Transmission of Onchocerciasis by *Simulium sanctipauli* 'Pra' form in the Upper Denkyira District, Ghana after mass ivermectin treatment.

A thesis

Submitted to the Department of Clinical Microbiology, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, for the award of the degree of

M. PHIL. Clinical Microbiology

By

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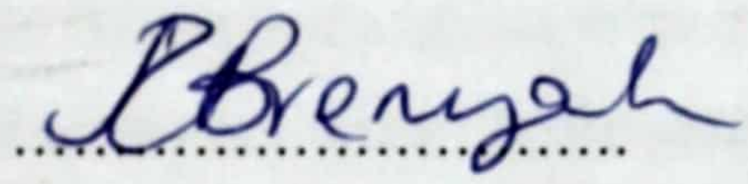
April 2003

Dedicated to the friends, families, and especially all individuals suffering from the misery of Onchocerciasis.

Certification

This is to certify that, this thesis is the candidate's own account of his research.



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ABSTRACT

The WHO has begun several control programmes to eliminate onchocerciasis. One of the programmes is the African Programme for Onchocerciasis Control (APOC). The aim of this project was to measure the impact of the mass treatment scheme in the Upper Denkyira District of Central Ghana.

Three vector sampling sessions (1909, 2132 and 1165 flies respectively) using human-biting method were conducted. Molecular biological analysis was also undertaken to identify the species of filarial larvae found in the vectors. Questionnaires were administered to determine the proportion of the population that was covered by the treatment. Larvae were reared to adult and their morphological characters compared with wild caught blackflies.

There was no significant reduction in the infection rates of the blackflies before and after the mass treatment. Infection rates per parous female blackflies were found to be consistently above 40% during all three sampling sessions. All larvae found in the vectors were *Onchocerca volvulus.* The vector was found to be an excellent host for Onchocerca volvulus with very low parasite mortality as they metamorphose from L1/L2 to the infective L3 stage. Vector infectiousness was two times higher in areas within 5km radius of the rivers than in areas more than 5km away (0.18 and 0.09 respectively). Less than 30% of the population participated in the treatment. Respondents who refused the drug administration gave the following reasons: travelled (3.9%), not important (5.8%), Not aware (10.6) unpleasant side effect (76.6%) and others (3.0%).

The mass treatment was not effective due to the aversion of majority of the population to ivermectin. Management of adverse reaction after ivermectin therapy is recommended. The 'Pra" form of Simuilium sanctipauli is highly antropophlic and efficient vector and this argues more for a semi-annual rather than an annual distribution of ivermectin.

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Acknowledgements

My special thanks go to Mrs Ruth Brenya, my supervisor and lecturer at the School of Medical Sciences, KNUST for her supervision, tolerance and encouragement. I acknowledge all her efforts with profound gratitude.

I am grateful to Prof. Rolf Garms, Hamburg for his advice, criticisms, and contributions. I also acknowledge the immense sacrifices he made in coming down to Ghana from Germany on two occasions to personally advice and instruct on the practical aspects of the work

My thanks go to Dr. Thomas Kruppa the Director of the KCCR, for his concern, advice and co-operation during my period of study, especially for the provision of laboratory facilities and other logistics without which this project would not have had the slightest chance of succeeding.

Thanks are due to the following persons who helped in various ways; Yaw Afrane, Mr. Kingsley Badu, Mr. Hubert Obiri-Yeboah, Ms. Serwaa Boateng, Ms Grace Mensa, Mr. Lincoln Gankpala and Mr Emmanuel Abbeyquay all the staff of the KCCR, Dr. Klutse and Dr. Demanya of Upper Denkyira District Hospital at Dunkwa and the fly catches from the villages studied the commitment to the study. I am also grateful to Dr. Christoff Berberich, Head of KCCR Laboratories, for his immense contribution especially in the molecular biology analysis.

Kwesi Kutin

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CHAPTER ONE

1.1.0 INTRODUCTION:

Onchocerciasis is a disease caused by the presence of the filarial worm *Onchocerca volvulus* in the dermis of the human host. The pathological manifestation of onchocerciasis is caused by the presence of microfilaria in the dermis of the human host. It has been estimated that 122.9 million people in the Americas, Africa and the Arabian peninsula are exposed to the disease and about 17.7 million suffer from it world wide with a greater majority-over 90%-living in Africa (WHO 1994). According to information available, approximately 270,000 people have been blinded by onchocerciasis, and another 500,000 have suffered considerable loss of sight resulting in at least partial disability (WHO 1987).

In Ghana, onchocerciasis is prevalent in the three northern regions and also in the Volta, Brong Ahafo and a small part of the Eastern region. In the Ashanti and Central regions onchocerciasis exists along the Pra and Ofin rivers, and along the Tano and Ankobra rivers in the Western regions (Ministry of Health Ghana, 2000).

Onchocerciasis is a leading cause of blindness and severe dermatitis, and in communities severely affected with the savanna form of onchocerciasis, 15% of the adult population can be blinded (WHO, 1998). Visual impairment is a major occupational and social obstacle which has been linked to a reduction of about ten years in the life span of the affected individuals (Kirkwood *et al.*, 1983; WHO,

1994; WHO, 1998). It is also a cause of depopulation in some fertile reverie valleys leading to the disintegration of the family fabric in the villages in such regions (WHO, 1994; 1998). In areas where blinding onchocerciasis is rare, onchocercal skin diseases occurs in up to 30% of the population and about 8 million people suffer from severe itching associated with dermal onchocerciasis (WHO, 1998). Other clinical features of relatively less importance, from the disease-burden perspective, are the onchocercomata (nodules); involvement of the lymphatic system and some associated systemic manifestation. (Anderson *et al.*, 1974a).

From 1974 to 2002 a comprehensive onchocerciasis control program known as the Onchocerciasis Control Programme (OCP) was undertaken under the sponsorship of WHO, UNDP, FAO, the World Bank, and various national ministries. This program which initially included seven countries of West Africa: Mali, Burkina Faso, Togo, Côte d'Ivoire, Benin, Ghana and Niger, was expanded to cover 11 West African countries. These later additions were Sierra Leone, Senegal, Guinea, and GuineaBissau (WHO 1987). The program focused on reducing the vector population of *Simulium damnosum* species complex to a critical level below which transmission did not pose a significant health or socio-economic problem (WHO, 1977. WHO, 1985). Between 1974 and 1989, such vector control was the only means of combating onchocerciasis. However, chemotherapy was latter incorporated into the scheme and the OCP was successfully phased out at the end of December 2002.

At the end of 1995, the African Programme for Onchocerciasis Control (APOC) was initiated for the control of the disease in those areas of Sub-Saharan Africa which were endemic with onchocerciasis but was not covered by the OCP. The main thrust of APOC was the establishment of an efficient and a self-sustaining system of community directed treatment with ivermectin (Mectizan). Ivermectin is a semisynthetic macrocyclic lactone developed by Merck and distributed free of charge to countries participating in APOC (Mectizan Donation Program 2001). It has been shown to have anti parasitic effect (Greene *et al.,* 1985) and that treatment with ivermectin results in a rapid and dramatic fall in skin microfilariae (Boussinesq *et al.*, 1995). Ivermectin has also been shown to have a deleterious effect on the release of microfilariae from the female uterus, so resumption of microfilaria production is delayed (Plaisier *et al.* 1995). Multiple treatment with ivermectin may also result in a rapid loss of vector infectiousness by *O volvulus* infected persons. Cupp *et al.*, (1989) reported that persons treated with ivermectin were significantly less able to serve as sources of infection to the blackfly vector than those who received no such treatment. Chemotherapy with ivermectin, to have the desired effect, will have to be sustained for 15-20 years, a period longer than the average life span of the adult worm, in order to achieve the elimination of onchocerciasis as a public disease (WHO, 1995 a).

For APOC to be successful, it is imperative that there should be an effective mechanism of measuring the impact of the mass treatment scheme on the

reduction of the skin microfilariae of the treated population. Sampling of vectoring *Simulium damnosum* s.l. affords a means of assessment in an excellent and non-invasive manner. Blackflies, because they feed indiscriminately, may sample a larger segment of the population, and also more randomly. Moreover, blackflies have been shown to be more efficient than conventional skin snipping in taking up microfilaria from the dermis of an infected person. For example Davis *et al.*, (1997) gave evidence that blackfies were able to ingest microfilaria from persons that were classified mf negative by the conventional skin snipping method. Thus it may be more accurate to estimate the level of infection in the population by the dissection of blackflies for *O volvulus* larvae than by the use of the more conventional skin snipping method. However, West African *S. damnosum* cytotypes exhibits some degree of zoophily (Seidenfaden *et al.*, 2001). It is conceivable that the blackflies would not to be infected with only *Onchocerca volvulus* but also with *Onchocerca* species of the animals on which they feed (Garms, 1985,1987). Some of these larvae can be distinguished morphologically from *Onchocerca volvulus* but there are other larvae, which can not be so easily distinguished. The infective larvae of bovine parasite *Onchocerca ochengi* are of particular interest not only because they are morphologically very similar to those of *O. volvulus* (McCall *et al.,* 1992), but also the definitive host (cattle) is always found to be in close proximity with humans. The presence of such larvae may lead to an overestimation of the filarial load in the population. It is therefore essential that to have a more precise estimation of the larval load in the population, a more refined diagnostic method which clearly distinguishes *O*

volvulus from other *Onchocerca* species must be coupled to the morphological method to clearly differentiate *O. volvulus* larvae from other *Onchocerca* larvae. Using the polymerase chain reaction (PCR) and DNA hybridisation, *O. volvulus* larvae can be distinguished from other *Onchocerca* larvae in black flies (Unnasch, 1987; Dissanayke and Piessens, 1992).

Since 1999, effort has been made at combating onchocerciasis through annual mass distribution of ivermectin in all onchocerciasis endemic communities in the Upper Denkyira District of the Central Region of Ghana. Village health workers who live within the communities carried out the drug distribution. It was envisaged that by treating sixty five percent of the eligible population annually for a period of 15 to 20 years, the microfilarial load in the population could be reduced to a level that would effectively disrupt transmission. However there is no program instituted to monitor the effectiveness of this mass treatment scheme in reducing the microfilarial load in the population after each round of treatment. Secondly, there is no baseline data on the infection rate in the vectors on which future evaluation of mass treatment scheme could be based. No entomological survey has been carried out to determine the transmission potential of the blackfly population in the area. It is necessary that the members of *Simulium damnosum* s.l. complex effecting transmission be properly identified because different species of the *S. damnosum* s.l. complex have been shown to respond differently to the reduction of microfilaria loads in the human population. The need to properly assess the biology of the vector population in the district was

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made more compelling by the discovery by Boakye and co-workers (1999) of a distinct IIIL-B sex chromosome differentiation among *Simulium sanctipauli* larvae breeding in the district. Such sex-chromosome differentiation is considered to be important in population sub-structuring leading to speciation (Procunier; 1982). This species, which was designated *Simulium sanctipauli* 'Pra' form solely on the basis of cytotaxomonic determination from larval material, had largely an unknown vectorial capacity and a largely uncharacterised adult morphology and behaviour. Though there was every reason to suggest that majority of the blackfly population in the area could have come only from the Pra and Ofin rivers, it was imperative to conclusively link the wild adult fly population to the larval population breeding in the two rivers. This larval population had hitherto been classified as the *S. sanctipauli* 'Pra' form. There was also the compelling need to identify the source of the vectoring blackfly population in the area to determine whether and to what extent there was a problem with re-invasion by flies from other areas which potentially could interfere with the intervention scheme.

1.2.0 The aims of the study

i) To determine the effectiveness of the mass treatment scheme in the Upper Denkyira District and also to establish a baseline data for the future evaluation of the mass treatment scheme.

ii) To describe and characterise the morphology and bioecology of the adult of the 'Pra' form of *Simulium sanctipauli*.

1.2.1 Specific Objectives

The specific objectives include:

i) To determine through the dissection of blackflies captured before and after the mass treatment scheme whether there was a perceptible drop in the infection rate after the intervention.

ii) To infer from the genetic composition of the larvae in the flies using Polymerase Chain Reaction (PCR) the source of the larvae.

iii) To determine whether wild caught blackflies were of the same species and form as the pupae from the main rivers Pra and Ofin by rearing pupae from the rivers to adult and by the comparison of their adult morphological characters to those of the wild caught flies.

iv) To determine the biology of the vector population including its dispersal pattern, its age spectrum and its ability to act as a vector.

CHAPTER TWO

2.0.0 LITERATURE REVIEW

2.1.0 Geographical distribution of onchocerciasis.

Onchocerciasis is endemic in 37 countries (WHO, 1998), 30 of which, including Ghana, are in sub-Saharan Africa. The African onchocercal belt lies between the latitudes 15 N and 14 S and extends from Senegal to the west of Ethiopia in the east (Kale, 1998). The northern-most occurrence is in Abu Hamed whilst the southern-most is Malawi. There are also onchocercal foci in six Central and South American countries (Brazil, Columbia, Ecuador, Guatemala, Mexico and Venezuela). There are also small foci in two districts of Yemen, in the Arabian Peninsula (WHO, 1976). The pattern of onchocerciasis in Africa can be divided into four broad categories (Anon, 1996): (1) the savanna woodland belt where the disease is of the blinding type; (2) the West and Equatorial African rainforest, where the disease is of the less 'blinding type' but characterised by severe skin lesions; (3) the Congo basin, characterised by a mixed pattern of severe blinding and less blinding onchocerciasis; and (4) the Eastern African highlands extending from Ethiopia to Malawi, where the disease is generally of the 'less blinding' type. There is a correlation between the strains of the parasite in a particular geoclimatic zone and the member of the *Simulium* sp. effecting the transmission. There are two fairly broad but distinct pathological patterns of onchocerciasis, particularly in West Africa, based on the disease's two predominant and major clinical complication: blindness and skin disease. In West Africa, blindness rates are significantly higher in hyperendemic communities in

the savanna than in communities with similar levels of infection in the rainforest (Anderson *et al.*, 1976; Dadzie *et al.,* 1989, 1990). Over most of East and Central Africa the clinical picture of onchocerciasis is not particularly severe. Local variations that have been reported include the high prevalence of severe eye lesions, particularly in the posterior segment, in the southern Sudan, where the vectors are known to be *S. damnosum* s.s. and *S. sirbanum*. Severe skin and lymphatic lesions have also been reported along the Uele River on the border between the Democratic Republic of Congo and the Central African Republic. Here gross elephantiasis of the scrotum and/or legs and also large number of head nodules is often present. (WHO, 1987).

2.1.1 Geographical distribution of onchocerciasis in Ghana

The two main types of onchocerciasis found in West Africa are represented in Ghana. The blinding type of onchocerciasis is found along the major rivers in the north of the country extending from the border with Burkina Faso in the north extending to areas in the Brong Ahafo region. Here the vegetation is of the savanna type. An isolated focus is also found in Asubende along the Pru River where the disease is of the blinding type. The forest strain of the disease which is manifested more by severe dermatitis and rarely by blindness is found in the south of the country along major rivers of Pra, Ankobra, Ochi, the Tano, Ofin and Bonsa rivers (Ministry of Health Ghana, 2000).

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2.2.0 Various manifestations of the disease.

2.2.1 The prepatent and incubation periods

The prepatent period, i.e., the period between infection with infective larvae and the production of detectable microfilariae by fertilized adult female worms varies from 7 month to more than 2 years; most commonly it is 12-15 months. The clinical incubation period i.e., the time from invasion of infective larvae to the development of clinical signs, is generally longer than the prepatent period.

2.2.2 Eye lesions.

Microfilariae enter the eye by passing along the sheaths of the ciliary vessels and nerves from under the bulbar conductive directly into the cornea and via the nutrient vessels into the optic nerve and via the posterior perforating ciliary vessels into the choroid (Rodger, 1959). Dead microfilariae in the cornea and the ciliary body give rise to foci of inflammation. The earliest effect in the cornea itself is the development of small foci of cellular inflammation developing around demised microfilariae. These cause the characteristic 'snowflake keratitis' and are potentially wholly reversible lesions. Sclerosing keratitis is a permanent opacification that develops progressively. It consists of chronic inflammatory cells, with eosinophils and neutrophils, in a fibrovascular pannus of the superficial stroma near the level of Bowman's membrane. Redistribution of pigment may occur, and subepithelial bullae can develop overlying the chronic inflammatory

infiltrate. Vascularization, fibroblast proliferation, and chronic inflammatory infiltrates may occur deep in the stroma as well as superficially; limbitis with perivascular plasma cell cuffs is usually present. A florid interstitial keratitis has been described in human corneas heavily infected with microfilariae, and this may be an early stage of sclerosing keratitis. The shift from acute inflammation to chronic inflammation and pannus may result from the summation of many individual acute reactions to microfilariae in the cornea; autoimmune mechanisms also may play a role, since microfilarial antigens may be retained within the cornea (WHO, 1987)

2.2.3 Skin disease

The skin lesions associated with onchocerciasis results from inflammatory reactions around damaged or disintegrating microfilariae. Antigens released by microfilariae probably damage vessels and connective tissue in the skin and, therefore, the clinical manifestations vary according to the microfilarial density in the skin, the immune responsiveness of the host, and the duration of infection.

Most infected people complain of pruritus that is intermittent at the beginning, but later become severe and permanent, sometimes so severe that it causes insomnia and reduces working capacity. Pruritus is not necessarily related to microfilarial density and some people do not complain of any itching despite the fact that they have a significant microfilarial load.

Visible skin changes include an alteration in the pigmentation with areas of hyper- and hypopigmentation. Hypopigmented macules can, at times be

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confused with the early sign of leprosy. Other patients may develop subacute relapsing or chronic inflammatory skin disease. Severe itching and scratching may lead to superficial excoriation and crusts. Secondary bacterial infection may develop sometimes leading to ulceration and, rarely, sepsis (WHO, 1987).

After years of chronic infection, with or without visible inflammatory lesions, atrophy of the skin develops. In severe cases the thin epidermis has a shiny fragile appearance that has been described as parchment-like. The appendages of the skin are reduced and the skin is dry. The normal dermal structure is replaced by scar tissue, and the alteration of the collagenous and elastic fibres leads to loss of elasticity. A spotty depigmentation (*leopard skin*) is a striking feature on the shins (WHO, 1987; Murdoch *et al.*, 1993).

Chronic hyperactive, localized onchodermatitis, often called *sowda*, was first described in Yemen, but it also occurs in Africa and America. People with *sowda* present with intense itching, usually asymmetrical, well-circumscribed onchodermatitis with papules, and often with pustules and crusts, oedema, parchydermia, darkening of the skin and considerable enlargement of the local lymph nodes. Microfilariae density is usually low and restricted to the area of the skin lesion. These patients have high levels both of blood eosinophils and antifilarial antibodies. *Sowda* usually occurs in children and young persons, for whom it represents not only a severe physical disease but also a severe psychological problem that often require therapy.

2.2.4 Other conditions

Several studies have shown that both men and women with onchocerciasis weigh less than an uninfected cohort. People with onchocerciasis also report more musculoskeletal pains (Lamp, 1967). Epilepsy and dwarfism with delayed sexual development have been associated with onchocerciasis in hyperendemic areas (WHO; 1987).

2.3.0 The parasite

Human onchocerciasis is caused by infection with thin helminthic worm, *Onchocerca volvulus*. The adult female worm measures 30-80 cm in length by 0.25-0.45mm in diameter while the male measures 2-5cm by 0.1-0.2mm. The adult worms are mostly found coiled up in nodules in the subcutaneous tissues. Some adults lie free in the subcutis, or aggregate in deep tissues as non-palpable worm bundles (Nnochiri, 1964; Duke, 1970,Duke *et al.*, 1991). The adult female worm has a life span of 12-15 years out of which 9-11 are reproductive (Kale, 1998). The adult female worm produces millions of microfilariae (mf), living embryos, that migrate from the nodules to invade the skin, eye and other tissues in the body causing most of the clinical manifestations of onchocerciasis. About 700-1600 mf are released per female worm per day into the human host (Schulz-Key, 1988; WHO, 1995 b). The microfilariae have a life span of 6-12 months (WHO, 1995 b; Ottesen, 1995; Duke, 1990). Microfilariae ingested from the skin by blood feeding *Simulium* vectors develop without multiplication over a 6 –12

day period into an infective larvae (L3). Soon after ingestion the mf appear in various parts of the body organs but only those that are able to find their way into the flight muscle can mature into infective larvae; mf that fail to escape from the stomach die and disintegrate (Omar and Garms, 1977, Crosskey, 1990). In the flight muscles, the mf metamorphose into a first stage (L1) then to a second and finally to the third (L3) larvae. The L3 larvae then migrate to the head. There are a number of different strains of the parasite worldwide. In West Africa, there are two main strains that infect man; the West Africa Savanna form associated with blindness and, the forest strain which is less associated with blindness but causes severe skin lesions (WHO, 1995b).

2.4.0 The vector

2.4.1 Various Vector species.

The vector of *O. volvulus* is a member of the family Simuliidae in the genus *Simulium*. Although there are over 700 *Simulium* sp. world-wide, only a few are known to transmit *O. volvulus*. The main vectors in West Africa are the *S. damnosum* s. l. complex. The Onchocerciasis Control Programme (OCP) identified nine species of the complex from West Africa as vectors of onchocerciasis. These were *S. damnosum* sensu stricto, *S. dieguerense, S. konkourense, S. leonense, S. sanctipauli, S. sirbanum, S. soubrense, S. squamosum, and S. yahense*. Three of these species were found to consist of two chromosomal variant population: *S. konkourense* 'Konkoure' and 'Menankaya' forms (Boakye *et al*., 1993), *S. sanctipauli* s.s (Kashan and Garms,

1987; Guzelhan and Garms, 1991) and 'Djodji' forms (Surtees *et al.*, 1988) and *S. soubrense* 'Chute Milo' (Boakye *et al.*, 1993), and 'Beffa' forms (Meredith *et al.*, 1983). Generally, each species is associated with the main savanna or forest habitats characterising the major climatic zones of West Africa and with particular river types. The geographical distribution of species also depended on the season. *Simulium squamosum* subcomplex has been found to be associated with rivers draining mountainous areas in both the forest and savanna zones (Boakye *et al.*, 1998). It was identified from the headwaters of the major rivers in Guinea and Sierra Leone and on the tributaries of the Sassandra River in Côte d'Ivoire. In the east, it was mainly found in forested and mountainous areas on rivers draining into the Volta Lake (Boakye *et al.*, 1998). The eastern form of *S. squamosum* extended considerably into the savanna during the rainy season and was the dominant species at most breeding places (Boakye *et al.*, 1998). By contrast, in the west, *S. squamosum* was found only in mountainous regions during the rainy season but was more widely distributed in the dry season (Boakye *et al.*, 1998). The other member of the *S. squamosum* sub-complex is *S. yahense*. This is a dark variant of S. squamosum found perennially along a wide band parallel to the coast. They breed principally in small permanent forest rivers. It s prevalence was recorded by Boakye *et al.* (1998) around the Sierra Leone-Guinea boundary and also in rivers draining into the eastern section of the Volta Lake in Ghana and Togo.

The *S. damnosum* subcomplex made up of *S. damnosum* s. s. and *S. sirbanum* is arguably the most widely spread members of the *S. damnosum* complex

(Garms & Vajime, 1975; Quillevere *et al.* 1982). Boakye *et al.* (1998) recorded *S. damnosum* s. s. as the most prevalent member of the complex found in the savanna-forest transition habitats in Côte d'Ivoire, Ghana, Togo and Benin they however found it to be rare in northern sections of West Africa. Its distribution however, was skewed to the north in the rainy season where it was found breeding even in minor tributaries. *S. sirbanum* occurred in small as well as large rivers and it was the most widely distributed species of the complex in the OCP zone. Although it was the main target of OCP larviciding, it is still abundant and was recorded to be present in samples collected from all major river basins in the OCP area (Boakye *et al.* 1998).

The *S. sanctipauli* subcomplex, made up of *S. sanctipauli* and *S. soubrense*, breeds exclusively in large forest rivers and was the predominant members of the subcomplex in the forest. *S. sanctipauli* s. s. has two broad but distinct distribution in West Africa. *S. sanctipauli* 'Djodji' form was confined eastwards of the Volta Lake in Ghana and in the Djodji mountain areas of Togo. This group extended northwards during the rainy season (Garms *et al.*, 1989). The other form of *S. sanctipauli*, *S. sanctipauli* s. s was identified from large rivers in the forest areas from the east of the Volta Lake in Ghana extending to Sierra Leone The 'Beffa' form of *S. soubrense* (Meredith *et al.*, 1983) has been identified in Togo and Benin (Boakye *et al.*, 1998) and was observed by Post (1986) in Nigeria around the border with Benin. The 'Chute Milo' form of *S. soubrense* is restricted to Guinea, with a core area on the Rivers Milo and Niandan (Boakye *et al.*, 1998). The 'konkoure' form of *S. soubrense* was restricted to the Fouta

Djallon area of Guinea and Guinea-Bissau and the Menankaya form to Guinea and Sierra Leone. Both forms (the 'konkoure' and 'menankaya') had a wider distribution in the dry season (Boakye *et al.*, 1998). *S. leonense* has been identified predominately in Sierra Leone and occasionally in Guinea (Boakye *et al.*, 1998). Boakye and co-workers also identified a few samples from Liberia. *S. leonense* was found to breed in both large and small rivers.

The *S. neavei* group includes all Simulliids in which the larvae and the pupae live in phoretic association on river crabs. They occur widely but sporadically in Africa from Ethiopia southwards to Malawi and eastwards through the Zaire River basin to Cameroon (WHO., 1987). In many foci, *S. neavei* s.l. occurred in sympatry with *S. damnosum* s. l. In West Africa up to Liberia, the species *S. ovazzae* is found in phoretic association with the fresh water crab *Libeonactes latidactylus.* In West Africa, the savanna *O. volvulus* is transmitted mainly by *S. damnosum* s.s., and *S. sirbanum* while the forest variety is transmitted by *S. yahense, S. sanctipauli* and *S. squamosum*. (WHO, 1995 b).

2.4.2 Vectors in Ghana

S. damnosum s.s. and *S. sirbanum* are the predominant vectors of onchocerciasis in the north of Ghana. Two cytotypes of the *S.sanctipauli* Vajime and Dunbar subcomplex occur in Ghana east of the Volta Lake. The 'Djodji'form of the *S. sanctipauli* (Surtees, 1986) is confined in the dry season to the Asuakwawkow and Dayi river systems in the mountainous forest along the Ghana/Togo border (Cheke and Denke., 1988). Boakye *et al.*, (1999) in a major

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taxonomic revision of the blackfly species in the forest regions of Ghana and Côte d'Ivoire recorded the presence of cytological variants of *S. sanctipauli* s.s on the major rivers in these countries. On the Pra river and its tributaries (River Anum and River Ofin) are found the 'Pra' form of *Simulium sanctipauli.* The 'Pra' form as well as the 'Comoe' form of *S. sanctipauli* is found on the Tano River and its tributaries.

2.4.3 General features of the larvae

In common with the larvae of other Diptera, larval blackflies have no legs. Their general form is worm-like, but they are among the most recognized insect larvae because of certain obvious structural features. The outline shape is very constant and shows three principal features: a pair of large *cephalic fans* on the head, and a 'false feet' or pseudopods and a posterior circlet. The cephalic fans are feeding organs and the prolegs organs of attachment and locomotion. When the fans are spread like open hands in the flowing water, to catch any small passing objects travelling downstream with the current, they are conspicuous appendages- visible to the unaided eye; shut, they are less apparent. The pseudopods are hollow fleshy extensions of the wormy body crowned with rows of radially arrange hooks. One, the *thoracic proleg*, is positioned on the underside immediately behind the head, while the other, the *abdominal proleg,* forms the hind end of the body. The hooks of the abdominal proleg constitute the *posterior circlet* . The postrior circlet secretes a silk mass onto the substrate which when hardens serves as anchorage for the larvae.

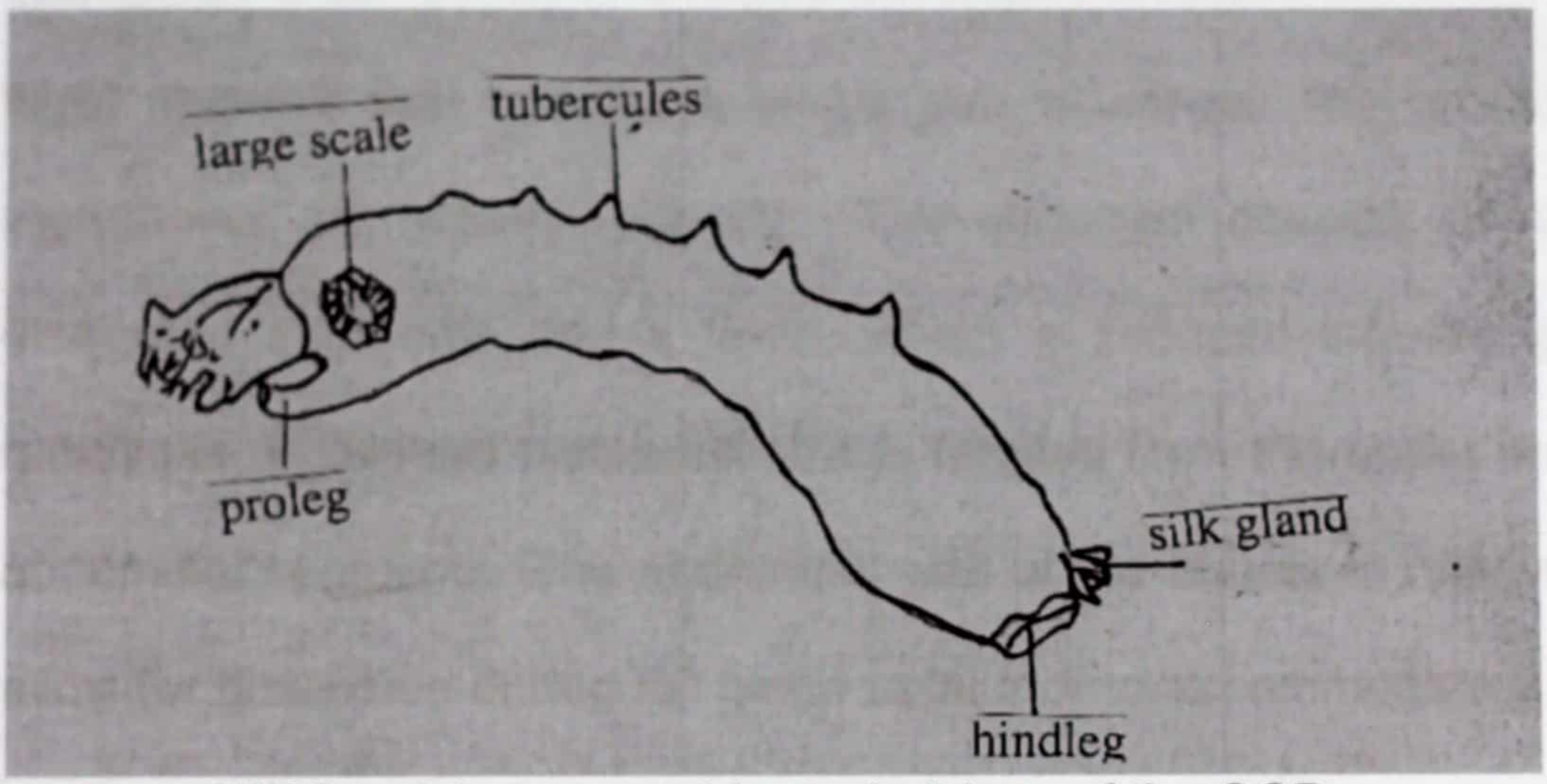
The prolegs are the truly diagnostic feature of Simuliidae because they are present at every stage of larval development and occur in every species of the blackfly in the world- irrespective of variations there may be in other structures. The cephalic fans on the other hand, are less strictly diagnostic because they are missing in the larvae of a few aberrant species and do not appear in definitive form in larvae of some normal species until the earliest larval moults have taken place.

The general body shape presents with a head, which is a much more strongly defined body region than the thorax and abdomen, which are externally separated by slight constrictions of the body outline. The thorax is wider than the head and the abdomen long and clubbed. Both head and thorax seen from above are barrel-shaped, except that a more ovoid head shape occurs in species with aberrantly modified mouthparts. The abdomen has eight segments but segmentation is distinct only on the narrower basal part, where five segments can usually be clearly recognized because they are separated by definite constrictions of the body. Although there are strong similarities in the body shape of all larval blackflies, there are also important variations. Some variations include a sudden bulging out at the midpoint of the lower abdominal surface in *Ectemnia* of North America and the occurrence of large dorsal tubercles on the middle segments of the abdomen in many larvae of the tropical African *Simulium damnosum* complex (Crosskey; 1990).

In larvae of some species the body surface bears variously modified cuticular hairs and scales (*macrotrichia*) articulated to sockets in the cuticle. These are

normally confined to the upper side of the posterior part of the abdomen, but sometimes they are present dorsally along both thorax and abdomen. More rarely, as in the larvae of *S. damnosum* complex, in which there is a covering of upright black scales, they are distributed more or less evenly over the cuticle (Crosskey; 1990).

Plate 1: Showing the morphology of larval *Simulium damnosum* s.s.



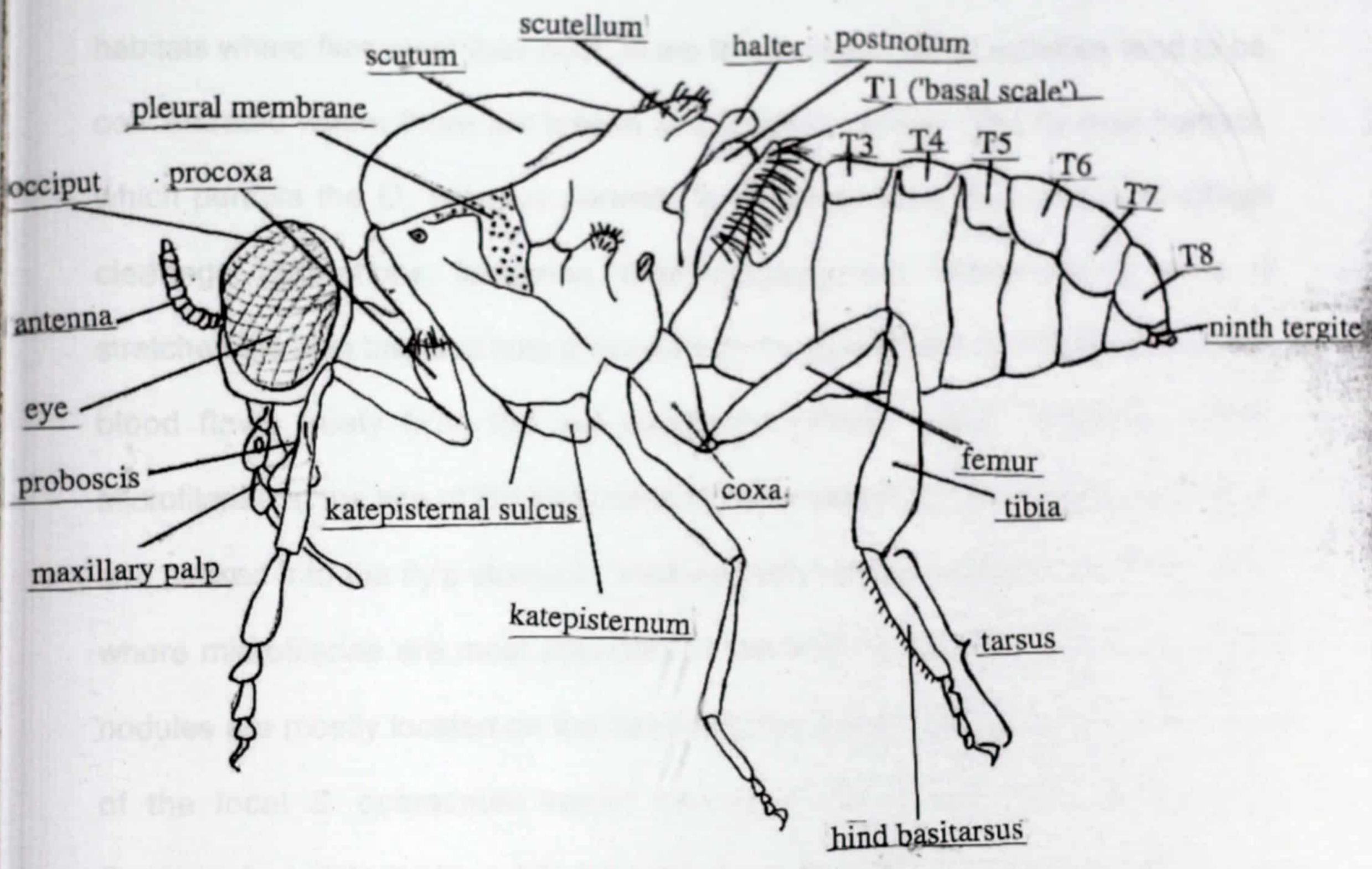
courtesy WHO training manual for technicians of the OCP

2.4.4 Morphology of the adult vector

The adult blackfly has a distinctive general appearance characterised by a relatively stout body, broad wings, short antennae, and a short downward pointing proboscis with long slender palpi. The head is almost circular in facial view. The ventral head surface is mainly a large opening (oral cavity) from which the proboscis is suspended. The proboscis is short stout cylindrical structure comprising of two closely integrated elements, the syntrophium and the labium.

The syntrophium comprises of six stylets: a labrum, a pair of mandibles, a pair of laciniae, and the hypopharynx. Each of these stylets is armed at their tips with teeth or spines, which together form the biting and cutting apparatus of the fly. The labium lies behind the syntrophium and partly encloses it. Within the head lie also a pair of pumps (the cibarium and the pharynx) with which the fly sucks up blood and plant nectar. The eyes of the blackfly consist of two large compound eyes composed of very numerous individual light receptors called ommatidia. The thorax is composed of a hugely developed mesothorax, which houses large flight muscles that operate a single pair of wings. The prothorax and the metathorax are greatly reduced. The abdomen consists of nine distinctly elongated segments and a tenth, which is reduced into the plates of the terminalia. Hardened plates called the tergites form the upper surfaces of the abdominal segments. The abdominal wall of the female is relatively flexible to allow for distension during full blood uptake or to accommodate a fully matured egg batch (Crosskey, 1990).

Plate 2: *Elements of the morphology of the female blackflly.*



courtesy WHO training manual for technicians of the OCP

2.4.5 Feeding habits of the vector

Blackflies feed on a variety of substances. Some are heamatophagous (blood feeders) whilst a few are non-heamatophagous. The non-heamatophagous

species are auto genous relying on proteins stored from the larval stage. Of the blood feeding types, a variety of animals-birds, mammals or man-may serve as host. Some species feed exclusively on one host or the other but others, probably the majority, feed indiscriminately though, they may have a predilection for a particular species or a group of related species. Blackflies bite by day and in the open. Woodlands, grasslands, cultivated fields, lakes and rivers all serve as habitats where flies meet their host. In the forest region biting activities tend to be concentrated where there are breaks in the forest canopy. The fly-man contact, which permits the *O. volvulus* parasite to be transmitted thus, occur in village clearings, plantations, footpaths, river crossings etc. When the fly bites, it stretches the skin taut and cuts a wound with its saw-tipped mandibles into which blood flows freely from the cut capillaries (Wenk, 1962; Sutcliffe, 1985). Microfilariae at the site of the bite can thus be released into the oozing blood pool and sucked into the fly's stomach. Vectors prefer to feed on regions of the body where microfilariae are most abundant in the skin. In Southern America where nodules are mostly located on the head and the upper torso, 70% of all the bites of the local *S. ochraceum* vector occurs on the upper part of the body. Conversely, in West Africa, biting by the *S. damnosum* complex is most intense below the waist level where most nodules are located (Duke, 1981, Duke & Beesley, 1958). The uptake of microfilariae is also promoted by "microfilariae concentration". There appears to be some chemical substances in the saliva of the vector, which are attractive to *O. volvulus* and serve to attract the parasites into the blood pool created by the fly (Duke, 1970; Shelley *et al.*, 1979).

Haematophagous blackflies feed on a multiplicity of hosts and none has been found to feed exclusively on man. However, *S. ochraceum*, the vector of onchocerciasis in Guatemala has been shown to exhibit super-anthropophily, feeding on man even when other hosts are equally available (Dalmat, 1955). In West Africa, savanna species of the *S. damnosum* complex tend to exhibit a greater degree of anthropophily by comparison than their forest siblings (Bellec & Hebrard, 1984).

2.4.6 Flight and migration of the vector

All blackflies have fully developed wings, which appear capable of sustained flight and most do fly to obtain food, find a sexual partner, to find a blood meal or to find a resting- place. There are usually two peak periods in the daily flight activity. There is an early morning peak and another peak in the late afternoon or early evening (El Bashir *et al.*, 1976; Roberts & Irving-Bell 1985). Roberts and Irving-Bell (1985) also reported that in eight West African *Simulium* species studied, parous and nulliparous flies flew at different times of the day; parous flies were more active in the morning in some species but in the evening in others. The flight pattern in the female flies tends to be affected by their physiological states. Duke (1975) observed that the dispersal range of the parous and nulliparous flies differed in the forest and savanna regions of Cameroon. He observed in the savanna regions, a preponderance of nulliparous flies inland

away from their riverine breeding sites. The parous flies tended to mainly stay relatively close to the breeding site, or moved along the banks of the breeding river. A few parous flies however dispersed inland to the same distance as the nulliparous flies. However, nulliparous female flies of the forest species tended to stay closer to the breeding sites whilst the parous ones dispersed further inland.

In tropical Africa, blood sucking female flies seem at times to follow people and their pets on bush paths and forest tracks connecting villages and farms. But even then the flight path is probably more guided by the host movement than by the track and path as such (Crosskey, 1990). Movement along river courses is however regular and well documented. It is assumed that in their dispersal along watercourses, blackflies use the reflectance of the water to guide their paths however, humidity may also have a role to play in this endeavour (Crosskey, 1990). Flight along large rivers seems to be quicker than along streams probably because larger expanses of water seems easier to track than small ribbons of water which can easily be hidden under vegetation (Crosskey, 1990).

Long ranged dispersal of *Simulium* over land usually involves a complex mix of climatic and biological conditions. Long ranged dispersal of blackflies probably starts as short goal-oriented flights that are accidentally disturbed by gusty air conditions which causes the flies to lose control of their height and course and so they are passively deflected onto unintended tracks as 'migrants' (Crosskey, 1990). A long distance migration however will involve a lot of active flying since still flies are unlikely to stay aloft for very long (Garms & Walsh 1986).

In West Africa, and more so in the savanna and semi-savanna than in the forest areas, the position of the Intertropical Convergence Zone (ITCZ) which causes mass air movements is an important factor in blackfly dispersal over long distances. In the dry season when the ITCZ is over the Gulf of Guinea, the suction pressure created over the sea sets adrift savanna flies to invade deep into the forest areas. Conversely when the ITCZ moves northwards in the wet season, there is a reversal of movement of flies from the forest into the savanna (Crosskey, 1990).

2.5.0 Various intervention programmes

2.5.1 The Onchocerciasis Control Programme (OCP)

From 1974 through to 2002, there was a comprehensive onchocerciasis control programme, which was initially centred in seven countries of West Africa: Ghana, Niger, Côte d'Ivoire, Benin, Togo, Mali and Burkina Faso. The programme was under the sponsorship of WHO, the World Bank, UNDP, FAO and the ministries of health in the participating countries. The Onchocerciasis Control Programme in West Africa (OCP) began its activities in January 1974. Its objective was to eliminate onchocerciasis as a disease of public health importance and as an obstacle to socio-economic development. The basic strategy was to interrupt the transmission of the blinding strain of *O. volvulus* for a period longer than the longevity of the adult worm in the human host-about 14 years, (Plaiser *et al.*, 1991). The interruption of transmission was achieved by destroying the larval stages of the vector through the aerial application of selective insecticides to

infested rivers. The temephos 'Abate' was the main insecticide used from the start of the programme upto1980. There after, the development of resistance in the vector population necessitated use of other insecticides whenever this was found to be necessary.) The first aerial insecticide treatments began February 1975, in areas where the incidence of blinding onchocerciasis was highest. Later, the area covered was gradually extended to 654000 sqm, spreading over the seven countries by the end of 1977. However it soon became apparent that the border of this area was infiltrated by infective blackflies originating from regions outside the OCP areas (Garms *et al.*, 1979). To give permanent protection in the original OCP area and to control the vector in the basins where the re-invading *Simulium* originated, the hyperendemic regions were identified and larvicidal treatment was given (Walsh, 1981). This resulted in two extensions to the original OCP area, one to the west, comprising the basins of south-eastern Guinea and northern Sierra Leone (western extension), and a second to the south and east, comprising the southern basins of Côte d'Ivoire. Benin, Ghana and Togo (south-eastern extension). The installation of vector control operations in the extension areas was completed towards the end of the 1980s, while all basins of the original areas were still being treated. The larviciding coverage then reached a peak with >40000 km sq. of river being treated over an area of 10000000 km sq., over nine countries of the OCP. In 1995, as a result of the satisfactory results achieved, most larviciding was discontinued in the original OCP area. After 1995, larviciding in the extension areas was combined with the mass distribution of the microfilaricidal drug ivermectin. This strategy of combined drug treatment

and vector control was continued up to the end of 2002 when the programme was brought to a conclusion. A few residual foci of infection will remain, however, after the conclusion of the OCP, both in the controlled areas and in the extension areas. This was mainly due to operational failures and inaccessibility of breeding sites.

2.5.2 OCP in Ghana

The Upper West, Upper East and the Northern region were included in the original phase of the OCP. The Brong Ahafo and Volta regions were included in the southern extension of the programme in 1987.

2.5.3 The African Programme for Onchocerciasis Control (APOC)

In 1995, a new programme called the "African Programme for Onchocerciasis Control" (APOC) was launched. Its objective was to establish within a period of twelve (12) years, an effective and self-sustaining community based ivermectin treatment throughout the remaining endemic areas of Africa (Remme *et al.*, 1989). Several studies have demonstrated the effectiveness of ivermectin in preventing the development and progression of onchocercal lesions (Paque *et al.*, 1991a). When taken, ivermectin leads to a rapid fall in the number of skin mf, followed by the maintenance of very low levels over a period of several months. Thereafter, there is a gradual increase in the skin mf to reach a peak. Ivermectin has a partially reversible effect on embryogenesis. Rodriguez-Perez *et al.*, (1995) working in an endemic area in southern Mexico reported a drastic reduction in

the prevalence of skin infection and the mean microfilarial skin density after five semi-annual treatments with ivermectin. Ivermectin interrupts the transmission of the parasite by reducing the ingestion of the microfilariae by its *Simulium* vector. The effect of the single dose treatment was noticeable after six month. Treatment with ivermectin at the hyperendemic focus at Asubende, Ghana, showed that a reduction of 68% - 78% in the total skin microfilariae available for transmission could result in a corresponding decrease of 65%-85% in transmission in the first three post-treatment months (Remme *et al.*, 1989).

2.5.4 Ivermectin therapy in Ghana

In Ghana the Ministry of Health orders ivermectin from the Mectizan Donation Programme (MDP) with UNICEF collaboration. Ivermectin distribution was carried out in all nine onchocerciasis endemic regions. The Community Directed Treatment Initiative (CDTI) approach is implemented throughout the country. By this approach, village health workers are recruited from the communities and they are responsible for the distribution of the drugs. The ivermectin distributed in the communities are recorded in the community register. A staff member from the sub-district followed up later to collect and collate the data at the end of the distribution period in each district and calculate the coverage rate for each village and thus the whole sub-district. This was sent to the district and regional levels for further analysis. Results from each of the regions are sent to the national headquarters in Accra.

2.6.0 Mechanism of action of ivermectin

Ivermectin is a synthetic derivative of avermectin B1, which is a fermentation product of Streptomyces avermitilis (WHO, 1987; Cambell, 1991). Its mechanism of action against *O. volvulus* has not been fully established. However, its microfilaricidal effect is thought to result from ivermectin's activity in enhancing both the pre-synaptic release of gamma-ammino butyric acid (GABA), and the post-synaptic enhancement of GABA-binding to a chloride channel-linked receptor. Maintenance of the chloride channel in an open state leads to muscular paralysis, and subsequent death of mf by immune responses or by other mechanisms (WHO, 1987). Ivermectin is given as a single dose of 150/kg of body weight once or twice a year (WHO, 1995 a; BNF, 1998).

2.7.0 Mazzotti reactions after antifilarial treatment

Reactions after treatment for all filarial infections including onchocerciasis are common and have led to failure of many a control programme (Ottesen *et al.,* 1987). In onchocerciasis, the adverse reactions after treatment with microfilarial drug diethylcarbamazine citrate (DEC) can be so severe that the drug is no longer recommended for treating this disease (WHO, 1995 a; Taylor & Hoerauf, 1999). Ivermectin, the present drug of choice is also associated with significant adverse reactions that affect compliance with the necessary repeated treatment rounds (Cooper *et al.,* 1999). The major side effects are fever, pruritus, prostration, and postural hypotension, which is the most severe side effect (Awadzi *et al.*, 1990). In onchocerciasis, this cluster of reactions has been termed

the Mazzotti reactions in honour of the man who first described them in 1948 in Mexico: Mazzotti (WHO, 1987; Boatin *et al.*, 1998). The pathology of these reactions is poorly understood (Cooper *et al.*, 1999), what is clear however is that, the response is due more to the host's response to the rapid death of mf than to the toxicity of the drug itself (Turner *et al.*, 1994). It has been shown quantitatively that these post-treatment responses are directly proportional to the pre-treatment mf densities of the affected patients (Francis *et al.*, 1985; Kumaraswami *et al.*, 1988).

2.8.0 Development of DNA probes

In 1985, a procedure was developed which revolutionised the field of molecular genetics. The procedure, known as the Polymerase Chain Reaction (PCR), allows the rapid amplification of gene sequences represented once or more in a large complex mixture of genes. The result is that within a few hours a particular genomic region can be amplified 1-100 million times (Saiki *et al.*,1985). Due to the high resolution obtained and the way in which interpretation is facilitated, direct DNA analysis using PCR has proven to be an extremely accurate and powerful tool in taxonomy and evolutionary genetics. Exploiting this technology, techniques have been developed which allow unequivocal identification of the major species within the genus *Onchocerca*.

The discovery of a family of repetitive sequence composed of 150 base pairs in the genotype onchocerca made possible the development of DNA probes for parasite of *Onchocerca* type. Several probes were isolated and developed from

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plasmids. In 1989, the sequence pOvs 134 which hybridizes with the DNA extracted from all *O. volvulus* strains was isolated. In 1993, two oligonucleotide probes OVS-2 and OCH were developed. OVS-2 recognizes all isolates of *O. volvulus* with a high degree of sensitivity but does not hybridize with DNA obtained from other *Onchocerca* species. OCH can differentiate *O. volvulus* from *O. ochengi*. (Harnett *et al*., 1989; Meredith *et al*., 1989; Perler and Karam 1986; Shah *et al*., 1987; Zimmermann *et al*., 1992; Zimmermann., 1993).

CHAPTER THREE

3.0.0 MATERIALS AND METHODS

3.1.0 Study area

The study was conducted in the Upper Denkyira District in the Central Region of Ghana. The Ofin and the Pra rivers flank the area at its northern border and it is drained extensively by the tributaries of these rivers. Extensive cocoa farming has reduced the original moist evergreen forest vegetation to a secondary forest dominated by cocoa trees interspersed with forest trees standing at heights of between 15 to 60 metres. The remaining forest is spotted and scattered. Large swathes of bamboo trees flank the banks of the rivers Ofin and Pra occasionally dipping into them to serve as breeding surfaces for the blackflies. The area is characterised by average temperature of 26-30°C and an annual rainfall of 800-1200mm. Cocoa farming constitute the basis of the local economy however other crops such as oil-palm, cassava, maize, plantain, rice, and orange are cultivated on smaller scales. The population consists predominantly of indigenous as well as migrant farmers particularly from the Northern, Ashanti, Central and the Greater Accra regions. Majority of the migrant population are second and third generation migrants with a rather stable occupancy of the area.

3.1.1 Village selection methods

In total eleven villages and three road-side clearings were selected from within a 14 km radius from the main rivers of Ofin and Pra. In all three catching sessions were held from November 2001 to November 2002 for each of the main sites viz. Buabinso, Akropong, Foso, Ayebiawhe, Assaman and Awisem. The first catching session (pre-treatment sampling) took place in November 2001 before the mass treatment undertaken in February and March of 2002 whilst the second session (2 month post-treatment) took place in April and May 2002, two months after the mass treatment scheme. The November 2002 collections involved nine occasional sampling sites namely, Awisem Road, Kontokrom, Kontokrom Road and Buabin. The rest were Buabin Camp, Zion Road, Zion No.1, Praprababida and Kumakrom. These temporary sites were selected for their varied degree of remoteness from the main rivers and were sampled to aid in the determination of dispersal pattern of the flies. In general these temporary sites were sampled for few hours.

Prior to the start of sampling, a meeting was arranged with the opinion leaders and senior committee members of each of the selected community and the aim of the work was explained to them. The intended methods were described and demonstrated to them. Each meeting ended with permission being given to conduct the research followed by a tour of the village its vicinity in order to select suitable sampling location.

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conduct the research followed by a tour of the village its vicinity in order to select suitable sampling location.

3.1.2 Catching sites

Village: Buabinso:

Catching point: catching was in a clearing in a cocoa plantation about 600m off the village. A footpath that connects the village to a hamlet on a hill overlooking the village transverse this cocoa plantation.

Village: Akropong:

Catching point: catching was carried out in a small mixed farm on a footpath that led from the village to the Ofin river. It was about a kilometre from the village and about twice that distance from the Ofin River. A bend in the watercourse at this portion produces numerous rapids, which served as breeding sites.

Village: Ayebiawhe:

Catching point: catching was carried out at the edge of a cocoa plantation about 50metres from the village

Village: Foso:

Catching point: the catching site was by the edge of a moribund cocoa farm along a footpath that led from the village to the Ofin river. It was about 400 metres from the village and about 50 metres from the river.

Village: Assaman:

Catching point: catching was carried out about a kilometre from the village by the edge of the river Ofin and at a point used as a crossing place. About 50 metres up river were several rapids that served as breeding sites for the blackflies.

Village: Awisem:

Catching point: catching was at a point between the Pra river and the village at the edge of a cocoa farm. It was about a kilometre from the village about half that distance to the river. There were numerous rapids at this portion of the river.

Temporary catching sites

Village: Kontorkrom:

Catching point: catching was at the outskirts of the village. The village was about 7 kilometres from the Ofin River.

Village: Buabin:

Catching point: this was a village about 10 kilometres from the Ofin River. Catching was carried out about 100 metres from the edge of the village at a clearing by a groove.

Village: Buabin Camp:

Catching point: catching was carried out at a point on a footpath through a cocoa plantation. It was about 20 metres from the outskirts of the village. Buabin Camp was about 13 kilometres from the Ofin River.

Village: Praprababida:

Catching point: Collection was carried out in a clearing at the edge of a thicket and about 200 metres from the village. This was a village about 8 kilometres from the Ofin River.

Village: Kumakrom:

Catching point: Collection was in a cocoa plantation about a kilometre from the village and at the banks of a tributary of the Pra River. This was a village about 14 kilometres from the Ofin river.

Village: Zion No.1:

Catching point: Collection was done right within the village. This was a village about 7 kilometres from the Ofin River.

Village: Zion Road: (this did not represent a village but was used to designate a point on the road to the nearest village, Zion No. 1)

Catching point: this was a point about 3 kilometres from the Ofin River and on a road leading to a cluster of villages. Collection was in a clearing by the edge of a thicket.

Village: Kontorkrom Road: (this did not represent a village but was used to designate a point on the road to the nearest village, Kontokrom)

Catching point: catching site was in a cocoa plantation at a point on a road to Kontorkrom. It was about 6 kilometres from the Ofin River.

Village: Awisem Road: (this did not represent a village but was used to designate a point on the road to the nearest village, Awisem)

Catching point: catching was at a point on the main road from Awisem to Dunkwa. It was about 4 kilometres from the Pra River and about the same distance from Awisem.

3.2.0 VECTOR SAMPLING

3.2.1 Larval sampling

Several portions of the Ofin and the Pra, and their tributaries were prospected for the presence of rapids that could serve as breeding grounds for the blackflies. Use was made of the local fisherfolks' knowledge of the river by asking them to help to locate portions of the river where, according to local parlance, 'it made noise'. Sampling was carried out at Awisem, Kyekyewere Buabinso and Assamang.

3.2.2 Rearing of pupae to adult flies

Pupae collected from the rapids were immediately wrapped in plastic bags and placed in a cooling box. Back in the main field laboratory, the pupae were transferred into a cup. A thin transparent plastic bag with one end closed was fixed onto the cup and fastened with elastic bands leaving an air space above. Wet cotton was wrapped around the cup to keep the inside moist. The set up was left in warm moist place with the plastic end exposed to light. This was left for between three to five days for the flies to hatch. Hatched flies were left to live for a day for their features to harden. Flies were killed by placing the whole set-up in a deep-freeze. The flies were then aspirated into 80% ethanol to be used later for morphological measurements.

3.2.3 Adult sampling

One catching site was used per each village throughout the investigations. These sites were selected after extensive prospecting to determine sites in the village where fly densities were found to be high. Fly catchers were given extensive training and were selected for their diligence to the task of fly collection and their attractiveness to the flies. Two fly collectors placed at intervals of 50 meters from each other bared their legs to attract the flies. Flies were collected in transparent plastic tubes with stoppers as they settled on the bared legs of the catchers to feed (Walsh et al.,1978). Flies collected were kept in moist cotton bags and placed in a cooling box. Catching took place from 08.00 to sunset with an hour's break from 12.00 to 13.00. At the end of the day these cooling boxes with the flies were sent immediately to the KCCR field laboratory at Dunkwa.

All together, three catching sessions were held from November 2001 to November 2002 for the main sites viz. Buabinso, Akropong, Foso, Ayebiawhe, Assaman and Awisem. The first catching session (pre-treatment sampling) took place in November 2001 before the mass treatment undertaken in February and Match of 2002 whilst the second session (2 month post-treatment) took place in April and May 2002, two months after the mass treatment scheme. The November 2002 collections involved nine additional sites viz. Awisem Road, Kontokrom, Kontokrom Road and Buabin. The rest were Buabin Camp, Zion Road, Zion No.1., Praprababida and Kumakrom. These additional sites were selected for their varied degree of remoteness from the main rivers.

3.3.0 Dissecting methods

Flies from the day's catch were collected from the various catching sites at the end of the day and brought to the KCCR field laboratory at Dunkwa. On arrival at the laboratory, they were kept in moistened cotton sacs wrapped with plastic sheets and kept in a refrigerator. The flies were dissected next day. Flies were killed by first placing them in a deep freezer for about ten minutes to immobilise them. They were then placed in drops of Phosphate Buffer Saline (PBS). The abdomen was drawn out under a dissecting microscope in order to determine from the appearance of fat-body, the colour of the malpighian tubules, the stretchability of the ovary and, most important of all, the presence of egg-relics whether the fly was parous or nulliparous.

Parous flies had scanty fat-body (Duke, 1967) whilst the abdomen of a nulliparous flies have voluminous, multilobular, semi-opaque fat-bodies. The malpighian tubules of the parous flies were pale relative to those of the nulliparous flies. By placing the tips of the dissecting pins at opposite ends of the ovary and by the gentle pulling of it apart, the stretchability of the ovary could be determined. Parous flies had ovaries that could be stretched a great deal whereas the ovaries of the nulliparous flies broke easily. On stretching the ovaries, it could be determined by the presence or absence of egg-relics, which presents as opaque, globular bodies on one side of the ovules, whether or not

the fly had under gone a gonotrophic cycle. This was of the most definitive diagnostic value.

Flies from the 8 month post-treatment sampling session identified as nulliparous were kept in 80% Ethanol and stored in a refrigerator and were used later for morphological identifications. Those flies that were identified as parous were separated into three- head, thorax and abdomen in separate drops of PBS on the same slide. The flies were then dissected by the meticulous teasing out of each of the portion and then examined under the dissecting microscope for the presence of filarial parasites (Garms & Cheke, 1985). The number, distribution and stage of development of the parasites were recorded and the slide passed on to an assistant who also re-checked it. Where discrepancies were observed, the slide was checked for the third time and that was taken as the final count.

For the third sampling session,filarial larvae were separated from the rest of the fly and kept on a different slide. These slides were labelled, dried and kept in a refrigerator and were used later for PCR. Other phenomena such as the presence of mermithids (parasites of the Simulium vector) and blood in the abdomen were also recorded.

3.4.0 MORHOLOGICAL MEASUREMENTS

3.4.1 Larval measurements

The larvae were characterised on the bases of the degree of prominence of their tubercles and also by the sizes of their scales. Very prominent tubercles in conjunction with large scale were diagnostic of *S. squamosum* and *S. yahense*

whilst the presence of moderate tubercles and large scales indicated *S. damnosum* or *S. sirbanum.* The presence of scanty dorsal tubercles and scanty large scales is diagnostic for *S. sanctipauli* and *S. soubrense.*

3.4.2 Measurements of the adult flies

1. The length of the antenna and thorax were measured under a dissecting microscope and the ratio of the thorax to antenna length calculated. These measurements are positively correlated for all cytospecies (Garms, 1978). The regressions for each have a similar gradient *S. damnosum/S.sirbanum* have the shortest antennae, *S. soubrense/S.sanctipauli* the longest and *S. squamosum* are intermediate.

2. The colour of the setae of the vein (wing tufts) was scored with the aid of a dissecting microscope with the incident light adjusted to avoid reflections or shadows. The colour was scored on a numerical scale ranging from 01 to 05. The code 01 represented all wing tuft being pale which was characteristic of the savanna cytospecies *S. damnosum/S. sirbamnum*. The codes 02 and 03 were used to represent a mixture of wing tuft colour with a predominance of pale tuft given 02 whilst 03 was given to a higher degree of dark hairs. The codes 04 and 05 were assigned to flies with predominately dark wing tufts with 05 being given to the flies with all dark wing tufts. The flies with the codes 04 and 05 were characteristic of *S. soubrense/S. sanctipauli* (Kurtak *et al* .,1981).

3. The colours of the setae of the ninth tergites were also scored on a scale of 1 to 3. Flies with entirely pale setae on the ninth tergites were assigned a code of 1whilst those with a mixture of pale and dark setae given a code of 2 and the code 3 were given to flies with entirely dark setae. Flies with entirely dark ninth tergit setae were characteristics of the savana species of *S. damnosum/S. sirbamnum*. The code 2 is characteristic of *S. soubrense/S. sanctipauli* and the code 3 assigned to *S. yahense* (Dang & Peterson, 1980).

4. The arculus of the wing was scored on a numerical scale of 1 to 3. The code 1 was assigned to flies with pale arculus characteristic of the savanna flies-*S. damnosum/S. sirbanum*. The code 2 was given to flies with brown arculus characteristic of *S. squamosum*. The code 3 was given to flies with dark arculus which is characteristic of the forest species *S. Sanctipauli/S. soubrense* (Garms *et al.*, 1982).

5. The scutellar hairs were scored on a numerical scale of 1 to 3. Flies with entirely pale scutellar hairs were scored as 1. Flies with a mixture of pale and dark hairs were scored as 2 whilst those with exclusively dark hairs were scored as 3. S. *damnosum/ S. sirbanum* have exclusively pale scutella hairs. *S. sanctipauli/ S. soubrense* also have mostly pale or a mixture of pale and dark scutella hairs but *S. yahense* has completely dark scutella hairs (Dang & Peterson, 1980).

6. The setae on the costa were also scored as 1 for flies with entirely pale costa setae, 2 for those with a mixture of pale and dark and 3 for those with exclusively dark costa setae. *S. damnosum/ S. sirbanum* as well as *S. squamosum, S. sanctipauli/ S. soubrense* have pale costa setae though a few dark hairs may present on the costa of *S. sanctipauli/ S. soubrense. S. yahense* on the other hand has exclusively dark costa setae (Garms *et al.*, 1982).

3.5.0. DNA amplification

DNA extraction and amplification potentially presents with problems of inhibition by other cellular components such as RNA and proteins. To overcome this problem, several sequential steps are usually needed to remove these inhibitors.

3.5.1 Lysis

This step aids in the break up of cell membranes and the release of cell components into solution. The slides with the dried filaria larvae were again examined under a dissecting microscope to ensure that they still had the worms on them. 60µl of a lysis buffer from Hybaid Limited (Middlesex, UK) was pipetted onto the slide and allowed to stand for a couple of minutes to allow the larvae to dislodge from the slide. With the aid of a dissecting microscope, the larvae were then transferred with a micropipette into a 2ml-reaction tube containing another 60µl of lysis buffer. This was subsequently freeze-thawed by submersion in liquid

nitrogen for a couple of seconds and then quickly incubated at 68°C for 10 minutes.

3.5.2 De-proteinization

This step was to aid in the removal of proteins from the mixture. 180µl Chloroform was added to the mixture and then gently mixed by inversion until the two phases formed an emulsion. The two phases were then separated by centrifugation at 14,000 rpm for 2 minutes at room temperature. Proteins are preferentially dissolved in the chloroform phase whilst DNA dissolve in the upper aqueous layer.

3.5.3 DNA Precipitation

This step preferentially precipitate DNA whilst most proteins remain in solution The upper aqueous surface containing the genomic DNA was transferred with a pipette into a new 2ml reaction tube and the DNA was precipitated by the addition of 180µl ddH20 and 20µl Precipitation Buffer from Hybaid Limited (Middlesex, UK). After mixing gently by inversion at room temperature, DNA was isolated by centrifugation at 10,000 rpm for 2 minutes at room temperature. The supernatant was discarded.

3.5.4 DNA Re-suspension

This step was to aid in the further purification of the DNA precipitated during the previous steps.

The precipitated DNA was re-suspended by the addition of 60ul of re-suspension buffer from Hybaid Limited (Middlesex, UK).

3.5.5 DNA Precipitation

225µl of 100% Ethanol was added to the re-suspended DNA and mixed gently by inversion. The sample was centrifuged at 10,000 rpm for 10 minutes at room temperature. The supernatant was discarded and the pellet was rinsed with 70% Ethanol. The Ethanol was discarded and the DNA was dissolved in 50µl of ddH2O.

3.5.6 DNA amplification

A 50µl PCR reaction mixture containing 1 unit standard *Taq* DNA polymerase (Amersham Pharmacia Biotec Inc), standard PCR buffer (Amersham Pharmacia Biotec Inc), 200µM dNTPs, 4mM MgCl2 and 40 pM each of the oligonucleotides S3 and S4 (Fischer et al., 1997) with the inserted restriction sequence (S3: TGTCTGCAGA TCAATTTTGC AAAATGCG, PstI underlined and S4: TGTAAGCTTA ATAACTGATG HIND III underlined) as primers. After an initial denaturation for 5 minutes at 94°C, the reaction mix was subjected to three cycles consisting of 1minute at 92°C, 1 minute at 60 °C and 2 minutes at 72 °C, followed by 34 cycles with a diminishing annealing temperature of 55 °C and a

final elongation step of 8 minutes at 72 °C, using the PERKIN ELMER CELTUS DNA Thermal Cycler. Several dilutions of the DNA extracts were subjected to PCR to overcome the inhibitory effect of remaining fly compounds on PCR (Fischer *et al.,* 1997).

3.5.7 Agarose gel electrophoresis

In order to visualise and interpret the results of the PCR reaction it is necessary to separate the PCR products using electrophoresis. Agars gel (2%) were prepared by combining 2.4g of agarose with 120ml of 1X TBE buffer containing 3μ ethidium bromide stain which was then heated in a microwave oven for 3 minutes. After leaving to cool for 5-10 minutes, the solution was poured into mini-gel trays (already containing gel combs to provide 32 slots) and allowed to set (taking approximately 30 minutes).

Finally 15 μl of each PCR product was mixed with 5 μl of Blue G buffer and loaded into slots provided by the removal of the gel combs. In the first and seventeenth lane of each gel, a known molecular weight marker was loaded with the remaining 30 of each occupied by PCR product from a positive fly. The gel tray was then connected to a power source at 50-80 volts for 1 hour. All gels were visualised and photographed on an ultraviolet transilluminator. Images were captured on a black and white film.

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3.5.8 Hybridization with O. volvulus and O. ochengi specific oligonucleotides

To differentiate *O. volvulus* from *O. ochengi*, each PCR product was hybridised separately to two different digoxygenin (DIG)-labelled DNA probes one specific for *O. volvulus* and the other for *O. ochengi*.

5µl of *O. volvulus* specific digoxygenin (DIG)-labelled DNA probe solution were added to 10µl of each PCR product in a 1.5ml tube. This was incubated at 94˚C for 10 minutes for the denaturation of the DNA. The denatured PCR product was centrifuged at 14000rpm for 10 seconds to bring down vapour from the lid of the tube. This was again incubated for 30 minutes at 37˚C for the hybridization of oligonucleotides probes to the PCR products. The procedure was repeated for each sample with *O. ochengi* specific digoxygenin (DIG)-labelled DNA probe. 5µl of each hybridized product were pipetted on to an *Onchocerca Species Detection Panel* designed to detect the presence of *O. volvulus* and *O. ochengi*. Each panel was then immersed for 10 seconds in a 1.5ml tube filled with chromatographic buffer and then left to dry for about 5-10 minutes on a bench. Positive samples were indicated by the formation of two bands on the panel. A sample (worm) is indicated to be *O. volvulus* if the hybridisation of its PCR product with *O. volvulus* specific digoxygenin (DIG)-labelled DNA probe produces a positive result. On the other hand it is *O. ochengi* if it forms the positive bands on hybridisation with *O. ochengi* specific digoxygenin (DIG)-labelled DNA probe.

All oligonucleotides used were custom syntheses from Roth (Karsruhe, Germany). Digoxygenin was detected with the DIG detection kit from La Roche-Boehringer (Mannheim, Germany)

3.6.0 Method of questionnaire administration.

Villages to be sampled were divided into 4 quarters and each quarter assigned to trained personnel. Use was made of the household listing code developed and used by the Ghana National Population Council during the 2000 National Population and Housing Censors. Houses in the communities were assigned numbers during the said exercise. In each quarter, the administrator of the questionnaire started with the house with the lowest number and proceeded by including every third house. A maximum of four persons from a house was included in the sampling. In a situation where an eligible house was vacant the next house in the series was included in the sampling. The inclusion criteria were those individuals 6 years or older or had lived in the community for the last 5 years prior to the exercise. It was one of the aims of the exercise to sample approximately 100 respondents per village.

Questions addressed pertained to the ages of the respondents, their height, the number of tablets given and the number of tablets that were actually taken by the respondent. The period between the time of issuance of the drug and it consumption was also assessed. For those respondents who did not take the treatment, the reason for their non-compliance was also assessed. The time frame addressed was the twelve months proceeding to the exercise.

3.7.0 Ivermectin Mass Distribution in the District.

Ivermectin is distributed annually in the Upper Denkyira District. It is disbursed from the district capital Dunkwa to the sub-districts through the Senior Field Technicians, who in turn disbursed them to the villages to the Community Based Surveillance Volunteers. These volunteers who have been trained in basic health delivery techniques are in charge of the distribution of the drugs at the community level.

3.8.0 Statistical analysis

Statistical analysis was carried out with the Statview package. ANOVA was used to test for homogeneity in the sizes of adult flies from different areas which was normally distributed. All other data were of negative binomial distribution and non-parametric tests were applied to them. The Chi square test was used to test for significance of the differences in the parity rates of flies from the different sampling sessions and sites. The Kruskal Wallis test and the Willcoxon Rank Sum tests were used to test for homogeneity in infection rates of the blackflies.

CHAPTER FOUR

4.0.0 RESULTS

4.1.0 Pre-control and post-control infection rates

Fly catches were conducted in 6 villages within the study area on three different catching sessions in the Upper Denkyira District of Ghana between October 2001 and November 2002. The first sampling was carried out from the end of October of 2001 to the second week of November of the same year. Mass ivermectin treatment was conducted in the district in February and March of 2002. The second and third sampling sessions (2 month post-treatment and 8 month post-treatment) were conducted in April/May and October/November of 2002 respectively. During each sampling session, pre-treatment, 2 months and 8 months after the treatment, 1909, 2132 and1941 *S. damnosum* s. l. females, respectively, were captured. Details of the dissection are given in Appendix 1. (Table 1). Mean parity rates were similar throughout the three sampling periods ($P > 0.05$, $\chi 2$). Two hundred and ninety nine (46.4% of the parous population) females were found to be infected or harbouring various stages of *O. volvulus* larvae during the pre-intervention period, of this, 270 (41.9% of parous females) carried L1/L2 larvae. Two and eight months after ivermectin mass treatment, the numbers of infected parous females were 268 (43.8%) and 250 (42.2%), respectively, and the numbers of females with L1/L2 were 248 (40.5%) and 240(40.5%) respectively(Table 1). The number of parous females with L3 in the head was 44 (6.8%) before the mass treatment and 36 (5.9%) and 39(6.6%) two

and eight months after treatment. Infection with L1/L2 larvae was especially considered since these are less affected by mortality and fly dispersion.

Plate 3: *L1 larvae found in the flight muscles of dissected fly.*

The specimen were stained with Mayer's 'hamalaum ' to enhance contrast.

Plate 4: *L3 larvae found in the flight muscles of dissected fly.*

The specimen were stained with Mayer's 'hamalaum ' to enhance contrast.

Table. 1. *Number of female flies caught, the numbers infected, mean parity rates, total infection in parous flies, infection with developing stages (L1/L2) and infection with infective larvae (L3).*

Sampling session	No. of flies captured	infected parous females (%)	Mean parity rates	females with L1/L2 (%)	females with L3 (%)
Pre-treatment	1909	299 (46.4)	33.7	270 (41.9)	44 (6.8)
2mth post - treatment	2132	268 (43.8)	28.7	248 (40.5)	36 (5.6)
8mth post - treatment	1165	250 (42.2)	35.8	240 (40.5)	39 (6.6)

Result

There were no significant differences between the geometric means of infection in the flies from the different villages during each sampling session ($P > 0.05$ Kruskal-Wallis test). This permitted the pooled results of the various sampling sessions to be compared directly. There was no statistically significant reduction in the total larval load of flies two and eight months after the ivermectin mass treatment intervention treatment ($P = 0.64$ and $P = 0.93$ respectively, Willcoxon rank sum test). No reductions were observed in the L1/L2 larval load over the same period.

Fig. 1 *Graph showing the comparison of the geometric means of the developing infections (L1/L2) and infective larvae (L3) during the three sampling sessions.*

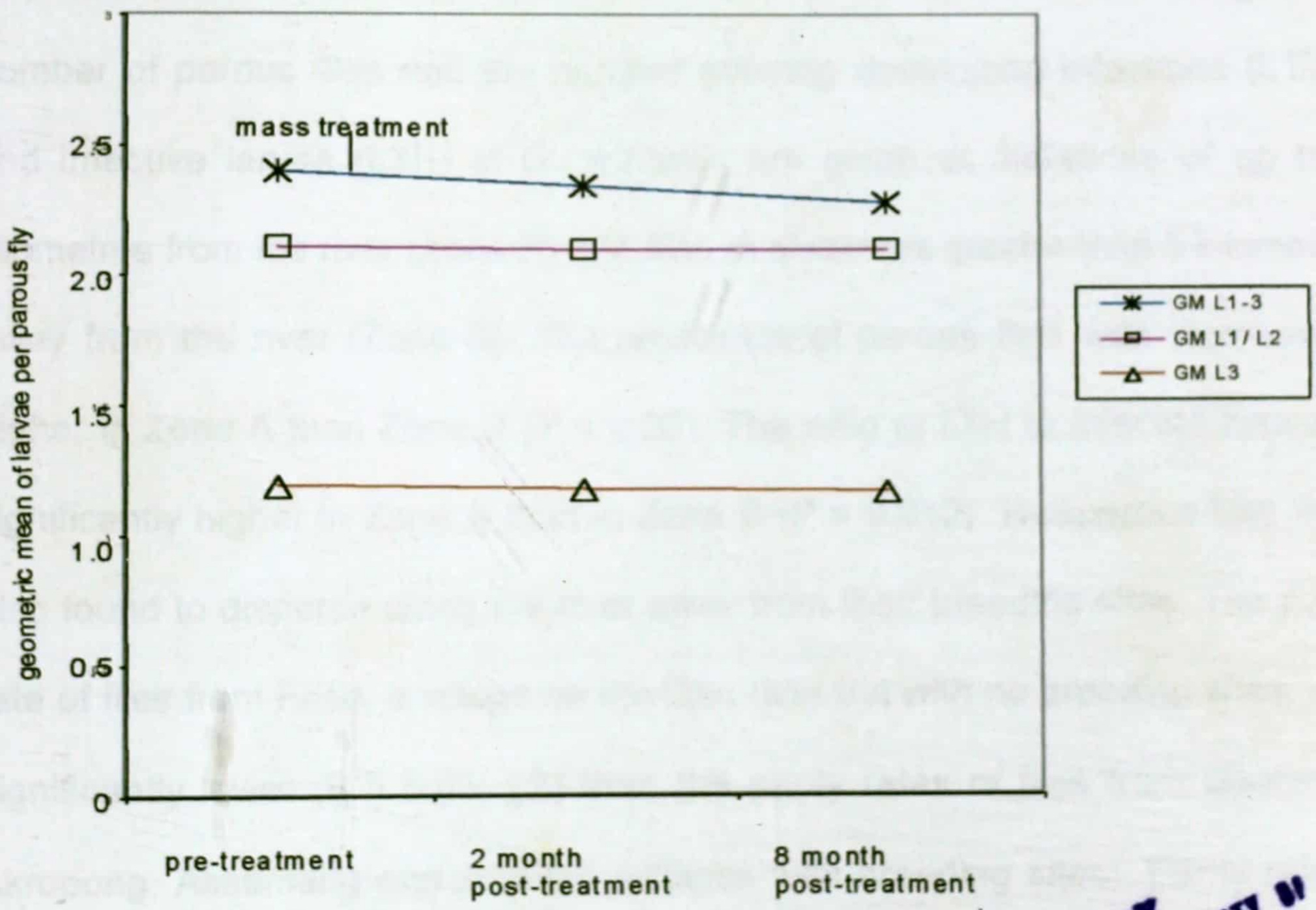

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Table 2. *Geometric means of L1/L2 (GM L1/L2) total L3 (GM L3) and total infection (GM L1-3) in parous female flies captured during the three sampling sessions.*

Sampling session	GM L1/L2	GM L3	GM L1-3
Pre-treatment	2.11	1.19	2.39
2month post treatment	2.09	1.17	2.34
8month post treatment	2.09	1.16	2.27

4. 2.1 Differential dispersal of *S. sanctipauli* 'Pra' form.

Comparison of parity rate and rate of infection with reference to distance from breeding sites was calculated (Table. 4) to further elucidate the biology and dispersal pattern of *S. sanctipauli* 'Pra' form. The number of flies caught, the number of parous flies and the number carrying developing infections (L1/L2) and infective larvae (L3H) of *O. volvulus* are given at distances of up to 5 kilometres from the river (Zone A) and also at distances greater than 5 kilometres away from the river (Zone B). The proportion of parous flies was significantly higher in Zone A than Zone B (P = 0.02). The ratio of L3H to infected flies was significantly higher in Zone A than in Zone B (P = 0.012). Nulliparous flies were also found to disperse along the river away from their breeding sites. The parity rate of flies from Foso, a village on the Ofin river but with no breeding sites, was significantly lower (P = 0.03; $\chi 2$) than the parity rates of flies from Buabinso, Akropong, Assamang and Awisem (villages with breeding sites). Parity rate of

flies from Foso was however found to be similar to parity rates of flies captured at distances greater than 5 kilometres inland (P = 0.12). Furthermore, the ratio of L3H to infected flies was significantly greater (0.02) near the river than away from it (Table 3.). There was however no difference between the ratio of L3H to total number of infected flies between Fosu on one hand and Buabinso, Akropong, Assamang and Awisem (villages with breeding sites) on the other (P = 0.23; $\chi 2$).

There was also no difference between the ratio of L1/L2 to the total number of infected flies between flies captured within 5 kilometre distance and those captured at distances greater than 5 kilometre.

Table 3. *Numbers, parity and the infections by the different stages of* O. volvulus *in flies caught at various distances away from the rivers during the 8-month post-treatment sampling session.*

Distance from river	Number of flies						
	Total	Parous (%)	infected with larvae	with L1/L2	with L3H	with L1/L2 per infected fly	with L3H per infected fly
≤ 5km Zone A	910	417^a (45.8)	175^a	167^a	32^a	0.95^a	0.18^a
> 5 km Zone B	890	175^b (19.7)	75^a	73^a	7^a	0.97a	0.09^b

Figures with different superscripts are significantly different (P < 0.05). Figures with the same superscript are not significantly different (P> 0.05).

Table 4. *Numbers, parity and the infections by the different stages of* O. volvulus *in flies caught in 5 villages on the rivers Pra and Ofin during the 8 month post-treatment sampling session*

Distance from river	Total number of flies						
	Total	Parous (%)	infected with larvae	with L1/L2	with L3H	with L1/L2 per infected fly	with L3H per infected fly
Awisem	130	71 (55)	36	35	5	0.97	0.12
Assamang	122	85 (70)	39	37	10	0.92	0.26
Foso	191	28 (15)	10	10	2	1.00	0.20
Akropong	256	100(39)	41	40	6	0.98	0.15
Buabinso	238	89 (37)	27	25	2	0.93	0.07

4.3.1 Age spectrum of biting population

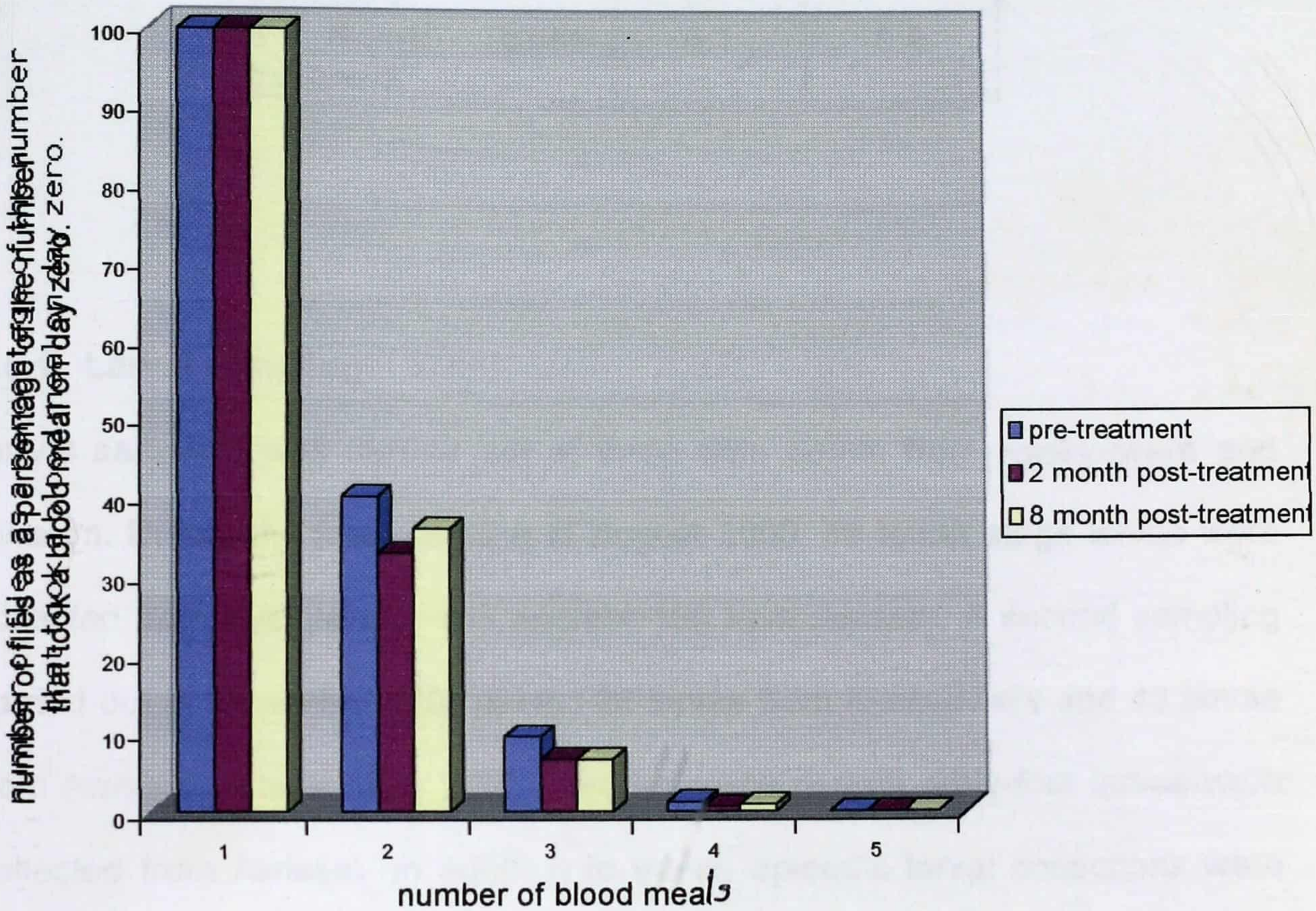
Table 5. *Number and the proportions of flies coming for their first (1ST B-M), second (2ND B-M), third (3RD B-M), fourth (4TH B-M) and fifth (5THB-M) blood meals for the three sampling sessions*

Sampling session	Number of flies coming for				
	1ST B/M (0-2) days	2ND B/M (3-5) days	3RD B/M (6-8) days	4TH B/M (9-11) days	5TH B/M (12-15) days
Pre-treatment	589	236	56	7	1
2 month post treatment	648	212	43	7	1
2 month post-treatment	572	206	38	7	1

The ages in days of the flies in each category are also shown.

The age spectrum of the fly population was undertaken based on the model proposed by Duke(1967a). This was done to determine the life span of *S. sanctipauli* 'Pra' form. The calculations involved are given in Appendix III. Duke (1967; 1968) postulated that flies had to take a blood meal within the third day of emergence and thence once every third day otherwise they perished. As only about 30% of all flies caught in this study were parous, it can be assumed that a minimum of 70% of the flies do not live beyond the third day. Flies infected with L1 and or with L2 are considered to be coming for a blood meal subsequent to the first whilst flies with L3 and double infections are considered to be coming for a meal subsequent to the second. The number of flies that survived from the first blood meal to the second was less than 40% during all three sampling sessions (Fig. 3). By the third blood meal, the percentage had reduced to less than 10% of the original number and by the 5th blood meal, the percentage had reduced virtually to nil (Fig. 3). Flies coming for their first blood meal were deemed to be between 0 and 2 days old (Duke; 1967a). Flies coming for a second blood meal were considered to be between 3 to 5 days old. By the 5th meal flies were considered to be between 12 to 15 days old (Table 7). The age spectrum showed very little variations with different sampling sessions.

Fig. 2. *Survival rate (%) of blackflies that initially took blood and the percentages that survived after subsequent blood meals during the three catching sessions.*



4.4.1 The Survival of *O. volvulus* parasites in *S. sanctipauli* 'Pra' form under natural conditions.

The geometric mean numbers of larvae per flies harbouring infections caught before the mass treatment were L1/L2, 5.9; and at the infective stage, 5.3 (Table 9). The numbers for flies harbouring infections at 2 months and 8 months after treatment were 6.1, 5.6 and 6.1, 5.8 respectively (Table 6)

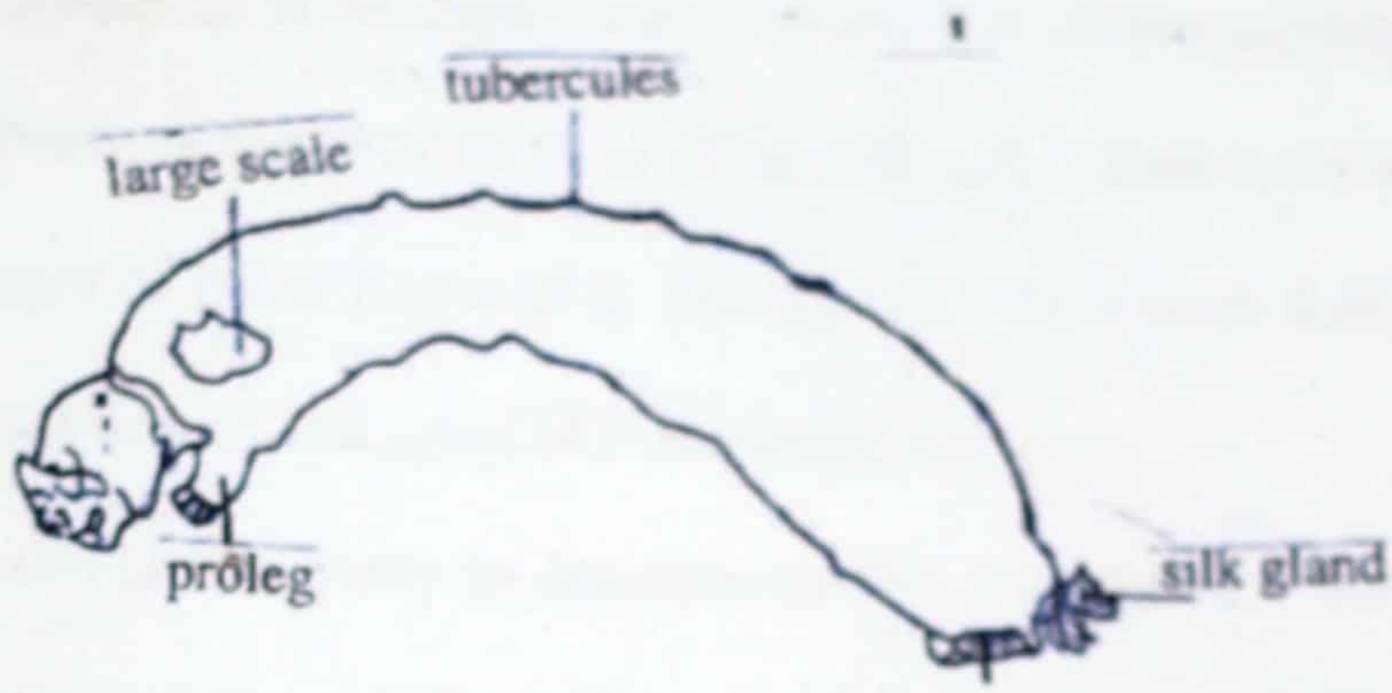
Table 6. The Geometric means of pre-infective (GML1/L2) and infective stages (GML3) of O. volvulus per infected flies during the three sampling sessions.

Sampling session	GML1/L2	GML3
Pre-treatment	5.9	5.3
2 month post-treatment	6.1	5.6
8 month post-treatment	6.1	5.8

4.5.1 Larval sampling

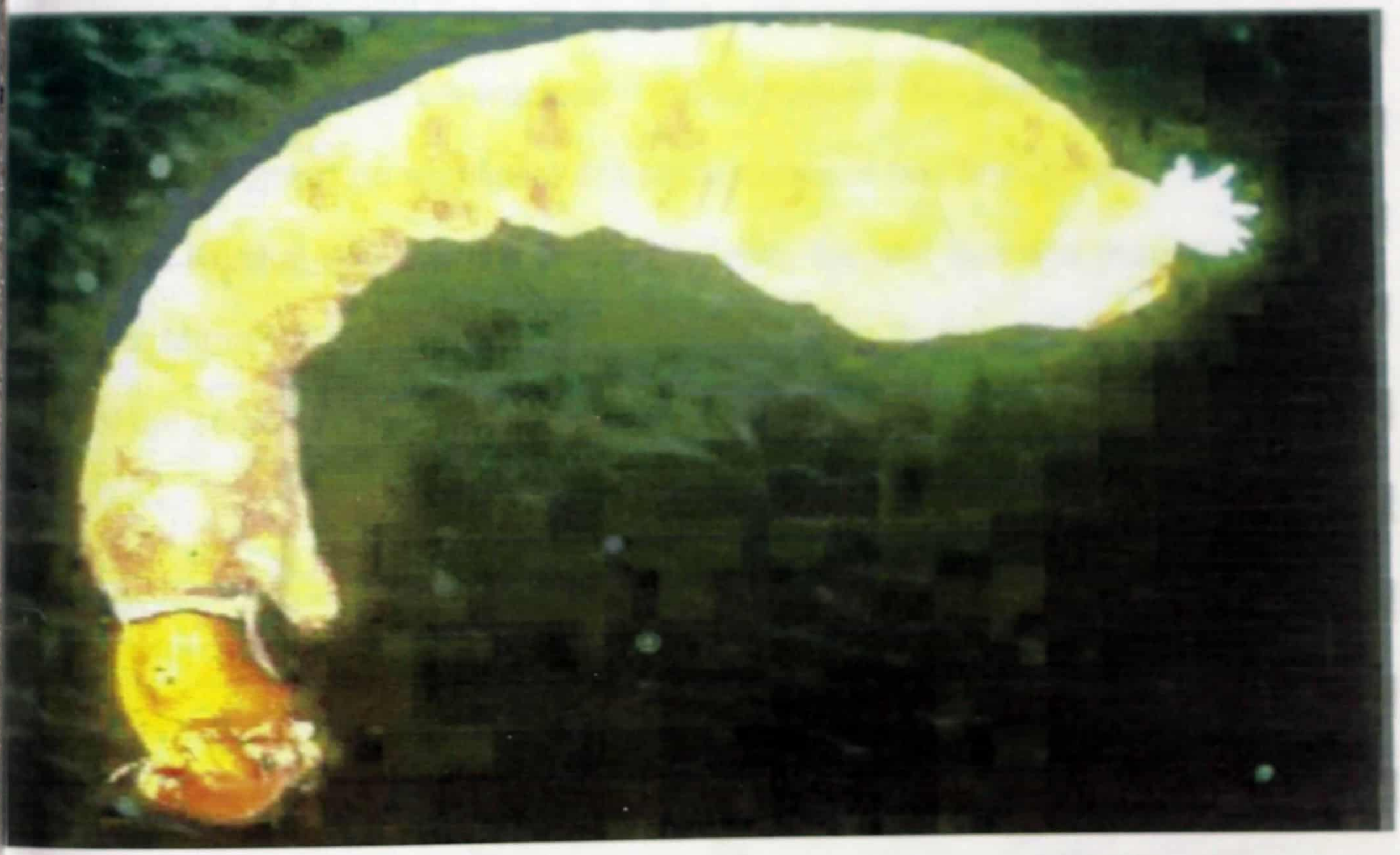
Larval sampling was carried out at three time points from Kyekyewere and Awisem. During the first sampling in August 2000, 58 fourth stage larvae were collected from Kyekyewere and another 106 from Awisem. A second sampling carried out in November 2001yielded 26 larvae from Kyekyewere and 43 larvae from Awisem. In November 2002 (third sampling period), forty-four larvae were collected from Awisem. In addition to these, episodic larval collections were carried out particularly in Buabinso and Assamang in August of 2001. No larval prospecting was carried out at Akropong. In all cases, the larvae examined presented with no tubercles and no large scales. This was in contrast to typical larval morphology of *S. sanctipauli*, which has very scanty, but nevertheless visible, tubercles, and large scales (Plate 6).

Plate 5: *Showing the morphology of the larvae of S. sanctipauali s.s*



courtesy WHO training manual for technicians of the OCP

Plate 6: *Showing the morphology of the larva of S. sanctipauali 'Pra' form.*



4.6.1 Morphometric analysis

Boakye et al., (1999) cytotaxonomically evaluated the status of the black fly population breeding at Awisem by examining the chromosomes of the larvae and described them as the 'Pra' form of *S. sanctipauli*. . This form was thought to be a genetic variant of other forms of *S. sanctipauli*. Their work focused exclusively on the larvae but not on the adult of this variant species.

It was the aim of this study to describe for the first time the morphological characteristics of the adult 'Pra' form of *S. sanctipauli* . The sizes of the flies captured from the different sampling sites were characterized by low variance (0.001). There was no significant difference between the lengths of the thoraxes (ANNOVA: P = 0.65) and antennae (P = 0.36) of the flies from the various sites. Table 7 shows that there was also no difference between the antennae and thoraxes of the wild flies and the flies hatched from pupae collected from the Awisem rapids (P = 0.9 antenna; P = 0.71 thorax). The ratio of thorax to antenna length depicted no significant difference between flies from different zones (P = 0.06; Kruskal-Wallis test) and also between the wild flies and reared flies (P = 0.5; Mann-Whitney U test). These result suggests that the wild caught flies were of the same stock as the reared ones.

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Plate 7: *Showing the adult of Simulium sanctipauli 'Pra' form at it resting position.*

Table. 7. *Table of the median thorax lengths, median antenna length and sample size of female* S. sanctipauli *'Pra' form caught during the 8 month post- treatment sampling session and reared pupae collected from the Awisem rapids in November 2002*

Source of fly	Sample size of female flies	Median thorax length	Median antenna length
Hatched from pupae	67	2.12	1.11
> 5km from river	188	2.13	1.11
≤ 5km from river	349	2.12	1.11

Data does not include measurement from savanna flies.

Wild caught flies as well as the flies reared from pupae were generally of the *S. sanctipauli* s.s morphologically with dark wing-tufts (05 on the scale of assessment given in materials and methods), pale scutella hairs (1) and dark arculus (3). They also presented with a mixture of pale and dark ninth tergit setae, which however, had more pale than dark setae (2). Setae of the costa were entirely pale (1). Also found with the wild catches were a few flies (12), whose setae were all pale and these were classified as 'savanna' types. These flies which generally were smaller in sizes had a median antenna and thorax lengths of 0.83 and 1.83 respectively. The mean ratio of their antenna to thorax length was 2.20

4.7.1 Mass treatment with ivermectin

The questionnaire approach was adopted to estimate the proportion of the community that were covered by the mass treatment in the 6 main villages. A total of 614 people from six villages were sampled and questionnaires administered. The sample size ranged from a low of 98 persons in Akropong to a high of 116 in Ayebiawhe. The overall coverage of the ivermectin mass treatment was about 24.4%, ranging from 0% in Buabinso to a high of 37% in Akropong (Table 8).

Table 8. *Mass treatment participation in the 6 villages as revealed by response to questionnaire.*

Village	No of respondents	No of treated respondents (%)
Buabinso	100	0 (0.0)
Akropong	98	36 (36.7)
Foso	99	28 (28.3)
Ayebiawhe	116	27 (20.7)
Assamang	100	32 (32.0)
Awisem	101	30 (29.7)
Total	614	150 (24.4)

There was a total rejection of treatment in Buabinso. The coverage in the 5 remaining villages where treatment took place was below 30 percent.

Table 9. *Showing the number of respondents (N) who did not participate in mass treatment and the reasons given for their non-participation.*

village	N	Not aware (%)	Travelled (%)	Not important (%)	Unpleasant side effect (%)	Others (%)
Buabinso	100	18 (18)	5 (5)	6 (6)	70 (70)	1 (1)
Akropong	62	0 (0)	2 (3.2)	1 (1.6)	57 (91.9)	2 (3.2)
Foso	71	3 (4.2)	0 (0)	5 (7.0)	63 (88.7)	0 (0)
Ayebiawhe	92	21(22.8)	3 (3.3)	2 (2.2)	63 (68.5)	3 (3.3)
Assamang	68	3 (4.4)	3 (4.4)	8 (11.8)	49 (72.1)	3 (4.4)
Awisem	71	4 (5.6)	5 (7.0)	5 (7.0)	52 (73.2)	5 (7.0)
Total	462	49 (10.6)	18 (3.9)	27 (5.8)	354 (76.6)	14 (3.0)

The percentages per N are given in brackets.

4.8.1 Amplification of O-150

Using the primers described in chapter 3, the PCR product of O-150 was amplified from filaria larvae obtained from 100 flies infected with L3. These were randomly selected from the total of 158 flies infected with L3. In addition, O-150 PCR products from 50 flies containing developing forms of larvae indistinguishable from *O. volvulus* were also amplified (Table. 10). Amplification was achieved from samples collected from all catching sites and from all sampling sessions.

4.8.2 Hybridisation of PCR products

All the 150 samples subjected to the PCR were hybridized to the *O. volvulus* specific DNA probe and also to the *O. ochengi* specific DNA probe, regardless of whether an amplification product was visible on the agarose gel or not. DIG-detection kit from Boehringer (Mannheim, Germany) was used to separate *O. volvulus* from *O. ochengi.*

Out of the 95 detectable PCR obtained from flies with L3, 96 hybridized with the *O. volvulus* specific DNA probe whilst none did so with the *O. ochengi* specific DNA probe. Of the 50 flies that contained developing larvae, 34 produced detectable PCR product whilst 35 gave positive reaction on hybridisation *O. volvulus* specific DNA probe.

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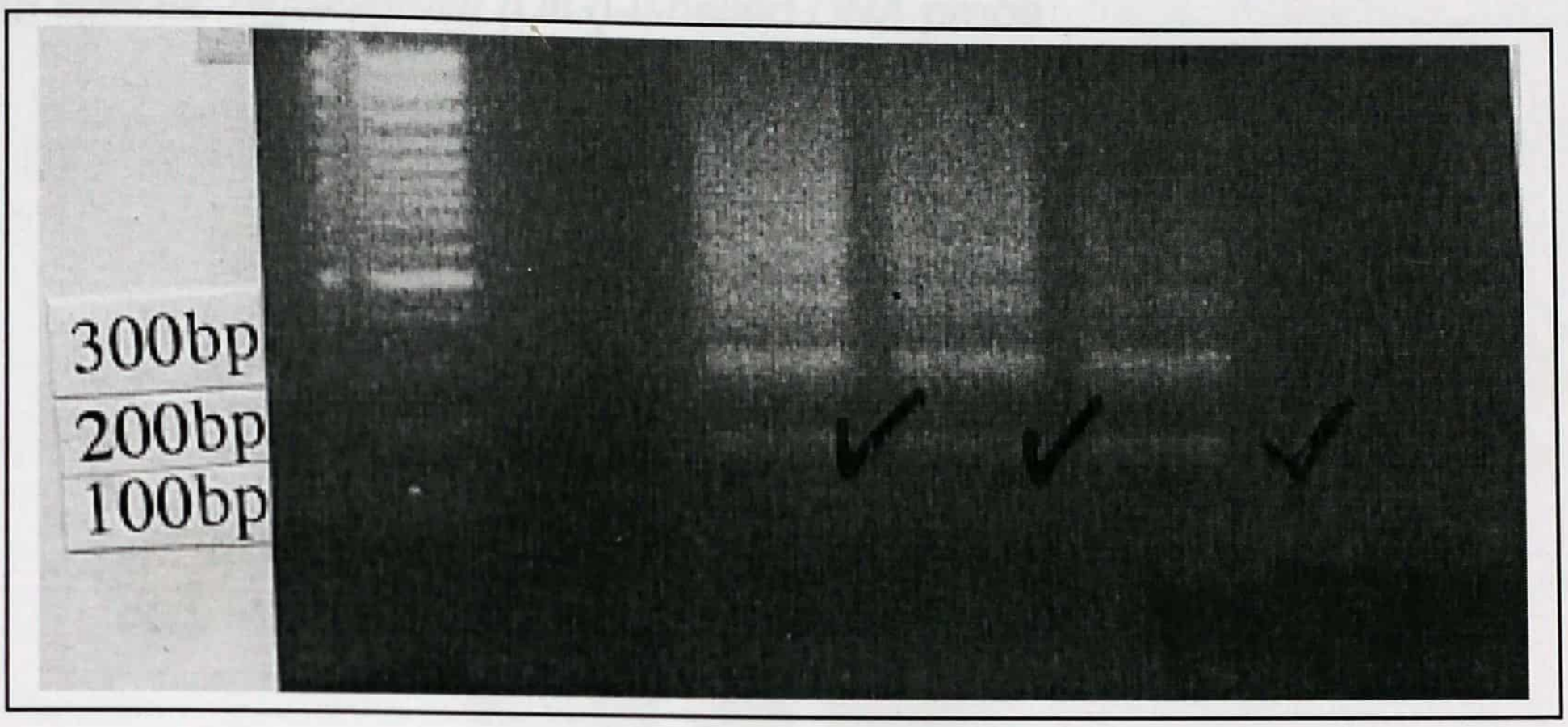
Plate: *O-150 ploymerase chain reaction (PCR) products in O. volulus infection in blackflies .The PCR products were run on 2% agarose gel and stained with ethidium bromide.* (lane 1 *100-bpair ladder;* lane 2 to 5 *genomic O. volulus DNA;* lane 6 *negative control*).

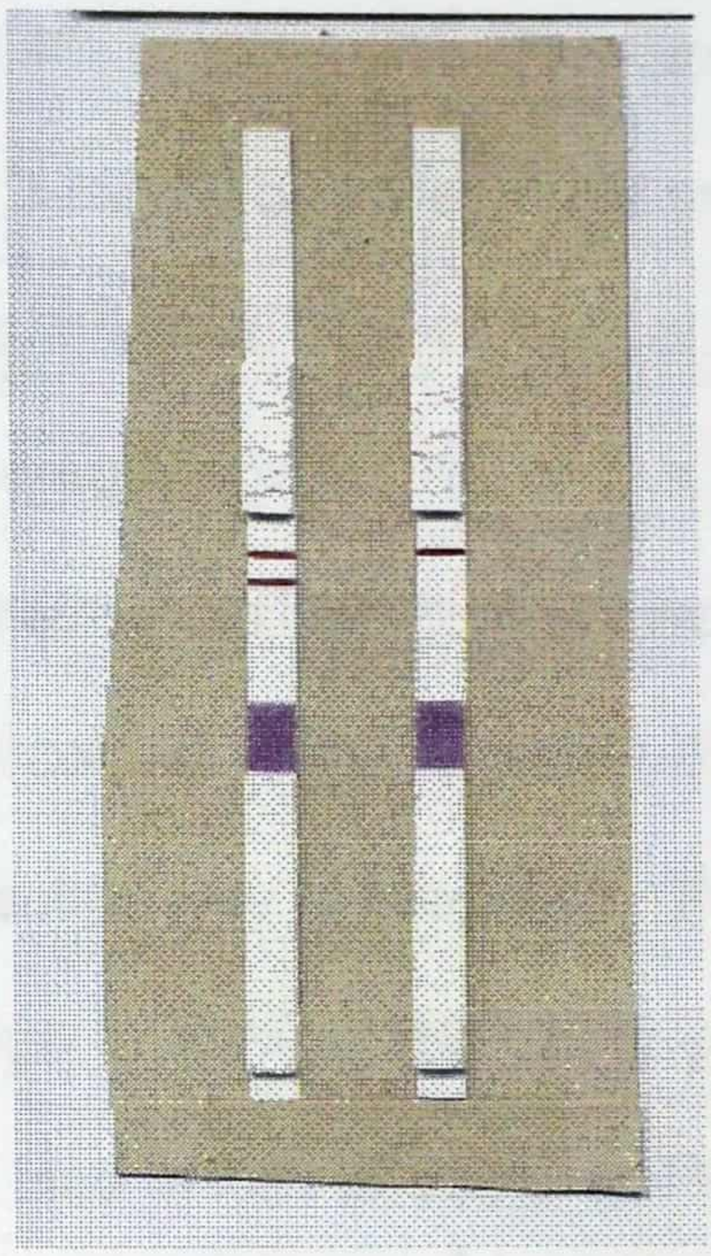


Picture shows typical 150bpair tandem repeats.

Table. 10 *Presence of Onchocerca volvulus (Ov+) determined by hybridisation of species specific oligonucleotides to amplification products (PCR+) obtained from filaria larvae from dissected S. sanctipauli captured from the Upper Denkyira District of Ghana between October 2001 and November 2002.*

Sampling session & Village	No. of blackflies containing									
	Larvae total		L1		L2		L2 thorax		L3 head	
	PCR +	Ov+	PCR +	Ov+	PCR +	Ov+	PCR +	Ov+	PCR +	Ov+
Pre-treatment	351	352	2	2	5	5	5	5	32	33
2 months post-treatment	299	302	1	1	3	4	6	6	30	30
8 months post-treatment	311	313	1	1	5	5	4	4	33	34

Plate: *Showing Onchocerca Species Detection Panel. Left panel indicates positive reading after hybridization with O. volvulus specific digoxygenin (DIG)-labelled DNA probe. Right indicates negative reading after hybridization with O. ochengi specific digoxygenin (DIG)-labelled DNA probe*



CHAPTER FIVE

5.0.0 DISCUSSION

5.1.0 Pre-treatment and post- treatment infection rates.

During the pre-treatment sampling session, 1909 blackflies were collected and examined out of which 46.4% of the parous flies were infected with *O. volvulus* larvae. The figures for the 2-month post-treatment sampling were 2132 blackflies with 43.8% of the parous infected. Out of a total of 1165 blackflies collected during the 8-month post-treatment 40.5% of the parous flies were infected. Results from the questionnaire administration showed that a less than 30% mass treatment coverage, as recorded in the treated villages, can not bring about a significant reduction in infection rate of blackflies. Previous studies revealed that a mass treatment coverage of 60% of the study population resulted in drastic reductions in the infection load in flies (Rodriguez-Perez *et al.*, 1995, Davis *et al.*, 1997). However there has not been a systematic study aimed at determining the level of mass treatment coverage below which no noticeable drop in the infection rates in the vector could be discerned. There was no dramatic reduction in the infection rates at 2 and 8 months after mass treatment in villages where mass treatment was undertaken and these rates were similar to that of Buabinso where no mass treatment (30%) took place. It is therefore reasonable to assume that 30% treatment coverage is insufficient to result in a significant drop in the vector infection rate.

5.2.0 Differential dispersal of *S. sanctipauli* 'Pra' form

The nulliparous flies dispersed selectively inland and away from the breeding sites whereas the parous and infective flies were proportionally more abundant near the river than inland. This observations stand in stark contrast to that of Duke (1975) who reported that nulliparous population of forest species of *S. damnosum* s. l. did not disperse selectively inland.

There appears to be no hindrance to the dispersal of infective flies inland to distances up to 10 kilometres as infective flies were captured as far off the river as at Kumakrom a distance of about 10 km from the nearest river. The ratio of the number of flies infected with L1/L2 to the total number of flies infected was the same for flies collected within the 5 km radius of the main rivers as well as for flies collected from more that 5 km away from the rivers (Table 6). However, the ratio of the number of flies infected with L3 in head to the total number of flies infected was significantly higher for flies collected within the 5 km radius of the main rivers than for flies collected from more that 5 km away from the rivers (Table 6). Since in all probability flies with L1/L2 larvae are relatively younger than flies with L3 infections, it seems probable, that the differential dispersal of *S. sanctipauli* 'Pra' form is largely age-dependent and not related to infection with *O. volvulus,* with older flies staying closer to the rivers.

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5.3.0 PCR and Dipstick results.

The result presented here clearly confirms the work of Fischer *et al*(1997) who demonstrated that blackfly dissection, combined with PCR and subsequent nonradioactive DNA probing is a suitable procedure for identifying *O. volvulus* in vectors. Detectable PCR products could not be amplified from all samples analysed. Non-amplified samples were obtained mainly from blackflies that mainly contained one to three L1 larvae or three L3 larvae. In these cases, the non-amplification was attributed to faulty DNA extraction procedure since all larvae identified by microscopy were morphologically identical to *O. volvulus*. There was not the possibility that non-amplified DNA were from species other than an insufficient DNA extraction was assumed. However it can not be excluded that some blackflies may have contained filarial species other than *O. volvulus* and the primers failed to amplify O-150. This possibility can be rejected because all examined filaria larvae were morphologically indistinguishable from *O. volvulus*. Toe and co-workers (1994) used a similar PCR assay and reported that in the OCP area, only 77% of the samples containing infective L3 from vectors produced detectable PCR products and discussed a number of possible explanations. These included the loss of larval samples during the experimental manipulations involved in preparing them for PCR, or that inhibitors were carried through the DNA preparation process that prevented successful PCR amplification in some samples. In this study, there was a close positive correlation between the detection of PCR by gel electrophoresis and detection of

hybridized PCR products to species specific oligonucleotide probes by *Onchocerca Species Detection Panel*. However there was consistently a slightly higher detection rates with the panels than with gel electrophoresis (Table 12). This could have resulted from a difference in the sensitivity of the two assays with the panels having a higher sensitivity than the gel electrophoresis.

Most reported cases of *O. ochengi* infections were made in the savanna flies, *S. damnosum/sirbanum* (Duke, 1967b). The status of *O. ochengi* infection in the forest flies has been less well investigated. The vector-parasite complex postulates that the savanna-dwelling black fly species can be efficient vectors for the savanna strain *O. volvulus* and that the forest-dwelling black fly species act as efficient vectors for the forest strain. It is possible that this postulate holds true for *O. ochengi* infection, and that cattle imported from the north of Ghana, and other Sahel countries may harbour *O. ochengi* which may fail to develop in the predominantly forest fly populations. Alternatively the absence of *O. ochengi* could be a result of lack of infection in the cattle. Since no assessment was carried out to determine the presence of infection in the cattle population we can only speculate on this.

The prevalence of filaria infection in the parous flies in the area was more than 40%. This finding together with the fact that no other filarial species other than *O. volvulus* could be clearly identified in the blackflies suggests that the *S. sanctipauli* 'Pra' form is highly anthropophilic and also a very efficient vector of human onchocerciasis. In contrast most studies on *S. sanctipauli* in West Africa reports of a rather high degree of a zoophilic tendency and poor vectorial

competence (Garms, 1973b; Quillevere *et al.*, 1977a). The reason for the observed discrepancy could imply that a new sub-species of *S. sanctipauli* was encountered during the study as indicated by the rather atypical dispersal patterns of the fly populations encountered in this study as well as their rather uncharacteristic larval morphology. This supports the work of Boakye and co-workers (1999) who speculated that the IIIL-B chromosomal differentiation shown by larvae collected from Awisem was important in population sub-structuring leading to speciation (Procunier, 1982). However the dramatic changes in the habitat as a result of upsurge in farming activities due to an explosion in the human population size might have caused a paucity of other vertebrate hosts of the fly resulting in a closer man-fly contact. The population size of the study area in the 1950s was not available but it is reasonable to assume that the population size has exploded since then in tandem with the national figure which increased from a little below 5 million in the 1950s to about 18 million in 2002. Taken together, it can be concluded that very few, if any, filarial species other than *O. volvulus* is transmitted by *S. sanctipauli* 'Pra' form in the Upper Denkyira District of Central Region of Ghana.

5.4.0 Result of ivermectin coverage

Various trials on the efficacy and the acceptability of ivermectin demonstrated that mass scale treatment with ivermectin was both practicable and capable of reducing the microfilarial load in the population (WHO; 1987). A critical examination of these trials however reveals that they were conducted under conditions that can be considered atypical of the conditions likely to be encountered in most West African communities where the organisational structures are virtually non-existent. An assumption was made that the delivery and distribution of the drug at the community level would be comfortably absorbed into the national health delivery system at a marginally extra cost. It was also assumed that side reactions due to ivermectin therapy (thought of as a mere inconvenience) should not pose a significant threat to the success of the mass treatment scheme (WHO; 1987).

The study revealed that the delivery of ivermectin to the community level was rather easily accomplished. Every village health worker interviewed indicated that the drugs were submitted in sufficient quantities. However, the assumption that problems arising from side effects of ivermectin therapy could be easily overcome proved to be unfounded. More than 70% of the respondents who refused treatment cited adverse reactions to ivermectin in an earlier treatment

round (either by themselves or by someone known to them) as the reason for their non-compliance (Table 11). There is little doubt that adverse reaction due to ivermectin therapy when properly managed by adequately trained health personnel need not significantly derail the mass treatment campaign. However in most of West Africa where health facilities are over stretched or non-existent this may represent an impossible undertaken. In the light of the finding of this study, a critical look must be taken of the decision by APOC to completely devolve responsibility of the mass treatment scheme to the governments and Ministries of Health of the participating countries.

5.5.0 The Survival of *O. volvulus* parasites in *S. sanctipauli* 'Pra' form under natural conditions.

The geometric mean numbers of larvae per flies harbouring infections caught before the mass treatment were L1/L2, 5.9; and at the infective stage, 5.3 (Table 8). The numbers for flies harbouring infections at 2 months and 8 months after treatment were 6.1, 5.6 and 6.1, 5.8 respectively. The number for the third stage larvae probably errs on the lower side as it can not be excluded that some flies did bite some vector collectors before being caught a process that could result in the shedding of some L3 larvae. It appears therefore that once the parasites evolve from the microfilariae to the L1/L2 stage, their mortality rate is much reduced. The *S. sanctipauli* 'Pra form is therefore an excellent host for *O. volvulus*

5.6.0 Age spectrum of biting population

According to the proposed model (Duke, 1967), flies infected with L1 and L2 were assumed to be coming for a bite subsequent to an earlier infective one whereas flies infected with L3 larvae were coming for their third or subsequent blood meal. Flies with both L1 and L3 infections were assumed to be coming for a blood meal subsequent to fourth. Duke (1967; 1968) gave evidence that nulliparous black flies had to take a blood meal within three days of emergence and thence at least once every fourth day or perish. Flies that had obtained blood meals adequate to trigger ovarian development did not come to feed again until they had digested the blood, matured their ovaries, and oviposited-processes which, at the earliest were not completed before the end of day 2 (Duke, 1967). It can thus be estimated that L1 larvae are approximately 3 days old and L3, 6 days old. L2 larvae are considered to be 4 to 5 days old. For simplicity, nulliparous flies were assumed to bite on day zero and may return for a subsequent blood meal with its parasite at any stage of development up to the infective larvae stage (Duke, 1968). In practice, the time that a fly might return for a subsequent blood meal can be estimated from the stage of development of its parasites. Since there is no reason to assume that infection with *O. volvulus* larvae significantly affect the behaviour and survival pattern of the flies, the

assumption can be made that the age distribution of the infected population would reflect that of the entire population.

It can be deduced from the result presented that the 'Pra' form of *S. sanctipauli* has a rather short life span with flies having a maximum life expectancy of about 15 days (Table 6). Less than 10% of parous flies lived to a stage long enough to support infective larvae. This may seems to present a major impediment in their transmission ability. However the fairly large population size of the flies and their rather high infection rate, an indication of excellent transmission ability, seems capable of compensating for this short life span.

6.0.0 Recommendations

Various trials on the efficacy and the acceptability of ivermectin demonstrated that mass scale treatment with ivermectin was both practicable and capable of reducing the microfilaria load in the population (WHO; 1987). A critical question is whether ivermectin will be well accepted at the community. It was also assumed that side reactions due to ivermectin therapy (thought of as a mere inconvenience) should not pose a significant threat to the success of the mass treatment scheme (WHO; 1987). However, the assumption that problems arising from side effects of ivermectin therapy could be easily overcome proved to be unfounded. More than 70% of the respondents who refused treatment cited adverse reactions to ivermectin (either by themselves or by someone known to them) in an earlier treatment round as the reason for their non-compliance (Table 9). These findings contrasted sharply with those of Biritwum et al (1997) who reported the main reasons for people not taking the necessary treatment rounds in a work carried out in Ghana, Benin and Togo as absence (54.5%); non-eligibility (12.2%); refusal (2.6%) and shortage of drugs (1.9%). The weight of evidence gathered from several well-supervised trials with ivermectin showed those adverse reactions after treatment with ivermectin poses no serious or life-threatening side effects. It was also demonstrated that when these side-effects occurred, they regressed after a few days without treatment or just with the

administration of analgesics (Greene et al., 1985; Paque et al., 1989; Paque et al., 1991a and Paque et al.1991b). The reason for this contrasting finding may be traced to the fact that in the Upper Denkyira District, and also in most parts of Ghana, ivermectin distribution was carried out mainly by the village health workers who had not the expertise in handling compliant resulting from adverse reaction to the drug. The implication is that the mass treatment scheme must be, as much as possible, accompanied by trained health providers capable of dealing with the adverse reactions. This will undoubtedly increase the cost of the mass treatment scheme. However this extra cost may need to be borne only during the first few years of the mass treatment scheme as it has been reported that repeated treatments resulted in increasingly milder reactions (Paque et al.1991). There is little doubt that adverse reaction due to ivermectin therapy when properly managed by adequately trained health personnel need not significantly derail the mass treatment campaign.

The bio-ecology of the 'Pra' form of *S.sanctipauli* revealed it to be a very highly antropophilic fly with an excellent ability to host *O. volvulus*. These features argue more for a semi-annual mode of ivermectin mass distribution as against the annual distribution as is the case presently. It was also shown that the intensity of infection of *O. volvulus* in the flies was twice as much near the river than 5km away from it. This suggests that semi-annual treat, if found to be expensive, could be limited to villages

that skirt the rivers whilst the annual distribution is continued for villages 5km of the river.

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APPENDIX I

IVERMECTIN MASS TREATMENT COVERAGE DETERMINATION QUESTIONAIRE

DATE _____

CODE No. _____

VILLAGE _____

AGE _____

HEIGHT _____

1. DID YOU GO FOR THE TABLETS YES ☐ NO ☐

IF NO GO TO 6

2. DID YOU TAKE THE TABLETS YES ☐ NO ☐

3. HOW MANY TABLETS WERE YOU GIVEN 1 ☐ 2 ☐ 3 ☐ 4 ☐

4. HOW MANY OF THE TABLETS DID YOU TAKE 1 ☐ 2 ☐ 3 ☐ 4 ☐

5. IN WHICH MONTH WE RE YOU GIVEN THE DRUG _____

APPENDIX I

6. WHY DID YOU NOT TAKE PART IN THE TREATMENT

i) NOT AWARE _____

ii) TRAVELLED _____

iii) NOT IMPORTANT _____

iv) UNPLEASANT SIDE EFFECT _____

v) OTHERS (SPECIFY) _____

APPENDIX II

Infections with *O.volvulus* in *Simulium damnosum* s.l in the Upper Denkyira District from the pre-treatment and the 2 month post-treatment sampling sessions

Nov -2001																							
Place	caught	NP	Par	diss	L1	L2	L3T	L1/L2	L3TA	L1-3	L1	L2	L3H	L1/L2	L3TA	L1-3	par	L1/L2	L3T	L1-3	L1-2	L3T	1000p
Awisem	261	153	108	108	429	94	22	523	24	569	43	19	7	57	4	58	41.4	52.8	6.5	53.7	9.2	3.1	204
Assamang	347	230	117	117	226	61	64	287	43	394	40	15	8	49	9	54	33.7	41.9	6.8	46.2	5.9	8	547
Fosu	379	292	87	87	223	113	29	336	46	411	25	16	4	34	11	41	23	39.1	4.6	47.1	9.9	7.3	333
Ayebiawhe	379	296	83	83	134	87	22	221	46	289	22	12	5	32	8	37	21.9	38.6	6	44.6	6.9	4.4	265
Akropong	308	170	138	138	335	79	35	414	23	472	49	16	10	62	9	67	44.8	44.9	7.2	48.6	6.7	3.5	254
Buabinso	235	124	111	111	266	25	36	291	34	361	32	8	10	36	8	42	47.2	32.4	9	37.8	8.1	3.6	324
Total	1909	1265	644	644	1613	459	208	2072	216	2496	211	86	44	270	49	299	33.7	41.9	6.8	46.4	7.7	4.7	323

May -2002																							
Place	caught	NP	Par	diss	L1	L2	L3T	L1/L2	L3TA	L1-3	L1	L2	L3H	L1/L2	L3TA	L1-3	par	L1/L2	L3T	L1-3	L1-2	L3T	1000p
Awisem	216	113	103	103	261	19	4	280	1	285	39	4	1	40	1	41	47.7	38.8	1	39.8	7	4	39
Assamang	181	79	102	102	420	61	141	481	62	684	43	15	16	49	12	57	56.4	48	15.7	55.9	9.8	8.8	1382
Fosu	554	430	101	101	221	79	26	300	16	342	35	15	4	42	6	47	18.2	41.6	4	46.5	7.1	6.5	257
Ayebiawhe	402	301	101	101	230	4	12	234	9	255	39	3	4	39	5	40	25.1	38.6	4	39.6	6	3	119
Akropong	271	164	103	103	373	77	74	450	40	564	39	16	9	40	9	44	38	38.8	8.7	42.7	11.3	8.2	718
Buabinso	508	406	102	102	234	46	6	280	4	290	36	7	2	38	3	39	20.1	37.3	2	38.2	7.4	3	59
Total	2132	1493	612	612	1739	286	263	2025	132	2420	231	60	36	248	36	268	28.7	40.5	5.9	43.8	8.2	7.3	430

APPENDIX II

Infections with *O.volvulus* in *Simulium damnosum* s.l in the Upper Denkyira District from the 8 month post-treatment sampling sessions

Nov - 2002						No. of larvae					No. of flies with						%	% parous flies with			larvae/fly		L3H
Place	Caught	NP	Par	diss	L1	L2	L3T	L1/L2	L3TA	L1-3	L1	L2	L3H	L1/L2	L3TA	L1-3	par	L1/L2	L3T	L1-3	L1-2	L3T	1000p
Awisem	130	59	71	71	332	23	50	355	39	444	29	9	5	35	5	36	55	49.3	7	50.7	10.1	10	704
Assamang	122	37	85	85	247	28	48	275	27	350	34	7	10	37	10	39	70	43.5	11.8	45.9	7.4	4.8	565
Fosu	191	163	28	28	17	7	6	24	1	31	6	4	2	10	1	10	15	35.7	7.1	35.7	2.4	3	214
Ayebiawhe	228	184	44	44	98	8	7	106	5	118	14	4	2	15	2	16	19	34.1	4.5	36.4	7.1	3.5	159
Akropong	256	156	100	100	346	32	36	378	9	423	37	7	6	40	3	41	39	40	6	41	9.5	6	360
Buabinso	238	149	89	89	283	40	8	323	8	339	22	3	2	25	3	27	37	28.1	2.2	30.3	12.9	4	90
Buabin	45	31	14	14	78	14	0	92	0	92	8	1	0	8	0	8	31	57.1	0	57.1	11.5		0
Buabin Camp	125	89	36	36	60	43	4	103	1	108	11	5	1	14	1	14	29	38.9	2.8	38.9	7.4	4	111
Kuntorkrom Rd	122	101	21	21	59	7	1	66	2	69	8	2	1	8	2	8	17	38.1	4.8	38.1	8.3	1	48
Kuntorkrom	14	13	1	1	18	0	0	18	0	18	1	0	0	1	0	1	7	100	0	100	18		0
Kumakrom	75	53	22	22	94	12	3	106	1	110	10	1	1	10	1	10	29	45.5	4.5	45.5	10.6	3	136
Praprababida	271	236	35	35	125	3	2	128	6	136	14	1	2	15	1	16	13	42.9	5.7	45.7	8.5	1	57
Zion	10	8	2	2	6	0	0	6	0	6	2	0	0	2	0	2	20	100	0	100	3		0
Zion Road	50	34	16	16	30	0	6	30	3	39	7	0	2	7	2	8	32	43.8	12.5	50	4.3	3	375
Awisem Road	64	36	28	28	103	8	37	111	6	154	12	4	5	13	3	14	44	46.4	17.9	50	8.5	7.4	321
Total	1941	1349	592	592	1896	225	208	2121	108	2437	215	48	39	240	34	250	30	40.5	6.6	42.2	8.8	5.3	351

APPENDIX III

Methods used to calculate the age spectrum of the fly population (after Duke 1967)

A) assumptions made

a) the nulliparous flies forming the basis of each model are considered as biting on day 0.

b) Parous flies with single infections showing L1/L2 stage larvae are coming for their second blood-meal.

c) Parous flies with L3 larvae are coming for their third or subsequent blood-meal.

d) The observed distribution by days of flies on the second blood-meal will be parallel by any group of flies coming for any subsequent blood-meal.

e) Of all the infected flies with developing infections (i.e., flies on their second or subsequent blood-meal), the proportion harbouring a second and older infection with infective larvae represents the proportion which is in fact coming for a blood-meal subsequent to the second. Of the total parous flies apparently coming for their third blood-meal, a like proportion may be coming may be assumed to be coming in fact for a blood-meal subsequent to the third. Similar reasoning may be applied to flies apparently coming for subsequent blood-meals.

B) Data

i) Pre-treatment

Total no. nulliparous	.....	1265
Total no. parous	.....	644
Total no. with L1/L2	.....	236
Total with double infections	.....	34
Total no. with infection	.....	300
Total no. with L3 only	.....	30
Total no. with infective larvae etc (on 3rd or subsequent blood-meal)	.....	64

ii) 2 month post-treatment

Total no. nulliparous	.....	1520
Total no. parous	.....	612
Total no. with L1/L2	.....	212
Total with double infections	.....	29
Total no. with infection	.....	261
Total no. with L3 only	.....	20
Total no. with infective larvae etc (on 3rd or subsequent blood-meal)	.....	59

iii) 8 month post-treatment

Total no. nulliparous	.....	1349
Total no. parous	.....	592
Total no. with L1/L2	.....	206
Total with double infections	.....	35
Total no. with infection	.....	251
Total no. with L3 only	.....	10
Total no. with infective larvae etc (on 3rd or subsequent blood-meal)	.....	45

Calculations (based on figures for pre-treatment session only).

1) *numbers and distribution of nulliparous flies.*

Corresponding to 320 infected parous flies dissected, there are

$$\frac{300 \times 1265}{644} = 589$$

nulliparous flies coming to take their first blood-meal on infected persons. These are considered to be flies between 0 – 2 days old.

2) *Number of flies on second blood meal.* This is known from data for flies harbouring L1/L2 infection as being 236. These are flies considered to be 3 –5 days old.

3) *Distribution of flies on third or subsequent blood-meal.* Of all the infective flies, the number coming for a blood-meal subsequent to the third is

$$\frac{30 \times 64}{236} = 8$$

therefore, 64 – 8 = 56 flies were on their third blood-meal. (assumption e). These were considered to be 6 –8 days old.

Of the remaining 8 infective flies, the number coming for a blood-meal subsequent to the third is

$$\frac{30 \times 8}{236} = 1$$

therefore, 8 – 1 = 7 flies were on their third blood-meal. (assumption e). These were considered to be 9 –11 days old.

The single fly remaining is considered to be 12 – 15 days old.

APPENDIX IV

Table 1. *Table showing the coordinates (latitude and longitude) for the various sampling site.*

village	latitude	longitude	Distance from river km
Buabinso	05°57'53" N	001°44'53" W	1
Akropong	05°55'49" N	001°41'66"W	1.5
Foso	05°55'45" N	001°36'41"W	0.5
Ayebiawhe	05°52'58" N	001°37'04"W	5
Assaman	05°54'55" N	001°30'47"W	1
Awisem	05°53'28" N	001°32'92"W	1
Awisem Road	05°53'30" N	001°34'23"W	3.5
Kontokrom Road	05°53'28" N	001°43'46" W	4
Kontokrom	05°53'28" N	001°43'72" W	6
Buabin	05°52'48" N	001°45'24" W	9
Buabin Camp	05°51'07" N	001°42'22" W	8
Praprababida	05°51'60"N	001°40'46" W	6
Zion No. 1	05°51'68" N	001°38'93" W	7.5
Zion Road	05°11'30" N	001°05'30" W	6
Kumakrom	05°49'68" N	001°39'81" W	11

APPENDIX V

\Table 2. *Showing the codes used to denote colours of the setae of the wing tuft, ninth tergites, arculus of wing, scutella and costa of corresponding species of blackflies.*

Colour code	Setae of	species	No caught
Wing tuft			
01	pale++++	*S. damnosum/sirbanum*	12
02	pale+++/dark+	*S. squamosum*	
03	pale++/dark++	*S. squamosum*	
04	pale+/dark+++	*S. squamosum*	
05	dark++++	*S.sanctipauli/soubrense/yahense*	5970
Ninth tergites			
01	pale++	*S.damnosum/sirbanum/ squamosum*	12
02	pale+/ dark+	*S.sanctipauli/soubrense*	
03	dark++	*S.yahense*	5970
arculus			
01	pale++	*S. damnosum/sirbanum*	
02	pale+/ dark+	*S. squamosum*	
03	dark++	*S.sanctipauli/soubrense/yahense*	5970
scutella			
01	pale++	*S.damnosum/sirbanum/ squamosum*	12
02	pale+/ dark+	*S.sanctipauli/soubrense*	
03	dark++	*S.yahense*	
costa			
01	pale++	*S. damnosum/sirbanum*	12
02	pale+/ dark+	*S.sanctipauli/soubrense*	5970
03	dark++	*S.yahense*	

APPENDIX VI

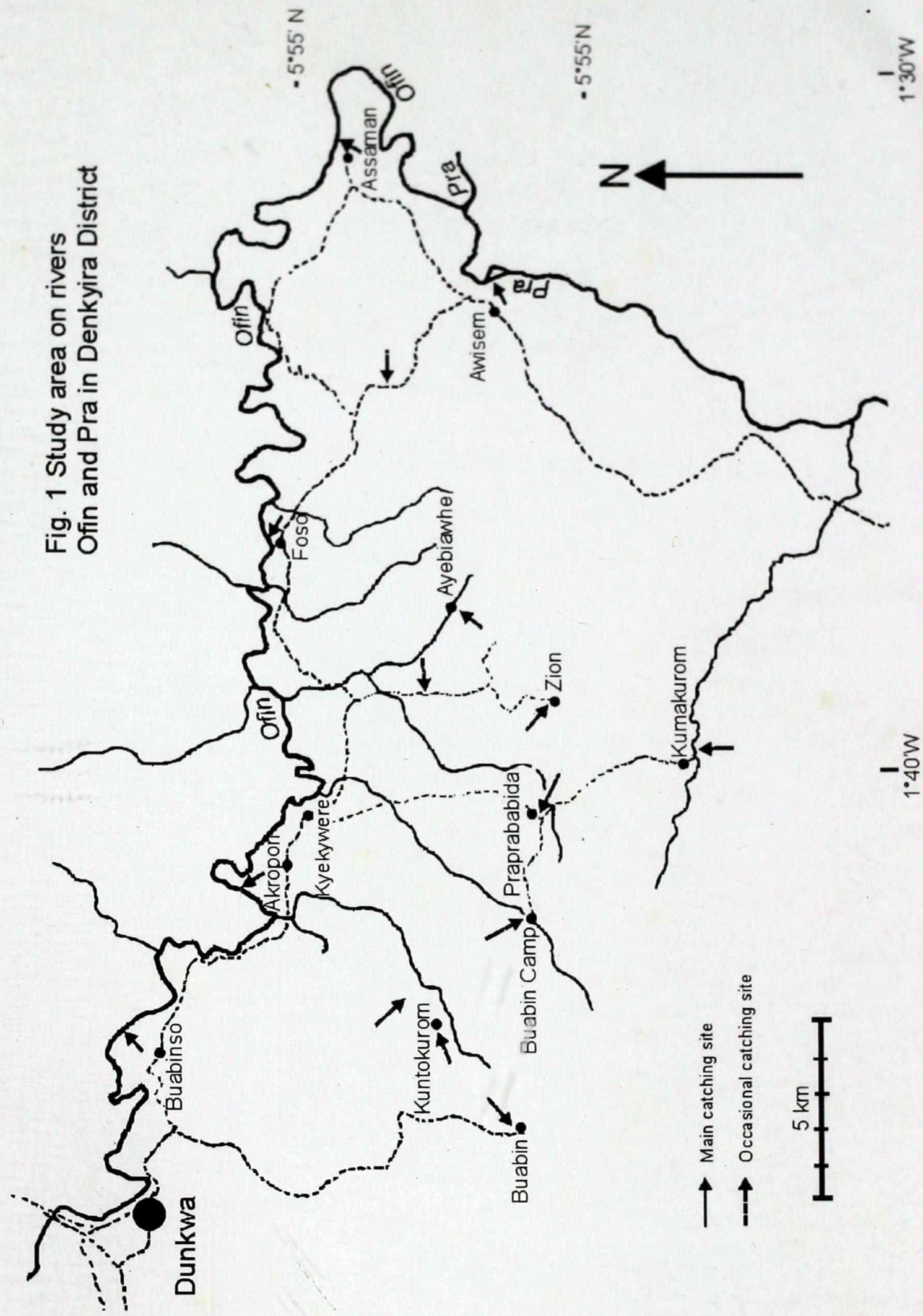

Fig. 1 Study area on rivers Ofin and Pra in Denkyira District