EFFECT OF DOXYCYCLINE TREATMENT ON *Onchocerca volvulus* WORMS THAT RESPOND POORLY TO IVERMECTIN

By

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DECLARATION

I hereby declare that this submission is my own work towards the MPhil and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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DEDICATION

I dedicate this thesis to my father, the late Mr. Kwabena Osei Mensah and my auntie, the late Mrs. Akosua Opong-Onyina.

ABSTRACT

Onchocerciasis is a significant public health concern especially in sub-Saharan African countries. Despite more than 20 years of ivermectin mass treatment programmes in endemic areas in the Pru and Lower Black Volta river basins, cases of recrudescence of infections in individuals suggestive of sub-optimal responses of *Onchocerca volvulus* to ivermectin have been reported. In this study, the effect of doxycycline (a licensed tetracycline) therapy on onchocerciasis patients responding sub-optimally to previous multiple treatments with ivermectin was assessed. A total of 149 onchocerciasis-infected volunteers were treated with either 100 mg/day doxycycline (n = 73) or matching placebo (n = 76) for 6 weeks. All study volunteers were allowed to take 2 rounds of 150 μg/kg ivermectin during the study period. Microfilaridermia levels of all study volunteers were monitored at pre-treatment, 12 months and 20 months post-treatment using skin biopsies. At the end of the study, there was a highly significant difference ($p < 0.0001$) in the number of microfilaridermic volunteers between the doxycycline and placebo groups, with the doxycycline group showing a drastic reduction from 65.7% to 2.9% whiles the placebo group showed a marginal increase from 63.2% to 69.0%. There was also a highly significant difference ($p < 0.0001$) in the microfilarial geometric mean load of volunteers between the two groups, with the doxycycline group showing a drastic reduction from 1.3 to 0.2 whiles the placebo group showed a marginal increase from 1.3 to 1.7 despite 2 treatment rounds of ivermectin during the study period. Doxycycline therefore proved to be effective in this study and is thus recommended as a front-line therapy for use in clearing microfilariae from individuals who seem to be responding sub-optimally to repeated ivermectin therapy.

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INTRODUCTION

1.0 Background

Onchocerciasis, commonly known as 'River Blindness' still remains a significant public health burden in developing countries, especially in sub-Saharan Africa. Onchocerciasis in humans is caused by the filarial nematode parasite, *Onchocerca volvulus*, and is one of the leading infectious blinding disease agents of the developing world (WHO, 2001; Thylefors *et al*., 1995), second only to Trachoma. The infective larvae of the parasite is transmitted by *Simulium spp* (blackflies) that breed in fast flowing rivers and streams (Opoku, 2000; WHO, 1995; Duke, 1990**)**.

The prevalence of infection and disease in a community is related to proximity to riverine breeding sites of the blackflies with the highest burden of infection and disease in communities adjacent to rivers (Taylor *et al*., 2010). Microfilaridermia rises with age until around 30 years, after which infection profiles vary between geographical region and sex, with higher rates of microfilaridermia and morbidity reported in men than in women (Little *et al*., 2004). Filipe and colleagues in 2005 attributed this variation in infection profiles to heterogeneity in age and differences in gender exposure to the vectors.

Throughout Africa, 120 million people in more than 34 countries are at risk of onchocerciasis, with over 37 million people being infected (WHO, 2007, 2001; Hoerauf, 2006; Basáñez *et al*., 2006). The WHO in 1995 estimated that 500,000 people were visually impaired and another 270,000 people were completely blind due to onchocerciasis.

The infective larvae (L3) of the parasite has the potential to develop into the adult filariae which have an average life expectancy of 10 years, during which period they have the capacity to produce millions of microfilariae (Habbema *et al*., 1990). The presence of these microfilariae in the skin of infected individuals is responsible for physical manifestations

such as dermatitis, skin atrophy and inflammation in the eye, with over half of the infected individuals presenting with skin disease (Hoerauf *et al*., 2009; Thylefors *et al*., 1995; WHO, 1995).

The effort to eliminate onchocerciasis as a public health problem has evolved over the decades. The Onchocerciasis Control Programme (OCP) which was launched in 1974 and officially ended in 2002, aimed at controlling the breeding of blackflies and hence the disease in 11 West African countries including Ghana, through larvicide spraying of fast flowing rivers and streams (WHO, 2010a; Thylefors *et al*., 1995; WHO, 1995). In 1987, ivermectin (Mectizan®), produced by Merck and Co. was introduced and free distribution by mobile teams for the treatment of onchocerciasis began (MDP, 2009).

Current control programmes including the African Programme for Onchocerciasis Control (APOC) rely on mass administration of the microfilaricidal drug, ivermectin, which has the potential to reduce microfilarial loads in infected humans and thus, transmission by the insect vectors (Molyneux *et al*., 2003; Remme, 1995). Simulation studies using the ONCHOSIM model for onchocerciasis transmission suggests that prolonged, high coverage (>80%) programmes giving ivermectin at 6 month intervals have a high probability of eliminating the infection (Winnen *et al*., 2002; Habbema *et al*., 1996; Plaisier *et al*., 1990).

Ivermectin is known to lower the microfilarial load in affected individuals and temporarily sterilize adult female filarial worms, thereby reducing transmission and mitigating the clinical manifestations of the infection (Awadzi *et al*., 1999; Goa *et al*., 1991). However in reality, while community microfilarial loads (a measure of the public health importance of the disease) can be reduced to nearly zero, there are well documented situations where transmission is continuing even after 10-12 years of ivermectin mass treatment (Borsboom *et al*., 2003). This situation pertains because female filarial worms resume their fertility after

temporary interruption by ivermectin treatment, coupled with the fact that at best, the drug has little or no macrofilaricidal activity (Hoerauf *et al*., 2009; Duke, 2005; Gardon *et al*., 2002; Kläger *et al*., 1993). It is therefore imperative to develop a safe macrofilaricidal and/or permanently sterilizing drug to control onchocerciasis, especially since studies in Ghana have already identified *Onchocerca volvulus* populations in whom the sterilizing effect of ivermectin is lost (Awadzi *et al*., 2004a).

In 2004, Awadzi and colleagues reported sub-optimal responses in endemic populations exposed to many treatment rounds of ivermectin. In Ghana, studies into populations that respond poorly to ivermectin found that a large proportion of adult female worms retained full embryonic production ability and consequently significant microfilaridermias persisted despite a history of multiple treatments with ivermectin (Awadzi *et al*., 2004a, 2004b). These studies have caused concern among scientists in the filarial community, because there is no other safe drug approved for the treatment of onchocerciasis.

Tetracyclines have been shown to have anti-filarial activity through destruction of *Wolbachia* from studies conducted in human and bovine onchocerciasis (Hoerauf *et al*., 2000a; Langworthy *et al*., 2000). They have been shown to kill adult worms of *Onchocerca ochengi*, a filariae of cattle that is the closest relative of *Onchocerca volvulus* (Gilbert *et al*., 2005; Langworthy *et al*., 2000).

Doxycycline, a member of the class of tetracyclines, was introduced as a novel chemotherapeutic principle targeting *Wolbachia* endobacteria in filarial worms a decade ago (Hoerauf *et al*., 2003b, 2001, 2000a). Hoerauf and colleagues in 2001 found that 100 mg/day of doxycycline given for 6 weeks led to *Wolbachia* depletion and sterilization of female filariae for at least 18 months and most likely even longer. There was also a reduction in the proportion of living adult female and male worms at 18 months, albeit the number of extirpated worms were too low to indicate a macrofilaricidal effect.

Taylor and colleagues in 2005, as well as Debrah and colleagues (2006a) demonstrated a macrofilaricidal activity of doxycycline against *Wuchereria bancrofti*, also a filarial parasitic infection. This finding was later established in *Onchocerca volvulus* (*O. volvulus*), albeit at a higher daily dose of 200 mg/day of doxycycline, even after a 20 months observation period (Hoerauf *et al*., 2008a).

Since doxycycline is already licensed for use in humans and its safety has been proven, it is a potential candidate to augment ivermectin treatment in those circumstances where ivermectin alone is insufficient, such as in areas where there is a faster repopulation with microfilariae despite numerous treatment rounds with ivermectin.

1.1 Rationale

In Ghana, in the Pru and Lower Black Volta river basins where vector control had been applied for 20 years and ivermectin mass treatment had been administered since 1987, cases of recrudescence of infections in individuals suggestive of ivermectin-resistant *Onchocerca volvulus* has been reported (Gardon *et al*., 2002; Plaisier *et al*., 1997).

Resistance to antihelminthics including ivermectin is already a major problem in veterinary parasitic nematodes (Eng *et al*., 2006; Coles, 2006; Coles *et al*., 2006, 2005; Kaplan, 2004). In humans, although as yet there are no confirmed reports of resistance in *Wuchereria bancrofti* to ivermectin (Fernando *et al*., 2011), several reports indicate possible resistance in the case of *Onchocerca volvulus* to ivermectin (Osei-Atweneboana *et al*., 2007; Awadzi *et al*., 2004a, 2004b). The most serious problems of antihelminthic resistance occur in *Haemonchus contortus*, a nematode parasite of sheep, which has been widely studied in order to try to elucidate the mechanisms of ivermectin resistance in nematodes (Eng *et al*., 2006).

In humans, recent reports of poor parasitological responses of *O. volvulus* to ivermectin (Osei-Atweneboana *et al*., 2007; Awadzi *et al*., 2004a, 2004b; Ali *et al*., 2002), the drug of

choice for mass treatment of onchocerciasis, as well as genetic evidence of ivermectin resistance selection on *O. volvulus* (Ardelli *et* al., 2006a, 2006b; Eng and Prichard, 2005; Ardelli and Prichard, 2004) gives cause for concern. Several studies have implicated a number of genes found in filarial nematodes including β-tubulin, multidrug resistant protein (MDR1) and P-glycoprotein-like protein (PLP), in *O. volvulus* resistance to ivermectin (Kudzi *et al*., 2010; Bourguinat *et al*., 2008, 2007; Eng *et al*., 2006; Eng and Prichard, 2005; Ardelli *et al*., 2006a, 2006b, 2005; Ardelli and Prichard, 2004). These transmembrane proteins are known to transport a wide variety of substrates, including drugs such as ivermectin, across the cell membrane (Bourguinat *et al*., 2008). Selection for ivermectin-resistant single nucleotide polymorphisms (SNPs) of these genes have been shown to occur after repeated ivermectin treatment in several endemic populations, serving as a hindrance to the effective control of onchocerciasis.

Grant in 2000, asserted that though it was equally likely that resistance selection would act on macrofilariae (adult worms) as on microfilariae, the consequences of macrofilarial resistance might be more serious for *O. volvulus* control than is selection for microfilarial resistance. This assertion by Grant becomes very relevant considering a number of facts. Firstly, that adult female *O. volvulus* could live for up to 15 years whiles most microfilariae have a maximum lifespan of 2 years, meaning that effective macrofilariae control would be more beneficial as well as have a longer lasting impact on onchocerciasis control efforts. Secondly, since *O. volvulus* has an indirect life cycle which requires that not all stages occur in a single host, effective control of macrofilariae would in turn lead to disruption of the production of the next generation of microfilariae, thereby halting the transmission cycle.

Data from studies by Awadzi and colleagues, 2004a and 2004b, strongly suggest that ivermectin resistance is developing in adult female *O. volvulus*, although ivermectin still

remained effective against microfilariae. This could have serious consequences for current onchocerciasis control programmes in Ghana.

It is therefore necessary that safe and efficient drugs which either kill adult worms or lead to long-term sterility are used to augment ivermectin treatment, if onchocerciasis is to be controlled effectively. Although the macrofilaricidal activity of doxycycline against *Onchocerca volvulus* has been established (Hoerauf *et al*., 2008a), it remains to be determined whether individuals living in endemic communities where ivermectin-resistant adult *Onchocerca volvulus* infections occur will respond to doxycycline treatment.

1.2 Aim

To assess the effect of doxycycline on onchocerciasis patients in whom repeated ivermectin treatment has failed to prevent a faster repopulation with microfilariae.

1.2.1 Specific Objectives

1) To assess the effect of doxycycline on microfilarial loads in onchocerciasis patients in whom repeated ivermectin treatment has failed to prevent a faster repopulation with microfilariae.

2) To assess the viability of doxycycline therapy in individuals living in communities where repeated ivermectin treatment has failed to prevent a faster repopulation with microfilariae.

Chapter 1 - LITERATURE REVIEW

Onchocerciasis - Host, Parasite and Vector Dynamics

Female blackflies in the Dipteran taxonomic family Simuliidae are the only vectors of human onchocerciasis in West Africa (Boakye *et al*., 1998). In Ghana, at least 6 sibling species of the *Simulium damnosum* Theobald complex have been identified as epidemiologically important in onchocerciasis transmission (Kutin *et al*., 2004; Opoku, 2000). Blackfly biting activities which occur mostly in the morning and afternoon are affected by factors such as light intensity, clouds, seasons and temperature (Opoku, 2000; Alverson and Noblet, 1976; Saunders, 1976; Underhill, 1940). In a study in a Ghanaian community in 2000, Opoku attributed higher biting densities in the morning to the stimulating effects of the morning sunlight after inactivity in the night and the general lull in biting activities in the afternoon to suppressive mean temperatures of around 32 degrees Celsius.

Interactions between parasites and vectors are believed to contribute to observed epidemiological patterns in vector-borne infections (Basáñez *et al*., 2009). Basáñez and colleagues assert that this interaction also happens in onchocerciasis. They further state that possible co-evolution of the *Onchocerca-Simulium* complex may give rise to local adaptations with the potential to stabilise the infection. Studies have also shown that the monthly onchocerciasis transmission potential, which is a basic index for assessing the disease transmission by the vectors, is usually higher in the rainy season than in the dry season (Opoku, 2000; Cheke *et al*., 1992a). Interestingly, other publications hold the reverse to be true; that the transmission potential is rather higher in the dry season than in the rainy season (Achukwi *et al*., 2000; Cheke *et al*., 1992b). Although the abundance of water during the rainy season provides a conducive environment for breeding of the blackfly vectors and hence a likely increase in transmission potential, flooding of breeding sites would invariably reduce the fly population and hence affect transmission potential during this season. In the

dry season, the combined population of old and new flies could be responsible for the increase in the transmission potential. Experimental evidence shows the existence of 2 forms of onchocerciasis in West Africa: onchocerciasis of the savanna regions and that of the forest zones (Bryceson *et al*., 1976; Duke and Anderson, 1972). Consequently, Duke and Anderson in 1972 also provided evidence of the differences in pathogenicity in savanna and forest strains of *Onchocerca volvulus* when they discovered that microfilariae taken from savanna patients produced worse keratitis after inoculation into the eyes of rabbits than did microfilariae from forest patients.

Humans are the definitive host of *Onchocerca volvulus*. The human host is known to harbor various stages of the parasite, including the infective larvae, the migrating and developing pre-adult forms, the adult male and female worms and the microfilariae (mf) (Awadzi *et al*., 2004a). Most of the adult worms are found in subcutaneous nodules (onchocercomata) in humans (Awadzi and Gilles, 1992), where they produce millions of mf which reside predominantly in the skin and eye and cause most of the pathology synonymous with onchocerciasis (Awadzi *et al*., 2004a).

Basáñez and colleagues in 1994 found little evidence of saturation in *Onchocerca volvulus* uptake by the blackflies as the intensity of infection in human hosts increased. Models which have been adduced to explain the dynamics of transmission have found that a nonlinear relationship exist in terms of the dependence on densities of hosts, parasites and vectors to drive transmission (Basáñez *et al*., 2006; Soumbey-Alley *et al*., 2004; Basáñez *et al*., 1994).

Life Cycle of Onchocerca volvulus

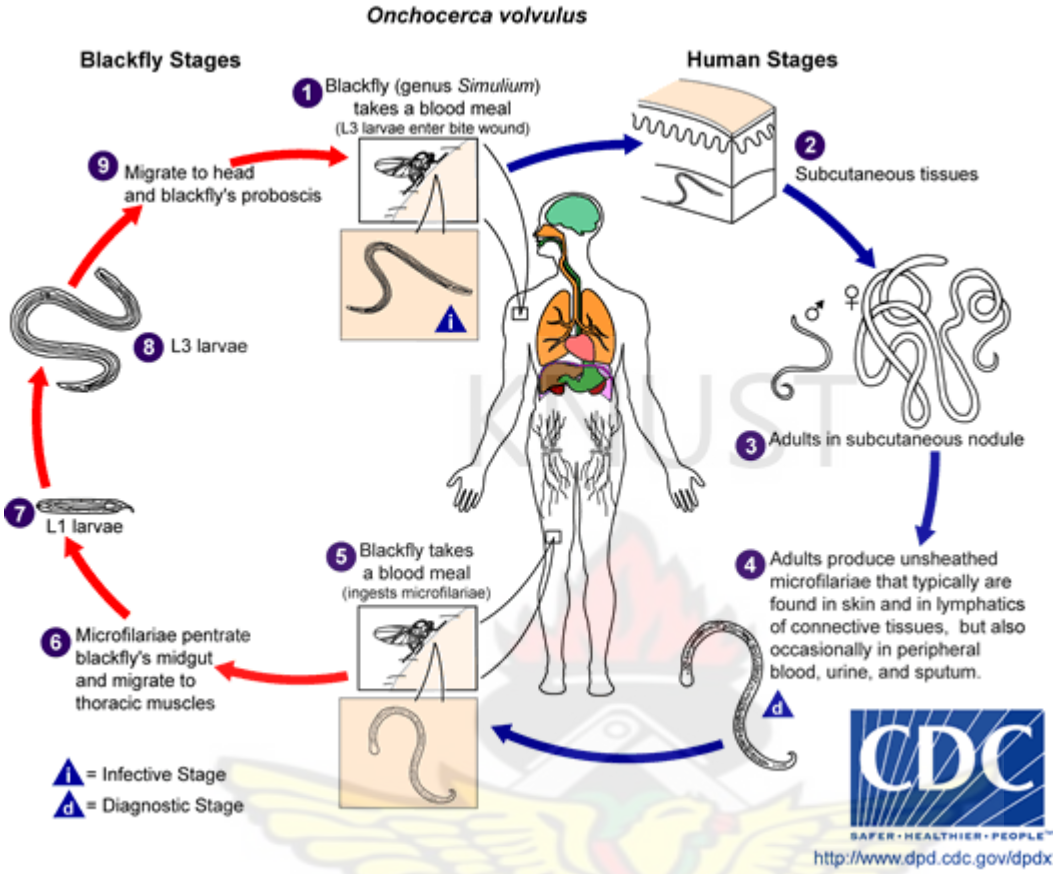


Figure 1.0: Life Cycle of *Onchocerca volvulus*

During a blood meal, an infected blackfly (genus *Simulium*) introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound. In subcutaneous tissues the larvae develop into adult filariae, which commonly reside in nodules in subcutaneous connective tissues. Adults can live in the nodules for approximately 15 years. Some nodules may contain numerous male and female worms. Females measure 33 to 50 centimetres (cm) in length and 270 to 400 micrometres (µm) in diameter, while males measure 19 to 42 millimetres (mm) by 130 to 210 µm. In the subcutaneous nodules, the female worms are capable of producing microfilariae for approximately 9 years. The microfilariae, measuring 220 to 360 µm by 5 to 9 µm and unsheathed, have a life span that may reach 2 years. They are occasionally found in peripheral blood, urine, and sputum but

are typically found in the skin and in the lymphatics of connective tissues. A blackfly ingests the microfilariae during a blood meal. After ingestion, the microfilariae migrate from the blackfly's midgut through the hemocoel to the thoracic muscles. There, the microfilariae develop into first-stage larvae and subsequently into third-stage infective larvae. The third-stage infective larvae migrate to the blackfly's proboscis and can infect another human when the fly takes a blood meal (CDC, Retrieved on 18th October 2010 at 6:45 am).

Clinical Manifestations of Onchocerciasis

People affected by onchocerciasis may either be asymptomatic or symptomatic (Egbert *et al*., 2005). Infected persons who show symptoms usually exhibit one or more of 3 general manifestations: (i) onchocercal dermatitis, (ii) ocular onchocerciasis and/ or (iii) subcutaneous bumps or nodules (onchocercomata), with the most serious manifestation consisting of eye lesions that can progress to blindness (CDC, 2008; Hagan, 1998). Clinical lesions occur in response to dead or degenerating microfilariae surrounded by macrophages, eosinophils, and neutrophils (Pearlman *et al*., 1999). It has been suggested that the release of *Wolbachia* from these dying microfilariae induces innate immune responses that contribute to these clinical manifestations (Keiser *et al*., 2002). Saint André and colleagues in 2002 also demonstrated the existence of a link between onchocercal blindness and *Wolbachia in vivo* in mice. They showed that the development of blindness in mice after injection of worm extracts is dependent upon *Wolbachia* as *Onchocerca volvulus* extracts depleted of *Wolbachia* does not induce blindness.

Onchocerciasis has also been described as a systemic disease that is associated with musculoskeletal pain, reduced body mass index, and decreased work productivity (Basáñez *et al*., 2006). These systemic disease manifestations may be due to the fact that microfilariae can invade many tissues and organs, as well as occur in blood and urine (Cox *et al*., 2005).

Heavy microfilarial infection is also suspected to be involved in the onset of epilepsy (Boussinesq *et al*., 2002) and the hyposexual dwarfism known as Nakalanga syndrome (Kipp *et al*., 1996).

Endemic communities in the western savanna woodlands of Africa have a high prevalence of blindness, whereas cutaneous symptoms are more prevalent in the rainforest and in the East African highlands (Duke, 1981; Woodruff *et al*., 1977).

Subcutaneous Nodules (Onchocercomata)

Subcutaneous nodules occur as fibrous nodules in the skin and subcutaneous tissues of onchocerciasis patients and are the least severe clinical manifestation of the disease (Awadzi and Gilles, 1992). These nodules harbor the male and female adult worms (macrofilariae), with the latter producing first stage (L1) microfilariae after fertilization by the former (Basáñez *et al*., 2006).

Palpable onchocercal nodules are asymptomatic subcutaneous nodules, scattered around the body over bony prominence and ranging in size from a pea to as large as a golf ball (Enk, 2006).

Onchocercal Dermatitis (Onchodermatitis)

Clinical features of this condition include itching (pruritus), papular and papulomacular rash, hanging groins, skin atrophy and depigmentation (Kipp and Bamhuhiiga, 2002; Hagan, 1998). Onchocercal dermatitis which is usually the first visible symptom of infection begins with intense itching and progresses to a manifestation of irritating papular rashes (Okoye and Onwuliri, 2007). Known as acute papular dermatitis, it presents with small pruritic papules that may develop into pustules or vesicles and often affects the face, the trunk, and the extremities (Okoye and Onwuliri, 2007; Enk, 2006).

Progression could lead to chronic papular dermatitis which presents as large, scattered, flat-topped papules and may result in hyperpigmentation and thickening of the skin which typically affect the shoulders, the buttocks, and the extremities (Okoye and Onwuliri, 2007; Murdoch *et al*., 1993). Further physical deterioration leads to lichenified onchodermatitis, resulting in mosaic patterns popularly known as "lizard skin", "crocodile skin" or sowda (Okoye and Onwuliri, 2007). Sowda occurs during the parasite-destroying phase of the infestation and is associated with a delayed hypersensitivity immune response, which is usually observed in patients with low microfilarial loads (Enk, 2006).

The late or advanced stages of this condition is characterised by depigmentation known as "Leopard skin", loss of elasticity and atrophy of the skin (Okoye and Onwuliri, 2007; Murdoch *et al*., 1993). Onchocercal depigmentation often affects the shins in a symmetrical pattern and is rarely associated with itching and excoriations (Enk, 2006).

Most patients in endemic communities are known to present with sub-clinical or intermittent dermatitis corresponding to acute papular onchodermatitis (Basáñez *et al*., 2006). However in populations where onchodermatitis is endemic, the most common skin manifestation is chronic papular onchodermatitis followed by onchocercal depigmentation and onchocercal atrophy (Hagan, 1998).

Ocular Onchocerciasis

Ocular onchocerciasis covers a wide spectrum ranging from mild symptoms such as itching, redness, pain, photophobia, diffuse keratitis, and blurring of vision to more severe symptoms of corneal scarring, night blindness, intraocular inflammation, glaucoma, visual field loss, and eventually blindness (Enk *et al*., 2003). Inflammatory reactions around microfilariae occuring in the eye is responsible for ocular onchocerciasis (Egbert *et al*., 2005). Ocular lesions, which result from the migration of microfilariae to eye tissues and the inflammatory

response invoked by their death, can involve all eye tissues except the lens, ranging from punctate and sclerosing keratitis (anterior segment) to optic nerve atrophy (posterior segment) (Taylor *et al*., 2010; WHO, 2010b; Basáñez *et al*., 2006). Punctate keratitis, which signifies initial involvement is transient and reversible with treatment, whereas long term infection results in sclerosing keratitis, iridocyclitis and inflammation in the anterior chamber and retinal epithelium (Taylor *et al*., 2010; Egbert *et al*., 2005). Lesions of the posterior segment may follow, including chorioretinitis, optic neuritis, and optic atrophy (Newland *et al*., 1991). Blindness may occur as a result of long term exposure to the microfilariae (Burnham, 1998).

Onchocerciasis Control in Ghana Today

About 3.2 million people are at risk of onchocerciasis which is endemic in all regions of Ghana, except the Greater Accra region (Taylor *et al*., 2009). Over 3000 communities in 66 districts are affected, with more than 240 communities in the Brong Ahafo and Ashanti regions being designated as Special Intervention Zones (SIZ) - these are areas of hyperendemicity within the Pru River basin that serve as foci of Community-Directed Treatment with Ivermectin (CDTI) (Taylor *et al*., 2009). CDTI was introduced in 1998, with about 3.4 million people treated with this approach between 2002 to 2007 (Taylor *et al*., 2009). From 2006, onchocerciasis control has been implemented in the context of the Neglected Tropical Diseases Control Programme (NTDCP), a 5-year programme designed to integrate and scale up delivery of preventive chemotherapy for 5 targeted neglected tropical diseases (NTDs) including onchocerciasis (NTD, 2007).

Chemotherapeutic Approaches to Onchocerciasis Control

Ivermectin is currently the sole drug approved by the World Health Organisation (WHO) for use in onchocerciasis control programmes (WHO, 2010a, 2010b; Taylor *et al*., 2010; Boatin

and Richards, 2006; Awadzi *et al*., 2003). Ivermectin is administered to all those aged five years or older, excluding pregnant women and those breastfeeding a child younger than one week old biannually or annually to reduce morbidity, disability and lower transmission (Tielsch and Beeche, 2004; Boussinesq *et al*., 1997; Collins *et al*., 1992). Over the years, several drugs including diethylcarbamazine, suramin, moxidectin, mebendazole, albendazole and the tetracyclines have been proposed in various studies as possible chemotherapeutic tools to eliminate the parasite (Hoerauf *et al*., 2003; Tagboto and Townson, 1996; Molyneux, 1995; Poltera *et al*., 1991; Francis *et al*., 1985; Thylefors and Rolland, 1979).

Activity of Ivermectin in Onchocerciasis Control

Ivermectin has broad antiparasitic activity against nematodes (Enk, 2006). It is a potent microfilaricide which has also been shown to partially interrupt embryogenesis after frequent application (Pfarr and Hoerauf, 2006; Awadzi *et al*., 1999). Dadzie and colleagues in 1991, showed that annual ivermectin treatment is adequate to control onchocercal ocular disease even in populations with very high endemicity levels. Other studies have also shown that ivermectin is effective in alleviating dermatological symptoms associated with onchocerciasis (Whitworth *et al*., 1996).

The general observed pattern in ivermectin usage is a marked reduction of microfilarial loads shortly after each treatment followed by a steady repopulation of the skin until a subsequent treatment round (Alley *et al*., 1994). Alley and colleagues also found that even a single treatment with ivermectin has a significant medium-term impact on microfilarial loads, with microfilarial counts stabilized around 55% of pre-treatment counts 2-4 years after a single treatment. At the recommended dose of 150 µg/kg, it neither kills nor permanently sterilises the adult worms (Awadzi *et al*., 1995a), although it has been shown to impair the ability of female worms to produce microfilariae (Kläger *et al*., 1996; Plaisier *et al*., 1995; Duke *et al*., 1992). Repopulation data of microfilariae from a study undertaken by Whitworth and

colleagues in 1996 suggest that adult female worms are still alive and fecund after repeated ivermectin treatment, strongly underlining the need to continue treatment to cover the lifespan of the female worms (Enk, 2006). Even in single doses as high as 1600 µg/kg, ivermectin was no more effective than the standard dose of 150 µg/kg; at best, it only leads to a mild-to-modest macrofilaricidal effect after repeated standard doses (Awadzi *et al*., 1999).

Although ivermectin is generally well tolerated, adverse effects associated with this drug appear 1 to 2 days after treatment and correlate with microfilarial loads; with high mf loads corresponding to substantial adverse effects such as pruritus, urticaria, dermatitis, fever, myalgia and oedematous swelling of the limbs and face (Taylor *et al*., 2010). A major difficulty however arises with ivermectin treatment of onchocerciasis in areas co-endemic for loaisis (Taylor *et al*., 2010; Boussinesq and Gardon, 1997), especially since patients with high *Loa loa* microfilariae loads may develop encephalitis due to the rapid killing of the microfilariae (Boussinesq *et al*., 2001; Gardon *et al*., 1997).

Activity of Diethylcarbamazine (DEC) in Onchocerciasis Control

Diethylcarbamazine, also known as diethylcarbamazine-citrate (DEC-C) was the established microfilaricide for the treatment of filariasis since it was discovered in 1948 (Taylor *et al*., 2010; Awadzi and Gilles, 1992). Although DEC has moderate macrofilaricidal effect (Pfarr and Hoerauf, 2006), it is inferior to ivermectin in its ability to eliminate high parasite loads without producing severe reactions or ocular deficiency and sustaining the suppressive effect on skin and ocular microfilariae for prolonged periods (Awadzi and Gilles, 1992; Awadzi *et al*., 1986; Diallo *et al*., 1986; Greene *et al*., 1985). Consequently, DEC is rather used in lymphatic filariasis control programmes, especially since in regions co-endemic for onchocerciasis, it induces strong local inflammation including ‘Mazzotti reactions’ in patients

with ocular microfilariae attributable to microfilariae death (Taylor *et al*., 2010; Awadzi and Gilles, 1992).

Activity of Suramin in Onchocerciasis Control

Suramin is currently one of the few officially recognized and highly effective macrofilaricides (Thylefors and Rolland, 1979). Awadzi and colleagues (1995a), reported that even at 2 years post-treatment, suramin had almost totally eliminated both ocular and skin microfilariae, albeit at a physiological cost (renal malfunction) to some patients. Nonetheless, examination of the subcutaneous nodules of these patients led to the discovery of an embryotoxic effect from 6 weeks, a lethal effect on male worms from 3 months and on female worms from 6 months after treatment (Awadzi *et al*., 1995a). Suramin is however strictly limited to supervised application, usually in a hospital setting, because fresh solutions have to be injected intravenously over several weeks with adverse (even fatal) consequences possibly occurring (Hoerauf *et al*., 2000). Suramin is thus considered too toxic and as such unsuitable for mass drug administration (Pfarr and Hoerauf, 2006).

Activity of Moxidectin in Onchocerciasis Control

Moxidectin, currently a trial drug (Townson *et al*., 2007), is also a highly effective microfilaricide whose half-life in humans is longer than that of ivermectin (Cotreau *et al*., 2003). However, it is structurally so similar to ivermectin that it may not be considered as an alternative against ivermectin-resistant worms (Freeman *et al*., 2003; Shoop *et al*., 1993), especially since moxidectin has the same method of action and binds to the same sites as ivermectin (Taylor *et al*., 2010). Moxidectin also does not seem to be truly macrofilaricidal as animal models have shown (Trees *et al*., 2000).

Activity of the Benzimidazole Carbamates in Onchocerciasis Control

The benzimidazole carbamates which include mebendazole and albendazole are known to differ in their effects on *Onchocerca volvulus* (Awadzi, 1997). Mebendazole has microfilaricidal effects and is toxic to developing embryos surrounded by an egg shell but not the stretched microfilariae, whiles albendazole has no microfilaricidal activity but is toxic to all intra-uterine stages, possessing important chemosterilant properties which are enhanced by administration with a fatty breakfast (Awadzi, 1997; Awadzi *et al*., 1994). Albendazole is usually given in combination with ivermectin in filariasis control programmes. However, Awadzi and colleagues reported that the combination of ivermectin with albendazole produces no additional effects against *Onchocerca volvulus* when compared to ivermectin administered alone (Awadzi *et al*., 2003; Awadzi *et al*., 1995b).

Antibiotics as Novel Chemotherapeutic Agents against Onchocerciasis

A novel approach using antibiotics to target the bacterial endosymbiont of the *Onchocerca volvulus* parasite aims at identifying superior chemotherapeutic alternatives to currently known antihelminthic drugs (Hoerauf, 2008; Johnston and Taylor, 2007; Taylor *et al*., 2005; Hoerauf *et al*., 2001). The rationale for this new approach is the antibiotic targeting of *Wolbachia* - the bacterial endosymbiont of filarial parasites which is essential for worm development, fertility and survival and an inducer of inflammatory disease pathogenesis (Taylor *et al*., 2009). The principle for this approach stem from earlier findings in animal models as well as in human onchocerciasis and lymphatic filariasis where depletion or a more than tenfold reduction of the *Wolbachia* endobacteria in adult female worms precede female worm sterility (Hoerauf *et al*., 2003) and worm death (Hoerauf *et al*., 2008a; Debrah *et al*., 2007, 2006a).

Studies using azithromycin administered alone for 6 weeks at 250 milligrams per day (mg/day) or 1,200 mg/week found this antibiotic not suitable for treatment of human onchocerciasis (Hoerauf *et al*., 2008b). Azithromycin was considered because it can be given to children and also used in areas with rural health standards since the weekly regimen of 1200 mg is already being administered to HIV-infected individuals as prophylaxis against infections with atypical mycobacteria (Hoerauf *et al*., 2008b; Sendi *et al*., 1999). Hoerauf and colleagues however recommended that daily azithromycin treatment for onchocerciasis should be studied in combination with other drugs and with other doses.

Studies using rifampicin alone administered for 2 or 4 weeks at 10 milligrams per kilogram of patients' body weight per day (mg/kg/day) showed promising results (Specht *et al*., 2008). Rifampicin was also considered because just like azithromycin, it can also be given to children (Specht *et al*., 2008). Specht and colleagues found that *Wolbachia* levels were reduced significantly after 2 weeks of rifampicin treatment, with embryogenesis and microfilariae production also being reduced after 4 weeks of rifampicin treatment; thus, rendering rifampicin an antibiotic with anti-wolbachial efficacy in human onchocerciasis. Specht and colleagues concluded that although rifampicin treatment is less effective than treatment with doxycycline, it may be considered as an alternative therapy especially in children or further developed for combination therapy.

Perhaps, the most effective antibiotics for anti-wolbachial targeting in onchocerciasis currently are the tetracyclines, with doxycycline being the most preferred and most effective (Hoerauf *et al*., 2009, 2008a, 2003b, 2001; Hoerauf, 2008).

Doxycycline as an Anti-Filarial Chemotherapy in Onchocerciasis Control

A series of field trials against onchocerciasis and lymphatic filariasis have demonstrated that 4, 6 and 8 week courses of the antibiotic doxycycline deplete the bacteria and result in the long-term sterility and most importantly, eventual death of adult worms (Hoerauf, 2008;

Debrah *et al*., 2007; Johnston and Taylor, 2007; Debrah *et al*., 2006a; Taylor *et al*., 2005; Hoerauf *et al*., 2001).

Studies in onchocerciasis showed that a 6-week course of 100 mg/day of doxycycline resulted in long-term (beyond 24 months) sterilization of female worms and an absence of skin microfilariae (Hoerauf *et al*., 2003b; Hoerauf *et al*., 2001; Hoerauf *et al*., 2000a). A 5-week course of doxycycline at 100 mg/day (Hoerauf *et al*., 2009) and a 4-week course at 200 mg/day had equivalent results (Hoerauf *et al*., 2008a). At 21 and 27 months after doxycycline treatment, Hoerauf and colleagues reported an increased proportion of dead female worms (at 50% from the normal range of 15-20%) with these two regimens. A 6-week course of 200 mg/day doxycycline led to proportions of dead worms of 60% (Hoerauf *et al*., 2008a), or more than 70% if newly acquired worms were subtracted (Specht *et al*., 2009).

Doxycycline is thus the first drug with a substantial macrofilaricidal activity, and ultimately a microfilaricidal activity as well in onchocerciasis, and does not have the severe and often fatal adverse events that were associated with suramin therapy (Taylor *et al*., 2010). It is a safe drug as long as the contraindications are observed, and it is readily available in pharmacy and chemical shops in onchocerciasis-endemic areas (Hoerauf *et al*., 2009; Hoerauf *et al*., 2008a).

Mode of Action of Doxycycline

Doxycycline is a broad spectrum antibacterial agent belonging to the tetracycline group of drugs. It acts by inhibiting bacterial protein synthesis by binding to the 30s subunit of the bacterial ribosome. It acts on a variety of gram positive and gram negative bacteria including members of the family Rickettsiae to which *Wolbachia* belongs, and also on other classes of bacteria

(http://www.icm.tn.gov.in/drug%20formulary/ANTIINFECTIVE%20DRUGS.htm).

Safety of Doxycycline

Doxycycline is a licensed and safe drug for treatment of various bacterial infections and has been used in various human studies (Hoerauf *et al.*, 2009, 2008a, 2003b, 2001), and as long as contraindications are observed, it is safe.

Pharmacokinetics of Doxycycline

Doxycycline is administered orally, and readily and completely absorbed from the gastrointestinal tract (GIT), with 80-95% of the drug binding to plasma proteins. It has a relatively long biological half-life of 15-25 hours, and it is excreted in the urine and the bile. It is however not metabolized in the liver (http://www.icm.tn.gov.in/drug%20formulary/ANTIINFECTIVE%20DRUGS.htm).

Potential Interactions With Doxycycline

Milky products interfere with the absorption of doxycycline and could lead to a lower drug bioavailability.

Some Indications of Doxycycline

- Rickettsial infections like rocky mountain spotted fever, typhus fever, Rickettsial pox and tick fever.
- In the treatment and prophylaxis of cholera.
- Prophylaxis in *P. falciparum* malaria.
- Chlamydial infections - Trachoma, Psittacosis, Salphingitis, Urethritis and Lymphogranuloma venereum.

(http://www.icm.tn.gov.in/drug%20formulary/ANTIINFECTIVE%20DRUGS.htm).

Contraindications of Doxycycline

- Pregnant women.
- Lactating mothers.
- Children below 8 years.

(http://www.icm.tn.gov.in/drug%20formulary/ANTIINFECTIVE%20DRUGS.htm).

Doxycycline as an Anti-Wolbachial Agent in Onchocerciasis Control

Wolbachia is known to infect all life cycle stages of filarial worms, with the intensity of infection varying between the life cycle stages (Fenn and Baxter, 2004; McGarry *et al*., 2004). *Wolbachia* is found in the hypodermis and oocytes of adult worms and also in all embryonic and larval stages (Hoerauf *et al*., 2003a; Taylor and Hoerauf, 1999; McLaren and Worms, 1995; Kozek, 1977). It is transmitted transovarially (vertically) to the next generation (Pfarr and Hoerauf, 2006; Kozek, 1977).

Members of the tetracycline family including doxycycline are known to affect filarial worms by inhibiting the filarial larval molt (from L3 to L4) and their development *in vitro* (Rao, 2005; Rao and Weil, 2002; Smith and Rajan, 2000). Doxycycline, like any other antibiotic, generally acts on bacterial RNA polymerases, protein synthesis, and other processes and there are suspicions that they affect similar pathways in both worms and their *Wolbachia* (Rao, 2005).

In several *Wolbachia*-harbouring nematode worm infections, tetracyclines have multiple effects on worm growth and development, worm fertility (particularly female worm embryogenesis) and worm survival, with considerable reduction in circulating microfilarial numbers in microfilaremic animals (Rao, 2005; Langworthy *et al*., 2000; Hoerauf *et al*., 1999). In contrast, in animals infected with aposymbiotic *Acanthochelonema viteae* worms, which do not carry *Wolbachia* bacteria, similar long-term treatment with tetracyclines had no effect on worm biology and development; an indication of the important role *Wolbachia*

plays in the growth and reproduction of the filarial worms that harbor them (Rao, 2005; Hoerauf *et al*., 1999). Polymerase chain reaction (PCR) assays have also confirmed the reduction or clearance of bacterial-specific hsp60 and *Wolbachia* surface protein (WSP) after prolonged therapy with tetracyclines (Kramer *et al*., 2003; Hoerauf *et al*., 2000b; Bandi *et al*., 1999).

Initial trials with doxycycline saw the depletion of *Wolbachia* in *Onchocerca volvulus* worms and the extensive degeneration of embryos by 4 months post-treatment (Hoerauf *et al*., 2000a). Hoerauf and colleagues asserted that *O. volvulus* worms became sterile after the loss of *Wolbachia*, and this led to a significant reduction or in some cases no microfilaridermia at all in infected individuals. Subsequent studies also show that a combination therapy with doxycycline and ivermectin also remarkably reduced microfilaridermia following reductions in *Wolbachia* numbers in worms (Hoerauf *et al*., 2003b; Hoerauf *et al*., 2001).

Doxycycline may also be considered in areas that are co-endemic for loiasis and where ivermectin treatment has resulted in the occurrence of encephalopathy (Thomson *et al*., 2004). Doxycycline treatment however showed no effect on *Loa loa*, a filarial worm free from *Wolbachia* endosymbionts in a study conducted by Brouqui and colleagues in 2001.

Chapter 2 - METHOD AND MATERIALS

2.0 Description of Study Area

The study was undertaken in 13 communities in the Pru (Pru and Lower Black Volta river basins), Kintampo North Municipal, Kintampo South and Tain Districts in the Brong Ahafo region of Ghana. The four districts are endemic for onchocerciasis according to mapping carried out by the National Onchocerciasis Control Programme (NOCP) of the Ghana Health Service (GHS), with ivermectin mass distribution starting in 1987 in these districts. The major occupations of inhabitants of these communities include farming (both crop and livestock rearing) and charcoal burning. A teak plantation situated in the Tain District provides a source of employment to inhabitants of the Tainso community.

The major rivers found in these communities are the Pru, Tain and the Lower Black Volta river basins. These, especially River Pru and River Tain, are fast-flowing rivers which provide ideal breeding sites for the blackfly vectors of onchocerciasis.

Most houses in these communities are made of mud, with bamboo leaves and elephant grass for roofing. A notable exception to these mud-houses is found in New Longoro, where block-houses are predominant.

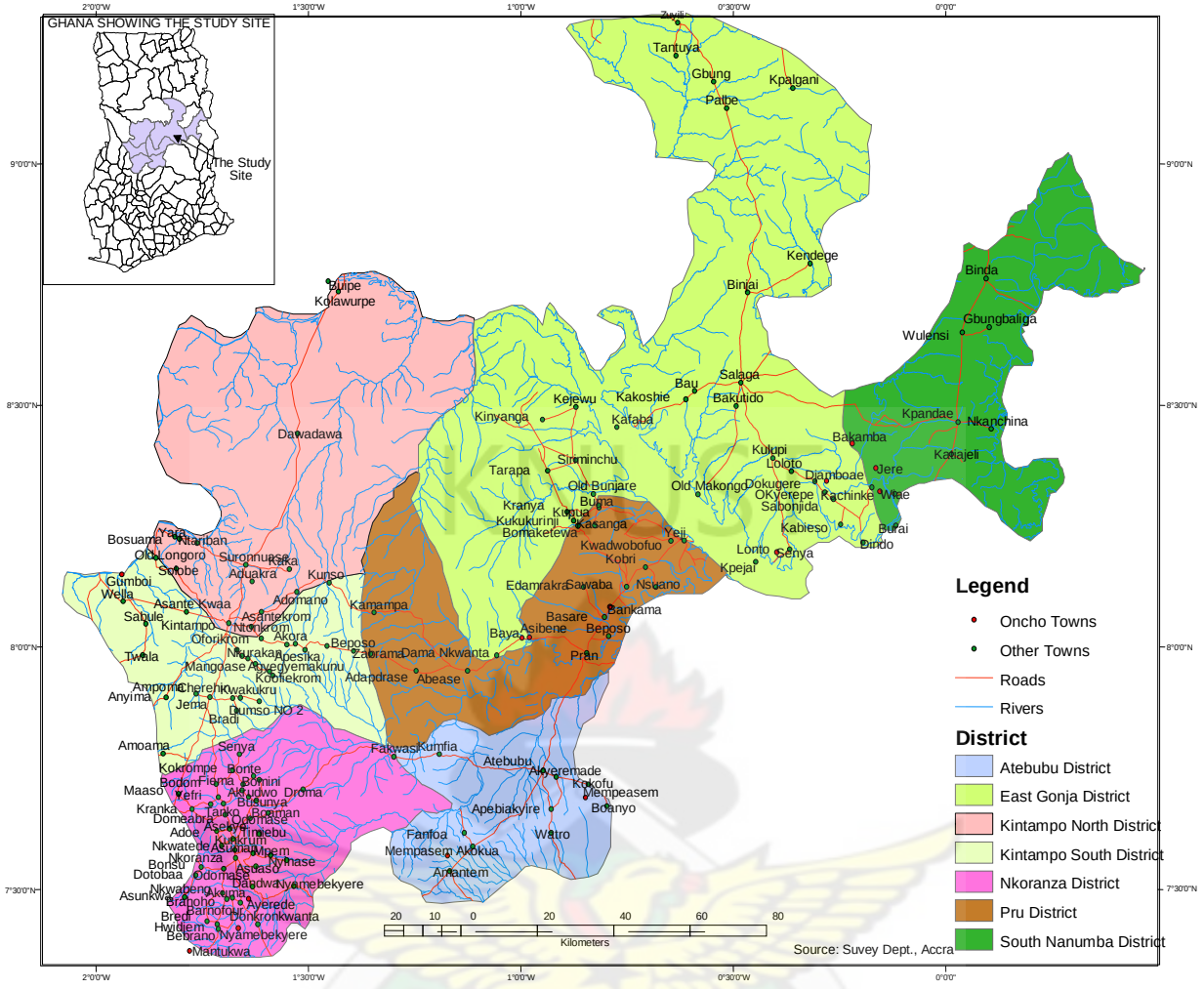


Plate 1.0 Map of the Study Area. Source: Survey Department, Accra.

2.1 Ethical Considerations

Ethical clearance was sought for the study and given by the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Sciences (SMS), Kwame Nkrumah University of Science and Technology (KNUST). Permission to conduct the study in the selected communities was sought from the Pru and Kintampo District Health Directorates.

Meetings were held in all participating communities with opinion leaders and inhabitants to explain the nature, purposes and procedures of the study in the Twi local language which was

the most common language of communication among inhabitants and non-inhabitants of the participating communities. Signed or thumb-printed informed consent was also obtained from each study volunteer.

2.2 Study Design

The study was a randomized double-blind, placebo-controlled trial conducted in endemic areas of Ghana (villages along the Pru and Lower Black Volta river basins) where some onchocerciasis patients who had been repeatedly treated with ivermectin over many years, but had not shown evidence of microfilarial reduction or elimination. Volunteers from communities in these areas were screened with the help of the OCP database for those having microfilariae in the skin despite multiple previous treatments, and/or reappearance of palpable nodules, raising clinical suspicion of sub-optimal response to ivermectin in these parts of the population (Awadzi *et al*., 2004b).

Volunteers determined to be eligible, based on the inclusion and exclusion criteria described in Section 3.2.1 and 3.2.2 were enrolled in the study. After providing written informed consent, volunteers underwent further eligibility screening, including medical history, physical examination, liver and kidney function testing and palpation of onchocercomata. Skin snips (skin biopsies) were also taken in order to determine the microfilarial (mf) load in the skin. Urine pregnancy tests were performed in female volunteers.

Study power calculations indicated that a minimum of 144 volunteers were to be enrolled and assigned to one of the treatment regimens (doxycycline or placebo).

Table 1.0: Treatment Design for 6 Weeks Daily Observed Treatment (DOT)

Treatment Arm	Number of Volunteers	Treatment Details
1)	72	100 mg/day Doxycycline
2)	72	Placebo Matching Doxycycline

2.2.1 Inclusion Criteria for Enrolment of Volunteers

i. Males and females from 18 to 50 years of age.

ii. Good general health without any clinical condition requiring long-term medication.

iii. Clinical manifestation of onchocerciasis assessed by palpation - in these areas of many rounds of ivermectin mass treatment, the presence of onchocercomata is one major indicator of worms that probably respond poorly to ivermectin.

iv. Minimum of 6 rounds of ivermectin over the years, prior to start of study.

v. Microfilaridermic volunteers assessed by skin snip technique (microfilariae > 10 mf/mg skin) 9 months after the last of a minimum of 6 rounds of ivermectin treatments (cross-checked with the local OCP record books as well as personal interviews with volunteers), or reports of moderate to severe adverse events following the last ivermectin administration (Awadzi *et al*., 2004b). These two indicators have been used to detect sub-optimal responses to ivermectin.

vi. Minimum body weight of 40 kilogrammes.

vii. Normal renal and hepatic profiles (aspartate aminotransferase (AST) [0-40 IU/L], alanine aminotransferase (ALT) [0-45 IU/L], gamma glutamyl transpeptidase (γ-GT) < 60 IU/L), creatinine [53-126 µmol/L] measured by dipstick chemistry (Reflotron®).

viii. Willingness to participate in the study as evidenced by the signing of the informed consent document.

2.2.2 Exclusion Criteria for Enrolment of Volunteers

i. Pregnancy determined by positive urine human chorionic gonadotropin (β-hCG), and assessed using hCG One Step Pregnancy Test Strip (Urine) from *ACON* Laboratories Inc. (San Diego, USA).

ii. Lactating and breast-feeding volunteers.

iii. Evidence of clinically significant neurological, cardiac, pulmonary, hepatic, rheumatological, or renal disease by history, physical examination, and/or laboratory tests.

iv. Behavioural, cognitive or psychiatric disease that may affect the ability of the volunteer to understand and cooperate with the study protocol.

v. Laboratory evidence of liver disease (AST), γ-GT and/or (ALT) greater than 1.25 times the upper limit of normal of the testing laboratory.

vi. Laboratory evidence of renal disease (serum creatinine greater than the upper limit of normal of the testing laboratory.

vii. Abuse of alcohol or illicit drugs by volunteer during the past 6 months.

viii. History of severe allergic reaction or anaphylaxis.

ix. Intolerance to doxycycline.

2.2.3 Assessment of Renal and Hepatic Profiles of Study Volunteers

Clinical biochemistry tests were performed to assess volunteers' kidney and liver functions using stick-technology by Reflotron® (Boehringer Mannheim, Germany). All tests were performed at the KCCR field laboratory at the Prang Health Centre.

Blood samples were collected from each volunteer and centrifuged to separate plasma from

blood cells. About 500 microlitres (µl) of each volunteer's plasma was pipetted into a(n) 1.8 millilitre (ml) eppendorf tube bearing the volunteer's identification details. For each test (AST, ALT and γ-GT), 30 µl of plasma was pipetted onto respective Reflotron® test sticks (strips) using a Reflotron® pipette according to the protocol provided by the manufacturer.

2.3 Study Procedure

2.3.1 Randomization of Study Drugs

Individuals were randomly assigned to either the doxycycline or the placebo group using computer-aided randomization (StatView® software). Blinding was assured by the exclusion of persons involved in randomization or tablet packaging in any clinical or laboratory assessments.

2.3.2 Study Drugs and Treatment Regimen

Patients received either 100 mg/day doxycycline capsules or placebo for 6 weeks. Doxycycline capsules or placebo capsules matching doxycycline capsules were provided by Docpharm Incorporated® (Pfintzal, Germany). Drugs were distributed *ad personam* by study clinicians and drug intake monitored on a daily basis.

Patients were advised to avoid the intake of milky products at least 4 hours before and after the drug intake by a study clinician.

2.3.3 Follow-up Examinations of Volunteers after Treatment

Volunteers were re-examined at 12 and 20 months post-treatment to determine skin microfilarial levels. At 4 and 12 months post-treatment, all study volunteers present were given the standard dose of 150 µg/kg ivermectin. At 12 and 20 months post-treatment, skin biopsies were taken in order to determine each volunteer's skin microfilarial load.

2.4 Parasitological Analysis

2.4.1 Determination of Skin Microfilarial Loads

Skin microfilarial loads of study volunteers were assessed before treatment, and at 12 and 20 months after treatment using skin biopsies taken from the left and right iliac crests to determine the number of microfilariae per milligram of skin. Skin biopsies are currently the "gold standard" in detecting the presence of *Onchocerca volvulus* microfilariae in infected populations. Although invasive, skin biopsies are a very specific method and as such are in line with the World Health Organization (WHO) strategy on the need for surveillance methods to be highly specific even at the cost of low sensitivity (Guzmán *et al*., 2002).

2.4.2 Assessment of Skin Microfilarial Loads Using Skin Biopsies (Skin Snips)

About 100 µl of normal saline (0.9% NaCl) was pipetted into a 96-well round bottom microtitre plate (Cellstar®, Greiner Labortechnik, Germany) labelled with the volunteers' identification codes. The skin (left and right sides of the iliac crests) was cleansed using 70% alcohol. The cleansed areas were allowed to dry.

Using a sterilized Walzer punch, a bloodless piece of skin was taken from both left and right iliac crests. The snipped skin was immersed into physiological saline in the microtitre plate and the snipped area was dressed by covering with a plaster. The punch was sterilized using 4% Incidin (4 ml Incidin topped up to 100 ml with potable water) for 20 minutes. The wells containing the snips were covered with a cellotape to avoid the contents of the microtitre plate drying up or pouring during transportation. The snips were incubated overnight at room temperature to allow the emergence of microfilariae into the saline solution.

Using the pipette, the normal saline around the skin snip was collected after thorough mixing, placed on a clean slide and examined under a light microscope for microfilariae using the 10X objective lens with the condenser iris closed sufficiently for good contrast. The number

of microfilariae observed were counted with the aid of a tally counter and the results recorded.

The skin snip was removed from the microtitre well, blotted on a paper towel and then weighed using a Sartorius electric balance (Göttingen, Germany). The weight was also recorded. The microfilarial density for each volunteer was calculated by dividing the average number of microfilariae by the average weight (in milligrams) of the skin snips (Guzmán *et al*., 2002).

Plate 2.0 Taking a skin snip (Left). Set-up of materials used for snipping (Right).

2.4.3 Investigation of Intestinal Helminth Infections in Study Volunteers

Each volunteer was given a plastic container and made to provide fresh stool (faeces) samples no more than 10 millilitres (ml) in volume. Each stool sample was assessed for infection with intestinal helminths the same day it was collected. Stool examinations were done before treatment and at 20 months post-treatment.

The concentration technique for stool analysis was used in the preparation and examination of each stool sample.

2.4.4 Concentration Technique in Stool Analysis

About 10 ml of 10% v/v formol-saline (formalin and isotonic saline solution) was added to approximately 1 gram of freshly passed stool in a plastic container labelled with the volunteers' identification number (ID) and the mixture emulsified using an applicator stick. A 400 µm mesh size surgical gauze was fitted into a funnel and the funnel placed on top of a 15 ml falcon tube (centrifuge tube) labelled with the volunteers' ID.

The stool-formol-saline suspension was poured through the surgical gauze filter into the falcon tube until the 7 ml mark was reached. The filter, together with the trapped lumpy residue, was removed and discarded.

About 3 ml of petrol was added to the filtered suspension in the falcon tube and shaken for about a minute to mix well. The falcon tube containing the suspension mixture was centrifuged at 900 g (600 rpm) for 3 minutes. After centrifuging, the fatty plug (debris) was loosened with an applicator stick and the supernatant poured away by quickly inverting the falcon tube.

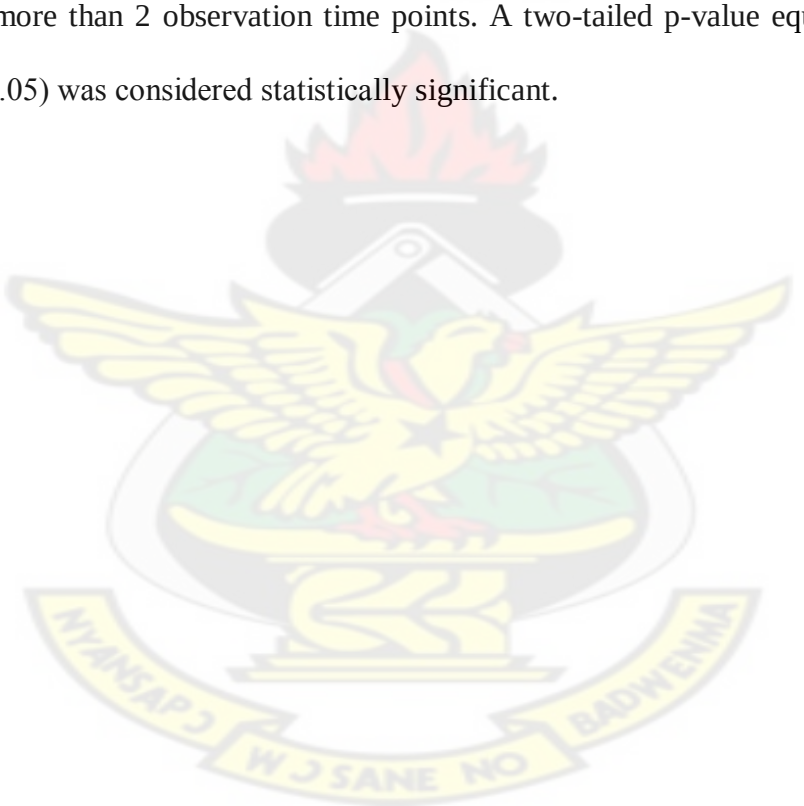
The falcon tube was replaced in its rack and the fluid on the sides of the tube was allowed to drain down to the sediment. The sediment-fluid mixture at the bottom of the tube was mixed well and a drop from this mixture was transferred onto a labelled microscope slide using a Pasteur pipette. A coverslip was placed on the drop and the whole area under the coverslip was examined using the 10X and 40X objective lenses of a light microscope for ova and larvae of intestinal helminths.

Slides positive for ova and larvae of any of the intestinal helminths were recorded.

2.5 Statistical Analyses

Statistical analyses were done using StatView®, Microsoft Excel® and GraphPad Prism®

software programmes. Descriptive statistics were used to obtain general descriptive information such as the mean and standard deviation from the data. One sample analysis (chi square test) was used to compare two proportions or groups. One-way ANOVA (Analysis of Variance) was used to test group means of demographic data. Logistic regression analysis was used to determine if any association existed between intestinal helminths co-parasitism with *Onchocerca volvulus* in an individual. For non-parametric data sets, analyses were done using Mann-Whitney *U* test for unpaired data that were not normally distributed, Wilcoxon Signed Rank test for paired samples that were not normally distributed and Friedman Test for data sets from more than 2 observation time points. A two-tailed p-value equal to or lower than 0.05 ($p \leq 0.05$) was considered statistically significant.



Chapter 3 - RESULTS

3.0 Summary of volunteer participation, treatment and drop-outs

Two hundred and eighty four volunteers turned up for the initial examination leading to recruitment and 116 were excluded based on the inclusion and exclusion criteria defined in sections 3.2.1 and 3.2.2. The remaining 168 volunteers affected by onchocerciasis and unresponsive to ivermectin were enrolled into the study and subsequently randomized into either the doxycycline or placebo treatment arm. Nineteen of these volunteers (representing 11.3%) dropped out due to a variety of factors. One hundred and forty nine volunteers completed the treatment and were followed up at 12 and 20 months post-treatment. Seventy six volunteers (51%) received placebo and 73 volunteers (49%) were treated with 100 mg/day of doxycycline for 6 weeks. Of the 149 treated volunteers, 96 (64.4%) were microfilaridermic (Mf-positive) with the remaining 53 (35.6%) having no skin microfilariae at pre-treatment. Of the 96 pre-treatment microfilaridermic volunteers, 48 (50.0%) were in the placebo treatment arm, with 48 (50.0%) also being in the doxycycline treatment arm. Of the 53 pre-treatment amicrofilaridermic volunteers, 28 (52.8%) took placebo and 25 (47.2%) took doxycycline.

One hundred and eleven volunteers, representing 74.5% of the total number of volunteers who completed the treatment were males, with the remaining 38 (representing 25.5%) being females.

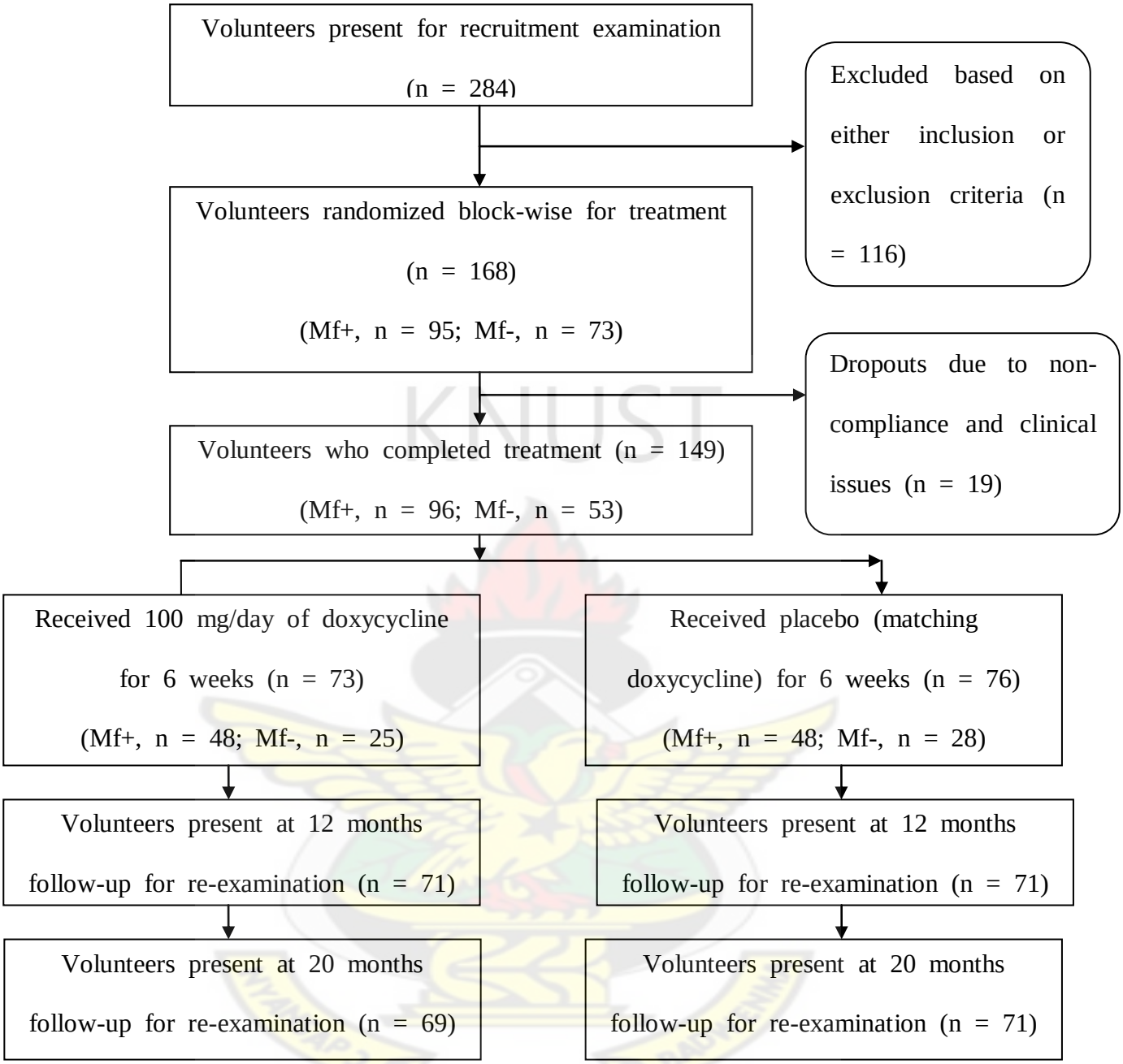


Figure 2.0: Flowchart of Onchocerciasis Volunteer Participation.

One hundred and forty nine volunteers completed the treatment, with 73 and 76 receiving doxycycline and placebo respectively for 6 weeks. The total number of volunteers present at 12 and 20 months post-treatment were 142 and 140 respectively.

Table 2.0: Demographic Data at Pre-treatment

Study Volunteers	**Doxycycline**	**Placebo**	**Total**	***p*-value**
Number at treatment start	85	83	168	
Number who completed treatment	73	76	149	**0.1738#**
Male	58	53	111	
Female	15	23	38	
Age in years (mean ± SD)	35.8 ± 9.2	40.4 ± 8.9	38.2 ± 9.3	**0.2354***
Weight in kilograms (mean ± SD)	59.4 ± 8.7	56.7 ± 7.9	58.0 ± 8.4	**0.2386***

 # - Chi Square Test; * - One-way ANOVA. SD = Standard Deviation

There was no statistical significance (p = 0.1738) in the number of males and females in both the doxycycline and placebo groups who completed treatment as Table 2.0 shows. There was also no statistical difference in the age and weight of volunteers as shown in Table 2.0 in both doxycycline and placebo groups (p = 0.2354 and 0.2386 respectively).

Table 3.0: Adverse Events Associated With Study Drugs

Adverse Events	**Doxycycline**	**Placebo**	**Total**	***p*-value (Fisher's exact test)**
Number of Volunteers With Adverse Events	41 (43.6%)	53 (56.4%)	94 (100%)	**0.0928**
Number of Adverse Events per Volunteer:				
1	26 (48.1%)	28 (51.9%)	54 (100%)	
2	9 (34.6%)	17 (65.4%)	26 (100%)	
3 or more	6 (42.9%)	8 (57.1%)	14 (100%)	

Doxycycline was well tolerated with minor adverse events. Adverse events reported included headache, fever, itching, waist pains, stomach pains, diarrhoea, joint pains, body pains, chest pains, bloody stools, nausea, vomitting and dizziness. Ninety four of the 149 volunteers complained of adverse events during the period of drug administration. From Table 3.0, a higher proportion of volunteers in the placebo group (53) compared to 41 in the doxycycline group reported these adverse events. Majority reported a single adverse event (54 volunteers) during the period of drug administration. Fourteen volunteers reported 3 or more adverse events per volunteer during this period; 6 in the doxycycline group and the remaining 8 in the placebo group. One volunteer in the doxycycline group complained of bloody stools, which after assessment by the study clinician was graded as mild and having no effect on the daily activities of the volunteer. No volunteer with adverse events was asked to discontinue treatment. There was no significant difference between the doxycycline and placebo groups ($p = 0.0928$) as shown in Table 3.0.

Table 4.0: Co-infection With Intestinal Helminths in Study Volunteers

Study Community	**Number of Volunteers with Intestinal Helminth Co-infection**					
	Schistosomiasis		***Ascaris***		***Hookworm***	
	Pre.	*20 months*	Pre.	20 months	Pre.	20 months
Abua	0	0	0	0	4	14
Bita	0	0	0	0	1	5
Adjaraja	0	0	0	0	1	4
Beposo	0	0	0	0	3	17
Mantukwa/ Domeabra	0	0	0	0	2	3
Tigamgam	0	0	0	0	11	18
Bolga Nkwanta	0	0	0	0	3	4
Prang	0	0	0	0	0	3
New Longoro	0	0	0	0	0	1
Tainso	0	0	0	0	0	0
Nyire	0	0	0	0	1	1
Fawomang	0	0	0	0	0	0
Ayorya	0	0	0	0	1	0
Total	0	0	0	0	27/147	70/138
p*-value					**0.0187**	

Pre. = Pre-treatment; 20 months = 20 months post-treatment. * - Chi Square test

From Table 4.0, the only intestinal helminth detected at both pre-treatment and 20 months post-treatment was hookworm, with 27 out of 147 volunteers (18.4%) and 70 out of 138 volunteers (50.7%) respectively infected. No *Schistosoma mansoni* or *Ascaris lumbricoides* was detected at these time points. There was a statistically significant increase (p = 0.0187) in

the number of volunteers with hookworm co-infection from pre-treatment to 20 months post-treatment.

Table 5.0: Infection With Hookworm in Treatment Groups at Study Time Points

Treatment Group	Number of Volunteers	
	Pre-treatment	20 months
Doxycycline	14	35
Placebo	13	35
Total	**27**	**70**

The numbers of hookworm-infected volunteers in both treatment groups at pre-treatment were almost equal; 14 in the doxycycline group and 13 in the placebo group as can be seen in Table 5.0. At 20 months the numbers of hookworm-infected volunteers were equal; 35 in the doxycycline group and 35 in the placebo group.

Table 6.0: Association Between Hookworm and Microfilaridermia

Study Time Point	Doxycycline Group	Placebo Group	Between Groups
Pre-treatment	$r^2 = 0.007$	$r^2 = 0.009$	$r^2 = 0.008$
(*p*-value)¶	**0.5299**	**0.5267**	**0.3782**
20 months	$r^2 = 0.012$	$r^2 = 0.001$	$r^2 = 0.003$
(*p*-value)¶	**0.5462**	**0.7245**	**0.4583**

r^2 = Coefficient of determination. ¶ - Logistic Regression

At pre-treatment, there was no association between hookworm infection and microfilaridermia within (r^2 = 0.007 and 0.009 in the doxycycline and placebo groups respectively) and between both groups (r^2 = 0.008) as Table 6.0 shows. There was also no

statistically significant difference within (p = 0.5299 and 0.5267 in the doxycycline and placebo groups respectively) and between both groups (p = 0.3782). A similar trend of no association and no statistical significance within and between the two groups was also observed at 20 months post-treatment as can be seen in Table 6.0.

Table 7.0: Microfilaridermia Status of Volunteers at Study Time Points

Doxycycline (100mg/day) Group	**Pre-treatment**	**12-months follow-up**	**20-months follow-up**
Microfilaridermia			
No. of MF- positive/total no. of patients (%)	48/73 (65.7%)	7/71 (9.9%)	2/69 (2.9%)
Placebo Group			
No. of MF- positive/total no. of patients (%)	48/76 (63.2%)	43/71 (60.6%)	49/71 (69.0%)
***p*-values**	**0.7408***	**<0.0001***	**<0.0001***

*** - Chi Square Test.**

At pre-treatment, 65.7% of volunteers in the doxycycline group were microfilaridermic compared to 63.2% in the placebo group as can be seen in Table 7.0. There was no statistical difference (p = 0.7408) in the number of microfilaridermic volunteers in both the doxycycline and placebo treatment groups. At the 12-months follow-up time point, only 9.9% of volunteers in the doxycycline treatment group remained microfilaridermic compared to 60.6% in the placebo group. Table 7.0 shows a highly significant difference (p < 0.0001) in the number of microfilaridermic volunteers between both groups at the 12-months follow-up time point. From Table 7.0, at the 20-months follow-up time point, there was also a highly significant difference (p < 0.0001) in the number of microfilaridermic volunteers between the

groups, with only 2.9% of volunteers in the doxycycline treatment group being microfilaridermic while in the placebo treatment group, 69.0% of volunteers were microfilaridermic.

Microfilarial Loads of Doxycycline and Placebo Groups at Various Time Points

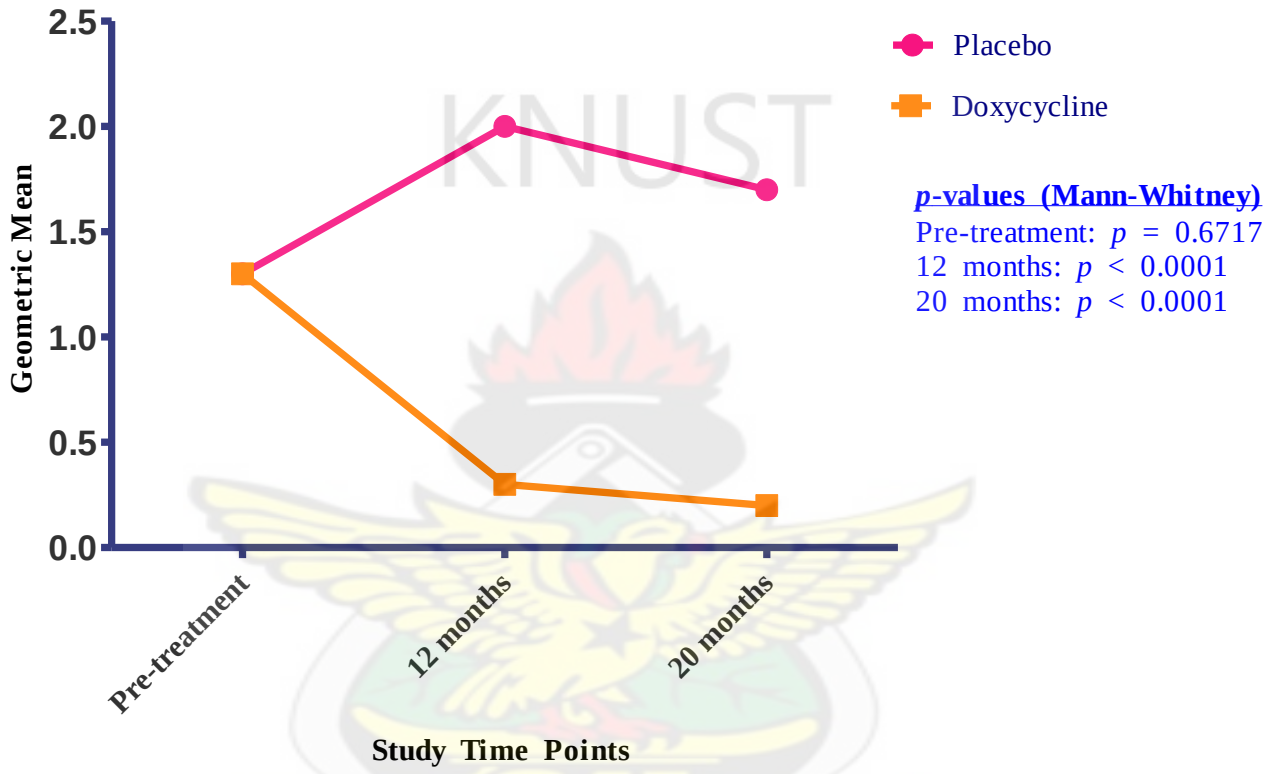


Figure 3.0: Microfilaridermia Through Study Time Points

At pre-treatment, there was no significant difference (p = 0.6717) in the microfilarial geometric mean load of volunteers in both the doxycycline and placebo treatment groups as is shown in Figure 3.0. However, at the 12-months follow-up time point, there was a significant difference (p < 0.0001) between the two groups, with the doxycycline group showing a drastic reduction from 1.3 to 0.3, whiles the placebo group showed a significant increase from 1.3 to 2.0 (Figure 3.0). At the 20-months follow-up time point, there was a significant difference (p < 0.0001) in the microfilarial geometric mean load of volunteers in both groups,

with the doxycycline group showing a further marginal reduction from 0.3 to 0.2, and the placebo group also showing a marginal reduction from 2.0 to 1.7 (Figure 3.0).

Table 8.0: Comparative Assessment of Microfilaridermia at Study Time Points

Treatment Group	Pre-treatment and 12 months¶	Pre-treatment and 20 months¶	Pre-treatment, 12 and 20 months§
Doxycycline	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Placebo	$p = 0.0189$	$p = 0.3750$	$p = 0.3337$

¶ - Wilcoxon Signed Rank Test; § - Friedman Test.

From Table 8.0, between pre-treatment and 12 months, significant differences in microfilaridermia levels were recorded in both the doxycycline and placebo groups, with microfilaridermia levels decreasing drastically in the doxycycline group whiles in the placebo group, a drastic increase was observed. Between pre-treatment and 20 months, a significant difference was recorded in the doxycycline group indicated by a drastic reduction in microfilaridermia. However, the placebo group showed no difference. From pre-treatment through 12 months to 20 months, there was a significant difference in microfilaridermia levels in the doxycycline group whiles there was no difference in the placebo group as occurs in Figure 3.0 and Table 7.0.

Chapter 4 - DISCUSSION

4.0 Tackling Sub-optimal Response to Ivermectin in Onchocerciasis Control

Recent evidence suggest that despite many years of treatment with ivermectin, significant microfilaridermias still persist in segments of onchocerciasis-infected populations (Awadzi *et al*., 2004a, 2004b). Various reasons have been adduced to explain this observation including the existence of occult but actively reproductive worms (Awadzi *et al*., 2004b), non-response of adult female worms in spite of adequate drug exposure (Awadzi *et al*., 2004a) and the presence of higher β-tubulin, multidrug resistance (MDR1) and P-glycoprotein-like protein (PLP) variant allele frequencies in sub-optimal responders (Kudzi *et al*., 2010; Bourguinat *et al*., 2008; Eng *et al*., 2006).

A meeting which was held in Ghana in April 2008 reviewed the status of onchocerciasis control in Ghana in light of evidence of poor-responsiveness to ivermectin, defined the research priorities for alternative drug and treatment regimes and control strategies to treat populations with existing evidence of suboptimal responsiveness and also defined research priorities for future control strategies in the event of the development of widespread ivermectin resistance (Taylor *et al*., 2009).

Since ivermectin (Mectizan®) is the only drug currently recommended for the treatment and control of onchocerciasis (Awadzi, 2003), it is of utmost concern when reports of possible resistance indicated by faster microfilarial repopulation dynamics (Osei-Atweneboana *et al*., 2007) and/or sub-optimal responses to this drug emerged.

In this study, doxycycline, a drug with macrofilaricidal activity and by extension a microfilaricidal activity as well (Taylor *et al*., 2010, 2005; Hoerauf *et al*., 2009, 2003b, 2001, 2000a; Debrah *et al*., 2007, 2006b) in onchocerciasis control, is used to tackle possible cases of resistance and/or sub-optimal responses to ivermectin.

The working definition for sub-optimal response in this study is the inability of ivermectin to reduce microfilariae levels as well as the number of microfilaridermic volunteers throughout the successive follow-up time points compared to pre-treatment states.

4.1 Adverse Events Associated With Study Drugs

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), defines a serious adverse event resulting from administration of a drug as "Any untoward medical occurrence that at any dose, results in death, is life-threatening, requires inpatient hospitalization or prolongation of an existing hospitalization, results in persistent or significant disability/incapacity and/or is a congenital anomaly or birth defect" (ICH, 1996). A modification to these criteria by the WHO in 1999 included important medical events that may not be immediately life-threatening, or result in death or hospitalisation, but may infact jeopardize the patient, or require intervention to prevent the more fatal outcomes (Awadzi, 2003).

No serious adverse event such as bloody diarrhoea were recorded with regards to doxycycline administration in this study. This is in conformity to previous studies (Hoerauf *et al*., 2009, 2001) where doxycycline administration was found to be well tolerated by human subjects. As a matter of fact, the results in this study (Table 3.0) show that more volunteers in the placebo group compared to the doxycycline group complained of adverse events, also in terms of number of complaints per volunteer during the period of drug administration. It can therefore be inferred that the adverse events reported by the study volunteers were most likely not related to the administration of the study drugs, but rather to other factors such as the volunteer's clinical state, therapeutic intervention and concomittant therapy (Awadzi, 2003).

4.2 Association Between Intestinal Helminths and Onchocerciasis

Onchocerciasis patients are believed to be more likely to have concurrent parasitic infections (Njoo *et al*., 1993). Although ivermectin, an antihelminthic, is used specifically for onchocerciasis control, it is also recommended for use in the treatment of gastrointestinal strongyloidiasis (Boussinesq, 2005; Taticheff *et al*., 1994). However, ivermectin provides no significant protection from infection, although it may reduce the intensity of infection with other intestinal helminths including *Ascaris lumbricoides*, *Schistosoma spp.* and *Trichuris trichiura*, with reductions in *Ascaris lumbricoides* being much more significant (Behnke *et al*., 1994; Taticheff *et al*., 1994; Njoo *et al*., 1993; Whitworth *et al*., 1991). In contrast however, hookworm egg production was found to rather increase after ivermectin treatment by Njoo and colleagues in a study in 1993.

Results from this study (Table 4.0) show that except hookworm, no other intestinal helminth was detected in the stools of study volunteers. This could be due to factors such as repeated ivermectin treatment being effective in clearing these helminths (*Ascaris lumbricoides*, *Schistosoma spp.* and *Trichuris trichiura*) as was reported in earlier studies (Behnke *et al*., 1994; Taticheff *et al*., 1994; Njoo *et al*., 1993; Whitworth *et al*., 1991). Secondly, these helminths could be less prevalent in the study communities, hence no observations were made of them in stool samples provided by the study volunteers.

The influence of other antihelminthics such as mebendazole in clearing these helminths except hookworm can be ruled out because in spite of having broad-spectrum antihelminthic effect, mebendazole in particular has specific activity against hookworm. Mebendazole usage by the study volunteers would have led to a reduction in prevalence of these helminths including hookworm (Grover *et al*., 2001). In such communities of repeated mass treatment with ivermectin, it is expected that prevalence and intensity of infection of these helminths

excluding hookworm become significantly lowered or almost non-existent as was seen in this study (Table 4.0).

The significant numbers of hookworm-infected volunteers at 20 months post-treatment compared to pre-treatment numbers observed in this study further supports a previous study by Njoo and colleagues in 1993, which found that production of hookworm eggs was found to increase after ivermectin treatment. As a consequence after 2 additional treatment rounds with ivermectin during the study period, prevalence of hookworm infestation is expected to increase in consonance with Njoo and colleagues' findings, as was observed in the stools of study volunteers at 20 months post-treatment (Tables 4.0 and 5.0).

The results in table 6.0 show that no direct association exists between hookworm infestation and *O. volvulus* infestation although they may co-exist in an individual; an observation also made by Mengistu and colleagues in a study in Ethiopia in 2002. The weak coefficient of determination (correlation) between and across the 2 treatment groups observed in this study (Table 6.0) gives credence to the assertion that no association exists between hookworm and onchocerciasis infections. In essence, the presence of *O. volvulus* in an individual does not predispose an individual to hookworm infestation; likewise the absence of *O. volvulus* does not prevent hookworm infestation.

4.3 The Effect of Doxycycline on Microfilaridermia Status of Volunteers

The results (Table 7.0) show a highly significant and consistent decline in the number of doxycycline-treated volunteers who remained microfilaridermic throughout the study time points. Hoerauf and colleagues in 2001 reported a similar trend in a study, albeit in their work ivermectin was administered at 2.5 months and 6 months post-treatment instead of the 4 months post-treatment as was the case in this study. They observed that at 12 and 19 months post-treatment, the number of study participants who were microfilaridermic had declined

drastically in the doxycycline-treated group. In the placebo-treated group, there was a marginal decline in the number of microfilaridermic volunteers at 12 months, however a subsequent increase was observed at 19 months post-treatment, consistent with the trend observed in this study.

Doxycycline is believed to cause sterility and subsequently degeneration and death of female worms between 6 and 27 months post-therapy (Hoerauf *et al*., 2008a; Hoerauf *et al*., 2003b), and by inference lead to an amicrofilaridermic status in treated individuals as was observed in most volunteers in the doxycycline group throughout the follow-up time points. In this study, although no definite explanations can be adduced to explain the microfilaridermic status of the 2 doxycycline-treated volunteers at 20 months post-treatment, a few probable explanations may hold. It is possible the microfilariae in these 2 volunteers were already present at pre-treatment or were acquired post-doxycycline treatment, and since doxycycline does not directly kill microfilariae (Korten *et al*., 2007) and ivermectin may be acting sub-optimally (Osei-Atweneboana *et al*., 2007; Awadzi *et al*., 2004a), it is probable that some microfilariae escaped destruction by the combined doxycyline plus ivermectin therapy. However, with the knowledge of the slow or sequential killing of filariae by doxycycline (Korten *et al*., 2007) and given a longer observation period (a few more months after the 20th month post-treatment), it is possible these 2 doxycycline-treated microfilaridermic volunteers may become amicrofilaridermic (Hoerauf *et al*., 2009). It is worthy to note that when skin biopsies were taken from these 2 doxycycline-treated microfilaridermic volunteers 4 months later (24th month post-treatment), they were both found to be amicrofilaridermic.

Repeated treatments with ivermectin is known to impair the ability of female worms to produce microfilariae (Kläger *et al*., 1996; Plaisier *et al*., 1995; Duke *et al*., 1992; Chavasse *et al*., 1992) as well as affect adult worm vitality (Gardon *et al*., 2002; Duke *et al*., 1992). Consequently, the combined effects of repeated treatment with ivermectin on microfilariae

and adult worms should result in the disappearance of microfilariae from the skin (Guderian *et al*., 1997). However, the results from this study show that despite the fact that each volunteer had taken a minimum of 6 rounds of ivermectin prior to enrolment, and given that each volunteer was administered ivermectin at 4 and 12 months post-treatment on a DOT (directly observed treatment) basis, more volunteers in the placebo group had become microfilaridermic at the end of the study compared to pre-treatment (Table 7.0).

4.4 The Effect of Doxycycline on Microfilaridermias of Volunteers

The results from this study show that mean microfilaridermias were significantly lower in doxycycline-treated volunteers than in placebo volunteers at the 20-months follow-up time point. This is consistent with observations in earlier studies (Debrah *et al*., 2006b; Hoerauf *et al*., 2003b; Hoerauf *et al*., 2001; Hoerauf *et al*., 2000a), where the combined treatment of 100 mg doxycycline per day and ivermectin led to profound and significant reductions of microfilaridermia. Doxycycline depletes the *Wolbachia* endosymbionts of the *O. volvulus* worm leading to an interruption in embryogenesis and microfilariae production and consequently death of the adult worms. The blockage of embryogenesis as a result of *Wolbachia* depletion is known to still occur even 18 months after doxycycline therapy and is directly responsible for the interruption in microfilariae production by the female adult *O. volvulus* (Hoerauf *et al*., 2003b, 2001, 2000a). Although ivermectin is still an effective microfilaricide, repopulation with microfilariae even after 2 treatment rounds with the microfilaricide is faster and higher in the placebo group as occurs in Figure 3.0. It is therefore believed that the profound and significant reductions of microfilaridermia in the doxycycline treatment arm can solely be attributed to the activity of doxycycline.

At pre-treatment, both doxycycline and placebo groups had the same microfilarial geometric mean load. Whiles the doxycycline group showed a consistent, profound and significant reduction in microfilarial loads at the follow-up time points, the placebo group showed an

inconsistent but significant increase in microfilarial loads through the follow-up time points compared to pre-treatment levels.

There was also an interesting observation made in the placebo group where the microfilarial geometric mean load was higher at the 12-months follow-up time point compared to the 20-months follow-up time point. This is particularly curious since ivermectin had been given to all study volunteers less than 8 months preceeding both follow-up time points and also considering the fact that all study volunteers had taken a minimum of 6 rounds of ivermectin before treatment. This observation thus confirms findings by Osei-Atweneboana and colleagues in 2007 of a faster microfilarial repopulation dynamic after ivermectin treatment, but it is in direct contrast to observations made by Plaisier and colleagues in 1995, where they found that annual treatment with ivermectin causes an irreversible decline in microfilariae production of approximately 30% per treatment. Such a contradiction could only occur if *Onchocerca volvulus* worms, both microfilariae but especially adult female worms, were to be responding sub-optimally to ivermectin.

4.4.1 Relatively Low Levels of Microfilaridermia Observed

A study by Bourguinat and colleagues in 2007, which investigated the relationship between β-tubulin genotype (a genotype linked to ivermectin resistance in nematodes) and female *O. volvulus* worms found that there was a significant selection for β-tubulin heterozygotes in these female worms. They found that in ivermectin-naive populations, the frequency of female worms who were β-tubulin homozygous were far higher and in addition, these worms were much more fertile. However, after a minimum of 4 treatment rounds with ivermectin, they observed that there was selection for β-tubulin heterozygotes, and as a consequence the frequency of β-tubulin heterozygous worms were far higher than their homozygous

counterparts. Equally important, they also found that these β-tubulin heterozygotes were less fertile than the homozygotes.

Such selection by ivermectin most likely accounts for the relatively low, but persistent microfilaridermia observed in this study.

4.5 Onchocerciasis control - The Case for Doxycycline Usage

Grant in 2000, speculated that non-responsive adult worms would have a greater influence on the effective use of ivermectin than non-responsive microfilariae because the longer lifespan of the adult worms would mean a longer time for infection to persist. In a study by Awadzi and colleagues (2004a), microfilariae from nearly all sub-optimal responders were found to be sensitive to ivermectin. This finding therefore excluded the possibility that persistent microfilaridermias despite multiple treatment with ivermectin are the result of resistance developed by the microfilariae or an incompetent immune system in the host (Ali *et al*., 2002). The results of this study therefore does not question the ability of ivermectin to act as an effective microfilaricide. Rather what is likely to be happening is that despite multiple treatments with ivermectin, some adult female worms continue to remain viable and unresponsive. As such microfilariae production by ivermectin-unresponsive adult female worms continue unabated, leading to the persistence of microfilariae in the skin.

Doxycycline showed a macrofilaricidal activity and consequently reduced microfilaridermia significantly in this study as was the case in previous studies (Hoerauf *et al*., 2009, 2008a, 2003b, 2001, 2000a; Specht *et al*., 2009; Debrah *et al*., 2006b). More importantly, doxycycline proved highly effective in situations where ivermectin seemed to be working sub-optimally, as was evidenced by the drastic reduction in microfilaridermia and microfilaridermic volunteers in the doxycycline group, while in the placebo group there was an increase in microfilaridermia and microfilaridermic volunteers even after the

administration of ivermectin during the study period. The ability of doxycycline to act effectively on *O. volvulus* worms that respond sub-optimally to ivermectin in individuals who would have otherwise become reservoirs for continued transmission in endemic communities will be of immense benefit to onchocerciasis control efforts. As this study has shown, doxycycline usage will lead to a significant reduction in parasitaemia and hence make it more feasible in the quest to interrupt transmission, reduce morbidity and eliminate onchocerciasis as a public health concern in Ghana.