EFFECTS OF LONG-TERM COMMUNITY TREATMENT WITH IVERMECTIN ON *ONCHOCERCA VOLVULUS* INFECTIONS IN HUMANS AND THE *SIMULIUM DAMNOSUM* VECTOR IN PARTS OF KADUNA STATE

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DECLARATION

I declare that the work in the dissertation entitled "Effects of Long-Term Community Treatment with Ivermectin on *Onchocerca volvulus* Infections in Humans and the *Simulium damnosum* Vector in Parts of Kaduna State" has been performed by me in the Department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

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Signature Date

CERTIFICATION

This dissertation entitled "EFFECTS OF LONG-TERM COMMUNITY TREATMENT WITH IVERMECTIN ON *ONCHOCERCA VOLVULUS* INFECTIONS IN HUMANS AND THE *SIMULIUM DAMNOSUM* VECTOR IN PARTS OF KADUNA STATE " by Sunday ISIYAKU, meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

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ABSTRACT

This study assessed the impact of long-term treatment with ivermectin in Kagarko, Lere and Kauru onchocerciasis foci of Kaduna State by parasitological, entomological and economic evaluation of the onchocerciasis control programme including its cost effectiveness. Epidemiological evaluation of infection status was done by parasitological examination of skin snips of individuals in 10 communities in Kagarko LGA of Kaduna State, where there was an 80.6% administrative coverage after 4 – 10 rounds of ivermectin treatment. Of the 973 (56% of total population) people examined only 3 (0.31%) were positive for microfilariae and none had ocular microfilaria. A total of 24,937 *Simulium damnosum* were caught in two rivers, Rivers Galma and Gurara in Lere and Kagarko foci respectively. No breeding site was identified in Kauru focus. The flies were analyzed for the presence of *O. volvulus* DNA using the O-150 PCR assay which detected 23 positive pools out of the 250 pools (100 flies/pool). The prevalence was calculated using the Poolscreen 2.0. The prevalence of infective flies (L3 in the head) was 0 – 1.5/1,000 flies in R. Galma and 0.38 – 0.83/1,000 flies in R. Gurara while prevalence of infected flies (L1 and L2 in the body) was 1 – 1.9/1,000 flies in R. Galma and 0.4 – 0.5/1,000 in R. Gurara (p=0·0095%, 95% CI of 0.58-3.76 in head and body pools). The prevalence of infective flies was higher than 0.05% prevalence rate used as a standard to determine suppression of transmission. The economic evaluation indicated that the programme was cost effective and the control of the disease resulted in improved economic productivity. This study provides further evidence to strengthen the potential for elimination of onchocerciasis and in the development of guidelines for elimination of the disease in Nigeria and elsewhere.

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LIST OF ABBREVIATIONS

ATP:	Annual Transmission Potential
ACER:	Average cost effectiveness ratio
APOC:	African Programme for Onchocerciasis Control
APOD:	Acute Papular Onchodermatitis
ATP:	Annual Transmission Potential
ATR:	Atrophy
DDH_2O:	Diodinised Distilled Water
CBA:	Cost-benefit analysis
CDI:	Community-directed intervention.
CDTI:	Community-Directed Treatment with Ivermectin
CEA:	Cost-effectiveness analysis
CI:	Confidence Interval
CSA:	Committee of Sponsoring Agencies
CYC:	Current Year Cost
CYCP:	Current Year Cases Prevented
DALYs:	Disability Adjusted Life-Years
dNTP:	Deoxyribonucleotide triphosphate
DTT:	Dithiothreitol
ERR:	Economic rate of return
FCT:	Federal Capital Territory
FLH:	Fly Larvae in Head
HB:	Hybridization buffer
LGA:	Local Government Area

LOD:	Lichenified Onchodermatitis
MC:	Marginal Cost
MCER:	Marginal cost effectiveness ratio
MDA:	Mass Drug Administration
MF:	Microfilariae
NGDOs:	Non-Governmental Development Organisations
NPC:	National Population Commission
NVP:	Net present value
OCP:	Onchocerciasis Control Progarmme
OEPA:	Onchocerciasis Elimination Programme for the Americas
OND:	Optic Nerve Disease
OSD:	Onchocercal Skin Disease
PCR:	Polymerase Chain Reaction
PHC:	Primary Health Care
PYC:	Previous Year Cost
PYCP:	Previous Year Cases Prevented
QALYs:	Quality Adjusted Life-Years
REA:	Rapid Epidemiological Assessment
REMO:	Rapid Epidemiological Mapping of Onchocerciasis
RPM:	Revolutions per minute
STP:	Seasonal Transmission Potential
SSPE:	Saline-sodium phosphate-ethylenediaminetetracetic acid
Taq:	Thermus aquaticus
TBS/Tween:	Tris-buffered Saline and Tween
WHO:	World Health Organisation

CHAPTER ONE

INTRODUCTION

Onchocerciasis, also known as "river blindness" caused by *Onchocerca volvulus* is a chronic parasitic infection with a public health and socioeconomic problem of considerable magnitude in many sub-Saharan African countries. The disease affects about 17 to 18 million people in 37 countries of the world, with 123 million being at risk of infection (WHO, 1997). In Nigeria, onchocerciasis is widespread and it is a cause of blindness in most rural communities. Of all the countries of the world, Nigeria has the largest number of persons with onchocerciasis, accounting for about a third of the global prevalence with about seven million Nigerians infected, 1.5 million blinded and about 40 million at risk of infection (WHO, 2001). In Nigeria, *O. volvulus* is transmitted primarily by the blackfly vector, *Simulium damnosum* complex (WHO 1995; Adewale *et al.,* 1999; Opara, 2005)

The *Simulium damnosum* Theobald complex are the only vectors of *Onchocerca volvulus,* filarial parasite causing the human onchocerciasis in West Africa (Ibeh *et al.,* 2006). Onchocerciasis disease may be mild (dermatitis) or severe leading to visual impairment and blindness; it is caused by the human immune response to microfilaria released by female adult worms as they move across subcutaneous tissue and spread throughout the body. Humans are the only known reservoirs (Gonzalez *et al.,* 2009).

The prevalence of human onchocerciasis has been observed to be directly related to the presence and abundance of its vector, *S. damnosum sensu lato (s.l.)* (Crosskey 1991).

Onchocerciasis control is currently nearly exclusively based on large-scale treatment with ivermectin (Eberhard *et al.,* 2008). Annual or six-monthly treatment of all eligible

members of high risk communities effectively controls the disease as a public health problem (Abiose *et al;* 1993, Cousens *et al.,* 1997; Brieger *et al.,* 1998; Afework *et al*., 2012). Following the donation of the drug free of charge for as long as needed by the manufacturer in 1987, large-scale ivermectin treatment programmes were established in nearly all endemic areas in Africa where over 99% of all cases in the world are found (Thylefors, 2008). The African Programme for Onchocerciasis Control (APOC) was established in 1995 to support the establishment of community-directed treatment with ivermectin (CDTI) in all areas where onchocerciasis was a public health problem in 19 African countries (Amazigo, 2008). The CDTI strategy has been very successful in ensuring sustained high treatment coverage and by the year 2010 some 75 million people at risk were treated annually with ivermectin in the APOC countries (APOC, 2010). The remaining 11 endemic African countries had been supported since 1975 by the Onchocerciasis Control Programme in West Africa (OCP) (Boatin, 2008). The Onchocerciasis Control Programme was based on vector control to which ivermectin treatment was added in 1988. Following the successful completion of the vector control activities and the closure of the OCP programme in 2002, large-scale ivermectin treatment using the CDTI strategy has been maintained by these countries themselves. As a result of these sustained ivermectin treatment activities, nearly all endemic areas in Africa are under annual ivermectin treatment and the control of onchocerciasis as a public health problem has already been achieved in the majority of these areas (APOC, 2011). Following this success, the principal question became how long these treatments needed to continue considering that it was initially ten years and later extended to 15 to 20 years; and whether in the long term it would ever be possible to eliminate onchocerciasis infection and transmission with ivermectin treatment so that treatment could be safely stopped.

Epidemiological models predicted that elimination would be feasible in the long term (Winnen *et al.,* 2002) and in the Americas where most onchocerciasis foci are small and most vectors relatively inefficient, elimination has been set as the objective by the Onchocerciasis Elimination Programme for the Americas (OEPA) (Sauerbrey, 2008; Gustavsen *et al.,* 2011). However, in the absence of empirical evidence on the feasibility of elimination in Africa, most experts doubted that elimination would be possible in the African continent where onchocerciasis is highly endemic over vast areas, and where the vectors are highly efficient and some vector species can migrate over long distances (Dadzie *et al.,* 2003; Hopkins *et al.,* 2005). In order to determine this, Traore *et al.* (2012) provided the first proof of principle of elimination with ivermectin treatment in endemic foci in Africa; the study was carried out in Mali and Senegal. Recent epidemiological survey by Afework *et al.* (2012) showed that after 15–17 years of ivermectin treatment in two onchocerciasis foci in Kaduna State, prevalence in communities had fallen to zero level in all communities.

Humans acquire infections after being bitten by black flies of the *S. damnosum* species complex carrying infective larvae of *O. volvulus* (Yameogo *et al.,* 199). Therefore, surveillance on infectivity status of the *Simulium* vectors biting along the river systems is a vital component in monitoring the transmission level of onchocerciasis which thus gives a non-invasive method of measuring the success of the control measures (Opara *et al.,* 2005). The classic method of capture-and-dissection of adult flies for infection has been the traditional technique of monitoring the dynamics of vector infectivity. Different infectivity rates can be found at different sites and it is recognized that different cytospecies of *S. damnosum* complex living in different biotypes may carry

heavier *O. volvulus* infective worm loads than others (Leveque *et al.,* 2003; Opara *et al.,* 2005).

Entomological evaluations present several advantages as a means of monitoring the effect of onchocerciasis control programmes. The prevalence of infected flies is an important epidemiological tool to help decision makers to adopt new control strategies in a certain area. Even though this could be accomplished by traditional entomological methods, the techniques are cumbersome and time-consuming, preventing the large scale application of the parasitological approach. Molecular methods based on PCR have proved to be sensitive and specific to address this problem bringing, the advantage of the possibility of performing the analysis in pools of insects, leading to a high throughput application. Perhaps most important, measurement of transmission levels is the most direct method of assessing a programme's progress toward the major strategic goal of preventing transmission. Unfortunately, in areas where control is successful, the prevalence of infected flies is quite low, meaning that a very large number of flies must be examined to accurately measure transmission levels. Poolscreen PCR-based methods represent a way of efficiently examining the large numbers of flies necessary to accomplish this goal. In previous laboratory and field-based pilot studies, the O-150 Poolscreen PCR has been shown to produce estimates of infection that were not statistically different from those obtained from traditional dissection (Yamèogo *et al*., 1999; Rodríguez-Pérez *et al*., 1999).

Recently this molecular technique has been explored as an adjunct to dissection in estimating the infectivity of adult female flies caught in the wild (Yameogo *et al.,* 1999). The O -150 Polymerase Chain Reaction (PCR) pool screen technique can detect a single infected fly in pools containing up to 100 flies (Katholi *et al.,* 1995). This is

based on a mathematical model that calculates prevalence of infection in the black fly population based on the size of the pools screened and on the percentage of the negative pools found. This method allows separate estimates of the prevalence of the parasites in the head capsule (representing L3 from human or other vertebrate infection stage) and the rest of the body (representing the earlier developmental stages, i.e. the L1 and L2 larvae). Studies in Africa and Mexico showed that the 0-150 pool screen PCR assay generated the estimates of the prevalence of infected and infective flies in the vector population that were indistinguishable from those obtained by traditional dissection of the flies (Yameogo *et al.,* 1999; Rodriguez *et al.,* 1999).

Since the 1990s the PCR amplification of an *Onchocerca*-specific repeated DNA sequence (O-150) has been used to detect and identify *Onchocerca volvolus* (Meredith *et al.,* 1991, 1994; Zimmerman *et al.,* 1992; Unnasch and Meredith, 1996). However, in populations of vectors with low prevalence of infection, large numbers of flies would have to be processed to obtain an accurate estimate of infection which is not only time-consuming but expensive.

The distribution of ivermectin has dramatically altered the nature of onchocerciasis control. Existing economic analyses of ivermectin distribution programmes show that these programmes have a highly beneficial impact. Most analyses have estimated the economic benefits in terms of increased labour productivity as a result of reductions in blindness, and in terms of additional land-availability because of a reduced transmission of the parasite (Benton, 2004). Cost-effectiveness analyses of ivermectin distribution have found a cost of $14–$30 per disability-adjusted life-year prevented estimates comparable with other priority disease control programmes. However, the economic success of ivermectin distribution is sensitive to the fact that the drug itself has been

donated free of charge. Pending the development of an effective macrofilaricide, the distribution of ivermectin will remain a public health priority into the foreseeable future.

1.1 Statement of the Problem

Long-term treatment with ivermectin has been used in parts of Kaduna State for the treatment of river blindess, but the impact on human population and the resultant ocular disease associated with it is yet to be properly elucidated. It is also unknown if this has affected the ability of the blackfly to transmit *O. volvulus*. The cost effectiveness in terms of economics of the long-term control programme is yet to be determined.

1.2 Justification

Parasitological survey was focused in 10 communities within 10km from river Gurara in Kagarko LGA. The results of the baseline survey which provided the justification for the intervention were not available during the study; it was however confirmed that the communities were surveyed in the early 1990s before intervention but no records were available at the local and state government programme office.

Records available indicate that the communities should have had at least 10 – 15 rounds of annual treatment. One reason for this is that the adult onchocercal parasites live for about 14 years (Plaisier *et al.*, 1991). Another critical factor is the pre-control endemicity level which reflects the initial worm load and the pre-control intensity of transmission (Cousens *et al*., 1997; Duerr *et al.*, 2011).

The outcome of this study will provide useful information on the effectiveness of long term treatment of onchocerciasis with ivermectin on humans and the vector and the

associated economic impact. The study would also provide results, as useful indices, for the control/elimination of the disease.

1.3 Aim and Objectives

The aim of this study is to assess the long-term effect of community- based treatment with ivermectin on prevalence and transmission of the disease towards establishing the worthiness of the programme after years of repeated annual treatment with ivermectin in areas of Kaduna State. The objectives of the study were

i. To determine the prevalence of *Onchocerca volvulus* in human population after long-term treatment with ivermectin in parts Kagarko Local Government Area of Kaduna State

ii. To determine infection rate of *Onchocerca volvulus* in *S. damnosum* in a long term ivermectin-treated communities in parts of Kaduna State

iii. To determine the economic impact of long term treatment of onchocerciasis with ivermectin in affected communities

1.4 Hypotheses

- Long-term treatment with ivermectin does not affect the level of infection with *Onchocerca volvulus* in humans.

- Long-term treatment with ivermectin has no effect on infection of *Onchocerca volvulus* in *Simulium damnosum*

- Long-term treatment with ivermectin does not have any impact on the economic status of endemic populations in parts of Kaduna State

CHAPTER TWO

LITERATURE REVIEW

Onchocerciasis (or river blindness) is a parasitic disease caused by the filarial worm O*nchocerca volvulus,* a disease transmitted to humans by blood-sucking dipterous insects – the blackfly; *Simulium damnosum*. The most serious manifestations are blindness and debilitating skin lesions. The disease is often called "river blindness" because the blackflies that transmit the disease abound in riverside areas, where they breed in well oxygenated fast-flowing water, broken up by vegetation (Hougard *et al.,* 1997; WHO 1995).

Man is the only known animal reservoir. All populations are exposed if they live near the breeding sites and the clinical signs of the disease are related to the amount of exposure and the length of time the population is exposed. In areas of high prevalence first signs are in the skin, with chronic itching leading to secondary infection and chronic skin changes. Blindness begins slowly with increasingly impaired vision often leading to total loss of vision in young adults, in their early thirties, when they should be at their most productive and when, as a result, their children are forced to be permanently at their side to accompany them. Other effects include epilepsy and growth retardation and these are again most evident in communities with high prevalence (Hopkins and Boatin, 2011).

The history of onchocerciasis as a disease dates back to 1875 when John O'Neil first associated the *Onchocerca volvulus* microfilariae with an irritating popular dermatitis in Ghana. In 1893, Leuckart first described the adult worm of *Onchocerca* in subcutaneous tissues. Robles (1915) published findings on a new disease from Guatamala with associated subcutaneous lesions, dermatitis and microfilariae. In Sierra

Leone in 1923, Blacklock demonstrated that *S. damosum* was the disease vector (Davies and Crosskey, 1991; Forgione, 2002).

At the start of the 21st century onchocerciasis still posed a threat to public health in many tropical regions of Africa including Nigeria. It is the world's leading infectious causes of blindness. It is a public health problem in most sub Saharan countries in Africa and constitutes a serious obstacle to the socio-economic development in the rural areas of these countries (WHO, 1997). As at 2003, the WHO estimated that some 125 million people were at risk of this disease, 96% of who live in Africa. An estimated 18 million people who have the disease have dermal microfilariae and itching with 300,000 persons blind. However, further mapping of the disease in non-Onchocerciasis Control Programme (OCP) areas has shown that more people are infected in these areas than was thought; the total number of people infected is now estimated as 37 million (Noma *et al.*, 2002).

In Nigeria, the disease is highly endemic, occurring in 32 states Abuja, The Federal Capital Territory; it is estimated to be responsible for 200,000 cases of blindness (NOCP 2002). In Nigeria, *Onchocerca volvulus* is the main causative agent of onchocerciasis and is transmitted primarily by *Simulium damnosum* complex (WHO, 1995, Asien *et al.,* 2004; Opara *et al.,* 2005). The intensity of infection of human communities by *O. volvulus* itself is dependent on the density of infective biting vector (Opara *et al.,* 2005).

Although the disease is not normally associated with mortality, a few studies have shown that life expectancy of those blinded by onchocerciasis was greatly reduced and that mortality in blind adults on the average was three to four times greater than in the fully sighted individuals (Hopkins and Boatin, 2011).

The unprecedented donation of Mectizan (ivermectin) by Merck & Co Inc. to as many people as needed it, for as long as it was needed was a watershed in public health (Colatrella, 2008). This donation, which began in 1987, has not only revolutionized the strategies for control of onchocerciasis but has also led to other major donations by the pharmaceutical industry for the elimination of trachoma, lymphatic filariasis, and other neglected poverty-linked tropical diseases (Gustavsen *et al.*, 2009). The control measures are now having an impact; the risk of the infection is actually much reduced and elimination of transmission in some areas has been achieved ((Traore *et al.,* 2012; Afework *et al.,* 2012)

Over the last sixty years there have been enormous research and publications on onchocerciasis as a disease and the *Simulium* species complex, its life cycle and that of the parasite as well as morphology and taxonomy of the vectors and its impact. Recent research on the impact of treatment by various authors has demonstrated the potential for the elimination of the disease in Latin America (Rodriguez-Perez *et al.,* 2008; Gonzalez *et al.,* 2009) and most recently in Africa (Traore *et al.,* 2012; Afework *et al.,* 2012).

2.1 The Parasite

The adult form of *O. volvulus* is found in the human host in fibrous nodules or onchocercomata, many of which are found in the subcutaneous tissues, especially over bony prominences, although others are found deeper in the tissues. Female worms, which are considerably larger than the male (30–80 cm and 3–5 cm long, respectively), are found entwined around each other in the nodules and each nodule may contain from one to two male worms and two to three female worms, although larger nodules may exceptionally contain up to 50 worms. Whereas the females remain in the nodules, the

males are more mobile and may go between nodules. Females release 700–1,500 larvae or microfilariae (mf) each day (Forgoine, 2002). The vast majority of mf are found in the skin, some find their way to the eye and probably other tissues of the body (Abiose *et al.*, 1993). If they are not ingested by the vector, they live in the host from 6 to 24 months. In order to develop further the mf must be ingested by *Simulium* species where, after penetrating the wall of the mid gut, they migrate to the thoracic muscles where they undergo an increase in size into sausage-shaped larvae (Blacklock, 1926).

After a few days these sausage-shaped larvae elongate and become much more mobile becoming the pre-infective form and eventually migrating into the heamocoel and toward the head where they wait in the proboscis for the next time the *Simulium* requires a blood meal. This part of the life cycle in the fly takes around 10 days (6–12 days) (Little *et al.,* 2004). When the *Simulium* bites again, the infective larvae (L3 stage) migrate actively into the wound made by the bite and live in the tissues for a few days before going through a further larval stage (L4). After about a week they develop into juvenile worms (L5). It takes a further 7–15 months before these juvenile becomes mature and moves toward the nodules where mating takes place and the cycle begins again. The adult worms live for 10–14 years (Somorin, 1983)

The pathogenicity of *O. volvulus* varies with different strains of the parasite; for instance, the savannah strains of the parasite provoke much more blindness than the forest strains (Duke and Anderson 1972; Duke and Gardener 1976; Dadzie *et al.,* 1989; Remme *et al.,* 1989); even though both provoke skin changes. The different strains of *O. volvulus* are particularly well adapted to the vectors, forest strains being transmitted by vectors adapted to the forest areas and similarly, savannah strains by vectors adapted to the savannah (Duke, 1993; Duke *et al.*, 1996; Lewis and Duke, 1996; Anderson *et*

al., 1974). The strains of *O. volvulus* in the Americas are also quite pathogenic to the eyes adding to the theory that the disease was introduced into the Americas with the slave trade.

2.2 The Vector and its recognition

Blackflies are arthropods of the class insecta, order Diptera, suborder Nematocera. The genus *Simulium* contains important vectors species namely *Simulium damnosum s.l.*, *S. Naevei*, *S. ochraceum, S. metallicum and S. callidum* (Service, 1979). They are easily recognized by their stout bodies and prominent thoracic hump. They are black in West Africa but could be yellow, orange or reddish in Neotropical regions (Crosskey, 1979b; Service, 1979). Blackflies belonging to *S. damnosum* complex are further identified by the presence of dorsal hair crest on the fore-tarsus and the white band of the third barsi-tarsus while the larvae have abdominal tubercles and also scales which feature similarly on the prolegs, with pupae showing characteristic banana shape (Crosskey 1990).

Vajime and Dunbar (1975) listed the possible cytospecies in West Africa as *S. damnosum s.s*. (Sirba), *S. sanctipauli* (Bandama), *S. soubrense* (Soubre), *S. squamosum* (Bille) and *S. yahense* (Yah) which were very common and the last two, *S. sudanense* (Sudan) and *S. dieguerense* (Dieguera) which were rare. Four out of these have been recognized in the Nigerian fauna viz *S. damnosum* Theobald *sensu stricto*, *S. sirbanum* (Vajime and Dunbar, 1975), *S. squamosum* Enderlein and *S. sudanense* (Vajime and Dunbar, 1975; Crosskey, 1981). The presence of the first two in the Guinea Svanna areas of Abuja and Kainji, where vector control took place in the 1950s and 1960s was reported by Vajime and Dunbar (1975). Later, a population of *S. squamosum* was identified at Dalle south of Kaduna State and *S. damnosum s.s* as the dominant cytospecies at the Kafanchan waterfalls (Nock, 1985; Vajime and Gregory, 1990). Both

vectors were found to be responsible for the spread of onchocerciasis in those areas. More works have investigated the sibling species of *S. damnosum s.l.* on the basis of differences in the chromosomal inversions of the salivary glands. Akoh (1990) and Mafuyai, (1996) have confirmed the presence of cytospecies which Vajime and Gregory (1990) identified along River Kogum in Kaduna State.

Simulium damnosum sensu lato is a complex group of over 40 sibling species of blackfly distributed throughout the sub-Saharan Africa and the Arabian peninsula (Cupp, 1996). They are responsible for more than 90 per cent of onchocerciasis cases worldwide and 95 per cent of cases in Africa (WHO, 1995). About 54 species of *Simulium* have been reported to bite man, transmitting onchocerciasis (Adler *et al.,* 2004).

Nearly all simuliids that suck blood of man belong to the genus *Simulium s.l.* and this is the only genus with the man-biting members that is present in the human onchocerciasis-endemic areas. Davies and Crosskey (1991) described the blackly as stout with cigar-shaped segmented antenna. There are 11 segments without whorls of long hair. The mouth parts consist of a short inconspicuous, downwardly-directed proboscis. The wings are broad, colourless and without scales. Its thorax is characterised by a large membranous area found at the base of the wing on each side of the thorax. The legs are short and stout without scales and the hind legs has apical projection and a groove across the upper surface of the second tarsal segment. (Davies and Crosskey, 1991). Some species of *Simulium* such as *S. neavei* do not fly far from breeding sites. *S. damnosum* however can fly long distances (400–500 km) by flying with the prevailing winds in West Africa. (Garms *et al.*, 1979)

Generally, the *Simulium* try to feed as close to the breeding sites as possible. Populations living within 5 km of the breeding site are the most at risk although communities living up to 12–15 km may be affected. Communities living on the river's edge are more at risk than those a little further away. In fact, these communities very close to the breeding site to a certain extent "protect" the population living a little further away. This is one reason why blindness is relatively rare in towns, even if they are riverside communities. The *Simulium* rarely enters houses to bite, but it's preferred biting times are in the early morning and late afternoon. These are also times when populations who depend on the rivers for water, washing, and other activities are most likely to be at the river for these various activities. Those working in fields nearby or fishing in the river itself also tend to be less active during the midday heat (WHO, 1997).

2.2.1 Sibling species

In natural population it is very difficult to recognize the different species. For very similar species the term "group" is used, e.g. the *Simulium neavei* group, for sibling species, which cannot be distinguished according to morphological but only by other criteria the term "complex", e.g. the *Simulium damnosum* complex. Non-morphological criteria are offered by the banding pattern of large polytene chromosomes in the nuclei of the larval salivary gland cells and sometimes of malpighian tubules of adults (Davies and Crosskey, 1992).

Species differentiation is based on examination of the polytene chromosomes of the salivary gland of the larvae. The morphological and morphometric criteria that enable differentiation of most species or groups of female adults have also been identified.

2.2.2 Distribution of *Simulium*

The geographical distribution of these species does not correspond exactly to the phytogeographic zones of West Africa, although it is greatly influenced by local

climatic and ecological characteristics (Dunbar and Vajime, 1972; Crosskey, 1992). The *S. damnosum s.s* and *S. sirbanum* pair are found in the Savanna zone as far as 14° N. This is also the northern limit of the endemic area for onchocerciasis. However, the southern limits extend well beyond the Savanna zone. The species find their way into the forest zone along the large watercourses. The *S. sancitpauli* sub complex is forest-dwelling but to the north of the great dense forest, it is also found in the zone of light wet forests and, in places, even reached Savanna zones as far as latitude 10° N. *S. yahense* is limited to small forest watercourses. *S. squamosum* probably covers several different entities and is widespread in both forest and Savanna zones (Crosskey, 1992).

2.2.3 Life cycle and features of *Simulium*

The eggs of *Simulium* species are oval to subtriangular in shape. The eggs measure 0.1 – 0.4 centimetres long and are pale in colour. They are laid in clusters of hundreds, 100 – 600, on substrates in flowing streams by the females (Wenk, 1981). They may hatch within 1½ to 2 days if deposited near the surface of water. Hatching may be affected by temperature and water level. Several species deposit eggs in substrates well below the water surface, thus avoiding early hatching and desiccation (Service, 1979).

The mature larva has a characteristic dorsal "bump" and is usually about 5 – 13 millimetres long, depending on the species. It is sedentary for long periods, attaching itself to submerged vegetation, or rocks by the posterior hook – circlet. The free-moving head combs the water with its feeding brushes – the cephalic fans. When disturbed, larva can release its hold and be swept downstream for some distance at the end of silky strands. Some larvae are predacious while some may be cannibalistic. The larva may complete development in two weeks, depending on the species and temperature (Service, 1979).

The larva pupates after weaving its cocoon which is cone-shaped and dark. It achieves this after it has moulted six times as larva. Wanson and Henrard (1945) found that these six moults are completed in five days. The pupa has a pair of usually prominent filamentous or broad, thin-walled respiratory gills placed dorsally and ventrally, and used for taxonomic separation into species. Pupal period may last two to six days and does not usually depend on temperature. The empty pupal cases with gill filaments still attached remain enclosed in their cocoons and retain their taxonomic value after adults have emerged (Service, 1979).

The adult may emerge rapidly to the water surface in a protective bubble of gas which prevents it from being wetted or it may escape by crawling up partially submerged objects. *Simulium* species (plate I) are quite small, about 1.5 – 4mm long with transparent unspotted wings of about 2-3mm and with bristles on the thick anterior veins. The wings close like scissors when at rest. The thorax is humped and the head has a pair of large compound eyes which are dichotic (separated on top of the head) in the female but holoptic (not separated) in the male, and used for separating the sexes (Service, 1979; Crosskey, 1990). The antennae are short and stout, without long hairs on the eleven segments. The mouthparts are short and have five-segmented maxillary palps hanging downwards. The body and legs are covered with silvery hairs, but some species may have orange or yellowish colour. The abdomen is short and squat and covered with closely apprised fine hairs. The genitalia is not very conspicuous in either male or female (Service, 1979).

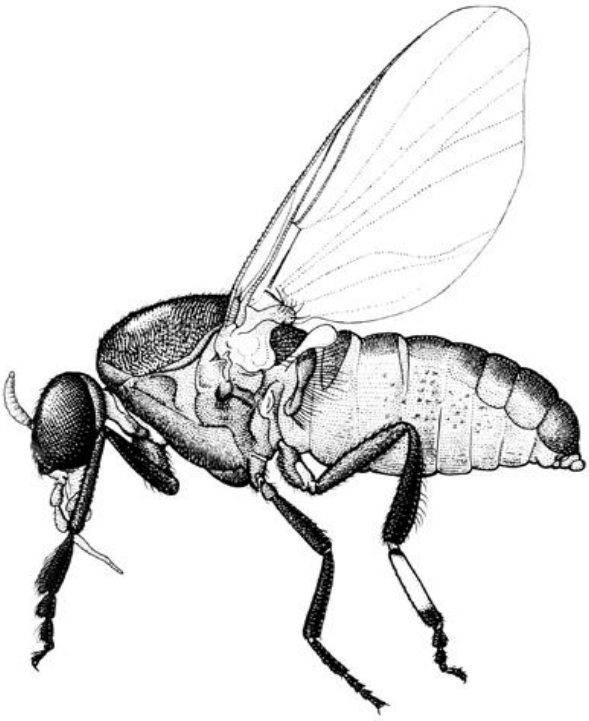


Plate I: **Schema of *Simulium* vector Blackfly (Source: Crosskey, 1991)**

Simulium species may fly considerable distances from emergence sites to feed and can be dispersed by winds. Adults can fly 3.2 – 4.8km or more (Soulsby, 1968). Some *S. damnosum* can be found biting 60 – 100km from their breeding sites (Service, 1979). At the beginning of rainy season, *S. damnosum* s. str. and *S. sirbanum* may migrate over considerable distances and repopulate rivers that had stopped flowing during the

preceding dry season (Renz, 1987a). This phenomenon was termed're-invasion' when it was first described for flies invading the controlled areas of the Volta river basin, (Garms *et al*., 1979). *S. damnosum s.l.* females have a maximum longevity of one month; the greatest actually reported being 23 days (Wenk, 1981).

The longevity is much greater in the Savanna grasslands than in the thick forest areas. Adult *Simulium* species are usually found biting on rocky or grass covered hills, several hundred feet above the breeding grounds. They are also found in open farmlands within easy access of shade trees (Crosskey, 1955).

Male adult *Simulium* species feed solely on plant juices and play no direct role in onchocerciasis transmission. The females are haematophagous and may absorb 1 mg of blood each meal. These blood meals influence the development of the ovaries, and the eggs are laid after three to five days. This is the geotropic cycle. Less than 24 hours after laying the eggs, the female takes a further blood meals and a new cycle begins. She may complete as many as five or more cycles in the course of her life. Since the maturation of the microfilaria into an infective larva takes seven to 12 days and the ovarian cycle lasts three to five days; the black fly can transmit the parasite only after the second meal following the one at which it became infected (Service, 1979).

Females that have not yet laid any eggs are called nulliparous, while those that have laid eggs are called parous. The respective percentages of these two categories, known as the parturition rate, can be used to assess the mean age of the black fly population. The *S. damnosum s.l.* females feed mainly on man, but in some areas they may also take blood meals from animals, particularly bovines, horses and small ruminants (Crosskey, 1990).

Activities of *Simulium* species slows down when the temperatures is below 17^{o} C and above 30^{o} C and during strong winds and rain. The females bite outdoors from sunrise to sunset and are particularly active at dawn and dusk, in cloudy weather and in shady areas. They bite close to the ground level and stationary person receives 95 percent of

the bites below waist level. The bite is not always noticed straight away but may be followed by severe itching. If the *Simulium* species are numerous, the inconvenience caused by their bites may make any kind of work impossible.

As in all the simuliids, only *S. damnosum* females are hematophagous. Male adult blackflies feed solely on plant juices and play no direct role in onchocerciasis transmission. (Hoc, 1996) The females are largely anthropophilic and may absorb 1mg of blood at each meal. Studies carried out showed that there are no preferred locations for resting sites and that the resting blackflies occupy the entire forest (Bellec and Hebrard, 1980).

The female *Simulium* species lives for up to four weeks. This female seeks a sugar meal (plant juices or nectars), then a blood meal and may thus ingest microfilariae if the meal is taken from a person infested with *O. volvulus*. Biting can be seasonal in tropical regions with prolonged dry seasons. In West Africa, biting activity of *S. damnosum* complex is all through the rainy season but ceases for several months in the long dry season of the northern Savannas when rivers stop flowing and resumes when the rivers run again and are recolonised by migrating flies. On the other hand, where the rivers are perennial biting activities occur year-round with continuous breeding (though not necessarily at the same intensity (Davies and Crosskey, 1991).

2.2.4 Feeding behaviour and transmission of disease

Host odour induces long-range attraction, followed by orientation to the carbon dioxide source and then a visual orientation to the preferred region of the host. Simuliids usually bite during the day (Service, 1979). When the simuliids suck blood from an infected human, microfilariae of *Onchocerca volvulus* are ingested, which penetrate the peritrophic membranes and the intestinal wall. In the haemocoel they migrate to the thoracic flight muscles, in which they grow and moult twice within six to nine days. The infective L3 larvae migrate to the labium. Movements of maxillae and mandibles during piercing of the skin and the increase of temperature by the blood induce the penetration of the L3 via the soft parts of the labium (Davies and Crosskey, 1991).

2.3 The causative agent: *Onchocerca volvulus*

The adult *O. volvulus* female lives in the human subcutaneous tissues, free or more often tangled in fibrous nodules (Schultz-Key, 1990). It is sexually active for 9 to 14 years. During its fertile life span it produces millions of very small embryos (250-330 microns, called microfilariae) which can survive for more than two years in the human skin. The microfilariae are the pathogenic stages which invade dermal tissues and cause severe disorder, including ocular tissues. They have to be carried from one person to the other by a female blackfly belonging to the *similium damnosum complex in* West Africa. Only females of *S. damnosum* bite man, and sometimes animals, as they need blood for the maturation of each batch of eggs laid. There is no known animal reservoir (Davies and Crosskey, 1991).

Once the blood arrives in the blackfly stomach most of the microfilariae are digested, but a few of them pass through the intestinal wall and reach the abdominal cavity, and the thoracic muscles where they transform (Bain, 1971); the microfilariae give rise to infective larvae, measuring 650 micros, which find their way in the mouth parts and may thus be transmitted to man during subsequent blood meal. The maturation cycle of the larvae in the blackfly takes about seven days at 27-30^{o}C. The number of infective larvae in blackfly is generally less than 10 and in most cases from one to three, while one female *S. damnosum* may ingest hundreds of microfilariae during one blood meal. When infective blackflies bite man they deposit the infective larvae in the skin. The larvae penetrate the skin superficial layers, moult, mate and cause formation of nodules which appear between seven to twelve months, sometimes up to three years (Davies and Crosskey, 1991).

2.3.1 Life cycle of *Onchocerca volvulus*

The life-cycle (plate II) begins when a female blackfly takes a blood-meal from a human host. A pool is pumped up into the blackfly, saliva passes into the pool, and infective *Onchocerca* larvae pass from the blackfly into the host's skin. The larvae then enter the host's subcutaneous tissue, where they migrate, from and lodge in nodules, and slowly and cluster together. It is thought that smaller male worms may migrate between nodules to mate. After mating, eggs in the female worm develop into microfilariae and leave the worm one by one. A female produce 1,000 microfilariae per day (Davies and Crosskey, 1991). Many thousands of microfilariae migrate in the subcutaneous tissue. When the microfilariae die, they cause skin rashes, lesions, intense itching and skin depigmentation. Microfilariae also migrate to the eye and can cause blindness. (Abiose *et al.*, 1994)

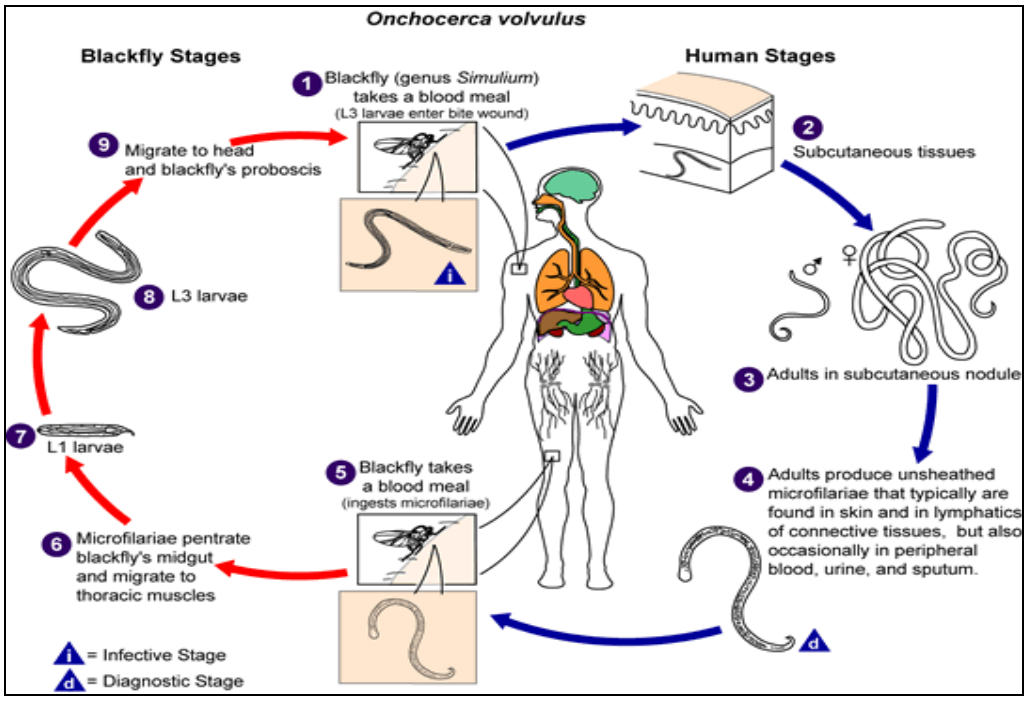


Plate II: Schema of the Life Cycle of *Onchocerca volvulus* in *Simulium* vector and Humans (Source: Centre for Disease Control http://www.dpd.cdc.gov.dpdx, 2013)

2.4 Feeding of Adult Blackfly

The morphology of the mouthparts of the blackfly is similar to those of the biting midges (Ceratopogonidae). Both the male and the female blackfly feed on plant juices but only the female such blood. Many species feed almost exclusively on birds

(ornithophilic) (Nelson, 1981; Crosskey, 1990) and others on non-human mammalian hosts (zoophilic) (Duke, 1967) but several species also bite man. Blackflies locate their host by sight and probably by odour (Service, 1979). The mouthparts do not penetrate very deeply in the host's tissues. The teeth of the labrum stretch the skin and the rasp-like action of the maxillae and mandibles cut through the skin and rupture the fine blood capillaries. A pool of blood is created just beneath the epidermis, possibly assisted by anticoagulating properties of injected saliva. The small pool produced is then sucked up by the fly. This method of feeding is ideally suited for picking up the microfilariae of *O. volvulus* which occur in man's skin and not blood.

The young *S. damnosum* which has not taken a blood meal and therefore has not laid eggs is referred to as nulliparous female, while the one which has taken at least a blood meal and has laid eggs is known as parous female. Blood meal of about 1ml which exceeds the weight of an unfed blackfly is taken at each feed, (Crosskey, 1958a). The duration of feeding varies from 1.5 to 6 minutes (Blacklock, 1926) and 4 – 6 minutes (Crosskey, 1955). A blood meal may take two to three days to digest in the tropics and up to eight days in non-tropical areas. Blackfly rests on the under-side of leaves near the ground during the hot part of the day. The adult of most species may live up to 3-4 weeks. It has been observed that the maximum number of completed gonotropic cycle in the fly's life span is six (Davies, 1961; Le Barre, 1966).

Wenk (1981) explained that in locating a suitable source of blood meal and relocating the preimaginal site for oviposition, blackfly may have to change its main flight direction several times: initially, away from the emergence place to find its vertebrate host, and after blood-feeding, in the contrary direction. The process has to be repeated for each ovarian cycle, up to six times in all. Since mating takes place only once, all

subsequent individual feeding and oviposition cycle are correspondingly simplified. Therefore transmission of parasites to vertebrate host depends on this blood-feeding/oviposition cycle. It has been estimated that a man exposed to infection over all the hours of day light for a year will receive 14,000 infective bites potentially transmitting 92,000 infective larvae (Duke, 1968a). Other works in the Cameroons and the Sudan, have covered the dynamics of transmission of onchocerciasis (WHO, 1987; Renz, 1987 a, b; Renz *et al.,* 1987c).

2.5 Relationships between Parasite, Vector and the Host Population

Onchocerciasis has had a significant impact on the populations in the savannah areas of West Africa. The close contact of man and the black fly poses not only medical problems for the communities that live near the river basin but also the bite of the flies causes intense irritation and a huge nuisance to the population (Crosskey, 1990). Communities are therefore compelled to abandon their villages and flee from the river basin for fear of acquiring the disease, and also to avoid the nuisance from the bite of the flies. Such movements have dire consequences on socio-economic development. Moving to areas removed from the rivers has likewise led to poverty because the soil is much poorer away from the fertile valleys and water is more difficult to find so agriculture is gravely affected. Productivity is also affected negatively, leading to diminished earnings and adversely affecting the supply of labour. A high prevalence of blindness in young adults leads to further deterioration and deepens the cycle of poverty. These young adults are inflicted at the time when they should be their most productive and when they have young families to care for and educate. Often the children are forced to lead their blind parents around rather than go to school. Civil conflict and insecurity in Africa (particularly in Central Africa and the Sudan) has led to

large population movements, often out of infected areas. Communities have complained of biting nuisance in areas populated by flies after the withdrawal of control measures, although the flies were uninfected and transmitted no disease. (Hougard and Seketeli, 1998).

Migration of the fly can also be significant. Movements of the *Simulium* have been mentioned above. It is possible that infected flies can be carried by winds into areas where the disease has already been controlled. *S. neavei* is found in forest areas in Central and Eastern Africa. The potamonautes crabs, on which the larva develop, like shady areas; with deforestation these crabs have disappeared in some areas and transmission of the disease has stopped as a result. Unfortunately however, *S. damnosum*, which prefers more savannah areas, has invaded some of these sites and once again transmission of the disease could become a problem (Duke, 1993).

2.5.1 Development of *Onchocerca volvulus* in *Simulium* and humans

The hematophagous vector *S. damnosum* species are active in the mornings and evenings and bite the legs, abdomen, head or ears. The microfilariae picked up from the blood meal migrate, piercing the gut wall, to develop inside the fly as the sausage first stage larvae (L1) and pass through the flight muscles as pre-infective second stage larvae (L2). Finally, they move to the head region in 6 to 10 days as the infective stage larvae (L3) (Bain, 1969; Soulsby, 1987). Double infections, stemming from larvae ingested at two subsequent blood meals could occur (Renz, 1987b). Seasonal variations in vectoral efficiency are likely to occur following changes in the species composition and in the dispersal pattern of the fly population (Renz, 1987a, b; Duke, 1975).

The third infective stage larva is injected into the body of man when the fly takes its blood meal and develops into an adult worm in the subcutaneous tissue. Little is known

about the development of the infective larvae into the fourth-stage forms and thence through to adult worms. Adult life span is likely to be related to the host's immune response and is considered between 10 and 12 years on the average (Mackenzie, 1987). The fertilized female adult (30 – 50cm long) discharges microfilariae which eventually reach the skin where once they attain a threshold concentration, they can be detected by microscopic examination of a skin snip (Remme, 1986). The components that pass from the uterus to the host include microfilariae, discarded egg shells and other uterine secretions. It is calculated in an African study, that a worm carried about 200,000 developing microfilariae (Schultz-Key *et al.*, 1980).

The microfilariae do not develop further in the mammalian hosts, and the life cycle of the parasite can only continue when the microfilariae enter the arthropod vector, otherwise they die after 6-30 months although Duke (1968a) suggested 1-2 years. In 1950, Ridley and Anderson reported the case of a European Administrative Officer from Nigeria with ocular onchocerciasis. Crosskey, a pioneer researcher in onchocerciasis, made several contributions from his study in the Northern parts of Nigeria especially in the Galma Valley area, Abuja and Kudaru which have led to several studies on the biology and control of *S. damnosum* Theobald (Crosskey, 1954; 1955; 1956; 1957 a, b; 1958a,b; 1960a,b; 1962b; 1973; 1979b; 1981a,b,c).

Eye lesions and blindness are the principal factors determining the public health and socio-economic importance of onchocerciasis (WHO, 1976). Several authors have emphasized that serious pathology in onchocerciasis is due to the cumulative effects of intense infection (Remme *et al*., 1986). In fact, most of the pathology is caused by the presence of dead microfilariae in the skin or more seriously in the eye (Duke, 1967).

2.5.2 Microfilariae (mf)

The principal symptoms of the onchocerciasis are due to the microfilariae (mf). Each female can produce up to 1,500 mf per day. Live mf do not really create a major problem. However, they start to die after 6 months, if not ingested by a *Simulium*. When mf die, they elicit an inflammatory reaction. Even if these reactions are small and localized, the death of a large number of mf each day (particularly in the skin), provoke widespread and chronic reactions. At first the reactions are reversible, but as the infection continues permanent changes occur. Recent evidence suggests *Wolbachia endobacteria* (symbionts of arthropods and filarial nematodes) may contribute to the inflammatory pathology associated with the disease (Taylor and Hoerauf 1999; Hise *et al.*, 2003; Saint Andre *et al.*, 2002)

2.5.3 The nodules (Onchocercomata)

These are firm, single or multiple, usually rounded bodies most often found on the bony prominences of the pelvic girdle, ribs (plate III) and head. They are essentially granulomatous reactions around adult worms formed possibly to protect the worms where they are lodged. They could be mistaken for lymph nodes. Nodules increase with age as does the number of worms within single granuloma where as many as 4-10 worms may be found (Mackenzie, 1987). Nodules vary in size and number and as many as 100 can be found in a patient (Ukoli, 1984).



Plate III: Typical nodule in an advance stage of onchocerciasis in a community in Lere Local Government Area.

Adult worms of *O. volvulus* measure 2700320 microns in length and about 5 microns in diameter. In Central America, nodules are found on the upper parts of the body usually connected with the biting habits of *Simulium cohraceum* and *S. metallicum* (Wilcocks and Manson-Bahr, 1972).

In Africa, nodules are found in the lower parts of the body from waist down and this also is related to the biting activity of *S. damnosum* complex and *S. naevei* complex. There are some exceptional cases where nodules have been found on the head (Fuglsang *et al*., 1976). The presence of microfilariae at the outer canthus has been significantly associated with the presence of head nodule. It has been suggested that as a working guide, a density of 10 microfilariae per milligram of skin at this site indicated serious risk to the eyes (WHO 1976; Abiose *et al*., 1993).

2.5.4 Dermal changes

The changes in skin are related in the main to two parasite stages, the microfilariae and to a lesser extent, the adults. Microfilariae lie freely in the subcutaneous tissues (Nnochiri, 1964; Israel, 1959). When they undergo destruction, papular rash often occurs on the skin and may become itchy (pruritus) and is commonly referred to as 'craw-craw' found on the buttocks and other body parts (Murdoch *et al.*, 1993). The first changes are due to itching and the lesions produced by energetic scratching. This is officially described as acute papular onchodermatitis (APOD), but the original description from West Africa of craw-craw somehow describes it more effectively (O'Neil, 1975). Sometimes patients use sticks or stones to scratch more vigorously. Trauma to the skin often leads to secondary infection, with localized edema and local lymphadenopathy. "Troublesome itching" has been shown to be one of the most important symptoms of onchocerciasis (Murdoch *et al.,* 1993) and has recently been recognized as affecting more than 50% of the populations in some communities in the rain forest belt where blinding onchocerciasis is relatively rare. A more chronic condition may develop and this includes dermal atrophy, degeneration of the dermal collagen, and thinning of the epidermis to produce a premature ageing of the skin,

together with loss of pigment (Leopard skin) (plate IV). Localized lesions are often characterized by intense itching, asymmetrical, well-circumscribed dermatitis with or without oedema (pachyderma) and darkening of the skin. In this case, there is a high level of microfilariae killing (Mackenzie, 1987). This state which is parchment – like is often referred to as lizard or crocodile skin. When the skin of the legs is involved, the leopard skin effect is produced. Edungbola *et al.* (1988) suggested using this spotty depigmentation of Leopard Skin (LS) as a rapid index for estimating onchocercal endemicity, especially when the use of other conventional diagnostic techniques becomes limited in making a rapid survey of onchocerciasis for a national programme or for mass treatment with ivermectin.



Plate IV: 'Leopard skin' patches of depigmentation on the shin as a result of positive for Onchocerciasis in Gurara, Kagarko Local Government Area

A further change that happens to the skin is atrophy (ATR) manifested by loss of elasticity. The skin of someone in their thirties or forties looks already like that of an old person. Swollen lymph nodes are found just under the skin in the inguinal region and the weight of these nodes drags down on the skin, if it has lost its elasticity,

creating the so-called “hanging groin.” In Yemen and the north of Sudan and in a small proportion of patients elsewhere there is a lichenified onchodermatitis (LOD), which typically affects young adults and is also called “Sowda” (Anderson *et al.*, 1973). The skin is itchy, thickened, and hyperpigmented. This gradually spreads and may cover large areas, particularly the lower limbs, but is often asymmetrical. Draining lymph nodes become inflamed, which usually results in lymphedema or thickening of the tissues.

2.5.5 Ocular changes

In 1915, Robles discovered the disease in Guatemala and associated the nodules containing the adult worm with development of eye lesions; years later. Ochoterena (1930) showed that the eye lesions were actually due to microfilariae. Many changes can occur in the eyes of infected individuals and these range from temporary alterations such as punctuate keratitis to permanent sclerosing keratitis and chorioretinal pathology. One of the two major changes involving the cornea, punctuate keratitis, is clearly seen to be associated with degenerating microfilariae in the anterior chamber and is considered as early development equivalent to the discrete paule reaction seen in the skin especially in children. It may cause itching and discolouration of the eye. Dead microfilariae are usually surrounded by ill-defined punctuate “fluffy” or “snow-flake” opacities (Budden, 1962; Thylefors, 1978; Duke, 1976; Abiose, 1993).

Late lesions may be separated with increasing severity as it is manifested in the cornea, anterior uveal tract, choroids and retina, and optic nerve. Sclerosing keratitis and iritis are caused by the presence of many microfilariae in the cornea. The lesion may become confluent, involving the whole corneal periphery and later the entire cornea and is

usually irreversible (plate V). This infection may lead to night blindness and reduced visual field (Mackenzie, 1987; Duke, 1976).

The development of 'pear-shaped' deformity of the pupil shows the presence and severity of iritis and iridocyclitis and possibly secondary cataracts (plate VI) and secondary glaucoma. In this situation, iris atrophy may set in. Living microfilariae have been seen attached to the capsule of the lens and are reported to remain there for a number of months. The changes in the posterior segment are perhaps the most important and can lead to reduction in visual acuity or reduced vision, although the extent of detectable retinal changes does not necessarily correlate with reduction in visual ability. In severe cases, the retina is abnormally transparent with its pigment heaped into masses. Changes in the optic nerve atrophy are common in advanced disease or following treatment and are usually signified by a large glaucomatous cup with peripheral visual field loss and severe visual handicap (Mackenzie, 1987; Abiose, 1993). It has been observed that the severity of ocular complications are due to different strains of the parasite in West Africa (Lewis, 1957; Budden, 1963). The pattern of transmission of *O. volvulus* differs considerably with areas. Some areas such as the rain forest provides a more constant infection over most of the year, contrasting with the very severe cases of blindness in the Savanna region caused by high transmission intensities (Le Berre, 1966; Budden, 1963). In rain forest areas therefore, blindness is usually due to posterior segment involvement (chorioretinitis and optic nerve disease) (Mabey, 1993).

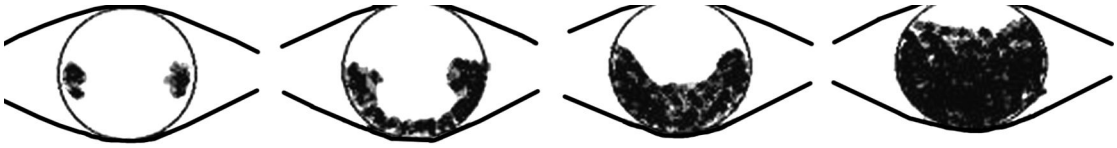


Plate V: Schema of the evolution of sclerosing keratits due to Onchocerciasis in the eyes (Source: Hopkins and Boatin, 2011)



Plate VI: Typical chronic ureitis with secondary cataract in the right eye and sclerosing keratits in the left eye (Source: Hopkins and Boatin, 2011).

2.5.6 The lymph node

Enlarged lymph nodes, either soft or firm are found in patients suffering from onchocerciasis but they are not commonly painful except after treatment. Clinical signs

are hanging groin and scrotial elephantiasis which are said to be common features in Africa. The condition may follow up in chronic cases where the lymph gland draining the skin enlarges to form hanging pockets due to loss of elasticity of the skin in the femoral region and may contain lymph nodules and is associated with hernia (Hopkins and Boatin, 2011)

2.6 Endemicity Levels

Endemicity is the permanent presence of a disease or pathogen agent in a given region, and its level is determined according to the prevalence of the disease or pathogen agent. In the case of onchocerciasis endemicity, levels have been defined according to the number of parasite carriers. However, regions with the same endemicity level can have quite different clinical and socioeconomic manifestations (WHO, 1995).

2.6.1 Hyper-endemicity

According to WHO (1995) hyperendmicity is characterized by the presence of more than 60% *O. volvulus* carriers in the population and an average of more than 10-15 filariae in positive skin snips. In Savanna areas, such percentages are generally accompanied by ocular onchocerciasis in 20% of patients and a blindness rate which can exceed 10%, representing 50% of males over 40 years of age. In this kind of situation the very survival of the rural communities is at stake and the fertile valleys are often unoccupied.

Not all communities with over 60% of onchocerciasis cases are in this situation. In the forest areas, prevalence rates of this order are not, or are only rarely, accompanied by any blindness (WHO, 2000). Moreover, human ecology minimizes the medical consequences of the disease in some societies, depending on the form of land occupancy and utilization and the way in which agricultural work is done.

2.6.2 Hypo-endemicity

In almost all villages where the prevalence rate is below 40% the disease has no social effects. In such communities:

- Fewer than 10% of the population have lesions caused by ocular onchocerciasis and less than 2.5% have irreversible eye lesions;

- The blindness rate is generally below 1%, unless there is another major endemic condition such as trachoma (WHO, 2000)

2.6.3 Meso-endemicity

Between these two limits, over than 40% or less than 60%, there are different kinds of situations. Here again; factors associated with human ecology may substantially modify the impact of onchocerciasis. The definition of meso-endemicity is mainly negative: it is a situation where the disease is socially recognizable but has not yet reached a level intolerable to the community (WHO, 2000).

2.7 Social Effects

Onchocercal skin disease (OSD) has been associated with a variety of psychosocial and economic effects. The disease also leads to stigmatization of affected persons and their families. Unsightly acute and chronic skin lesions and thickened and irritating skin limit the chances of adolescent girls finding marriage partners. Likewise, negative socio-cultural aspects of the skin disease (people worried that skin disease would affect their ability to interact socially, fear of being ostracized, a feeling of low esteem, and children more likely to be distracted in school due to constant itching) have now been recognized (Brieger *et al*., 1998). At the community level, studies have shown a reduced productivity due to the incessant itching, leading to increased poverty, increased expenditure on health in spite of a reduced income, all adding to the vicious

cycle of poverty that the disease creates; is apart from the effects of blindness. Many young people leave the villages for the towns and areas free of *Simulium*, increasing the level of poverty and destitution among the older people left behind and further contributing to the shortened life span of the blind (Prost and Vaugelade, 1981; Remme, 1995; Ikem and Onwuliri, 2007).

2.8 Control Measures

2.8.1 Control of the vector

The most effective way to control the vector is to attack the larval forms. They are easier to control because of their relatively easy-to-reach known habitats in fast-flowing rivers, with a flow rate of 5 metre/sec. The fly has a wind-assisted flight range of about 400 km, making the adult fly more difficult to control directly (Thompson, 1976).

Various methods have been used to control the vector, including attempts at environmental management. The mainstay for vector control has been the use of insecticides with the aim to interrupt transmission of the parasite. In the Onchocerciasis Control Programme (OCP) of West Africa the objective was to continue larviciding for a sufficiently long period during which time the human reservoir of the parasite would die out. For vector control to be effective larviciding needs to be carried out preferably weekly. The larval stages rarely take more than 7 days to about 12 days to change to the next stage. Ground and aerial larviciding are both used for control. Ground larviciding is best applied where this is feasible, such as in small foci, and where the type of vegetation allows it. Aerial larviciding on the other hand has been used more extensively to cover large tracts of river basins, which would otherwise be either impossible or very difficult to cover by ground larviciding. The formulation of insecticides used for large-scale treatment of rivers must meet a range of requirements.

They must be highly effective against the vectors, safe to the rest of the environment, including human and other life forms, with guaranteed supply for a long period and the cost should be low. They must be biodegradable but with a good carry downstream. Various insecticides are available. Temephos (AgrEvo France) is a cheap and efficient organophosphate with insignificant impact on the non-target fauna. Its use, however, needs careful attention given that insecticide resistance emerges very quickly with its application. Other environmentally friendly larvicides that have been used are the organophosphates, carbamates, pyrethroids, and the biological *Bacillus thuringiensis* serotype H14-B.tH14. These are used in rotation in accordance with river flow rate, to help avoid the emergence of insecticide resistance in the vector; to minimize adverse impact on non-target organisms, riverine flora and fauna; and to increase cost effectiveness (Hougard *et al.*, 1993).

2.8.2 Nodulectomy

Nodulectomy can remove some of the adult worms and has been used in some circumstances to try to control symptoms of the disease. Because they are often multiple, and many are also impalpable in the deep tissues, it is impossible to eliminate all adult worms by nodulectomy. Nodules may be removed for cosmetic reasons or because of annoyance due to their position, for example, around the waist where a belt might irritate. The logistical exercise of removing all palpable nodules from all patients in an endemic area would also be overwhelming, so this is not a strategy used for control **(**Schulz-Key, 1990).

Mass nodulectomies were carried out, particularly in the Americas. This made sense because the variant of onchocerciasis in the Americas was often associated with a preponderance of head nodules, which theoretically made it more likely that ocular

lesions would eventuate. However, the effectiveness of this strategy in the long term is questionable, given that a study in Ecuador showed a reappearance of nodules in hyperendemic areas (Guderian, 1988).

2.8.3 Control of the disease with medication

Chemotherapeutic control of onchocerciasis is presently aimed on the microfilaria. The first two drugs most used for treatment in the past were Diethlcarbamazine (DEC) or Hetrazan and suramin with both having detrimental effect on microfilariae of *O. volvulus* DEC reduces microfilarial level in the skin for 1 to 2 months after treatment, but the parasite usually re-establishes itself to pre – treatment level soon after this. Treatment is usually followed by adverse reactions such as pruritus and popular eruptions. DEC has been produced as eye drop and as skin lotion with little encouraging results (Mackenzie, 1987). Suramin the other drug which is an effective microfilaricide produces problems when used in the treatment of onchocerciasis also has macrofilaricidal effect. Suramin which is also known as Bayer 205®, Antrypol®, naprany1®, naphuride sodium, was introduced in 1920 for use in trypanosomiasis and it was subsequently found that the drug also acted on *O. volvulus.* These drugs must be used with caution especially when considering mass treatment. The microfilaria density must be reduced by treatment with DEC before administering Suramin (Ukoli, 1990).

In the late eighties a successful a novel, safe and highly effective microfilaricide (Ivermectin) which represents a very significant therapeutic advance in the field of filariasis, (WHO, 1983 – 1988) was developed. This drug is manufactured by Merck, Sharpe and Dohme of USA for efficient mass dosing. Effort is still needed for the production of macrofilaricidal drugs to take care of adult worms.

Ivermectin is currently the drug of choice for treatment of onchocerciasis following successful community trial in a few countries including Nigeria were it was successful (Abiose *et al*., 1993). It is an effective microfilaricide (kills microfilaria). It also has an effect on reproduction preventing the release of microfilaria by the adult female for approximately 4 months. When microfilariae are released the repopulation of the skin is very slow and after 12 months it reaches around 20% of the original level. There is also a macrofilaricidal effect. This effect was not thought to be particularly important but recent research shows that some of the early studies were not done in a closed system and some of the live adult worms found were possible worms from new infections rather than adult worms resistant to ivermectin. Ivermectin is contraindicated in children under 5 (less than 90 cm height or 20 kg weight), pregnancy and during the first week after delivery and also patients with chronic disease, or central nervous system diseases (Dominguez-Vazquez *et al.*, 1983). Also, Abiose *et al*. (1993), working in the Southern Guinea Savanna of Nigeria, observed that ivermectin would have to be given to all the affected population regularly for the life span of the adult worm – about 19 years. This was considered then a very difficult task especially as large groups of people are excluded from treatment for one reason or the other and others may refuse treatment. They pointed out that best hope for ivermectin will be a reduction in eye disease and blindness from onchocerciasis. They reiterated the suggestions of Braide (1990b) and Pond (1990) that for mass distribution of ivermectin, the responsibility lies with Government or any other organization that takes on the task of distributing the drug. During the elaborate field trial of the ivermectin drug for the control of optic nerve disease (OND), Abiose *et al.* (1993) suggested that ivermectin could reduce the incidence of onchocercal OND in subjects with loads of 10mf/mg by up to 88%. They

stated that onchocerciasis was not the only cause of OND in the communities where they investigated.

During the mass treatment of a human population with ivermectin in Liberia, Trips *et al.* (1990), reported a reduction of infective larvae L_3 by 81.7% to 89.3% and a reduction of all three stages L_1 L_2 L_3 by 93.4% to 95%, leaving yet a few percentage which landing/biting black flies will transmit since monthly transmission potential only showed a decrease from 22.9 to 5.8 (94.6%).

In the long run therefore, the combination of vector control plus treatment with ivermectin became necessary to effect significant and widespread reduction in the transmission of *O. volvulus* within and between affected communities (Cupp 1984). The ideal drug would be one that kills the adult worm causing no side effects. A drug, Moxidectin, is currently undergoing trials, but it is unlikely to be cleared for mass usage before 2014 or 2015 even if it does prove to be effective. Doxycycline also has macrofilaricidal effects by killing *Wolbachia*, an obligatory endo-symbiotic bacterium in some filarial parasite including *onchocerca*. If *Wolbachia* are killed, the female cannot reproduce and will eventually die. The problems with Doxycycline are that it has to be given for a prolonged period, at least a daily dose for 4 weeks, and it cannot be given to children under 12, which is a very significant proportion of the affected population in most endemic areas (Wanji *et al.*, 2009).

2.8.4 The role of ivermectin in disease control and elimination

The discovery of the microfilaricidal potential of ivermectin radically changed the prospect of onchocerciasis control by chemotherapy. Ivermectin is isolated from the soil-dwelling mold, *Streptomyces avermectilis*. This mold was discovered by Omura *et al.,* near a golf course in Ito, Japan, and sent to the Merck, Sharp, and Dohme

laboratories in 1974. In 1981, ivermectin was marketed as a broad-spectrum anthelmintic drug in veterinary medicine, particularly for the dog heart worm *Dirofilaria immitis.* In 1982, researchers at Merck, Sharp, and Dohme reported activity against *O. volvulus* (Aziz *et al*., 1982, Sutherland and Campbell, 1990; Omura and Crump, 2004).

The immediate concern then was whether or not ivermectin caused optic neuritis and secondary optic atrophy, as did DEC. A randomized controlled trial carried out in Kaduna State compared ivermectin with placebo and found that there was a significant difference in the incidence of optic neuritis between the two groups (Abiose *et al*,. 1993). This suggested that ivermectin reduced the incidence of optic neuritis in subjects with loads above 10 microfilariae per mg, but had little effect in those with lower loads. The implication was that sustained annual delivery of ivermectin could prevent a substantial proportion of onchocercal blindness (Guderian, 1988). In the same trial it was established that ivermectin did not precipitate optic neuritis within 2 weeks of ingestion, as did DEC (Murdoch *et al*., 1994). This trial and other established the safety and efficacy of ivermectin (Pacqué *et al*., 1991; Campbell and Benz, 1984).

2.9 Onchocerciasis Control Programme in West Africa

The Onchocerciasis Control Programme (OCP) in the Volta River Basin in West Africa was established as a regional programme in the early 1970s. Its prime aim was to eliminate onchocerciasis as a disease of public health importance in West Africa and to place participating countries in a position to control any recrudescence of infection should it occur. The program was executed by the World Health Organization (WHO) on behalf of the Committee of Sponsoring Agencies (CSA) (Food and Agricultural

Organization, United Nations Development Programme, the World Bank with a rich donor participation, and the WHO) and the 11 participating countries—Benin, Burkina Faso, Cote d'Ivoire, Ghana, Guinea Bissau, Guinea Conakry, Mali, Niger, Senegal, Sierra Leone, and Togo (Samba, 1994; Boatin, 2008).

The OCP started as a vertical control programme with its vector operations in 1975. At the close of the program in 2002, the operations covered an area of 1,200,000 km^2 of which 764,000 km^2 benefited directly from vector control. Over 50,000 km of rivers was surveyed and appropriately larvicided. The objective of vector control was to interrupt transmission of the parasite for long enough periods to allow the human reservoir to die out. Initially planned to last 20 years, the period for larviciding was reduced to 14 years upon new evidence, which suggested a shorter reproductive life span (12 years) for the worm. This period of vector control was further reduced to 12 years when larviciding was combined with ivermectin treatment in light of additional information from model predictions **(**Boatin, 2008**).** Vector control did not benefit the population already infected, or those who were already suffering the morbidity associated with the disease before larviciding started. However, as from 1987, when ivermectin treatment was added to larviciding in some areas, direct morbidity control became part of the programme strategy. The OCP is cited as one of the most successful large-scale control programme ever undertaken in the developing world.

Several reasons may be responsible for this success. First, there was a sense of common purpose by all concerned. There was a single objective that was developed by all, agreed upon, accepted, and was stuck to by the group concerned. It was as a "regional programme" that the participating countries, which bore the brunt of the disease burden, wanted. It is clear that given the long range of the vector and the complex nature and

heavy infrastructure involved in the operations only a regional approach was likely to succeed. All concerned devoted their attention in terms of time, funds, and in kind contribution to the OCP. Donor participation and funding was unflinching and the contribution through the free drug donation by Merck was remarkable. Furthermore, the programme was driven by motivated and competent staff and by operational research whose findings underpinned the strategies that were used. As the flies in the river basins were controlled, the communities returned to the previously abandoned villages **(**Boatin, 2008**).**

This, in several situations, was not done in a controlled fashion, creating a new challenge of intense pressure on the river basins and the land, although in some countries organizations were set up to coordinate this rehabilitation of abandoned land. Since the program has ended, flies have begun to reinvade the areas. Although these flies are no longer infected, and there is no risk of river blindness, the population in some areas is complaining of the "failure of the programme," as they suffer once again from the biting nuisance **(**Boatin, 2008**).**

2.10 African Programme for Onchocerciasis Control (APOC)

The African Programme for Onchocerciasis Control (APOC) was established at the end of 1995 to help control Onchocerciasis in 19 countries that were thought to be endemic for onchocerciasis in Africa outside of the OCP area. The principal strategy was mass distribution of ivermectin and the chief objective of the program was to set up sustainable ivermectin distribution that would continue when the program finished. A secondary objective was to eliminate the vector completely in the few sites where the conditions existed. One of the first activities of APOC was to map out not only where the disease existed but where the prevalence of the disease was sufficiently high to warrant mass treatment (Taylor *et al.,* 1992).

It was found that there is a fairly consistent relationship between the presence of nodules in the population and the overall prevalence of the disease, nodules being present in one third to one half of the population who are positive by skin snip. Fifty people resident in a community for at least 10 years can be examined and an estimation of the prevalence can be made dividing populations into hyper-endemic where mass treatment is urgent, meso-endemic where mass treatment is desirable and hypo-endemic where only individual cases with symptoms need treatment. Skin and eye disease is infrequent in hypo-endemic communities. This assessment by village called Rapid Epidemiological Assessment (REA) was further adapted using knowledge of the terrain and the population into a broader-based mapping system based on river valleys where a biased sample of villages were evaluated, according to strict criteria. Although this Rapid Epidemiological Mapping of Onchocerciasis (REMO) is not a detailed epidemiological study, it enables decisions to be made where mass treatment should be carried out (Ngoumou and Walsh, 1993).

Although some refinement is still needed in difficult areas, most of the 19 countries have been mapped and red areas (needing mass treatment) (plate VII) and green areas (not needing mass treatment) have been defined. Three of the 19 countries—Kenya, Mozambique, and Rwanda—only have hypo-endemic disease and are not therefore receiving MDA. Because of this need for a sustainable method of MDA, a low-cost distribution system was needed. Early trials using mobile teams were not sustainable and community-based approaches were required. Research showed that if communities were empowered to make their own plans of actions once they had been fully sensitized and had been trained in the necessary technical details, they were able to organize their own MDA with minimal input from the health services, which are often non-existent in remote communities. This led to a refinement of community-based approaches called Community-Directed Treatment with Ivermectin (CDTI) (Amazigo, 2008).

Once communities are informed, they undertake the following tasks of choosing a community distributor for training, conduct census to calculate Mectizan requirements, organize the collection of Mectizan from a health centre or other distribution point, organize a distribution method (house to house or fixed point, assist the distributor calculate the dose and distribute the Mectizan, organize transfer of patients with adverse events to health facilities for treatment, if required, note the treatment statistics and report to the health authorities, participate in community supervision and arrange appropriate recognition at the community level of those who have given their time to work for the distribution (Amazigo, 2008; WHO/APOC, 2009).

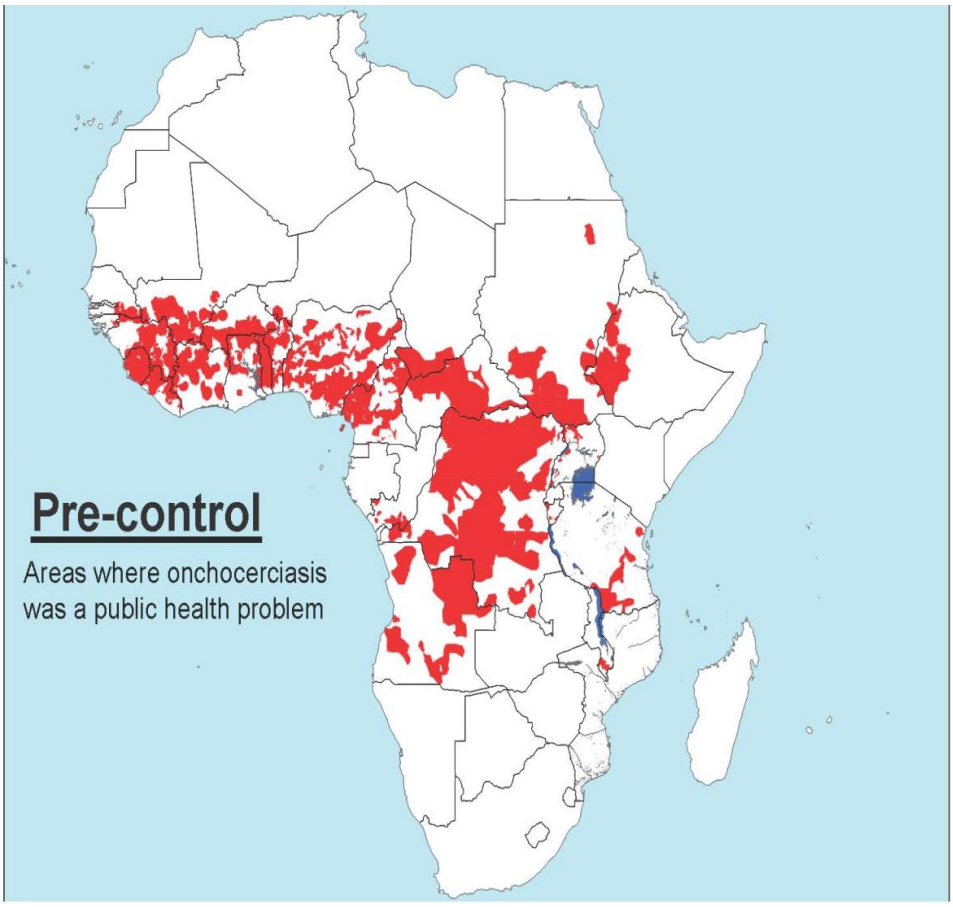


Plate VII: Onchocerciasis endemic areas in Africa (Source: APOC, 2010).

After 5 years communities should be ready to continue treatment alone, with minimal help and supervision from the primary health care (PHC) services, so although it takes time and effort with each community to initiate CDTI, once the system is functional, it becomes more and more sustainable. The development of CDTI has been one of the major advances in PHC over the last decade and has been the foundation for other community activities (WHO/APOC, 2009).

2.11 Community-Directed Intervention (CDI)

The strategy for onchocerciasis control until 1987 was entirely by controlling the vector. The community was hardly involved apart for the employment of some local people as fly catchers and participation in parasitological and epidemiological studies. With the introduction of ivermectin and Mass Drug Administration (MDA), different strategies were needed. Many onchocerciasis MDA programmes in Africa were begun initially by Non-Governmental Development Organizations (NGDOs) working in eye care; these MDAs were often a part of an integrated eye care programme, and Vitamin A distribution was added on to the MDA. In some areas MDA with Praziquantel for schistosomiasis (Bilharzia), and/or MDA including albendazole for lymphatic filariasis elimination were added on as well. Repeated MDA requires much more community participation and research has shown that if communities are empowered to organize their own treatment, they are fully able to do so, not just with ivermectin but also with other MDA. This co-implementation has now been fully tested and found to achieve better results than traditional methods using the health services in an approach called community-directed intervention (CDI). It has also been extended to other non-MDA interventions such as distribution of bed nets, and home treatment of malaria (WHO/TDR, 2009).

APOC was conceived as a control programme with two main objectives. First, the main objective was to set up sustainable program for MDA, with the eventual aim of eliminating onchocerciasis as a "public health problem." Second, another less important objective was to eliminate the vector of the disease where possible, which included a few small foci in non-OCP areas in Uganda, Tanzania, and the Island of Bioko (Equatorial Guinea) in the Gulf of Guinea. It was considered that onchocerciasis could not be eliminated in Africa using current tools (Dadzie *et al.*, 2003). Studies in Senegal and Mali have now shown that ivermectin could be stopped after 16 years, at least in some areas of savannah onchocerciasis (Diawara *et al.*, 2012; Afework *et al*., 2012). Some areas in Uganda where there has been a combined vector and ivermectin approach treatment is on the point being discontinued (Ndyomugyenyi *et al.*, 2007). Skin snip research in other foci of infection have indicated similar results after 14–18 years, but further studies are required to confirm the interruption of transmission. Results have not been uniform and are probably related to the intensity of the original infection as well as levels of treatment coverage. These studies have stimulated a major change in thinking, that onchocerciasis could be eliminated at least from some areas of Africa using ivermectin distribution. It is now conceivable to think of eliminating the transmission of onchocerciasis in western and eastern Africa, even though the central African region will remain a problem due to the problems caused by conflict and the presence of another filarial infection Loa-Loa (WHO/TDR, 2009; APOC, 2010).

Because ocular damage is mostly attributable to the microfilaria, it became necessary to find chemotherapeutic agents active against both the microfilaria and the adult worm (either singly or in combination). This is because the adult worms, often found coiled up in subcutaneous nodules, sometimes adjacent to deep muscular tendons, have a life span of about 10–15 years and continue to produce microfilaria. With confirmation of

the efficacy and safety of ivermectin as a microfilaricide, it became possible to plan a control programme with ivermectin, particularly in the non-OCP countries, which include Nigeria and the onchocerciasis-endemic countries in central and east Africa, as well as countries in Central and South America. APOC was set up in 1995 to eliminate onchocerciasis as a disease of public health importance in Africa. It expresses a renewed commitment to work towards the elimination of onchocerciasis in all countries where it is a public health problem. The challenges to the execution of the community directed treatment with ivermectin projects include timely collection of drugs, integration of community-directed treatment with ivermectin into existing primary health care services, strengthening of local health infrastructure, achievement of optimal treatment coverage, establishment and up-scaling of community self-monitoring, designing and implementing operations research locally, ensuring the adequacy of community-directed distributors, and increasing the involvement of local Non-Governmental Organizations (Amazigo *et al.*, 2002). Others are the achievement of financial sustainability and cost-recovery systems and effective advocacy. Coverage so far has varied from place to place. The following are not eligible to receive ivermectin: children five years or younger, pregnant women, and the obviously sick (Brieger *et al*., 2002).

However, coverage is deemed satisfactory where 90% of the annual treatment objective or 65% of total population is met, as is the case of communities in Uganda (Katabarwa *et al*., 2002). The annual treatment objective being the number of persons at risk of onchocerciasis in any given area of community-directed treatment with ivermectin who are estimated to require treatment. Adverse drug events have been reported with ivermectin, but these have been generally mild, except in areas where there are overlaps with *Loa loa* (Rothova *et al*., 1989)

In Sierra Leone, 28 (32%) of 87 patients had adverse reactions that required treatment with acetylsalicylic acid and antihistamines, but none of the observed adverse reactions were considered life-threatening. The incidence of the Mazzotti reaction during treatment of onchocerciasis with ivermectin runs at approximately 10%, which is much lower than that seen with DEC. Even so, nearly one-quarter of patients treated with ivermectin developed isolated fever or itching without the remaining constellation of symptoms. Because of its better safety profile, ivermectin has supplanted DEC as the drug of choice for the treatment of onchocerciasis (Ottesen, 1994). A significant relationship was found between the frequency and severity of side effects and the degree of parasite infestation. In Nigeria, a later study indicated that most of the reactions were experienced in the first round of dosing, and the frequency reduced with subsequent rounds. The most serious side effect noted in that study was joint pains (Oyibo *et al*., 2003).

2.12 Criteria for Elimination of Transmission of Onchocerciasis

The World Health Organization has produced criteria for certifying elimination of the transmission of the disease. These are not applicable in all circumstances and will have to be adapted to different epidemiological situations.

Transmission trials showed that mass treatment with ivermectin resulted in a major reduction in transmission of onchocerciasis in Africa (Remme *et al.,* 1989). However, after the first three rounds of treatment significant transmission continued. Simulation models that were calibrated using these early trial data predicted that interruption of transmission by ivermectin treatment alone would be difficult to achieve and that ivermectin treatment needed to be planned for a period of decades (Winnen *et al.,* 2002). Hence, ivermectin treatment programmes were established with a long time

frame in mind, with the exact duration to be determined on the basis of further evaluation of the long-term impact of mass treatment on transmission and the parasite reservoir.

With the closing down of the Onchocerciasis Control Programme (OCP) in West Africa at the end of 2002, all subsequent onchocerciasis control were transferred to the participating countries and almost entirely based on periodic mass treatment with ivermectin using the strategy of Community Directed Treatment with Ivermectin (CDTI) (Remme, 1995). The duration of treatment with ivermectin is not clearly spelt out, since there is no certainty that the drug alone can eliminate transmission. If it is possible to totally eliminate transmission by treatment alone then, to avoid recrudescence, control has to continue indefinitely or until alternative interventions (e.g. macrofilaricidal drugs (Remme, 1995) become available. Studies using the stochastic microsimulation programme ONCHOSIM (Plaisier, 1990), carried out by Winnen *et al*. 2002 and Remme (1989) indicated that prolonged high-coverage treatment programs with ivermectin would have a high probability of achieving elimination. However, many uncertainties remain about these model predictions, and much effort is needed to refine them.

In Africa, several field studies have achieved significant reductions in the transmission of infection by repeated annual mass treatments with ivermectin (Guillet, 1995; Boatin, 1998; Diawara *et al*., 2009; Afewwork *et al*., 2012; Traore *et al*., 2012). A simulation study, using the microsimulation model ONCHOSIM for onchocerciasis transmission and control, predicted that programmes combining vector control and the mass distribution of ivermectin would lead to the elimination of the infection from a community much more quickly than vector control alone (Plaisier, 1997). However, this

finding is not germane to today's control strategies because vector control ceased in most countries in 2002. Currently studies are focused on the model of epidemiological impact of community treatments with ivermectin alone. In order to make our results relevant to the African Programme for Onchocerciasis Control we consider areas of endemicity where there has never been any vector control.

The ONCHOSIM model, attempts to predict whether, and under what conditions, control strategies based on mass treatments with ivermectin may lead to the elimination of onchocerciasis. The factors investigated include the interval between treatments, coverage and the pre control level of endemicity (Borsboom *et al*., 2003)

In West Africa the Onchocerciasis Control Programme demonstrated that the prevalence and intensity of infection with *Onchocerca volvulus* could be reduced to insignificant levels through vector control (Remme *et al*., 1990). For economic reasons, however, it became apparent that neither vector control alone nor a combination of vector control and ivermectin treatment would provide a sustainable long-term solution for most areas of endemicity, where, consequently, reliance has to be placed solely on drug therapy for controlling or eliminating the disease. Although more effective alternatives to ivermectin alone, either by combining ivermectin with other drugs or macrofilaricides (Langworthy *et al*., 2000), hold promise. It was recently suggested that increasing the frequency of ivermectin administration from annually to six-monthly would increase the probability of eliminating the parasite in the long run (Richards *et al*., 2001), but this has been questioned for reasons of logistics and cost (Abiose *et al*., 2000 and Richards *et al*., 2001). In fact, this has been the strategy of the Onchocerciasis Elimination Programme for the Americas, following the successful interruption of transmission by treatment programmes in isolated foci in Guatemala and Ecuador

(Cupp *et al*., 1992; Collins *et al*., 1992 and Guderian *et al*., 1997). Afework *et al*., (2012) studied the impact of long-term treatment of onchocerciasis with ivermectin in Kaduna State, Nigeria, and thereby providedthe first evidence of the potential for elimination in the operational area of APOC.

The concept of a breakpoint is operationally important: it means that infection and transmission does not have to be completely zero before treatment can be safely stopped. This concept has been proven in practice: in Senegal and Mali there were still several mf- positive people in each of the three river basins but when treatment was stopped, there was no renewed transmission and infection. The same was observed in the Onchocerciasis Control Programme in West Africa where the prevalence of infection was still greater than zero in each river basin where vector control was stopped but, again, the cessation of control did not lead to renewed transmission and the infection died out (APOC, 2010; Traore *et al*., 2012).

There are four phases which indicate the transition between phases and changes in required interventions or surveillance activities. The four phases are critical periods in progress to elimination. In Phase 1 transmission of the disease continues but the annual transmission potential is gradually reduced due to an appropriate intervention (ivermectin treatment, vector control, or both). The Annual Transmission Potential (ATP) is the number of infective larvae that could be transmitted to an individual if all the flies biting him during 1 year were able to transfer all the load of L3s. This figure varies with vector differences and the strains of *O. volvulus*. An ATP of less than 100 is considered to be safe to protect from eye manifestations of the disease but must be brought down much lower to eliminate transmission. This is considered the “break

point" in modelling of the disease (Plaisier *et al.*, 1997; APOC, 2010; Traore *et al*., 2012).

As the treatment strategy continues, there will be no more transmission, either due to absence of flies using vector control or absence of infected bites if ivermectin treatment is used. In Phase 2, transmission has ceased and the adult population will age and will die off. It should be noted, however, that while some adults are alive, there is always the possibility of transmission, if control activities are suspended. It is in fact vital during this phase that control efforts continue to the end of the phase, if not all that has been gained will be lost (Hopkins, 2007). OCP using vector control, used 14 years as the most appropriate length of Phase 2. A similar period is proposed for ivermectin, but if multiple treatments per year are used, the adult worms will age and die much more quickly and this period could be shortened to 6 or 7 years. It should be noted that Phase 2 only begins when transmission has been suppressed because until that is achieved; there is always the possibility of introducing new filaria into the system. Annual treatments with ivermectin, especially when coverage has not been adequate, will not suppress transmission and so will delay the beginning of Phase 2. In Phase 3, treatment is stopped. The disease will die out under normal circumstances, even if all the parameters are not right down to zero, but regular surveillance is carried out to confirm that there is no recrudescence. It should be noted that during this phase there are surveillance activities that need to be continually funded (APOC, 2010).

Phase 4 covers a period of time when reinvasion could occur from another focus of infection, or alternatively due to massive movements of populations, for example, in a war situation, infected patients in sufficient numbers move to a new area where there are flies, so that transmission could recommence (Traore *et al*., 2012).

2.13 Guidelines for Elimination

The World Health Organization has produced guidelines to certify elimination (WHO, 2001).

2.13.1 Elimination of morbidity:

- Prevalence of microfilariae (Mf) in the cornea or anterior eye chamber <1%.

2.13.2 Elimination of transmission:

- L3 in flies <0.05% (0.1% in parous flies).
- Annual Transmission Potential (ATP) lower than 5–20 L3 per season.
- Absence of detectable infection in school children and antibody prevalence of <0.1%.

To certify elimination by WHO, this has to be country wide, for all foci of infection. Some modifications of these criteria are needed to adapt to different situations, for example, where flies have been eliminated by vector control methods (WHO, 2001).

2.14 Transmission Zones

This is an important issue for the Onchocerciasis elimination programme. Mass treatment is on-going in meso-endemic and hyper-endemic regions only. These are areas where skin disease and eye complications are present. In hypo-endemic areas only patients with symptoms are treated. Transmission is certainly taking place in some of these hypo-endemic zones, although it may not be important in some areas where the meso-endemic and hyper-endemic foci have been well controlled. These areas need to be fully investigated to see where transmission is occurring and what would be an appropriate treatment strategy. In many of the foci in APOC countries there is co-infection with lymphatic filariasis, another filarial disease that uses ivermectin together

with albendazole for an elimination program. Distribution of ivermectin may already be on-going in some of these hypo-endemic areas and programs must be coordinated. (APOC, 2010)

2.15 Frequency of Treatment with Ivermectin

A single dose of ivermectin will reduce Mf in the skin to negligible levels in a day or so. The adult females start to reproduce after 3–4 months and microfilaria can appear once again in the skin. However, as already mentioned, even after a year the worm load is around 20% of the pre-treatment levels. This has been the basis for suggesting annual treatment as a control measure. After a few rounds of annual treatment, levels of MF in the skin are so low as to already have an effect on transmission. If the objective is to change to the elimination of transmission, this may or may not be sufficient. Annual treatment has certainly proved effective in some circumstances and has eliminated the disease in some foci in West Africa. To move from Phase 1 to Phase 2 of elimination twice yearly treatment would be quicker. However, in programmes already well-established with annual treatment and already moving toward elimination, the logistical complications of changing strategy is probably not warranted (Aziz *et al.,* 1982; Sutherland and Campbell, 1988; APOC 2010).

2.16 Diagnostics

Skin snipping and fly dissection have been the mainstay of control diagnostics. Fly dissection is tedious, since many flies need to be dissected. However, for the flies, pool screening using polymerase chain reaction (PCR) is proving very effective and is very practical. Skin snipping becomes increasingly less sensitive as numbers of MF diminish. Also skin snipping is a painful procedure; to confirm the absence of the disease, it is recommended to check 3,000 children in the area. Two methods are useful,

but both have drawbacks. First, the DEC patch test is non-invasive, but the patch must remain on the skin until read the following day and health staff must be available to read the result. Serological tests such as the OV16 test are very useful but require laboratories some distance from the field to analyse the results. If OV16 could be developed into a simple card test carried out in the field and read in the field, it could become an ideal test (Unnasch and Meredith 1996; APOC, 2010).

2.17 Polymerase Chain Reaction (PCR) for Estimating Prevalence of *Onchocerca* infection in *Simulium* sp

A method based on the polymerase chain reaction (PCR) amplification of an *Onchocerca*-specific repeated DNA sequence (the O-150 PCR) was developed to identify *O. volvulus,* and to distinguish the blinding and non-blinding strains of the parasite (Meredith *et al*, 1991, Zimmerman *et al.*, 1992; Zimmerman *et al.,* 1993) This method has been successfully applied by the OCP to correct for the presence of animal filariae and non-blinding *O. volvulus* in calculations of the annual transmission potential for blinding Onchocerciasis (Toe *et al.,* 1994).

The O-150 PCR has also been shown to be capable of detecting a single infected fly in pools containing up to 100 flies (Katholi *et al.*, 1995). Furthermore, a mathematical model has been developed to allow one to calculate prevalence of infection in the black fly population, based upon the size of the pools screened and on the percentage of negative pools found. (Katholi *et al.*, 1995) The assay and algorithm have been shown to accurately predict the prevalence of infection in simulated black fly populations prepared by mixing known numbers of experimentally infected flies and uninfected flies (Katholi *et al.*, 1995).

This pool screening technique has proved to be a useful adjunct to the classic dissection method because it is capable of providing an early warning that transmission is occurring in a given area. If this is found, more elaborate studies can then be carried out in the endemic human population to better assess the situation. This could, in some cases, be the first step in the approach to the early detection of possible recrudescence. For the PCR pool screening assay to be of practical use, it must first be demonstrated that the method is capable of accurately predicting the prevalence of infection in natural vector populations. It is particularly important to demonstrate that the method can be used in areas that have been subject to successful control measures, where infection rates are low. The method has been used to distinguish infective from merely infected vector black flies. This is important since only the flies carrying the infective stage of the parasite are capable of contributing to transmission. This could be accomplished by restricting the analysis to the head capsules of the fly, in which only the infectious stage of the parasite is found. The PCR pool screen assay has proved to be a valuable addition to the tools for epidemiologic surveillance for *O. volvulus* infection (Yameogo *et al*., 1999).

In recent time, molecular technique has been explored as an adjunct to dissection in estimating the infectivity of adult female flies caught in the wild (Yameogo *et al*., 1999). The O -150 PCR pool screen technique can detect a single infected fly in pools containing up to 100 flies (Katholi *et al*., 1995). This mathematical model is able to calculate prevalence of infection in the black fly population based on the size of the pools screened and on the percentage of the negative pools found (Katholi *et al*., 1995). To achieve this, a method for the mass separation of heads and bodies of black flies was developed (Yameogo *et al*., 1999; Guevera *et al*., 2003). This method allows separate estimates of the prevalence of the parasites in the head capsule (representing L3 of from

human or other vertebrate infection stage) and the rest of the body (representing the earlier developmental stages, i.e. the L1 and L2 larvae).

The field studies in Africa and Mexico showed that the 0-150 pool screen PCR assay generated the estimates of the prevalence of infected and infective flies in the vector population that were indistinguishable from those obtained by traditional dissection of the flies (Opera *et al*., 2005; Rodringuez-Peres *et al*., 1999).

2.18 Economic Importance

Onchocerciasis programmes have successfully controlled transmission of this disease in central West Africa, at an annual cost per person of $0.58.26 As a result of the work of the OCP, improved health among the adult population and additional onchocerciasis-free land has led to increased agricultural and labour productivity generating an estimated $3.7 billion. The estimated economic rate of return for the OCP is 20% over a span of 39 years (Kim and Benton, 1995).

In its follow-on intervention, APOC which uses a drug therapy rather than insecticide spraying, demonstrated cost effectiveness for *ivermectin* use that was estimated at $14-$30 per DALY prevented (Waters *et al*., 2004). WHO has found that treatment cost for *ivermectin* is $0.57 per person, yielding a 17% economic rate of return. The prevalence of blindness was 0.40% in 1995 (385,000 cases); dropping to 0.28% in 2010 (265,000 cases). The APOC program in Uganda is on track to eliminate onchocerciasis at a cost of $0.78 per person. As a result of onchocerciasis programmes, uninhabited fertile areas along river banks, which were breeding grounds for flies spreading onchocerciasis, can now be utilized (Haddad *et al*., 2008). This is best illustrated by a World Bank evaluation of onchocerciasis control programmes, which looked at river-based communities that had abandoned land due to endemic blindness in their populations. It

found that once onchocerciasis was controlled in these areas, 25 million hectares of land was returned to agricultural production, enough to feed 18 million people (Brooker *et al.*, 2009).

2.19 Economics of Control Programme

The distribution of ivermectin has dramatically altered the nature of onchocerciasis control. Existing economic analysis of ivermectin distribution has shown that the programme has a highly beneficial impact. Most analysts have estimated the economic benefits in terms of increased labour productivity as a result of reduction in blindness, additional land availability because of reduced transmission of the parasite. The economic success of ivermectin distribution is sensitive to the fact that the drug itself has been donated free the costs of treatment is mainly covered by Non-Governmental Organisations. (Waters *et al.*, 2004)

APOC through CDTI has substantially lowered the per dose of ivermectin treatment. Onwujekwe *et al.* (2002) estimated the cost of treatment to be $0.17 and $0.13 per dose in 2 villages, Nike and Achi in Enugu State, Nigeria. These estimates include direct financial cost, opportunity cost, advocacy, community mobilisation and health education and training.

Economic analysis is used for documenting the costs of disease and the payoff to public health disease control programmes. Economic analyses of onchocerciasis and programmes to control it have used all of these techniques to quantify the relationship between programme cost and impact. There remains a wide variety among studies in terms of category of costs included and techniques to arrive at total cost estimates (Waters *et al.*, 2004)

According to Waters *et al.,* (2004) there are 3 types of economic analysis of disease control, which provide an overview of the principal economic evaluation of onchocerciasis control programmes. These are:

- Calculating the costs of illness – documentation of the costs of a disease is a key step in promoting treatment efforts. Costs can include lost wages, lost time, the costs of treating the illness, and the lost earnings of persons caring for sufferers of the disease.
- Cost-benefit analysis (CBA) – provides information on both the costs of the intervention and the benefits, expressed in monetary terms. Results are generally expressed as the difference between discounted benefits and costs (net present value), the economic rate of return, or the ratio of benefits to costs.
- Cost-effectiveness analysis (CEA) – provides information on the cost of the intervention and its effectiveness, where effectiveness is not expressed in monetary terms but rather by a defined metric..

2.19.1 Estimates of economic costs of onchocerciasis

Several studies have used cost-benefit analysis to calculate the Net Present Value (NPV) of Onchocerciasis control. NPV is the difference between the discounted present value of benefits and the discounted present value of costs. For this review, NPV is estimated as the present value of stream of net returns (benefits minus costs) of a project during its economic life. The net present value is determined by discounting expected future net returns at a rate which reflects the cost (interest rate) of borrowing funds or the likely return on a best alternative investment available for those funds (Boardman *et al*., 1996). A Positive NPV is an indicator of a successful investment.

The cost benefit analyses included in the review also provide an economic rate of return (ERR), otherwise known as the internal rate of return. The ERR is most often calculated

as the discount rate that sets the net present value of the stream of net benefits equal to zero. If the ERR is greater than the market interest rate or the cost of borrowing money, then the programme is determined to be an economically worthy investment . An ERR of 10% is considered by the World Bank as a standard for successful health programmes (Kim and Benton, 1995). The ERR will generally increase when long-time horizons are evaluated – because positive programmes impacts such as reducing blindness will continue to accrue after the completion of fixed period of programme inputs.

McFarland and Murray (1994) estimated that $195 million would be required for the OCP over10 years. They assumed that the programmes served 16.8 million person and that 500,000 cases were prevented annually and that subsistence income level was $150 resulting in economic benefits of $75 million annually. Additionally land-related benefits for the OCP estimated at $205 million over 50-year project life with a 5% discount rate resulting in a net present value of $85 million over a 10 year project time period. Land-related benefits include positive effects on the supply of labour as fewer workers are affected by Onchocerciasis and increased agricultural output as unusable land is reclaimed.

The 1997 economic impact analysis of APOC also led by Benton (1997) estimated that the APOC programme's costs over the time period 1996-2007 to be $161 million and $3131.2 million in 1996 using an annual deflator of 4%. These figures include all costs incurred by the donors, the beneficiary governments and NGOs but excludes the cost of the drug which is donated by Merck. This focuses on ivermectin distribution only. Benton's analysis calculated the value of preventing blindness as an increase of 20 productive healthy life-years discounted at #5. He used a disability weight of 1.0 for

blindness – in order words assuming that blindness completely prevents productivity. He found an NPV of $15.5 million and an ERR of 6% at a 10% discount rate for the 1996-2009 project period.

Haddix (1997) also evaluated the APOC programme using the same cost values as Benton (1997) and also excluding the costs of Merck. One difference between the two studies is that Haddix (1997) includes the benefits of both land and labour instead of solely focussing on labour. Haddix (1997) estimated that long-term NPV using a 3% societal discount rate to be $307 million, for an ERR of 24%, and a corresponding NPV of $87.6 million with a 10% discount rate.

2.19.2 Cost-effectiveness analyses

There are different analyses evaluating the cost-effectiveness of onchocerciasis control. McFarland and Murray (1994) calculated that on an annual basis in Africa onchocerciasis accounts for 640,000 Disability-Adjusted Life-Years (DALYs) – health life years lost due to disability and mortality. This represents 0.22% of the total disease burden for the region. They estimated that a programme to effectively address onchocerciasis on the continent would cost $19.5 million annually which would result in a cost per DALY of $30.47 if all onchocerciasis -related DALYs were eliminated

Post and Prescott (1984) calculated that the activities of the OCP in seven West African countries could add 1.1 million health life-years added over 20 year at a cost of $20 per healthy life-year added. They assumed that one case of blindness resulted in 23 healthy life years lost. Benton (1997) calculated that the APOC's activities would result in 10 million discounted healthy life-years being added in the time horizon of 1996-2017 equal to a programme cost of $13.52 per DALY prevented. By way of comparison, cost-effectiveness studies have found similarly wide ranges of estimates from $12 to

$30 for immunisation programmes; $1-$25 for micronutrient fortification; $25-475 for distribution of oral rehydration salts and $20-$50 for treatment of acute respiratory infections.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Kaduna State (10° 33′ N; 7° 75′ E), located in the middle belt of Nigeria with a projected population of 7.31 million (National Population Commission, 2006). The state shares boundaries with Katsina and Kano States in the north, Plateau State to the east, Nassarawa State to the southeast, Niger State to the west and Federal Capital Territory, Abuja (FCT) to the south. Some major rivers in the state include *Kaduna*, *Galma* and *Gurara* rivers. Vegetation is mainly Guinea savanna grassland, with few areas of forest mosaic. The climate consists of two distinct seasons: the dry season from November to March and wet season from April to October. The people are predominantly farmers and fishermen with a few engaged in petty trading (http://www.onlinenigeria.com/map.gif.) Kaduna State is meso to hyper endemic for onchocerciasis (NOCP, 1994; 2002).

The selection of the study area was based on the works of Afework *et al*. (2012) who had carried out an epidemiological survey in Lere and Kauru LGAs based on baseline surveys of 1988. In the 2008 post intervention survey, 6,062 persons were examined and none was found to be positive for microfilariae (Afework *et al.*, 2012).

Prior to the commencement of the study, prospection of river basins was done to determine availability of blackflies to ensure that both epidemiological and entomological studies can be carried out in the same area.

3.2 Study Design

The study was designed to evaluate entomological, parasitological and economic indices in some onchocerciasis-endemic communities after several years of mass ivermectin treatment. Data were collected in 3 transmission foci; one in Kudaru (Lere LGA), Galadimawa (Kauru LGA) and Gantang (Kagarko LGA). The entomological collections were along River Galma (8° 50′N; 10° 66′E) in Kudaru and River *Gurara* (7° 88′N; 9° 68′E) near Gantang (Figure 3.1). The parasitological survey was done in 10 communities in Gantang area while economic evaluation was in 33 study communities within the three transmission foci (Table 3.1). Afework *et al.* (2012) had earlier carried out parasitological evaluation of the fifteen communities in Kudaru and Galadimawa areas in 2008.

3.3 Ethical Clearance

Ethical clearance was sought and obtained from the Kaduna State Ministry of Health before the commencement of the study (Appendix 1). Informed consent was obtained from the local government authorities, communities and from where individuals were recruited for the study.

3.4 Collection of Blackflies

The entomological evaluation of residual transmission levels in the two foci of Lere and Kagarko was carried out in two rainy seasons to determine impact of lont-term treatment on the disease vector. Adult Blackflies were collected from selected breeding sites along River Galma (8° 50′N; 10° 66′E) in Kudaru and River *Gurara* (7° 88′N; 9° 68′) in Gantang of Lere and Kagarko LGAs, respectively.

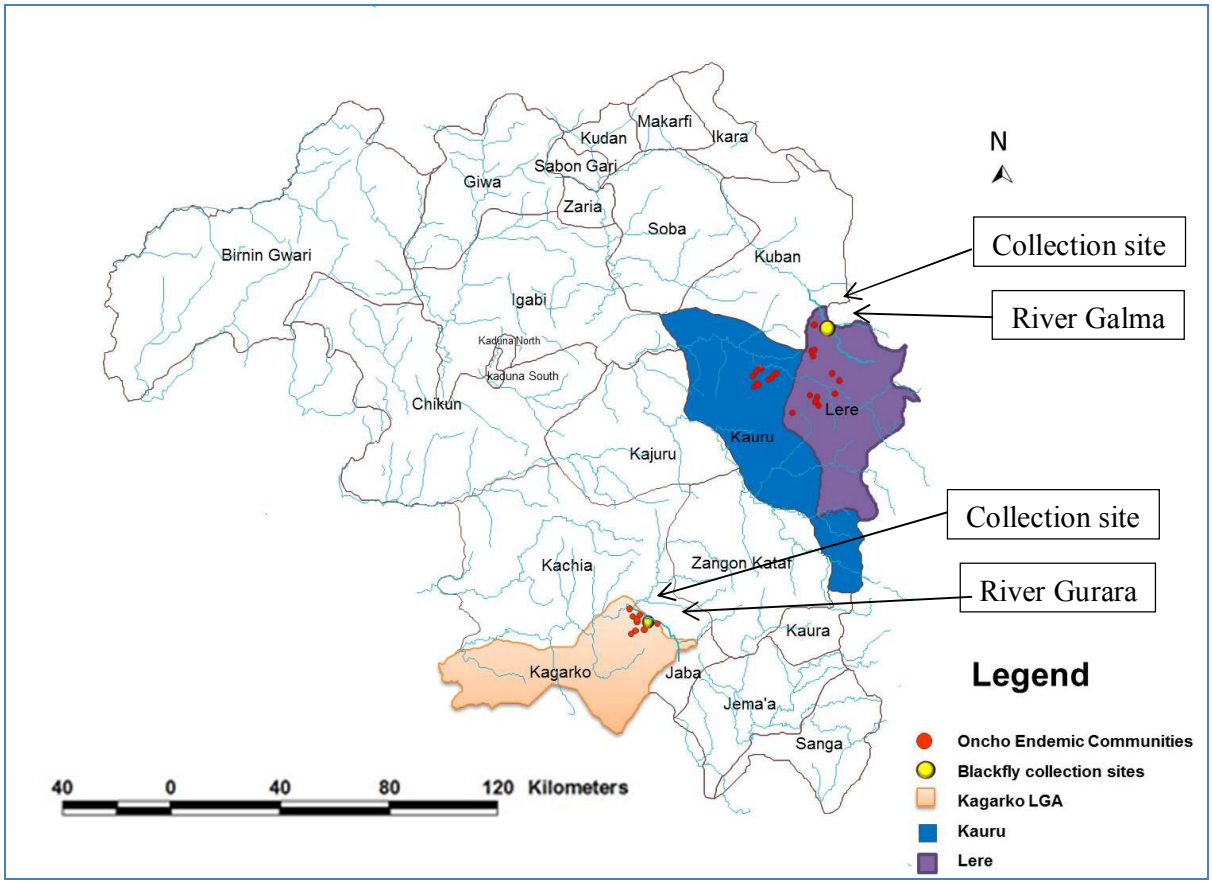


Figure 3.1: Three onchocerciasis transmission foci and fly collection points in Lere, Kauru and Kagarko Local Government Areas.

Table 3.1: Coordinates of communities in the the study area

LGA	Community	N (Latitude)	E (Longitude)
Kagarko	Atara	9° 68′	7° 85′
-10	Gurara	9° 67′	7° 88′
	Hayin Gantan	9° 64′	7° 94′
	Kasaru	9° 61′	7° 87′
	Kasaru Kukkok	9° 60′	7° 86′
	Kurmin Lemu	9° 66′	7° 86′
	Ruzai	9° 62′	7° 90′
	S/Garin Ung. Pah	9° 64′	7° 87′
	Ungwan Doya	9°66′	7° 88′
	Ungwan Pah	9° 65′	7° 88′
Kauru	Akansa	10° 43′	8° 27′
(10)	Galadimawa	10° 45′	8° 32′
	Garmadi	10° 44′	8° 28′
	Jankasa	10° 47′	8° 34′
	Kaguta	10° 44′	8° 28′
	Kubau	10° 49′	8° 29′
	Madam	10° 46′	8° 33′
	Sabon Layi	10° 47′	8° 33′
	Sayawa	10° 48′	8° 28′
	T/Wada Garmadi	10° 46′	8° 27′
Lere	Ashema	10° 48′	8° 53′
(13)	Dan Alhaji	10° 40′	8° 54′
	Kakidare	10° 34′	8° 40′
	Kudaru	10° 64′	8° 47′
	Kuduru	10° 40′	8° 45′
	Ung Buzu	10° 54′	8° 47′
	Ung. Pah Hausawa	10° 38′	8° 48′
	Ung. Pah Kurama	10° 38′	8° 48′
	Ung. Shawara	10° 37′	8° 49′
	Ungwan Tanimu	10° 40′	8° 48′
	Were I	10° 55′	8° 46′

Were II	10° 56′	8° 47′
Zarangi	10° 38′	8° 49′

The choice of the rivers for fly collection was to link the findings from the survey to the entomological transmission of the disease since this was not covered by Afework *et al* (2012). Prior to the commencement of fly collection, prospective visits were made to possible breeding sites in Birnin Gwari, Kauru and Lere LGAs, which were the foci surveyed by Afework *et al*., (2012). Rivers Kabuka in Kauru LGA and River Mariga in Birnin Gwari LGAs, which are within the endemic communities did not yield any fly catch nor was there indication of breeding by *S. damnosum.* The flies were therefore collected from two rivers, Galma in Lere LGA and Gurara in Kagarko LGA (Table 3.2).

Blood seeking female flies were collected using the human landing-bait method (Rodriguez-Perez *et al.,* 2004; Walsh *et al.,* 1978) from two main breeding sites in each location. The flies were collected during the rainy season (June – October) which is the peak of the *Onchocerca volvulus* transmission, for two years (2010 and 2011). This period was chosen based on pre-study visits in 2009, which showed little or no fly biting activity in the dry season months of November to April on account of very low water level, which does not support breeding in both rivers. Four volunteers were recruited for each location and treated with ivermectin before fly collection activities. Daily fly collections were made between 7.00 a.m. and 6.00 p.m. (Crosskey, 1990) (Appendix III) and carried out simultaneously in the 2 collection points (Appendix IV). To ensure that none of the captured flies had ingested microfilariae from the collectors, all flies showing evidence of recent blood meal were discarded (Davies and Crosskey, 1990). The field-collected flies were preserved in 100% isopropanol in tubes and transported to the laboratory for storage at room temperature. The flies, in pools of 100,

were processed for DNA (Rodriguez-Perez *et al.*, 2004; Post *et al.,* 1993) according to location, date and time of capture.

Table 3.2: Fly collection points

LGA	Endemic communities	Longitude (N)	Latitude (E)	Fly catching point	Longitude (N)	Latitude (E)
Lere	Kudaru	8° 47′	10° 64′	River Galma	8° 50′	10° 66′
Kagarko	Gurara	7° 88′	9° 66′	River Gurara	7° 88′	9° 68′

3.5 Molecular Studies

This was carried out at The Carter Center laboratory in Jos, Plateau State Nigeria using the protocol for O-150 PCR analysis of blackflies for *O. volvulus* infection for African blackflies (Unnasch, 2011) (Appendix I). The processes described below were undertaken to determine the infection of *O. volvulus* in the blackflies species collected

3.5.1 Isolation of heads from blackflies

The *S. damnosum* preserved in 100% isopropanol were separated in pools of 100 or less and rinsed in 95% ethanol and then allowed to dry by evaporation on filter paper with care taken to ensure that the flies were not desiccated completely and were devoid of contamination. The flies were then poured into clean dry 15 ml polypropylene conical centrifuge tubes and inserted into the fingers of a regular latex glove and refrigerated overnight at -70°C. The heads were snapped off the bodies by holding on to the sleeve of the glove with the tubes inside and gently snapping the fingers against the bench (Guevara *et al.*, 2003).

The flies were then collected from the tubes by re-suspending them in 95% ethanol and removing them with a wide-bore transfer pipet. The heads of the flies were collected by pouring the ethanol/fly mix through a mesh sieve (0.0278 inches) connected to a pan (ASTM E-11 specification, Fisher Scientific Co. USA) (Appendix V). The bodies were collected on the sieve while the heads passed through into a container. The flies bodies collected on the sieve were rinsed with 95% ethanol to ensure that all of the heads passed through to the pan. The excess ethanol in the pan was decanted using a narrow pipet and the heads allowed to dry at room temperature.

3.5.2 Preparation of deoxyribonucleic acid (DNA) from pools of heads and bodies

A pool of 100 heads and 100 bodies or less were used to extract the DNA of *O. volvulus* targeting a repeated sequence, the O-150 specific for the parasites of the genus *Simulium* (Meredith *et al*., 1991). According to Katholi *et al* (1995), the number 100 has been found to be the most successful for blackfly species in Africa compared to species from South America where pools of 50 were used because of their high protein content (Guevara *et al*., 2003). These were prepared in batches of 77 in 96-well plates with the top horizontal row empty and seven sham extractions (prepared controls) on the extreme right vertical column, these were carried through the process exactly as if they contained flies. This was necessary to ensure that the DNA extraction process remained free of contamination (Guevara *et al*., 2003). All the reagents used for the study were supplied by the Global Health Infectious Disease Research Programme, Department of Global Health, College of Public Health, University of South Florida through the Carter Center, Jos, Nigeria

The heads and bodies were placed in a 1.5ml snap cap micro centrifuge tube and rinsed three times in 95% ethanol. The ethanol was removed using a narrow-tip pipet and left for 10 minutes at room temperature for the ethanol to evaporate. 300ul of homogenization buffer (100mM NaCl, 10mM Tris-HCl (pH 8.0), 1mM EDTA, 0.1% SDS) was added to each tube containing the heads and bodies. 2µl of carrier DNA solution (250ng/µl salmon sperm DNA) was further added to the mixture and then homogenized with a plastic homogenizer until the contents of each tube was completely broken up. 100µl of Proteinase K (400ug/ml prepared in homogenization buffer; freshly made each day) was added to the extract and incubated at 55^{o}C for one hour after which

4µl of 1M Dithiothreitol (DTT) was added and boiled for 30 minutes and freeze-thawed (Gopal *et al.*, 2012).

The homogenate was extracted with 400µl of a 1:1 mix of phenol and chloroform. The top layer was transferred to a second tube and a second extraction with phenol/chloroform carried out. The aqueous layer from the second phenol extraction one time with 400µl of chloroform was extracted and 3 volumes of NaI solution was added and incubated in the refrigerator for 15 minutes. This was then carefully applied over the 96-well unifilter (Whatman GF/B or GF/C), which had been placed over the pump unit for each well. The vacuum pump was switched on and after all the solution had been drawn out through the filter, the pump was switched off. 500µl of ethanol was added to each well and the vacuum pump switched on. After all the ethanol had been drained through the wells the vacuum pump was switched off. The filter was placed over the 96 well collection plate and 50µl of sterile hot distilled water (55°C) was added to each sample and centrifuged (filter and plate) at 4000 RPM for 1 minute. 150µl of Nal was added to each well and incubated in a refrigerator for 15 minutes.

The stage from the extraction of the aqueous layer from the second phenol extraction to the draining of ethanol wash through the wells using the vacuum pump was repeated twice to ensure purification of the DNA. The DNA from the plate was eluted into 50µl of sterile distilled water by placing the filter over the 96 well collection plate and adding 50µl of sterile distilled water at 55°C to each sample and centrifuged at 4000 RPM for 1 minute. The DNA extract was stored at -20^{o}C (Unnasch, 2011).

3.5.3 Primers for *Onchocerca volvulus*

The primers, supplied by Department of Global Health, College of Public Health, University of South Florida, were dissolved in the same number of µl of PCR water as had nmol of primer to produce a 1mM stock solution and divided into aliquot of 10µl in screw capped microfuge tubes and stored at -80^{o}C. Before running the PCR, 490µl of PCR water was added for a 20µM working solution and stored at -20^{o}C. The primer sequences used were 1632: 5' GATTYTTCCGRCGAANARCGC 3'; 1633 biotin: 5' biotin-GCNRTRTAAATNTGNAAATTC 3' and N = A,G,C OR T Y = C OR T R = A OR G (Morales-Hojas *et al*., 2001)

3.5.4 Preparation of PCR reactions

Before commencing the PRC reactions 10-number fold of serial dilutions of the positive control DNA were prepared in order to determine the limit of detection of the assay for the positive control stock. A standard 50µl PCR reaction containing 1µl of the serial dilution of the positive control DNA plus 1.5µl of PCR water was run. 2.5µl of the highest dilution that reliably gave a PCR positive was used as the positive control in all subsequent experiments. For each complete ELISA plate, two positive control reactions (in wells A11 and A12) and 10 negative control reactions (in rows A1-A10) were set up. The negative control reactions contained 2.5 µl of PCR water in place of the DNA. In one of the positive control PCRs, 2.5 µl of the positive control DNA was added. In the second reaction, 2.5 µl of the PCR positive control DNA plus 2.5 µl of DNA from a preparation from a pool that had previously tested negative, or DNA prepared from a pool of 50 flies known to be uninfected. The first positive control indicated that the reaction was working as efficiently as possible, while the second positive control (the

one with the fly DNA) demonstrated that the fly DNA preparations were not inhibiting the PCR. Eighty four (84) PCRs were carried out on each ELISA plate (Unnasch, 2011).

3.5.5 PCR amplification

Polymerase Chain Amplification was performed using the modified PCR amplification protocol for the O-150 family (Katholi *et al*., 1995; Unnasch, 2011). A Master mix for 1,000 reactions was prepared by adding 3.45ml of deionized distilled water (ddh_2O) to 500µl of 10x Buffer, to which 500µl of 2mM each of dNTP and 125µl of 20µM 1632 primer and 125µl of 20µM 1633 biotin primer were added, and then 50µl of Taq polymerase to give a total volume of 4.75ml of the master mix.

To each 47.5µl reaction volume, 2.5µl complete DNA extract was added. Prior to this, a portion of the master mix was poured into the wells of a 96 well PCR plate in the PCR master mix area and sealed with a plate sealing strip. The PCR cycles were programmed to run 5 cycles for 1 minute at 94°C, 2 minutes at 37°C and 30 seconds at 72°C, then run 35 cycles for 30 seconds at 94°C, 30 seconds at 37°C, 30 seconds at 72°C and end with 6 minutes at 72°C (Unnasch, 2011). A BIO-RAD MyCycler TM Thermal Cycler PCR machine was used for the PCR amplification.

3.5.6 ELISA-based detection of O-150 PCR products

The PCR amplification products of *O. volvulus* were detected by PCR enzyme-immunosorbent assay (ELISA). The PCR was to ensure that there were enough copies of the target DNA sequence to detect a signal in the ELISA. It provided a second layer of specificity to the assay. This was more efficient than using an agarose gel to detect products (Unnasch and Meredith, 1996).

The ELISA-based detection of the amplified PCR products was carried out with a Spectra Max Microplate Reader Model Vmax (EMax Endpoint ELISA Microplate Reader). The flat-bottom ELISA plate (sourced from Global Health Infectious Disease Research Programme, Department of Global Health, College of Public Health, University of South Florida through the Carter Center, Nigeria) were coated with 100µl of 1µg/ml Strepavidin (dilute stock of 1 mg/ml 1/1000 in 1x coating buffer) for a minimum of 2 hours at 37^{o}C, after which the hybridization buffer was removed from the refrigerator and placed in a 37^{o}C water bath to dissolve. Sufficient TBS/Tween wash (2 litres per plate) was prepared and the substrate and amplifier from the kit diluted and thawed to room temperature. The Saline-sodium phosphate-ethylenediaminetetracetic acid (SSPE)/SDS buffer was prepared at 56^{o}C and a squirt bottle of TBS/Tween also heated to 56^{o}C. The plates were then washed 6 times with TBS/Tween in squirt bottle, emptying the plate on to a paper towel between washes. 20µl hybridization buffer (HB) was added to all wells and 5µl of undiluted PCR product (10% of the PCR reaction) was added to the appropriate wells and incubated for 30 minutes at room temperature. During this time the probe DNA (OVS2-FL) was diluted to 50 ng/ml in Hybridization Buffer (HB) (Unnasch, 2011).

After the 30 minutes of incubation the plates were washed 6 times with TBS/Tween. Thereafter 100µl 1M NaOH was added to all wells and incubated for 1 minute at room temperature. The plates were further washed 6 times with TBS/Tween and 50 µl OVS2-FL-probe diluted to 100ng/ml in HB was added to all wells and incubated for 15 minutes at 42°C and washed again 6 times with TBS/Tween. A 100µl pre-warmed SSPE/SDS buffer was added to all wells and incubated for 10 minutes at 56^{o}C. The BluePhos substrate was then warmed at room temperature before use. Equal volumes of

Solution A and Solution B (5ml of each for one plate) were mixed in 15ml conical tubes and used within 30 minutes of mixing. The conjugate (anti flouresecin Fab fragment) was prepared by diluting it 1/10,000 in antibody dilution buffer (i.e. 1µl Fab fragment plus 10ml dilution buffer) in readiness for use. The plates were then washed 6 times with TBS/Tween heated to 56^{o}C and 50 µl of the diluted anti-florescin Fab fragment added to each well. The plates were then incubated for 15 mins at 37 ^{o}C and washed again 6 times with TBS/Tween. 100ul of freshly mixed BluePhos Microwell substrate solution was then added to each well and tapped gently to mix; then incubated at room temperature for 20-30 mins. Thereafter, the appearance of a blue colour signified positive (Unnasch, 2011) (plate XIII).

To read the plates, one part of AP stop solution was diluted with 9 parts of high-quality water to make 1x stop solution at room temperature, i.e. for one plate 1 ml AP stop +9ml H_2O. 100ul of AP stop solution was added to each well to stop colour development at desired time and the plates were read at 650nm.

3.6 Assessment Community Treatment Coverage

One of the key criteria for selection of communities for parasitoligical evaluation is the attainment of 65% annual treatment coverage (APOC, 2010). This involves reviewing community treatment registers to identify the number of people treated as a percentage of the total population of each endemic community. This is an important criterion in determining if the community had consistently attained the WHO recommended minimum treatment coverage to qualify for parasitological survey (WHO, 2000).

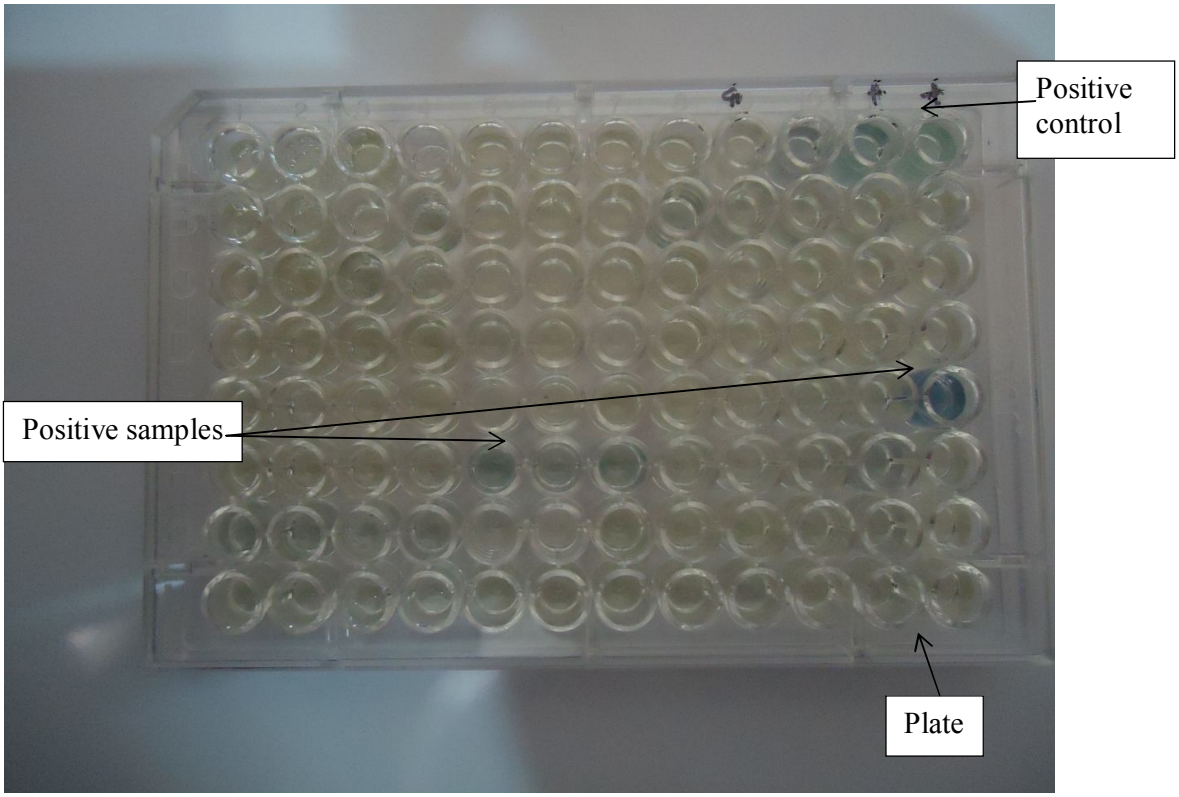


Plate XIII: ELISA positive (blue colour) for *Onchocerca volvulus*

Information about previous treatments records for ten communities in Kagarko LGA were obtained from Kaduna State Ministry of Health and verified during field visits. The communities were also selected because they had or should have had 10 - 15 rounds of annual treatment as recommended by APOC for a parasitlogical evaluation (APOC, 2010). The treatment history for the communities in Lere and Kauru LGAs have been reported (Afework *et al*., 2012).

3.7 Skin Snipping

Parasitological evaluation in humans was carried out in 10 communities in Gantang area by skin snip (Taylor *et al*., 1987; Nguyen *et al*., 2005) to detect infection. Two skin biopsies were collected from each community member who consented, from the left scapula and the other from the right buttock. The skin snips were taken using a Holth 1.5-2.0 mm bite *Stolz corneoscleral biopsy punch* (Indo-German Surgical Co.), placed on microscope slides with normal saline and then examined under an inverted microscope (x40) 30 mins after for any microfilariae. The contents of each slide were then transferred into 96 wells flat bottom micro titre plates for storage as described by Barbiero and Trpis (1984). The micro titre plates were then sealed with adhesive tape and incubated over night at room temperature and re-examined the following morning for microfilariae. Emerging *Onchocerca* microfilariae were counted and recorded and the density determined as number of microfilariae per cm square skin snip for each subject.

3.8 Ophthalmologic Study

Ocular examinations were carried out for all that were skin-snipped in the 33 communities in the study area. This was through visual acuity test, ocular opacities, onchocercal-specific keratitic lesions and prevalence of microfilariae in the anterior

chamber of the eye (Rodriguez-Perez *et al*., 2008). Visual acuity test were carried out using visual acuity chart at a distance of 6 meters to evaluate the vision of community members. Persons with vision 6/18 and above were subjected to further ocular examinations by an ophthalmologist using a Topcon Optical SL-3D slit lamp (Kogaku Kikai KK, Tokyo, Japan). The focus was on finding *O. volvulus* microfilariae in the cornea and/or the anterior chamber of the eye (Rodríguez-Pérez, 2008). Before the examination the patients kept a "head down position" (forehead in the lap) for 5 minutes to enable microfilaria to migrate to anterior chambers where they become visible. Observation of at least one microfilaria during the ocular examination was recorded as positive.

3.9 Assessment of Economic Impact

3.9.1 Measure of productivity

Structured questionnaires (Appendix 4) were administered on 396 household heads in 33 communities within the study area. Data collected included yearly income earned from the sale of crop and anima/animal products, income derived from other services provided, type and number of animal(s) reared, number of bags of goods produced, the monetary value of services rendered over the study horizon, demographic characteristics of famers, year of first treatment and duration of treatment wth ivermectin, and number of years in the productive/service activity. Based on data collected for a year on the individual household, yearly wage earned were computed (quantity of commodities multiplied by the price) and monetary value of service provided was added, and this was used as a measure of economic productivity. The information was used to assess the benefits expressed in monetary terms over a period of time.

3.9.2 Cost-benefit analysis

Cost-benefit analysis for the entire period could not be carried out because of the inability of community members to recollect benefits retrospectively and thus information collected was only for the year of the study.

3.9.3 Cost effectiveness

Desk review was carried out on project data base with focus on annual technical reports and financial contribution of partners -Kaduna State Ministry of Health, African Programme for Onchocerciasis Control and Sightsavers to determine implementation costs for the period of 2003 to 2009. The study was restricted to this period because there was incomplete information prior to 2003 and from the year 2010 the programme was integrated with control of lymphatic filariasis. These costs also excluded cost of drugs because they were donated free by the manufacturers. The costs of implementing the programme were identified and measured using activity-based data. Bottom-up approach was used to estimate the economic costs (where information on resource use and costs were available (Drummond *et al.*, 2003).

Major areas of resource use were training of health workers and community volunteers, community health education, advocacy and sensitization, transport, incentives/allowances, Information, Education and Communication (IEC) materials, radio jingles and capital items.

3.10 Data analysis

The entomological parameters analysed were infection in the vector population from the PCR/ELISA, which was calculated using algorithms contained in PoolScreen 2.0 (Katholi *et al.*, 1995; Carlos Vieira *et al.,* 2007) and the absence or near absence of

infective stage larvae of *O. volvulus* in the vector population as determined by ELISA (Unnasch and Meredith, 1996; WHO, 2001). Infection rate in the vector population was calculated from the proportion of body pools positive in the PCR/ELISA assay and expressed per 10,000 flies. Infective rate was estimated from the proportion of head pools positive in the PCR/ELISA assay and expressed per 10,000 flies. The Poolscreen® computer programme 2.1 was used to estimate both proportions and the 95% confidence intervals (CIs) surrounding these parameters (Yameogo *et al*., 1999). The prevalence of *O. volvulus* infection in the *S. damnosum* was determined based on the proportion of PCR-positive pools while the prevalence of infection in the vector population was determined at 95% Confidence Interval (CI) based on the algorithms of PoolScreen 2.0 (Katholi *et al.,* 1995).

Community treatment registers were analysed by determining the treatment range and mean treatment coverage of the communites (Brieger *et al*., 2011). For the the prevalence of skin infection with mf, two parasitoligical parametres were calculated to evaluate the endemicity levels in the population. Parasite prevalence was the percentage of individuals with mfpositive skin snip. Intensity of infection was the geometric mean microfilarial skin density (log [microfilarial skin density +1] including negative count of all subjects. The proportion of individuals positive to infection with mf in the conea and/or anterior chamber of the eye was calculated as the the number of positive individuals divided by the total number examined and expressed as percentage (Rodriguez-Perez *et al.*, 1999).

In determining productivity, the average output of individuals in the communities who have benefited from the programme for the year 2012 was calculated. The output was calculated by taking the monetary output of individuals in a year (2012) and dividing it by the number of man hours spent working (Walker, 2011).

Marginal Cost Effectiveness Ratio (MCER) is the change in cost/change in cases Prevented and Average Cost Effectiveness Ratio (ACER) is the cost of the programme divided by the cases prevented, were used to asses the cost effectiveness of the programme (see formulae).

$$\text{MCER} = \frac{\text{Total Cost of Oncho Programme Current -Total Cost of Oncho Programme Previous}}{\text{Total Prevented Cases Current - Total Prevented Cases Previous}}$$

(Walker, 2011)

$$\text{ACER} = \frac{\text{Total Cost of Onchocerciasis Programme}}{\text{Total Prevented Cases}}$$ (Walker,2011)

CHAPTER FOUR

RESULTS

4.1 Fly Collection

A total of 24,973 *S. damnosum* adult flies were collected along two rivers - River Galma and River Gurara over 60 collection days in the two rainy seasons (June to October) of 2010 and 2011 (Table 4.1). Average daily catch per person ranged from 34.88 to 78.18 for River Galma and 125.9 to 176.7 for River Gurara. The number of flies was higher in River Gurara (18,153) than the number from River Galma (6,784).

4.2 Polymerase Chain Reaction/ELISA

A total of 24,937 flies were screened in pools of 100 using of O – 150 PCR/ELISA and 32 of the 500 pools (250 each of head and body) were positive for *O. volvulus* (13 from River Galma and 19 from River Gurara) (Table 4.2).

Figure 4.1 shows the results of confirmatory test of the 54 positives PCR/ELISA products from the first screening (Appendix 2a-f), 23 samples were confirmed to be positive. Cut- off value for presence of infection or infectivity was computed as mean plus 3 standard deviation of the 10 negative control: Mean = 0.0604, SD = 0.1034, M+3SD = 0.0.09142, therefore Cut-off value was 0.1 (Unnasch, 2011).

Table 4.1: *Simulium damnosum* caught along Rivers Galma and Gurara

Characteristics	River Galma (Kudaru)		River Gurara (Gurara)	
	2010	2011	2010	2011
No. of fly catchers	4	4	4	4
No.of days worked	60	60	60	60
*Total Flies caught	2,093	4,691	7,553	10,600
Average daily catch per person days worked	34.88	78.18	125.9	176.7

*Total flies caught = 24,973

Table 4.2: Pool Prevalence of *Onchocerca volvulus* in pools of *S. damnosum* from

River Galma and River Gurara focus for 2010 and 2011

River	Year of collection	No. per pool	No. of pools	No. of pool positive (%)	Total No. of *S. damnosum*
R. Galma	2010	100	20	5 (25)	2,000
		<100(93)	1	0 (0)	93
R. Galma	2011	100	46	8 (17.4)	4,600
		<100(91)	1	0 (0)	91
R. Gurara	2010	100	75	9 (12)	7,500
		<100(53)	1	0 (0)	53
R. Gurara	2011	100	106	10 (9.4)	10,600
		<100	0	0 (0)	0
Total			250	32 (12.8)	24,937

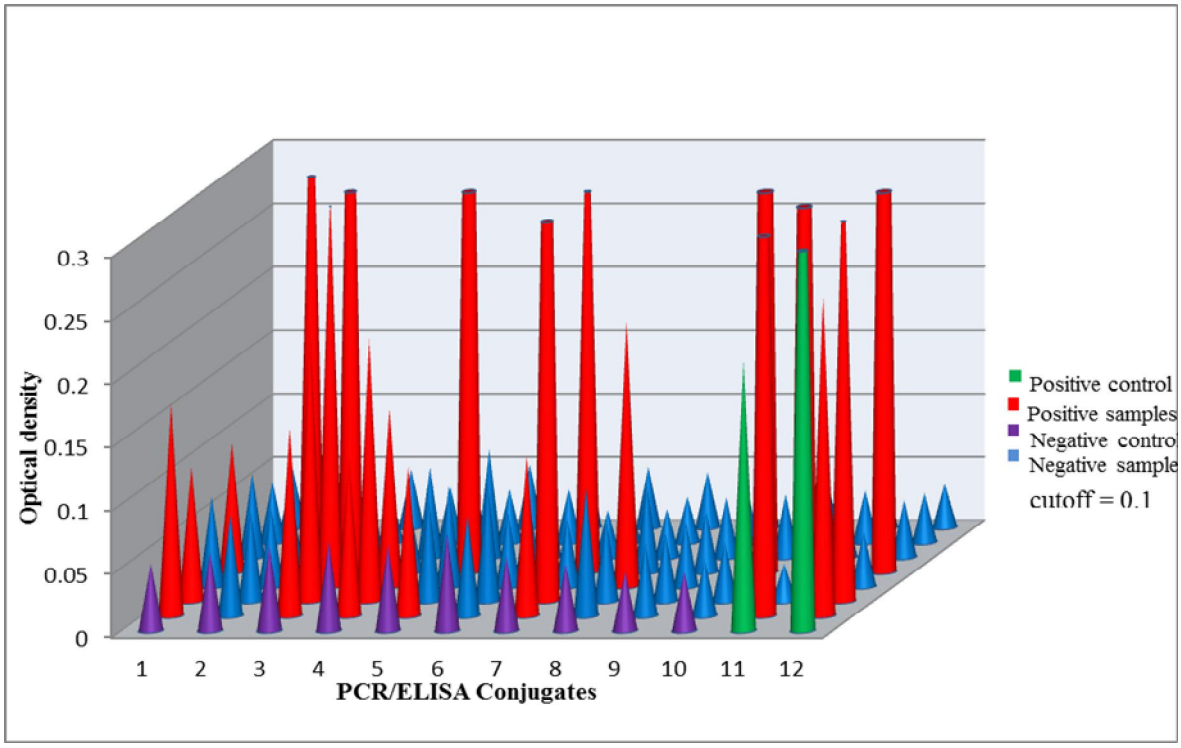


Figure 4.1: Results of PCR/ELISA Amplification (confirmation)

The prevalence of infective flies (calculated from the infection rate in pools of heads, representing flies carrying infective stage larvae in Table 4.3 (prevalence 0 – 1.5/1,000 flies in River Galma and 0.38 – 0.83/1,000 flies in River Gurara); the prevalence of infected flies (calculated from the infection rate in pools of bodies, representing flies carrying developing stages of the parasite in Table 4.4 (prevalence 1 – 1.9/1,000 flies in River Galma and 0.4 – 0.5/1,000 in River Gurara); and the overall prevalence of infection (calculated from the overall number of positives in either the head or body pools) are summarized in Table 4.5 (prevalence 1.87-2.7/1,000 flies in R. Galma and 1.2 to 1.27/1,000 flies in R. Gurara).

4.3 Assessment of Community Treatment Coverage

The reported treatment history for the 10 communities in Kagorko LGA as indicated in Table 4.6 was not complete, only 5 (50%) had the minimum 10 rounds of treatment suggesting that treatment has been inconsistent, which was blamed on the unavailability of adequate drugs. The mean treatment coverage was 80.6% (range 62.7-97.5%). The expected minimum treatment coverage for elimination is 80% and all the communities therefore had a lower range (below 80%) The upper range of 97.5% was high.

4.4 Parasitological Study in Humans

A total of 973 persons participated (Table 4.7), out of which 483 (50%) were males and 490 (50%) females. This represented 56% of the total population of the ten communities. All the participants had lived in the community for several years. The participation was almost equal among males and females and with non-participation of 44%. The non-participants included children below the age of 1 year. People of age 50+ participated more, participants of age group of 30 – 39 had the least attendance.

Table 4.3: Prevalence of *Onchocerca volvulus* in pools of heads of *S. damnosum*

River	Year of collection	Number of pools	Number of positive pools (%)	Prevalence of infected flies*
R. Galma	2010	21	3 (14.3)	**1.5** (0.30 - 4.49)
R. Galma	2011	47	0 (0)	**0** (0 - 0.41)
R. Gurara	2010	76	6 (7.9)	**0.83** (0.28 - 1.8)
R. Gurara	2011	106	4 (3.8)	**0.38** (0.10- 0.99)

*Number of infected flies per 1,000 flies The number in bold represents the estimated prevalence and the range in bracket, the 95% CI upper and lower limits around the point of estimate (Yameogo *et al*., 1999)

Table 4.4: Prevalence of *Onchocerca volvulus* in pools of bodies of *S. damnosum*

Community	Year of collection	Number of pools	Number of positive pools (%)	Prevalence of infective flies*
R, Galma	2010	21	2 (9.5)	**1.0** (0.11 - 3.5)
R. Galma	2011	47	8 (17)	**1.9** (0.75 - 3.76)
R. Gurara	2010	76	3 (3.9)	**0.4** (0.08 - 1.18)
R. Gurara	2011	106	6 (5.7)	**0.5** (0.20 - 1.29)

* Number of infective flies per 1,000 flies. The number in bold represents the estimated prevalence and the range in bracket, the 95% CI upper and lower limits around the point of estimate (Yameogo *et al*., 1999).

Table 4.5: Infection prevalence in *Simulium damnosum* (rate per 1,000 flies) in two *O. volvulus*-endemic foci along Rivers Galma and Gurara

Collection Site	Year	Number of *S. damnosum* examined	*S. damnosum* infective rate[1]	*S. damnosum* infected rate[2]	*S. damnosum* total infection rate[3]
R. Galma	2010	2,093	**1.0** (0.11 - 3.5)	**1.5** (0.30 - 4.49)	**2.7** (0.8 - 6.45)
	2011	4,691	**1.9** (0.75 - 3.76)	**0** (0 - 0.41)	**1.87** (0.75 - 3.76)
R. Gurara	2010	7,553	**0.4** (0.08 - 1.18)	**0.83** (0.28 - 1.8)	**1.27** (0.54 - 2.46)
	2011	10,600	**0.5** (0.20 - 1.29)	**0.38** (0.10- 0.99)	**1.2** (0.58 - 2.15)

1. Rate of infection (bold) and 95% confidence interval in parenthesis in head pools
2. Rate of infection (bold) and 95% confidence interval in parenthesis in body pools
3. Combined rate of infection (bold) and 95% confidence interval in parenthesis in head and body pools

Table 4.6: History of Ivermectin treatment in ten villages in the onchocerciasis foci of Gurara, Kagarko LGA from 1994 – 2010

Community evaluated	Year of First Treatment	Total No. of treatments	Mean Treatment Coverage and Range (%)
Gantan Gida	1994	13	82.8 (75.9 – 93.7)
Ungwan Pah	1994	11	80.3 (71.9 – 88.8)
Gurara	1994	10	84.8 (73.9 - 91.6)
Sabon Gari U/Pah	1994	7	80.9 (64.2 - 90.8)
Kurmin Lemu	1994	13	79.9 (74.8 – 90.9)
Atara	1996	4	80.2 (75.6 – 86.0)
Ruzai	1996	13	78.4 (62.7 - 89.3)
Kasaru	1998	8	80.9 (66.3 - 89.5)
Ungwan Doya	1998	5	77.5 (68.0 – 97.5)
Kasaru Kukuk	2001	5	75.3 (67.2 – 83.0)
Total	1994 - 2001	4-13	80.6 (62.7- 97.5)

Table 4.7: Age and sex distribution of onchocerciasis in Gurara foci, Kagarko LGA

Community (population)	Age in years (%)						Sex			
	1-9	10-19	20-29	30-39	40-49	50+	Male (%)	Female (%)	Total Examined N (%)	Total Population
Atara	30 (*34*)	26 (*29*)	11 (*12*)	12 (*13*)	8 (*9*)	3 (*3*)	44 (*49*)	45 (*51*)	89 *(58)*	153
Gantan Gida	29 (*30*)	10 (*10*)	12 (*12*)	19 (*8*)	18 (*18*)	10 (*10*)	45 (*46*)	53 (*54*)	98 *(40)*	242
Gurara	59 (*45*)	18 (*14*)	15 (*12*)	23 (*18*)	8 (*6*)	7 (*5*)	63 (*48*)	67 (*52*)	130 *(83)*	156
Kurumin Lemu	21 (*58*)	3 (*8*)	9 (*25*)	1 (*3*)	1 (*3*)	1 (*3*)	22 (*61*)	14 (*39*)	36 *(51)*	70
Kasaru	9 (*14*)	15 (*23*)	5 (*8*)	8 (*12*)	8 (*12*)	21 (*32*)	29 (*44*)	37 (*56*)	66 *(38)*	172
Ruzai	23 (*21*)	18 (*17*)	17 (*16*)	14 (*13*)	8 (*7*)	28 (*26*)	61 (*56*)	47 (*44*)	108 *(62)*	172
Kasaru Kukuk	29 (*37*)	17 (*22*)	16 (*20*)	8 (*10*)	4 (*5*)	5 (*6*)	36 (*46*)	43 (*54*)	79 *(63)*	125
S/Gari U/Pah	46 (*45*)	13 (*13*)	13 (*13*)	8 (*8*)	9 (*9*)	12 (*12*)	47 (*46*)	55 (*5*)	102 *(46)*	218
U/Pah	29 (*29*)	25 (*25*)	16 (*16*)	12 (*12*)	9 (*9*)	9 (*9*)	52 (*52*)	48 (*48*)	100 *(51)*	193
U/Doya	51 (*31*)	40 (*24*)	25 (*15*)	19 (*12*)	18 (*11*)	12 (*7*)	84 (*51*)	81 (*49*)	165 *(70)*	235
Total	326 (*34*)	185 (*19*)	139 (*14*)	124 (*13*)	91 (*9*)	108 (*11*)	483 (50)	490 (*50*)	973*(56%)*	1,736

The survey in the ten villages in Kagarko LGA had an overall participation from the villages of 56%, the least being 38% and 83% being the (Table 4.8). Of the 973 people examined, 79.5% were below the age of 40. After 4 – 13 rounds of treatment, only 3 (0.3%) were positive with *O. volvulus* microfilaria in the skin samples giving a community microfilarial load of 0.0026. There were no results of pre-treatment surveys to compare with this result. The positive cases were found in two age groups; 1 (0.8%) in 30-39 and 2 (1.9%) in 50. The highest Odd Ratio of 16.3019. The individuals between 50 years and above are 16.3 more infected with p=0 + (Table 4.9).

4.5 Ocular Examination

A total of 4,453 persons were screened for visual acuity in the 33 communities in three onchocerciasis-endemic foci, out of which 88.12% had good vision with 477 having visual impairment mainly due to refractive error; 1.17% were blind due to either cataract or glaucoma (Table 4.10). None had microfilaria in the conea and/or anterior chamber of the eye due to onchocerciasis.

4.6 Economic Impact

Table 4.11 presents the analysis according to the Local Government Areas for three economic activities - crop production, animal production and services-related economic activities such as trading, carpentry, clergy service and civil servants. Productivity/services rendered showed an ouput of □228,959,800, commodities accounted for □136,167,796 (59.5%), animal production accounted for □29,601,624 (12.9%) and other services □63,190,380 (27.6%). Kagarko accounted for □119,141,470 representing (52.0%), Kauru □43,277,725 (18.9%) and Lere □66,540,605 (29.1%) of the total monetary value over a period of twelve months

(2012) (Appendix XIV). The individuals could not provide information on the productivity output for previous years.

Table 4.8: Prevalence of onchocerciasis after 4-13 rounds of treatment in the onchocerciasis focus of Gurara area, Kagarko LGA

Village	Population of community	No. Examined	% Examined	No positive	Prevalence ss (%)	mf load *(mf/ss)
Atara	153	89	58	0	0	0
Gantan Gida	242	98	40	0	0	0
Gurara	156	130	83	2	1.54	0.0017
Kurmin Lemu	70	36	51	0	0	0
Kasaru	172	66	38	1	1.52	0.0008
Ruzai	1721	108	63	0	0	0
Kasaru Kukuk	125	79	63	0	0	0
Sabon Gari Pah	218	102	47	0	0	0
Ungwan Pah	193	100	55	0	0	0
U/Doya	235	165	70	0	0	0
Total	1,736	973	56	3	0.3	0.0026

*mf= microfilaria ss= skin snap

Table 4.9: Infection with *O. volvulus* in the different age groups

Age Group (Years)	No. Examined	No. Infected	% infected	Odd Ratio	Confidence Interval	Probability
1-09	326	0	0	0.282	0.0145-5.4759	0.4029
10-19	185	0	0	0.6049	0.0311 to 11.7625	0.7399
20-29	139	0	0	0.8515	0.0431 to 16.5754	0.9155
30-39	124	1	0.8	3.4431	0.3099 to 38.2563	0.3142
40-49	91	0	0	1.3731	0.0704 to 26.7934	0.8343
50+	108	2	1.9	16.3019	1.4657 to 81.329	0.0231
Total	973	3	0.3			

OR = 1 i.e. no difference between the group; OR>1 = positive association; OR<1 = Negative association

Table 4.10: Visual Acuity and Ocular Examination in parts of Kagarko, Kauru and Lere Local Government Areas

	No. of Villages	No. Examined	No. with Good Vision	%	No. with Visual Impairment	%	No. Blind	%	Microfilaria in Anterior Chamber	*Reason for blindness/Visual Impairment
Kagarko	10	973	926	95.17	34	3.49	13	1.37	0	Cat, RE, Glau.
Kauru	10	1,343	1181	87.99	142	10.57	20	1.49	0	Cat, RE, Glau.
Lere	13	2,137	1,817	86.05	301	14.09	19	0.89	0	Cat, RE, Glau.
	33	4,453	3,924	88.12	477	10.71	52	1.17	0	

*Cat= Cataract; RE= Refractive Error; Glau=Glaucoma

Table 4.11: Cumulative Household Economic Output (millions of naira)

Output	Local Government Areas			
	Kagarko (□) (%)	Kauru (□) (%)	Lere (□) (%)	Total (□)(%)
Commodities	71,014,608 (52.2)	28,474,060 (20.9)	36,679,128 (26.9)	136,167,796 (59.5)
Animals	26,365,062 (89.1)	1,712,385 (5.8)	1,524,177 (5.1)	29,601,624 (12.9)
Others	21,761,800 (43.4)	13,091,280 (20.7)	28,337,300 (29.1)	63,190,380 (27.6)
Total	119,141,470 (52.0)	43,277,725 (18.9)	66,540,604 (29.1)	228,959,800

Majority of programmes that are cost-effective are considered good investment only at a certain level, this mainly through comparing the cost of implementation *viz a viz* the number of cases prevented (Table 4.12). Marginal Cost Effectiveness Ratio was used to assess the optimal level of implementation of the control programme i.e. the level where the onchocerciasis programme was considered most effective at the lowest cost. The data collected was restricted to 2003 to 2009, because that was the period when complete data was available. The programme was expanded in 2010 to include lymphatic filariasis as part of integrated control. The cost from 2010 was therefore not included. Table 4.12 provides details on the important variables to find out whether the programme was effective and at what cost (cost per additional case prevented).

Table 4.12 shows that in 2004 the cost per additional case prevented was □19.09. From 2005-2007, the cost per additional cases prevented were negative values. In 2008, the cost per additional case prevented was □10.09 and peaked at □59.4 in 2009 showing an additional cost per case prevented compared with the previous years. The cost per case prevented in 2009 was above the period average (□3.60, □4.17 and □4.52) for 2006, 2007 and 2008). The average cost per case prevented for the total period (2003-2009) was □5.95.

Table 4.12: Cost of Intervention, onchocerciasis cases prevented and cost-effectiveness of the control programme (2003-2009)

	A	B	C	D	E=B/D	F=A/C
Year	**Cost of intervention (□)**	**MC=CYC-PYC (□)**	**Cases Prevented (Cases)**	**MCP=CYCP-PYCP (Cases)**	**MCER (□)**	**ACER (□)**
2003	7,027,486.70	-	1,045,031	-	-	6.72
2004	7,576,154.00	548,667.30	1,073,771	28,740	19.09	7.06
2005	7,107,990.79	-468,163.21	1,073,809	38	-12,320.08	6.62
2006	4,302,987.00	-2,805,003.79	1,196,356	122,547	-22.89	3.6
2007	4,847,731.00	544,744.00	1,163,667	-32,689	-16.66	4.17
2008	5,590,925.00	743,194.00	1,237,301	73,634	10.09	4.52
2009	11,987,755.00	6,396,830.00	1,344,931	107,630	59.43	8.91
2003-2009	48,441,029.49	-	8,134,866	-	-	5.95

MC= Marginal Cost; CYC= Current Year Cost; PYC=Previous Year cost; CYCP= Current Year Cases prevented; PYCP=Previous Year Cases Prevented (i.e.no. treated); MCER=Marginal Cost Effectiveness ratio (Change in Cost/change in Cases Prevented); ACER=Average Cost Effectiveness Ratio (Cost/Cases Prevented)

CHAPTER FIVE

DISCUSSION

This study shows there are still infective flies in both rivers Galma and Gurara. Of the four seasons of collection, only the 2010 collection along River Galma had a high infectivity (more than 1.0/1,000). Though this is higher than the accepted threshold of 1 Fly Larvae in Head (FLH) per 1,000 flies it could be attributed to fly migration because it was zero and below the threshold in 2011. It also signifies that there is man-vector contact but no active transmission. This confirms the findings by Afework *et a*l. (2012) in the area where there were no microfilariae were found in the skin snip of individuals' survey.

The infectivity in the flies caught along River Gurara which had a higher fly catch was below the vector infectivity threshold and thus suggests that onchocerciasis transmission levels were low as well. This is further confirmed by the low (0.3) prevalence of positive cases in the villages along River Gurara and could be due to cross border movement of the fly; this is consistent with other studies in West Africa (Traore *et al.*, 2012). The results indicate that possible transmission may already be below the threshold for the 2 foci, indicating an impact of ivermectin after 4 – 13 years of treatment.

The PCR-ELISA results show that the prevalence of infected flies were higher than the prevalence of infective flies (i.e., those with parasites in the head) and is consistent with previous studies (Guevara *et al*., 2003; Rodríguez-Perez *et al*., 2004). This confirms the

hypothesis that the prevalence of infected flies is the most sensitive indicator of parasite–vector contact, and thus body pool PCR/ELISA positivity can be used effectively to monitor the parasite–vector contact in communities subject to control.

It has been suggested that entomologic indicators of impact of ivermectin on transmission be based upon the prevalence of all forms of the parasite found in the vector, and not just infective larvae alone (Basanez *et al*., 1995; 1998).

The number of flies caught along River Galma for each year were below the recommended 6,000 per foci per year (APOC, 2010) they were however sufficient in conducting the study using PCR and do not affect the outcome of the study as confirmed by Diawara *et al*. (2009) who used 3,900 as minimum flies per catching point; since the focus is to establish the intensity of transmission, which is a function of host-vector contact and the prevalence of infection in the vector population (Katholi *et al.,* 1995).

The treatment coverage in the 10 villages in Kagarko LGA was at an average of 80.6% with a combined range of 62.7% - 97.5%. While the average treatment coverage was within the accepted rate; the range was above and below the recommended and acceptable range of 65% – 85%. This was not unexpected considering the poor record keeping observed in the village treatment registers. The treatment history varied from village to village with some having inconsistent treatment. It was evident that ivermectin treatment was not immediately introduced in all villages at the same time and as a result the number of treatment rounds as at the time of the study varied from 4 to 13 years. It is not uncommon to find inconsistent annual treatment in many communities mainly due to unavailability of or insufficient ivermectin. One of the key requirements for epidemiological surveys is to ensure consistent annual treatment of endemic communities with an annual therapeutic coverage of least 65% and also have a

baseline data which can be used to compare with survey results to determine impact (APOC, 2010). From an epidemiological point of view, the most significant criteria is that all first-line villages, located near the river and the vector breeding sites and which play a dominant role in onchocerciasis transmission (WHO, 1976) the accounted for the choice of the ten villages in Kagarko LGA, these are first line communities to River Gurara, which had breeding sites for *S. damnosum*.

The parasitological examination of the communities showed low prevalence of 0.3 %. This is indicative that ivermectin treatment of the communities was effective in killing the microfilariae. The ONCHOSIM model predicts that with such low endemicity levels, onchocerciasis elimination can be achieved after 10 to 15 years of annual ivermectin treatment with good (> 80%) treatment coverage (APOC, 2010). It is also indicative that there is no on-going transmission of the disease since the prevalence of microfilariae had fallen to a very low level. The low number (3) of people positive for microfilaria was significant towards the elimination of the disease. It further provides strong evidence on the feasibility of elimination of onchocerciasis infection in areas where treatment with ivermectin has been carried out for a number of years. The result is consistent with other studies in Mali and Senegal (Diawara *et al*., 2009; Traore *et al*., 2012) and parts of Kaduna State Nigeria (Afework *et al*., 2012). It is noteworthy that 5 (of the ten communities surveyed) had inconsistent treatment during the life of the programme suggesting that these communities had very low prevalence at the inception of the programme as there was no data on pre control endemicity level which reflects the pre-control intensity of transmission.

In this study, only 3 people were found positive in Kagarko LGA and none in the earlier study in Lere and Kauru LGAs (Afework *et al*., 2012). Of the 973 individuals skin

snipped, 79.5% were below the age of 40, which was expected considering that this group formed the majority base of the population. The age group with the highest infections are those 50 years and above showing progressive infection with increase in age. This trend is similar to earlier findings in Kaduna State by Abiose *et al.*, 1994 and in Oyo State (Akinboye *et al*., 2010) and Ebonyi State (Okonkwo *et al*., 2010). This study is based upon the hypothesis that ivermectin can reduce the community skin microfilariae load to a level that results in a reduction of transmission to a level below what is considered of public health importance. The absence of positive case in persons aged 1 – 30 suggests that ivermectin coverage has dramatically reduced the number of microfilariae available for transmission.

Of the 4,453 persons screened for visual acuity in the study area, 88.12% had good vision while 10.71% had visual impairment mainly due to refractive error. 1.17% were blind due to either cataract and glaucoma and no case of blindness due to onchocerciasis was observed. This is a significant achievement for the programme considering the negative impact this would have had on the study area. Abiose *et al* (1994) reported that substantial proportion of optic nerve atrophy without any other ocular pathology may be attributable to onchocercal infection and found this in parts of Kaduna State, Nigeria. This study did not record any individual with ocular opacities or microfilariae in the anterior chamber of the eye which could have associated the blindness to onchocerciasis. This is consistent with other studies that demonstrate that the anterior segment changes are onchocerciasis-specific and resolve completely after treatment thus preventing severe ocular pathologies (Winthrop *et al.*, 2006; Rodriguez-Perez *et al*., 2008). The microfilariae load of 0.0026 is very low and the ONCHSIM model predicts that with such endemicity levels the programme is moving towards attaining elimination of the disease (Winnen *et al.*, 2002).

Since there were no blindness due to Onchocerciasis in the study area, it proves that the communities have been protected from the debilitating effect of the disease leading to high productivity levels of the individuals; with the large cases prevented in the study area at low cost □5.95 suggests that the programme has been cost effective. There has been substantial economic impact in terms of augmented productivity, which is believed to have led to increased household-level welfare resulting from the elimination of the skin-related symptoms and onchocercal blindness; a great improvement compared to pre-control prevalence of onchocerciasis ranging from 22% - 93% in communities within the study area as reported by Abiose *et al.* (1994). It should be re-iterated that the increased productivity of non-blind and non-*Onchocerca*-infected individuals resulting from the APOC programme has not been considered by previous studies in the study area.

In 2004 the cost per additional case prevented □19.09. From the period 2005-2007, the cost per additional cases prevented was negative indicating savings in cost per additional case prevented rather than the cost incurred. This is greater cost-cost effectiveness relative to the other years. Thus, there was saving (instead of incurring) cost per additional case prevented. The savings amounted to □12,320.08, N22.89 and □16.66 per case prevented in the years 2005, 2006 and 2007 respectively.

There is a distinction between ACER and MCER in this study because while ACER shows the cost per case prevented for each year evaluated against its baseline option, MCER shows change in cost per change in case prevented over the time horizon and analytical horizon (2003-2009). This is relevant for public health action, the knowledge would help determine the cost elements requiring additional funding for the elimination of the disease.

This study has provided critical information about the onchocerciasis control programme in parts of Kaduna State which indicate that long-term treatment with ivermectin if sustained can potentially lead to the elimination of the disease. The programme has been cost-effective and improved the economic benefits of the communities and individuals in the study area.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Kaduna State was one of the sites where the ivermectin was successfully tried as a drug of choice for the control of Onchocerciasis; which had been a disease of public health importance. After almost two decades of the Onchocerciasis control programme in Kaduna State the study examined the impact of the programme through an epidemiological and entomological evaluations, the cost effectiveness of the programme and how it has affected economy of endemic communities.

The study has further provided the epidemiological evidence that there is potential for the disease to be eliminated if the minimum threshold for treatment is attained. Low prevalence suggests that the community directed treatment has the potential for elimination of the disease in the study area. There is also evidence of suppression of in transmission of the parasite by the vector and this is a good indication that there is a reduction in the transmission of the disease within the study area.

The economic analysis of the programme shows that the distribution of ivermectin in these areas has had a highly beneficial impact. It is considered to be very cost effective in terms of people reached and funding requirements. This has led to substantial economic benefit through productivity gained and the capacity to improve productivity. There were no identified cases of blindness or visual impairment due to onchocerciasis, an indication of the public health impact as it relates to blindness.

6.2 Conclusion

This study has highlighted the positive impact of Community-directed treatment with ivermectin which been on in Kaduna State for the past two decades.

This study demonstrated that long-term treatment with ivermectin has an impact on infections in humans in Kaduna State; it has interrupted transmission of *O. volvulus* by *S. damnosum* and signifies an important step towards possible elimination of the disease. The control programme has been cost effective leading to improved productivity and economic status of the communities. The results of this study could be a useful guide to the National Onchocerciasis Control Programme in developing strategies for the complete elimination of the disease onchocerciasis, in Nigeria.

6.3 Recommendations

There is the need to have a wider state survey to determine the prevalence of blindness due to onchocerciasis and the impact of the control programme to provide more evidence.

The National Onchocerciasis Control Programme in Nigeria is currently trying to develop guidelines for the elimination of the disease in Nigeria. This study would be useful as they go through that process. It is however important that good coverage studies are conducted to determine if the treatment coverage thresholds are being attained by communities.

The community directed intervention has turned out to be an effective strategy for mass drug administration which should be adopted as the nation focusses its attention on control of other neglected tropical diseases like lymphatic filariasis, trachoma, schistosomiasis and soil transmitted helminthes.

Government should improve on its record keeping particularly baseline results, treatment reports and financial expenditure on onchocerciasis control programmes. This will enhance disease control activities and provide baseline for impact assessment.

The African Programme for Onchocerciasis Control should improve support to programmes where the parasites still exist in Nigeria and elsewhere. The National Onchocerciasis Control Programme should review and improve the current health education strategies by using more electronic media (Television and Radio) to enlighten the public about the disease and control strategies. Health education messages should be produced in pamphlets and posters using local languages for dessimination in endemic communities.

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APPENDICES

Appendix 1: Ethical Clearance

MINISTRY OF HEALTH, KADUNA STATE

All Communications to be Addressed to:
THE HON. COMMISSIONER
Quoting Reference and Data
Tel: (062) 248084
(062) 248252

Independence Way,
P.M.B. 2014,
Kaduna,
Kaduna State, Nigeria.

MOH/ADM/122/VOL.II/560 **1st December, 2010**

The country director,
Sight saver International

RE:- REQUEST FOR ETHICAL CLEARANCE FOR STUDY OF EFFECTS OF LONG TERM COMMUNITY TREATMENT WITH IVERMECTIN ON ONCHOCERCA VOLVULUS INFECTIONS IN HUMANS AND THE SIMULIUM DAMNOSUM VECTOR IN PARTS OF KADUNA STATE.

I am directed to convey the approval of the Ministry to allow you to carry out the above mentioned research in parts of Kaduna State. This is borne out of Careful deliberation on the research proposal and the realization that the research is in no way harmful or invasive to the research subjects.

Please accept the assurance of our co-operation

DR ADO Z MOHAMMED
Director Primary Health Care
For: Hon. Commissioner

Appendix II: Protocol for O-150 PCR Analysis of Blackflies for *O. volvulus* infection (By Thomas Unnasch, 2011)

Isolation of Heads from African Blackflies

1. Begin with flies that have been preserved in isopropanol. Rinse the flies in 95% ethanol, and pour them out into a plastic container or on to a weigh paper. Allow the ethanol to evaporate until the flies appear to be dry. You can have up to 100 flies per pool. Do not allow the flies to dessicate completely, or the head purification will not work.

2. Place the flies in a clean dry 15 ml polypropylene conical centrifuge tube. You can do up to five tubes at one time.

3. Place the bottle at -70^{o}C, or in the vapor phase of liquid nitrogen for 30 minutes.

4. Place the tubes containing the frozen flies into the fingers of a regular latex glove. Snap off the heads by holding on to the sleeve of the glove and snapping the fingers against the bench.

5. Collect the flies from the bottle by resuspending them in 95% ethanol, and removing them with a wide bore pipet, such as a plastic transfer pipet. The amount of ethanol is not important, but try to get all of the fly material.

6. Pour the ethanol/fly mix through a 25 mesh sieve connected to a pan. The bodies will collect on the sieve, and the heads will pass through. Rinse the flies collected on the sieve to ensure that all of the heads pass through.

7. Decant the excess ethanol from the pan, using a narrow pipet. Allow the heads to dry at room temperature.

Preparation of DNA from Pools of Heads or Bodies

1. Begin with a pool of heads or bodies. The largest pool found to successfully work with this protocol is 100 heads or bodies. Plan to prepare the DNA in batches of 18 pools. In each batch, include two sham extractions. These are empty tubes that are carried through the process exactly as if they contained flies. These are necessary to ensure that the DNA extraction process remains free of contamination.

2. Place the heads or bodies in a 1.5ml snap cap microcentrifuge tube. Rinse the flies three times in 95% ethanol. Remove as much of the ethanol as possible using a narrow tip pipet, or pipetman.

3. Allow the ethanol to evaporate for about 10 minutes at room temperature.

4. Add 300ul of homogenization buffer (100mM NaCl, 10mM Tris-HCl (pH 8.0), 1mM EDTA, 0.1% SDS) to the tube containing the flies. To the mixture, add 2µl of carrier DNA solution (250ng/µl salmon sperm DNA). Homogenize with a plastic homogenizer. Homogenize until the flies are completely broken up.

5. Add 100ul of proteinase K solution (400ug/ml prepared in homogenization buffer; make fresh each day).

6. Incubate the extract at 55^{o}C for one hour.

7. Add 4µl of 1M DTT. Transfer the extract to a screw cap tube, and boil for 30 minutes.

8. Freeze-thaw the extract two times.

9. Extract the homogenate with 400µl of a 1/1 mix of phenol and chloroform. Transfer the top layer to a second tube, and perform a second extraction with phenol/chloroform.

10. Extract the aqueous layer from the second phenol extraction one time with 400µl of chloroform.

11. Measure the volume of the aqueous layer. Add 3 volumes of NaI solution. Incubate in the refrigerator for 15 minutes.

12. Place the 96 well unifilter (Whatman GF/B or GF/C) over the pump unit and carefully apply the solution from step 11 to each well. Switch on the vacuum pump. After all the solution have been drawn through the filter, switch off the vacuum pump.

13. Add 500µl of ethanol wash to each well and switch on the vacuum pump. After all the ethanol wash has been drawn through the wells, switch off the vacuum pump.

14. Repeat step 13 twice.

15. Place the filter over the 96 well collection plate, add 50µl of sterile distilled water at 55°C to each sample. Centrifuge the unit (filter+ plate) at 4000 RPM for 1 min.

16. Add 150ul of NaI solution to each well, and incubate in the refrigerator for 15 minutes.

17. Repeat steps 12-14. Elute the DNA from the plate into 50ul of sterile distilled water, as described in Step 15.

18. Store the DNA at -20^{o}C.

Reagents

Homogenization buffer:

100mM NaCl, 10mM Tris-HCl (pH 8.0), 1mM EDTA, 0.1% SDS

NaI Stock:

90.3g sodium iodide

1.5g $NaSO_3$ (Sodium Sulfite)

Place approximately 30ml of distilled water in a 150ml beaker. Slowly add the sodium iodide, allowing it to dissolve. Add additional water as needed to ensure that the sodium iodide dissolves. Be careful with the water addition, as the solution in it's final form is nearly saturated. Adjust the final volume to 100ml. Add the sodium sulfite, and stir for 10 minutes (not all of the $NaSO_3$ will dissolve). Filter the solution through Whatman #1 filter paper. Add an additional 0.5g of sodium sulfite to the solution, to ensure that the solution is saturated with sulfite. Store in the dark (amber bottle, or bottle covered with aluminum foil) at 4^oC. When using, ensure that the solution you remove does not contain any of the non-dissolved $NaSO_3$.

Ethanol Wash solution:

20X Stock = 2M NaCl, 200mM Tris-HCl (pH 7.5) 20mM EDTA

for 1 liter:

absolute ethanol (not denatured)	500ml
20X stock	50ml
distilled water	to 1 liter

Store at -20°C

Modified PCR amplification protocol for the O-150 family

Master Mix - for 100 reactions:

PCR water	3.45ml
10X buffer F	500µl
2mM each dNTP	500µl
20µlM 1632 primer	125µl
20µM 1633 biotin primer	125µl
Taq polymerase	50µl
Total volume	4.75ml

For each reaction, use 47.5µl of the master mix plus 2.5µl sample DNA.

Aliquot the master mix into the wells of a 96 well PCR plate in the PCR master mix area. Remove the plate to the DNA prep area and add the DNA samples. Seal the plate with a plate sealing strip.

PCR programme:

5 cycles:

1 minute @ 94oC

2 minutes @ 37oC

30 seconds @ 72oC

Then go to 35 cycles:

30 seconds @ 94oC

30 seconds @ 37oC

30 seconds @ 72oC

finish with 6 minutes @ 72oC

10X buffer F:

Final Concentration	Stock Concentration	Amount to Add
600mM Tris-HCl (pH9.0),	1M	30ml
150mM $(NH_4)_2SO_4$	1M	7.5ml
20mM $MgCl_2$	1M	1.0ml
PCR water	---	11.5ml

Primers:

Dissolve the primer in the same number of µl of PCR water as you have nmol of primer. This produces a 1mM stock solution. Aliquot the 1mM stock solution into 10µl aliquots in screw capped microfuge tubes and store at -80^{o}C.

Add 490µl of PCR water for a 20µM working solution. Store at -20^{o}C.

Primer sequences:

1632: 5’ GATTYTTCCGRCGAANARCGC 3’

1633 biotin: 5’ biotin-GCNRTRTAAATNTGNAAATTC 3’

N = A,G,C OR T Y = C OR T R = A OR G

ELISA based detection of O-150 PCR products

Preparation of PCR reactions:

Before beginning the protocol, set up a number of 10 fold serial dilutions of the positive control DNA in order to determine the limit of detection of the assay for the positive control stock you are using. Run standard 50µl PCR reactions containing 1 □l of the serial dilution of the positive control DNA plus 1.5µl of PCR water. Once you determine the highest dilution that reliable gives a PCR positive result, use 2.5µl of this dilution as your positive control in all subsequent experiments.

For each complete ELISA plate, plan to set up two positive control reactions (in wells A11 and A12) and 10 negative control reactions (in rows A1-A10). The negative control reactions should contain 2.5 µl of PCR water in place of the DNA. In one of the positive control PCRs, add 2.5 µl of the positive control DNA. In the second reaction, add 2.5 µl of the PCR positive control DNA plus 2.5 µl of DNA from a preparation from a pool that has previously tested negative, or DNA prepared from a pool of 50 flies known to be uninfected. The first positive control will tell you that your reaction is working as efficiently as possible, while the second positive control (the one with the fly DNA) will demonstrate that your fly DNA preparations are no inhibiting the PCR. You should be able to do 84 PCRs on each ELISA plate.

ELISA protocol:

1. Coat plates with 100µl of 1µg/ml Strepavidin (dilute stock of 1 mg/ml 1/1000 in 1x Coating buffer) for a minimum of 2 hours @ 37°C. or 4°C overnight.

2. Remove the hybridization buffer from the refrigerator and place in a 37°C water bath to dissolve. Ensure that there is sufficient TBS/Tween wash (2 liters per plate for the whole protocol) and that the substrate and amplifier from the kit are diluted and thawed to room temperature. Ensure that the SSPE/SDS buffer is at 56°C and that you have a squirt bottle of TBS/Tween also heated to 56°C.

3. Wash plates 6 times with TBS/Tween, emptying the plate on to a paper towel between washes. Use a squirt bottle loaded with TBS/Tween for the washes.

4. Add 20µl hybridization buffer (HB) to all wells.

5. Add 5µl of undiluted PCR product (10% of the PCR reaction) to the appropriate wells.

6. Incubate 30 minutes at room temperature. During this time dilute the probe DNA (OVS2-FL) to 50 ng/ml in HB.

7. Wash the plates 6 times with TBS/Tween.

8. Add 100µl 1N NaOH to all wells.

9. Incubate for 1 minute at room temperature.

10. Wash plate 6 times with TBS/Tween.

11. Add 50 µl OVS2-FL-probe diluted to 100ng/ml in HB to all wells.

12. Incubate for 15 minutes at 42oC.

13. Wash the plate 6 times with TBS/Tween.

14. Add 100µl pre-warmed SSPE/SDS buffer to all wells.

15. Incubate 10 minutes at @ 56^{o}C. Warm the BluePhos substrate at room temperature before use. Mix equal volumes of Solution A and Solution B (5ml of each for one plate) in 15ml conical tubes. Use within 30 minutes of mixing. Prepare the conjugate (anti flouresecin Fab fragment) by diluting it 1/10,000 in antibody dilution buffer (i.e. 1µl Fab fragment plus 10ml dilution buffer).

16. Wash the plate 6 times with TBS/Tween heated to 56^{o}C.

17. Add 50 µl of the diluted anti-florescin Fab fragment to each well.

18. Incubate 15 minutes at 37 oC.

19. Wash plate 6 times with TBS/Tween.

20. Add 100ul of freshly mixed BluePhos Microwell substrate solution to each well. Tap gently to Mix. Incubate at room temp for 20-30 min. Blue color will start to appear.

21. Dilute one part of AP stop solution with 9 parts of high quality water to make 1X stop solution at room temperature. i.e. For one plate 1 ml AP stop +9ml H_2O.

22. Add 100ul of AP stop solution to each well to stop color development at desired time.

23. Read plates at 650nm.

Data analysis:

Take the mean of all ten negative control wells and determine the standard deviation of the negative controls. Add a value equal to three standard deviations to the mean of the negative control wells.

We use two different cutoffs for the assay depending upon what the mean plus 3 SD of the negatives are. If this value is above 0.1, use the mean plus 3 SD as the cutoff. If it is below 0.1, set the cutoff at 0.1. The reason for this is that as you do the assay, your consistency will improve and the blank wells will begin to show very little variation. The major source of variation that you see when this happens arises from background contributed by the DNA samples themselves and not variation in the assay. If you get very tight low blanks, the cutoff will not be sufficient to account for this underlying source of variation, and you will score a number of wells as false positives. However, this underlying variation never exceeds 0.1, so 0.1 is the cutoff to use in this situation. Any well with an OD value below the cutoff is scored as negative and anything with a value above the cutoff is scored as a putative positive. When a putative positive is detected, repeat the entire assay, beginning with a new PCR reaction. Any sample that scores above the cutoff in two independent PCRs is scored as a confirmed positive.

Materials:

Microtiter plates: Immulon 2 round bottom 96 well plates. Dynex order# 3655.

Strepavidin: Jackson Immunoresearch

Coating Buffer: 50mM $NaHCO_3$, 2mM $Na2CO_3$. (4.2g Sodium bicarbonate, plus 0.211g Sodium carbonate per liter). Make the coating buffer fresh weekly as the pH goes off during storage. Store it at 4^oC

TBS/Tween: 1X TBS, 0.05% Tween 20.

Stocks needed:

10x TBS:

30.2g Tris base

43.8g NaCl

Dissolve in 350ml distilled water. Adjust pH to 7.5 with HCl. Adjust volume to 500ml

Store at room temperature

TBS/Tween For 2 liters:

10X TBS	200ml
Twen 20	1ml

Store at room temperature

Hybridization buffer (HB):

Stocks needed:

20X SSPE:

174 g NaCl

27.6g NaH_2PO_4

7.4g disodium EDTA

dissolve in 700ml distilled water. Adjust pH to 7.4 with NaOH. Bring volume to 1 liter.

Store at room temperature

10X Denhardt's:

0.2% (w/v) bovine serum albumin, 0.2% (w/v) polyvinylpyrrolidone, 0.2% (w/v) Ficoll 400.

Store at -20^{o}C

1% N-lauryl sarcosine:

For 100ml:

N-lauryl sarcosine 1g

dissolve in 80ml distilled water. Adjust volume to 100ml

Store at room temperature

For 500ml HB:

120ml 20X SSPE

50ml 10X Denhardt's

50ml 1% (w/v) N-lauryl sarcosine

1ml 10% SDS

Water to 500ml

Store at 4°C

1N NaOH:

Weigh out 0.4g solid NaOH. Dissolve in 8 ml distilled water. Adjust volume to 10ml.

Make fresh daily.

OVS2-FL probe

We order this from Operon (Fisher), although products from other suppliers will probably work as well.

5' AATCTCAAAAAACGGGTACATA-fl 3' where fl = flourescein.

Prepare a master solution at 200 □g/ml concentration. Store in the dark at -20°C.

Antibody dilution buffer:

0.4M NaCl, 0.1M Tris-HCl (pH 7.5) 0.5% (w/v) bovine serum albumin. Store at 4°C.

For 100ml:

4M NaCl	10ml
1M Tris HCl (pH 7.5)	10ml
BSA	0.5g
Distilled water	80ml

Dissolve BSA. Store at 4oC

SSPE/SDS Buffer:

1XSSPE, 0.1% SDS

For 200ml

20x SSPE	10ml
10% SDS	2ml
Distilled water	188ml

Store at 42oC sealed tightly. Shake bottle before use.

Anti-flourescein-AP Fab fragments:

Order number 1426338 from Roche Biochemicals

Blue Phos detection system:

catalog # 50-88-22 from KPL www.kpl.com

APStop solution catalog # 50-89-00 from KPL www.kpl.com

Appendix III:



Human bait catching blackflies at a collection site at River Galma

Appendix IV:



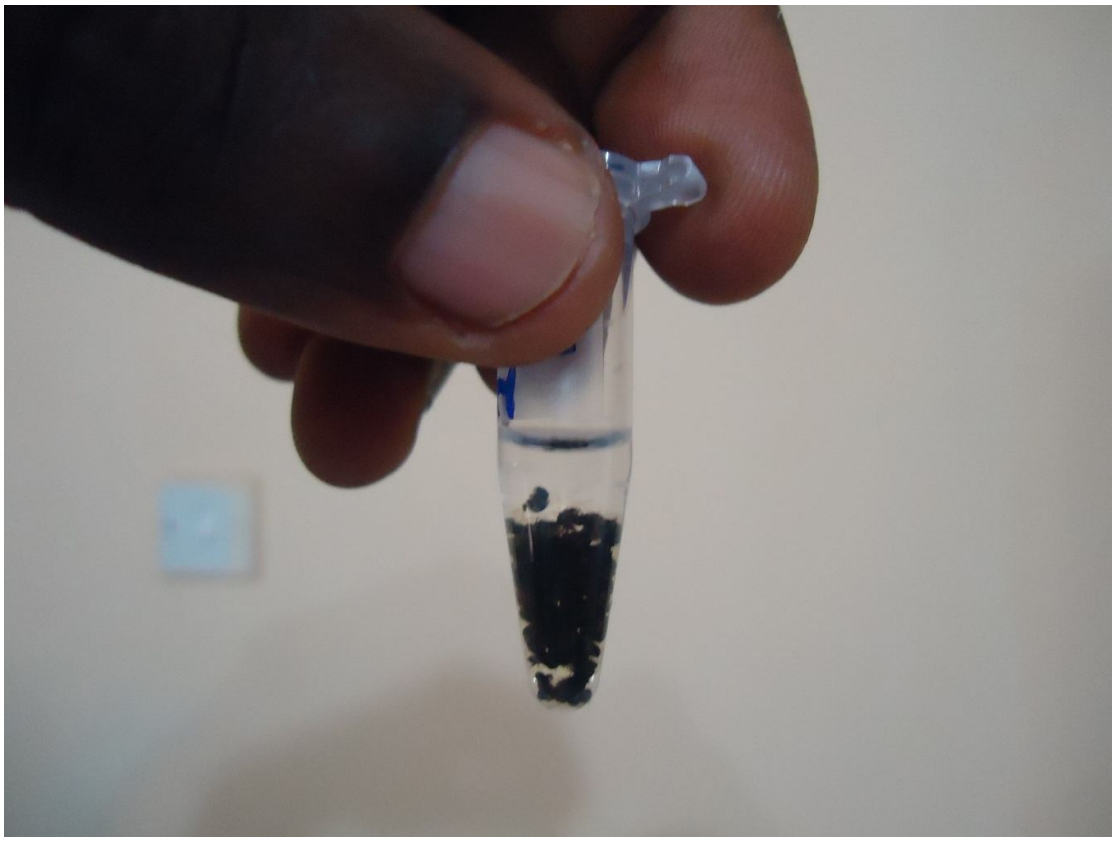
Simulium breeding site and fly collection spot along Galma River in Lere Local Government Area

Appendix V:



Sieve mesh used to separate the heads from the bodies of *S. damnosum*

Appendix VI:



Heads of *O. volvulus* separated from the bodies of *S. damnosum* before PCR

Appendix VII:



Collection of skin snip from a community member in Gurara community in Kagarko Local Government Area

Appendix VIII:



Microscopic examination of skin snip for microfilariae

Appendix IX:



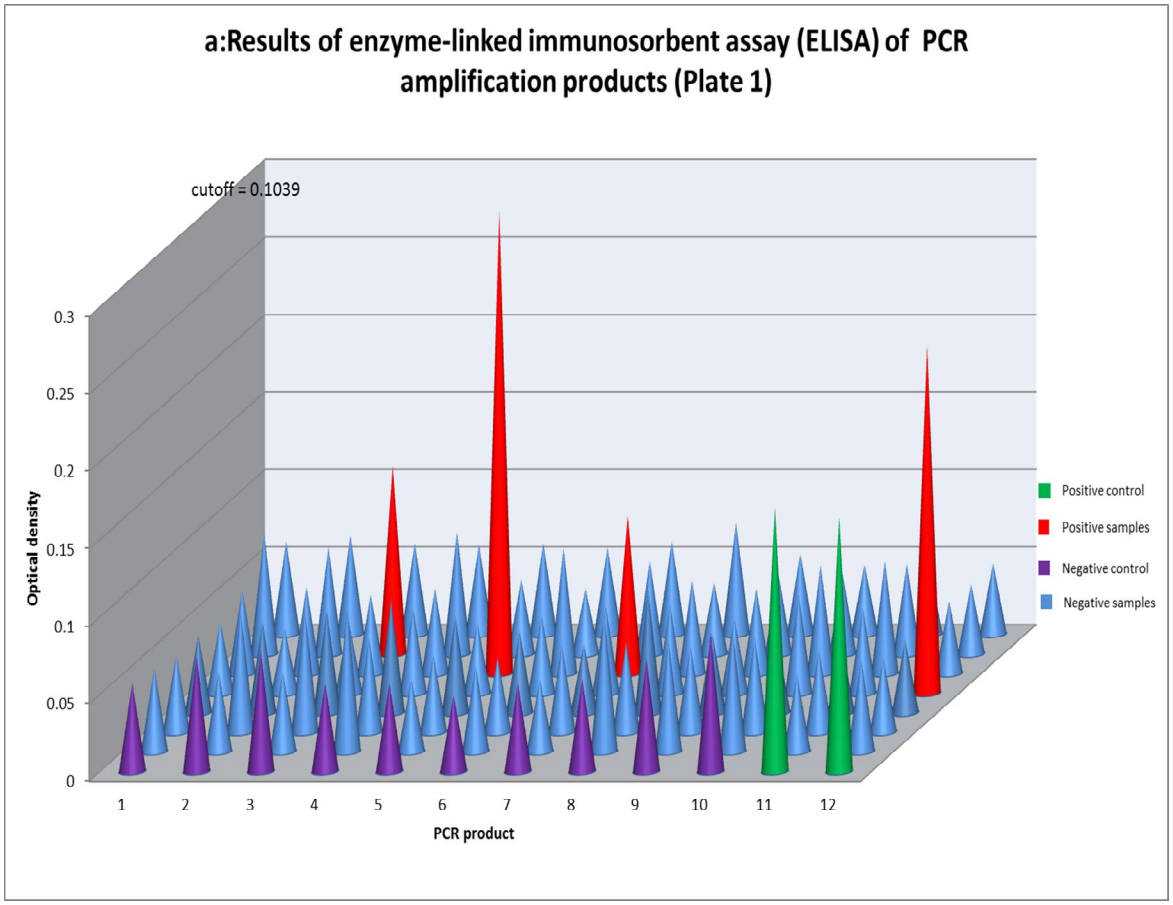
A female community member taking visual acuity test in Were I community, Kauru Local Government Area

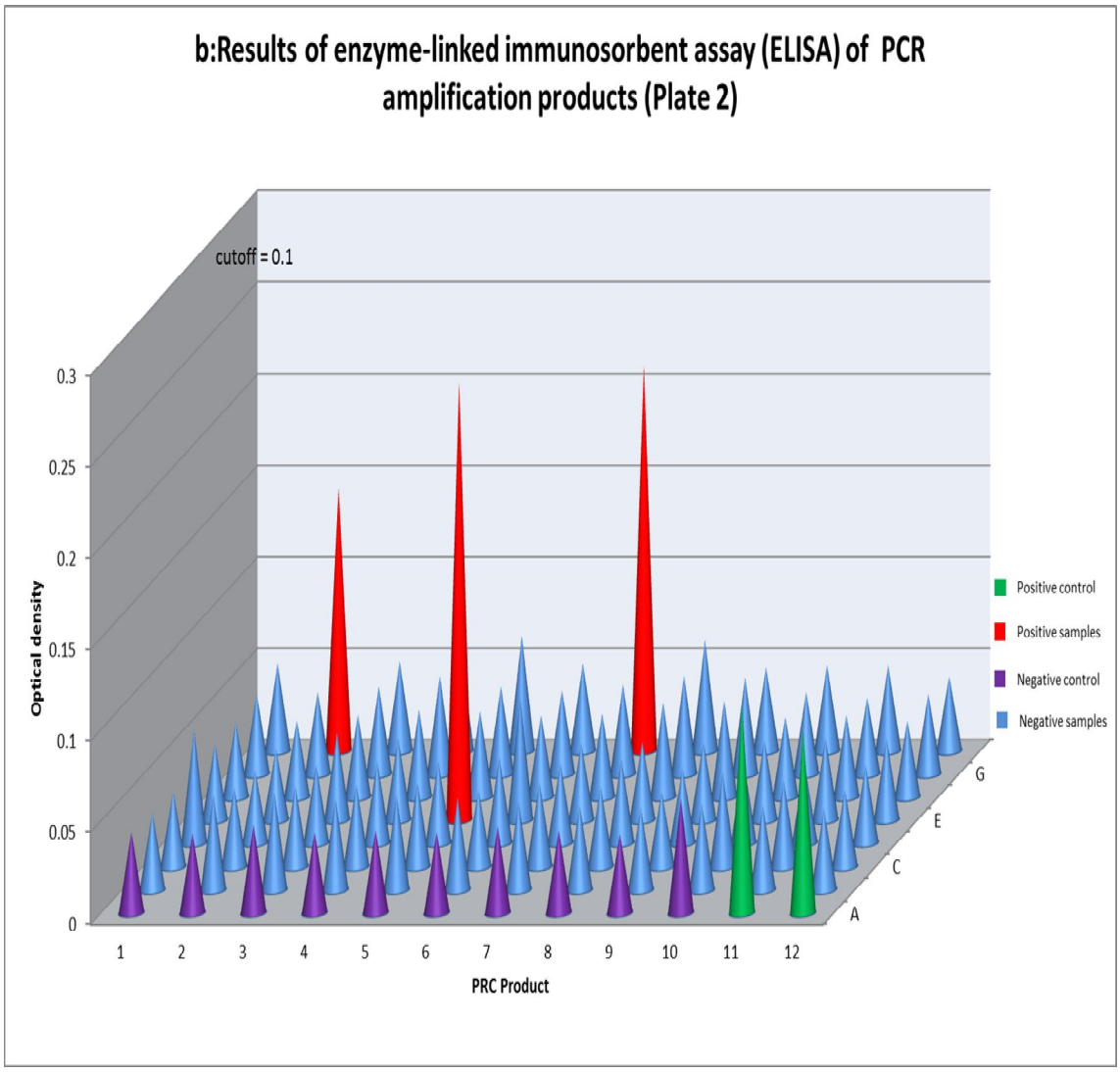
Appendix X:

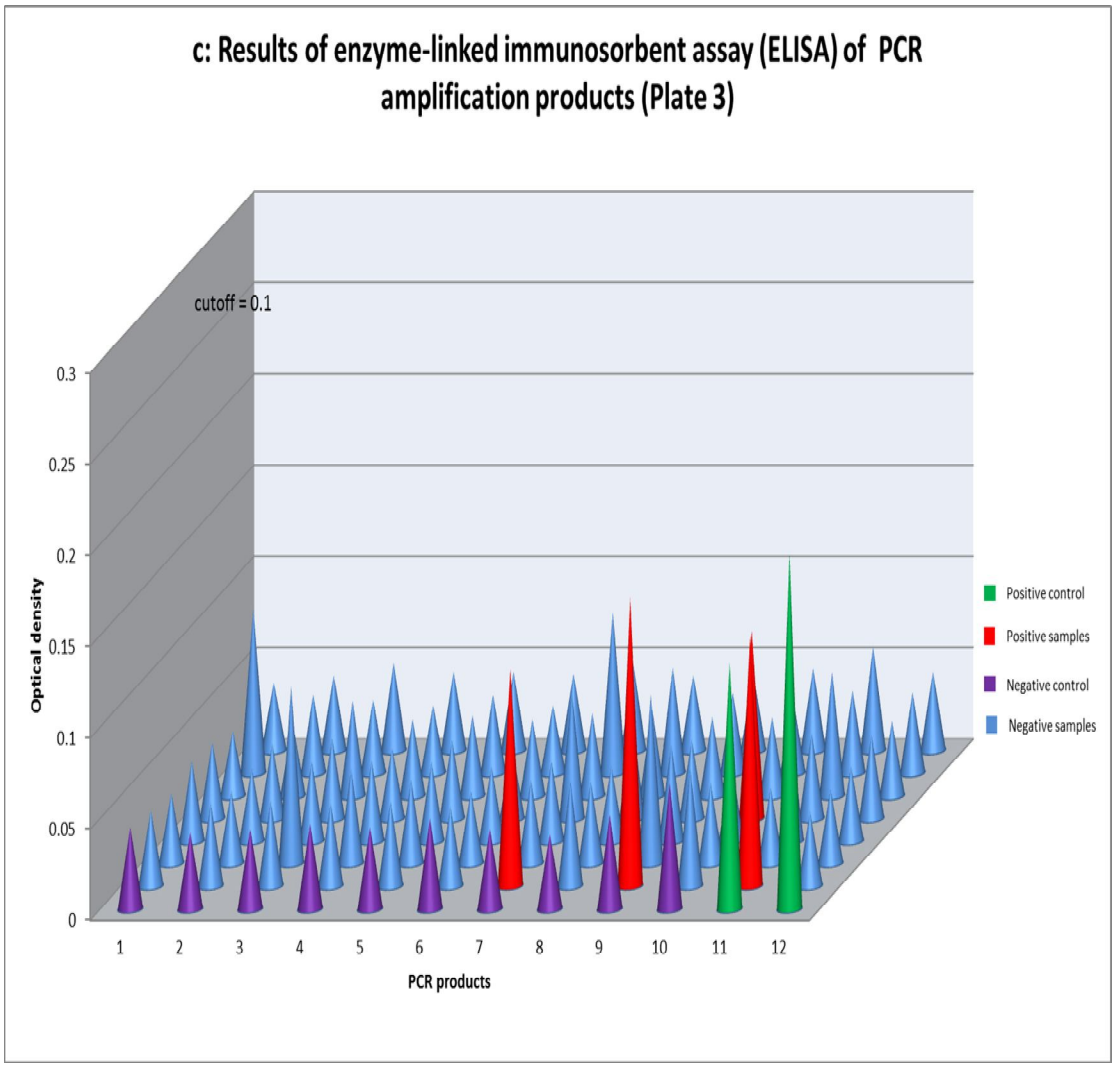


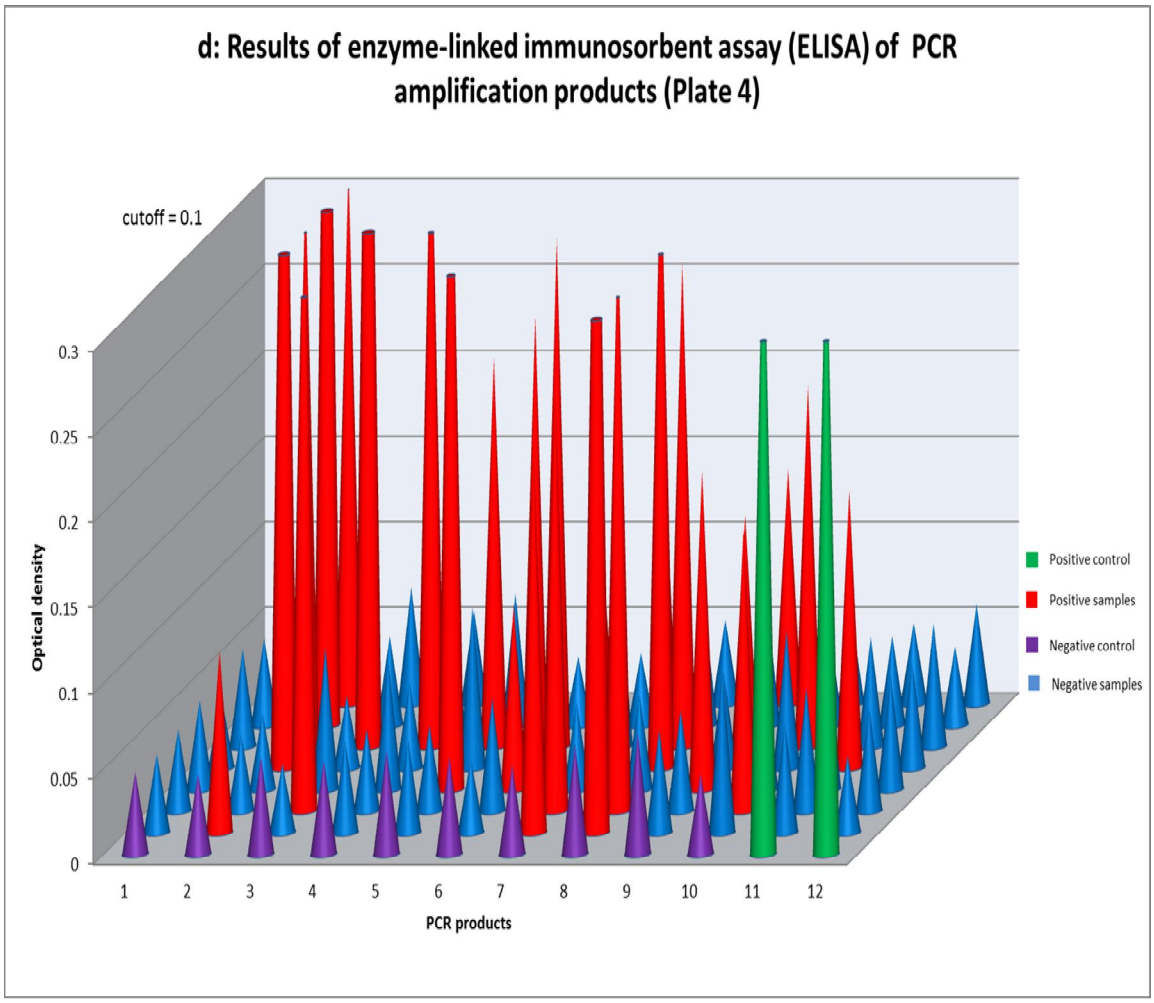
Administration of structured questionnaire to a household head on the benefits from the control programme

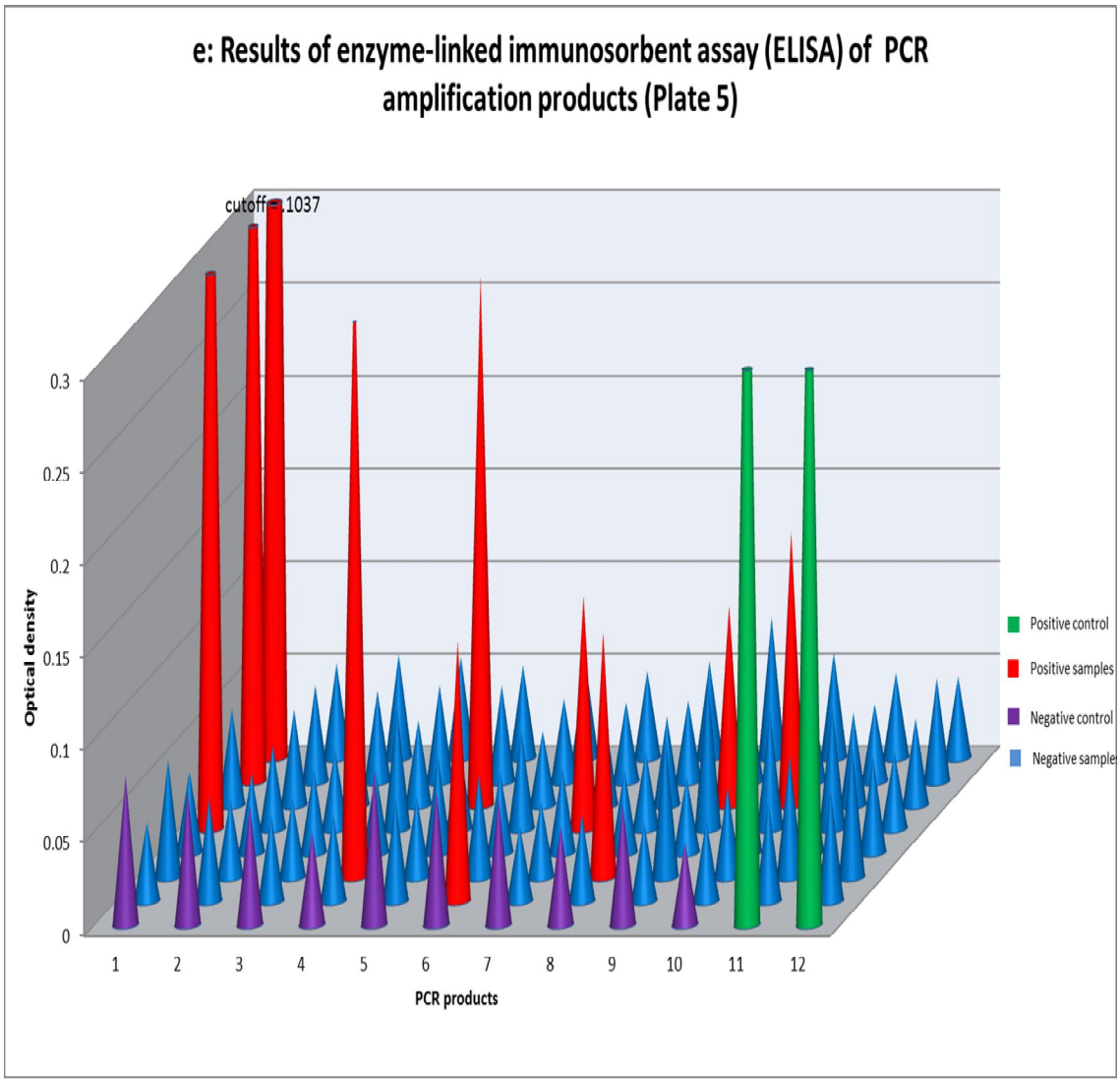
Appendix XI a – f: Results of Enzyme-linked Immunosorbent Assay (ELISA)/PCR Amplification Products

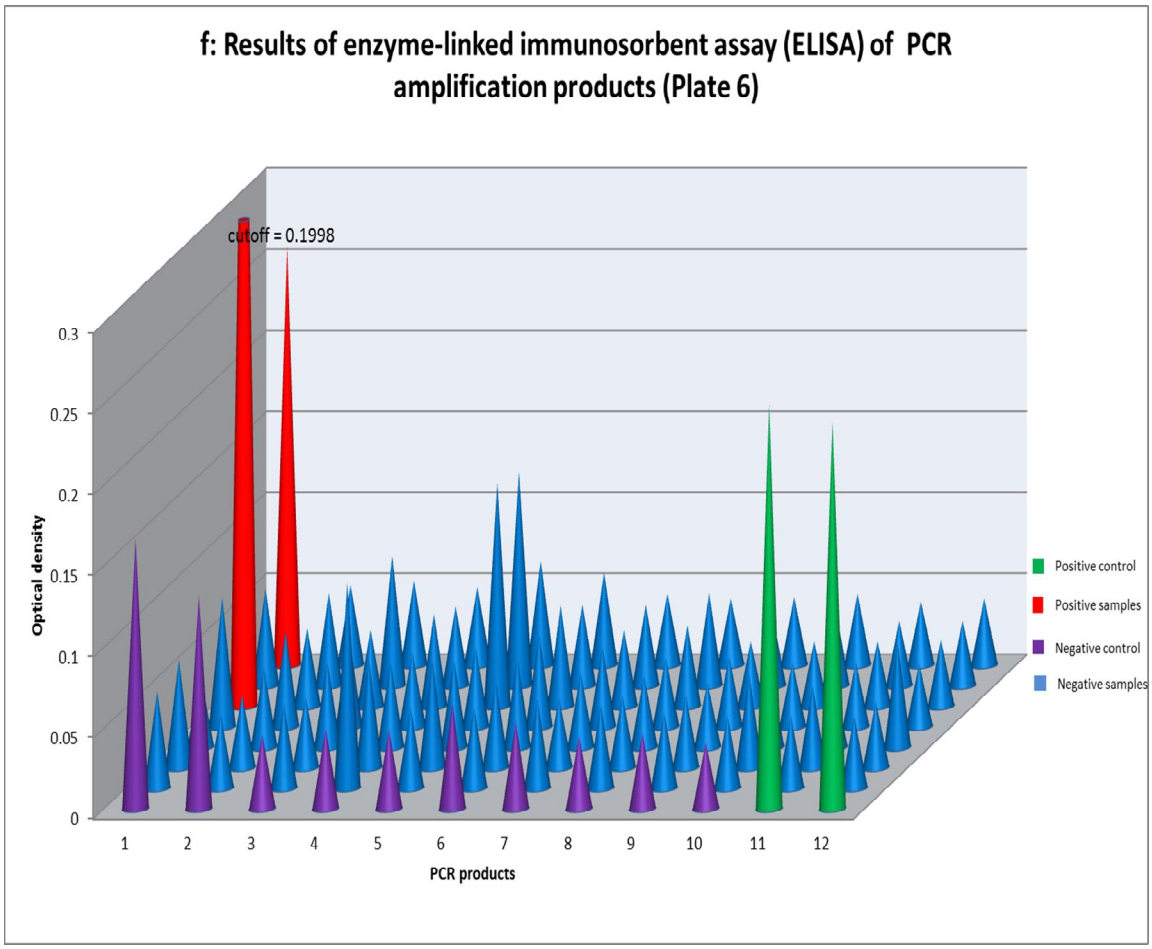



b:Results of enzyme-linked immunosorbent assay (ELISA) of PCR amplification products (Plate 2)
cutoff = 0.1
Optical density
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
10
11
12
PRC Product
A
C
E
G
Positive control
Positive samples
Negative control
Negative samples


c: Results of enzyme-linked immunosorbent assay (ELISA) of PCR amplification products (Plate 3)
cutoff = 0.1
Optical density
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
10
11
12
PCR products
Positive control
Positive samples
Negative control
Negative samples


d: Results of enzyme-linked immunosorbent assay (ELISA) of PCR amplification products (Plate 4)
cutoff = 0.1
Optical density
0
0.05
0.1
0.15
0.2
0.25
0.3
1
2
3
4
5
6
7
8
9
10
11
12
PCR products
Positive control
Positive samples
Negative control
Negative samples


e: Results of enzyme-linked immunosorbent assay (ELISA) of PCR amplification products (Plate 5)
cutoff = .1037
Optical density
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
10
11
12
PCR products
Positive control
Positive samples
Negative control
Negative samples


f: Results of enzyme-linked immunosorbent assay (ELISA) of PCR amplification products (Plate 6)
cutoff = 0.1998
Optical density
0
0.05
0.1
0.15
0.2
0.25
0.3
1
2
3
4
5
6
7
8
9
10
11
12
PCR products
Positive control
Positive samples
Negative control
Negative samples

Appendix XII: Visual acuity and ocular lesions in parts of Kagarko, Kauru and Lere LGAs

	Village	No. Examined	No. with Good Vision	%	No. with Visual Impairment	%	No. Blind	%	Microfilaria in Anterior Chamber	Reason for blindness/Visual Impairment
Kagarko	Atara	89	87	97.75	2	2.25	0	0	0	Refractive Error
	Gantan Gida	98	95	96.94	1	1.02	2	2.04	0	Cat. Ref. Error
	Gurara	130	128	98.46	0	0	2	1.54	0	Cataract
	Kasaru	66	51	77.27	11	16.67	4	6.06	0	Cat. Glau. Ref Error
	Kasaru Kukuk	79	78	98.73	1	1.27	0	0	0	Refractive Error
	Kurmin Lemu	36	34	94.44	2	5.56	0	0	0	Refractive Error
	Ruzai	108	98	90.74	9	8.33	1	0.93	0	Cat. Refractive. Error
	S/Gari U/Pah	102	100	98.04	1	0.98	1	0.98	0	Cat. Refractive. Error

	U/Doya	165	160	96.97	4	2.42	1	0.61	0	Glau.RE
	U/Pah	100	95	95	3	3	2	2	0	Glau.RE
Kauru	Akansa	93	84	90.32	7	7.53	2	2.15	0	Cataract
	Galadimawa	235	204	86.81	25	10.64	6	2.55	0	Cataract Glau
	Garmadi	155	127	81.94	23	14.84	5	3.23	0	Cat, Glau, Ref. Error
	Jankasa	118	110	93.22	7	5.93	1	0.85	0	Cataract
	Kubau Bauchi	196	187	95.41	6	3.06	3	1.53	0	Cat. Ref. Error Glau
	Kaguta	82	66	80.49	14	17.07	2	2.44	0	Cat.Glau, Ref.Error
	Madam	117	112	95.73	5	4.27	0	0	0	Refractive Error
	Sabon Layi	117	92	78.63	25	21.37	0	0	0	Refractive Error
	Sayawa	127	107	84.25	20	15.75	0	0	0	Refractive Error

	T/Wada Garmadi	103	92	89.32	10	9.71	1	0.97	0	Cat. Refractive Error
Lere	Ashema	162	148	91.36	13	8.02	1	0.62	0	Cat. Ref. Error
	Dan Alhaji	69	56	81.16	13	18.84	0	0	0	Ref. Error
	Kakidare	182	171	93.96	8	4.4	3	1.65	0	Cat. Ref. Error
	Kudaru	224	174	77.68	44	19.64	6	2.68	0	Cat. Ref. Error
	Kuduru	90	62	68.89	28	31.11	0	0	0	Ref. Error
	U/Buzu	141	126	89.36	15	10.64	0	0	0	Ref. Error
	U/Pah Kurama	142	110	77.46	31	21.83	1	0.7	0	Cat. Ref. Error
	U/Pah Hausawa	181	170	93.92	11	6.08	0	0	0	Ref. Error
	U/Shawara	375	299	79.73	74	19.73	2	0.53	0	Cat. Glau, RE
	U/Tanimu	77	62	80.52	14	18.18	1	1.3	0	Cat. Ref. Error

Were I	67	59	88.06	8	11.94	0	0	0	Ref. Error
Were II	167	131	78.44	33	19.76	3	1.8	0	Cat. Ref. Error
Zarangi	260	249	95.77	9	3.46	2	0.77	0	Cat. Ref. Error
	4,453	3,924	88.12	477	10.71	52	1.17	0	

Cat=cataract; RF= refractive error and Glau = Glaucoma

Appendix XIII: Questionnaire

Dear Respondent

In this questionnaire you will find questions regarding the Economic Impact of onchcerciasis in your community in general and you in particular. This is important so that the quantification of the benefits accruing to this community and you for a period of over 15 years or so are established. It is also imperative that you know what could have happened if the programme was not in place in the period under consideration. In other words, the losses and, or gains depending on whether the programme was in place or not is necessary. This is also essential so that you can quantify what the donors via Sight Savers have done to your communities in general and you in particular. This is also vital as it will encourage the international donor via Sightsavers and other donors to provide more progrmmes in your community and you in order to save sights. This is just an academic exercise for a Ph.D programme in trying to "**Assess the Economic Impact of Ochocerciasis in Lere and kauru LGAs**". You are assured that the information you will provide will be use strictly for this purpose. It is optional to participate in the study, so feel free to participate or ot out.

Thank You

Section I: Research Assistant

Date of Administration: ----------

Name of Research Assistant: ----------

Section II: Socio-Demographic Characteristics of Respondent

Name of LGA ----------

Name of Community ----------

Sex: (a) Male [ ] (b) Female [ ]

Age ----------

How many years ago since you first received mectizan in this community ---------- years?

How long have you been practicing the farming ---------- years?

How many years ---------- have you been in the farming profession?

Section III: Farming

What type of farming do you practice?

(a) Crop Farming [ ] (b) Poultry [ ](c)Animal Farming [ ](d) Fishing Farming [ ] Others (Specify) ----------

Farming

Farming Type	Land Size in Hectares	Type	Seasonal		Annual	
			Quantity	Earnings	Quantity	Earnings
Crop Farming						
	1.	1.Maize	1.	1.	1.	1.
	2.	2.Millet	2.	2.	2.	2.
	3.	3.Yam	3.	3.	3.	3.
	4.	4.Groundnut	4.	4.	4.	4.
	Others (Specify)	Others (Specify)				
	----------	----------				

Poultry Farming	**1.** **2.** Others Specify ----------	1.Broilers 2.Young Chick Others Specify ----------	**1.** **2.**	**1.** **2.**	**1.** **2.**	**1.** **2.**
Animal Farming	**1.** **2.** **3.** Others (Specify) ----------	1.Sheep 2.Goat 3.Cow Others (Specify) ----------	**1.** **2.** **3.** Others (Specify) ----------	**1.** **2.** **3.** Others (Specify) ----------	**1.** **2.** **3.** Others (Specify) ----------	**1.** **2.** **3.** Others (Specify) ----------
Fishing Farming						

Others Specify ----------						

Section IV: Others

Other Professions (Specify) ----------

How long have you been practicing the profession/occupation ---------- years?

Other Profession (Specify)	Semi-Annual		Annual	
	Quantity	Earnings	Quantity	Earnings
1.	1. 2.	1. 2.		
2.	1. 2.	1. 2.		
3.	1. 2	1. 2		

Thanks for cooperation

Appendix XIV: Value of Economic Activities in study area (in Naira value)

Kagarko		Kauru		Lere		
Commodity Production	**Value in Naira**	**Commodity Production**	**Value in Naira**	**Commodity Production**	**Value in Naira**	**Total Value**
Maize	6,826,750	Maize	7,663,325	Maize	9,203,400	23,693,475
Millet	3,613,250	Millet	825,375	Millet	3,678,150	8,116,775
Yam	2,079,800	Yam	241,850	Yam	1,297,450	3,619,100
Groundnut	4,557,000	Groundnut	0	Groundnut	0	4,557,000
Guinea Corn	5,578,500	Guinea Corn	0	Guinea Corn	0	5,578,500
Soya Beans	4,329,250	Soya Beans	9,193,250	Soya Beans	6,684,250	20,206,750
Beans	405,500	Beans	0	Beans	0	405,500
Ginger	35,972,538	Ginger	0	Ginger	0	35,972,538
Pepper	0	Pepper	0	Pepper	514,000	514,000
Rice	7,279,520	Rice	10,550,260	Rice	15,285,028	33,114,808
Cassava	372,500	Cassava	0	Cassava	0	372,500
Sugar Cane	0	Sugar Cane	0	Sugar Cane	16,850	16,850
LGA Sub Total	**71,014,608**		**28,474,060**		**36,679,128**	**136,167,796**
Animal Rearing	**Value in Naira**	**Animal Rearing**	**Value in Naira**	**Animal Rearing**	**Value in Naira**	**Total Value**
Fowl	782,000	Broiler	28,285	Broiler	13576.5	823,862
Goat	8,213,062	Goat	357,600	Goat	447850	9,018,512
Cow	17,370,000	Cow	1,317,000	Cow	1033500	19,720,500
Sheep	0	Sheep	9,500	Sheep	29250	38,750
LGA Sub Total	**26,365,062**		**1,712,385**		**1,524,177**	**29,601,624**
Service Provision	**Value in Naira**	**Service Provision**	**Value in Naira**	**Service Provision**	**Value in Naira**	**Total Value**
Trading	4,254,000	Trading	0	Trading	7195500	11,449,500

Carpentry	10,840,000	Carpentry	1,206,000	Carpentry	0	12,046,000
Civil service	2,153,400	Civil service	11,885,280	Civil service	8766400	22,805,080
Religious Activities	1,052,400	Religious Activities	0	Religious Activities	0	1,052,400
Driving	1,320,000	Driving	0	Driving	2892000	4,212,000
Timber operation	1,560,000	Timber operation	0	Timber operation	0	1,560,000
"Okada" riding	0	"Okada" riding	0	"Okada" riding	5670000	5,670,000
Others	582,000	Others	0	Others	3813400	4,395,400
LGA Sub Total	**21,761,800**		**13,091,280**		**28,337,300**	63,190,380
Grand Total	**119,141,470**		**43,277,725**		**66,540,605**	**228,959,800**

Source: Computed from Field Survey