 bp.
CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The application of molecular techniques have aided in the description of *O. volvulus* isolates from different geographical regions. The close examination of the tandem repeat O-150 DNA sequences in particular has been useful in the differentiation of *O. volvulus* from other *Onchocerca* species (Meredith et al., 1989; Zimmerman et al., 1993) as well as the separation of *O. volvulus* parasites into savannah and forest strains (Meredith et al., 1991).

This study produced results which suggest that all the *O. volvulus* isolates from the Nkwanta North district were savannah strains. This is not surprising given the current climatic condition prevailing in the Nkwanta North district which lies in the savannah-forest mosaic where both forest and savannah strains are sympatric. As a result of human activities, these areas have been seriously deforested. The rampant deforestation in Ghana was confirmed by Wilson et al. (2002), who observed that as a result of human activities there has been substantial increase in the proportion of savannah vectors of *O. volvulus* in southern part of Ghana and Togo. The increase in the savannah vectors according to them will lead to increased transmission of savannah strains of the parasites in deforested areas. Nkwanta North district is not spared the effect of deforestation, hence this outcome.

This finding is not consistent with the observations made by Dordor and colleagues (unpublished data) where they found both savannah and forest strains among black flies caught in some communities in the Nkwanta North district. However, they found the predominant
strain to be that of savannah; 7 out of the 9 isolates characterized were suggestive of savannah strains and the remaining 2 being forest strains. Given the mobile nature of the flies, the identification of both forest and savannah strains of *O. volvulus* is not strange as those vectors harbouring forest strains could be making occasional incursion into the district at the time of fly-catch. Again, this finding is not consistent with the findings of Oyibo *et al.* (2002) in a study they conducted in the Lade District (in Kwara state) which is in the forest-savannah transition zone of Nigeria on adult worms harvested from nodules. They found both savannah and forest strains of *O. volvulus* in the same individual. They explained this occurrence to be due to either the simultaneous transmission of both savannah and forest parasites or existence of a “hybrid” form of *O. volvulus*. Participants in the Nkwanta North district of Ghana being in the forest-savannah transition zone could also have been exposed to simultaneous transmission of both forest and savannah strains of the parasite, however, only the savannah strains were able to possibly establish infection in the individuals sampled. Given that nodules contain a number of adult worms, it is possible to have a mix of parasites of different strains due to individuals being exposed to bites of both savannah and forest vectors provided both parasites are able to establish infection in that individual.

By skin snip microscopy, this study revealed that more males than females were infected with *O. volvulus*. This result is consistent with the findings of Wogu and Okaka (2008) who in their study observed that more males (27.5%) suffer from onchocerciasis than females (20%). Similar observations were made by Uttah (2010) who found 39.2% of the study participants to be males and 34.9% females and Nmorsi *et al.* (2002) who found more males (49.4%) than females (33.3%). The explanation for this observation is that males are more exposed to the bites of the vectors of the disease either as they go about their occupational activities such as farming and
fishing or by living close to the breeding sites. Akinboye et al. (2010) attributed this observation to the fact that men are less clad as they go about their activities compared to females and so are more liable to bites of the blackflies. In the Nkwanta North district, farming is the predominant occupation in which males are expected to be engaged than females. This observation is a contradiction to what was found by Akinbo and Okaka (2010) who found more females (93.1%) infected than males (74.5%) infected by *Onchocerca volvulus* in a study conducted in Ovia Northeast LGA in Edo state of Nigeria. The observation by Akinbo and Okaka of more females being infected than males is corroborated by the PCR results in our study where more females (60%) than males (40%) were classified positive using DNA PCR. Nkwanta North district is inhabited mostly by Konkombas (GSS, 2014) and among these tribes females are also engaged in farming just as the men if not more. Females in these communities do farming in addition to going to the river side to fetch water or wash by the river side with the attendant frequent exposure to insect bites. Therefore the result was expected.

This study revealed that prevalence of onchocerciasis was highest among farmers than any other occupational groups by both microscopy and DNA PCR. Similar observations were made by Okoro et al. (2014) who found a significantly higher prevalence (combined prevalence of 22.2% in the two Senatorial Districts in Ebonyi State, Nigeria) among farmers than traders, civil servants, and students. This difference in prevalence is due to risk of occupational exposure to the bites of the vectors. Civil/public servants did not record any infection with *O. volvulus* either by microscopy or DNA PCR in this study. The reason for this low prevalence is that this category of individuals are mostly working indoors during the day which is normally the biting period for the black flies and so are less exposed to the bites of these flies.
The commonest skin manifestation among the participants in the communities studied was rashes/itches) and is most prevalent among the 31-40 age bracket. The possible explanation for this observation is that this category of people are mostly the active working group and so are more exposed to frequent bites as they go about their farming, fishing or water fetching activities which are major risk factors of *O. volvulus* infection.

From the gross examination of the eye using Snellen chart, visual impairment was observed among 8.3% of the study subjects mostly among those above age 51 years. This is consistent with the observations made by Kamalu & Uwakwe (2014) where they found that ocular manifestations of onchocerciasis was more prevalent among the 50-62 year group. Blindness, visual acuity of <3/60 (WHO, 2005) was detected among 0.5% of the participants examined compared to 0.75% found in 3 sites sampled in Asante Akim district in Ashanti region of Ghana (Taylor *et al*, 2009).

This study revealed skin snip microscopy prevalence which is lower than the data obtained from the 5 onchocerciasis sentinel sites in the Nkwanta North district in 2012 (unpublished data from NTDCP) which has the highest prevalence at 6.9%. This low prevalence can be attributed to the impact of continued onchocerciasis control activities in the communities since about 95 % of the participants had ever had treatment with ivermectin as part of the CDTI with the most recent distribution done just about a year before sample collection. This finding is also consistent with epidemiological data obtained in 2014 from Asubende where the prevalence dropped from 5.9 % in 2007 to 3.2 % in 2014 (GHS, 2015).
The prevalence of clinical signs of onchocerciasis is generally low in the communities studied which corresponds to the low microscopy prevalence. This low prevalence is a good testament of the impact of ivermectin treatment in the district, nevertheless, the presence of skin MF is suggestive of the continued disease transmission.

Skin snip microscopy has been the method of choice for monitoring onchocerciasis control activities in Ghana. The sensitivity of microscopy depends largely on microfilaria load (Taylor et al., 1987; Basanez et al. 2008). Therefore with the sustained IVM mass drug administration and the reduction in microfilarial load as evidenced in the 2014 epidemiological data (GHS, 2015) calls into question the utility of microscopy for disease mapping as elimination programmes are expanded to cover areas of low endemicity given that the PCR method in this study identified 12 more additional samples previously classified as negative by microscopy.

5.2 Conclusion

The results from this study suggest that Nkwanta north district is endemic for savannah strains of *O. volvulus*. The prevalence of the savannah strains in these communities may indicate a changing trend in vector population as a consequence of deforestation and climate change.

The predominant clinical manifestation found among the study subjects was skin rashes/itches. The generally low prevalence of clinical manifestations and skin snip microscopy is an indication of success of several years of control activities in these communities in spite of evidence of disease transmission in the area.
5.3 Limitations

Owing to logistical challenges, refraction, slit lamp examination and ophthalmoscopy examinations were not done on the participants.

5.4 Recommendations

Further molecular characterization studies should be done in the other communities in the region to determine strain type(s) prevalent in these communities using both forest and savannah probes.

Also, more extensive study should be carried out in these communities with full ophthalmology examinations done on the participants to determine the true extent of visual impairment.

Based on evidence of the PCR test detecting more skin snip positive samples than microscopy, PCR-based techniques or more sensitive tools should be employed for monitoring onchocerciasis control activities.
REFERENCES


https://www.cdc.gov/dpdx/onchocerciasis/index.html


Excerpt from Medicine.com, Inc (abstracts).


Murdoch, M.E. (2012). Onchocerciasis up to date


APPENDICES

APPENDIX 1

PARTICIPANT INFORMATION FORM

Note: To be read or translated to the study subjects in a language they understand.

Dear Participant,

Your permission is kindly being sought to take part in a research study to determine the strain of *Onchocerca volvulus* prevalent in your area. This study is being conducted by Rowland Adukpo, an MPhil candidate from the School of Biomedical and Allied Health Sciences (SBAHS). I am asking you to take part because you live in this area and so could have been infected by the parasite.

**What the study is about:** There is evidence that, at least, two strains of *Onchocerca volvulus*, the parasite which causes onchocerciasis exist in West Africa and are transmitted by different types of blackflies. They are also different in the severity of disease they cause. The savannah strain found in West Africa is associated with blindness in large proportions of individuals it infects but the forest strains on the other hand, have been found to be less likely to cause ocular disease, even in individuals with high parasite load. Reports from field officers working on onchocerciasis research project in the North Nkwanta district suggested that judging from the morphological features the microfilariae type present are most probably of the savannah type. However, the preponderance of the ocular manifestations that are usually associated with
infection with savannah strains is absent. The purpose of this study is to characterize the strain type of *O. volvulus* prevalent in Kpassa and its environs.

**What we will ask you to do:** If you agree to be in this study, we will ask you a few questions and then take skin snips from your buttocks. The skin snips will be examined for the presence of microfilariae. Further investigations will be conducted on the microfilariae.

**Risks and benefits:** The risk associated with the skin snipping includes small cut, pain, discomfort and possible infection. The laboratory scientists and clinicians will take care of any such complications. Results of the tests will be communicated to health authorities in the district for appropriate action.

**Voluntary participation and confidentiality:** Taking part in this study is completely voluntary. You are free to withdraw at any time from the study. The information you give us will be used only for the study and not in any way that will harm you. The records of this study will be kept private. In any sort of report we make public we will not include any information that will make it possible to identify you. Research records will be kept in a locked file; only the researchers will have access to the records.

**Contact:** If you have any questions concerning the study you may contact Dr. Simon K. Attah at skwakuattah@yahoo.com or at 0277 520813 or Rowland Adukpo at rowlu@yahoo.com or at 0243 485320.

You will be given a copy of this form to keep for your records.
APPENDIX 2

INFORMED CONSENT FORM

I…………………………………………………………of………………………………………………
hereby certify that the contents of the above information has been read by me/ interpreted to me
in the…………………….language by……………………………………………………………….

I have perfectly understood the same, and thereby appended my signature/mark (Right
thumbprint) to this consent form as an evidence of my agreement to participate in this project. I
will be given a copy of this consent form after it is completed and signed.

Signature or thumb print of Participant/Guardian ______________________

Date ______________________

Your Name __________________________________________________________

Signature of person obtaining consent __________________ Date __________________

Printed name of person obtaining consent __________________ Date __________________
APPENDIX 3

ETHICAL CLEARANCE

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

My Ref. No. ...............

Mr. Rowland Adakpo
Department of Medical Microbiology
School of Biomedical and Allied Health Sciences
University of Ghana
Korle-Bu

Dear Mr. Adakpo,

16th May, 2016.

ETHICAL CLEARANCE

Protocol Identification Number: CHS-Et/M.7 – P 3.7/2015-2016

The Ethical and Protocol Review Committee of the College of Health Sciences on the 28th of April, 2016 unanimously approved your research proposal.

TITLE OF PROTOCOL: “Strain Determination of Onchocerca volvulus prevalent in the Nkwanta North District of Ghana”

PRINCIPAL INVESTIGATORS: Mr. Rowland Adakpo

This approval requires that you submit six monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee’s duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till 31st March, 2017.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: [Signature]

PROFESSOR ANDREW A. ADJEI
CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE

CC: Provost, CHS
Dean, SBAHS
Head of Department

P. O. Box 52, Korle-Bu, Accra, Ghana • Tel: +233 (0) 302666103/244061270 • Fax: +233 (0) 302666762
• Email: eprc@chs.edu.gh / provost@chs.edu.gh • Website: www.chs.ug.edu.gh
APPENDIX 4

QUESTIONNAIRE

Study Number___________________________     Date_________________________

Section A: Personal Data

Date of Birth ___________________

Gender: [ ] M     [ ] F

Level of education attained

No formal Education [ ]

Primary [ ]

Secondary [ ]

Tertiary [ ]

Other (specify) [ ]

Occupation

Farmer (Crop or animal) [ ]

Fisherman [ ]
Hunter [ ]

Trader [ ]

Public/Civil servant [ ]

Other (Student/pupils) [ ]

For how long have you been living in this community?

Have you ever received treatment for onchocerciasis? Yes/No

If yes, when was the last time?

Section B: Assessment of clinical signs and symptoms of onchocerciasis

Does the subject have? Please tick as appropriate

<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodule(s)</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>Rashes/Itching</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>Leopard skin</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>Lizard skin</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>Ocular lesion/visual impairment</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>Blindness</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>Other (specify)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>