KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

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SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLOGY

EPIDEMIOLOGICAL STUDIES ON THE DISEASE AND VECTOR OF *MANSONELLA PERSTANS* IN THE ASANTE-AKIM NORTH DISTRICT OF GHANA

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BY

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY

IN THE DEPARTMENT OF CLINICAL MICROBIOLOGY, SCHOOL OF MEDICAL SCIENCES, COLLEGE OF HEALTH SCIENCES

DECLARATION

I declare that this submission is my own work towards the award of an MPhil and that to the best of my knowledge, it does not contain any materials previously published by another person nor material which has been submitted for the award of any other degree in any University, except where due acknowledgement has been made in the text.

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(Head of Department)	Signature	Date

DEDICATION

This work is dedicated to my wonderful father, Emmanuel Opoku Mintah for his immeasurable love, encouragement and support throughout my studies.

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ABSTRACT

Large parts of African and South American countries are colonized by *Mansonella* a very common but poorly described filarial nematode. Blood-sucking flies of the genus *Culicoides* are suspected to be the vector of *Mansonella perstans* but no study in Ghana has confirmed that it can transmit the parasite. An epidemiological survey was conducted in eight communities in the Asante-Akim North district of Ghana to determine the prevalence of *M. perstans*. A total of 1,216 residents comprising 601 males and 615 females with an age range of 9 years and above, participated in the study after an informed consent. All participants were examined for the presence of microfilariae. The average prevalence of *M. perstans* was 33.2% with the range between 1.6% and 72.3%. The prevalence of the parasite among the males was 57.2% and females 42.8%. Microfilariae were detected among all the age groups with the highest prevalence in the 30-39years age group. *Culicoides* flies were investigated in the study communities for *M. perstans*. In all, 2,194 *Culicoides* species (spp) were collected at the end of the study. Light trap collections gave diverse species where as HLC gave only one species. Investigations of seasonal abundance revealed a higher prevalence in the rainy season (58.7%). Biting activities had a peak period between 5-6pm. The monthly biting rates ranged from 83 bites per person per month to 2250 bites per person per month. Bebusu was the community with the highest monthly biting rate (2250). No *Culicoides spp* was found positive for *M. perstans* and its vector still requires identification. In conclusion, *M. perstans* has been detected in the AsanteAkim North District of Ghana with high prevalence. Hence, similar studies should be carried out in other parts of the country to determine the actual prevalence of the disease.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

CDC- Centres for Disease Control and Prevention

HLC- Human Landing Catch

L1- Larval stage one

L2- Larval stage two

L3- Larval stage three/infective larvae

MBR- Monthly Biting Rate

MF- Microfilaria

Mf/ml- Microfilariae per millilitre

NTD- Neglected Tropical Diseases

Spp- Species

CHAPTER ONE

INTRODUCTION

1.1 Background

Mansonella perstans formerly *Dipetalonema perstans* (Blacklock & Southwell, 1977; Cheesebrough, 1987) is a little known but widespread human filarial parasite in many parts of Sub-Saharan Africa and South America (Simonsen *et al*., 2011). It is a vector-borne filarial nematode, transmitted by tiny female flies of the genus *Culicoides* (biting midges) and is the causative agent of mansonellosis (Simonsen *et al*., 2011). The prevalence is often very high in endemic areas, including children (Nelson, 1965).

Mansonellosis is one of several filarial nematode infections for which humans are the definitive host (Simonsen *et al*., 2011). This puts it in the same group as several parasitic infections of importance to global health, including onchocerciasis, lymphatic filariasis, dracunculiasis and loiasis (Downes & Jacobsen, 2010). The known three agents that cause mansonellosis – *Mansonella perstans, M. streptocerca* and *M. ozzardi* – vary in features such as anatomy and periodicity, the vectors that transmit the agent to humans, the clinical signs and symptoms they cause, and the world regions where they are endemic (Downes & Jacobsen, 2010). Among these parasites, *M. perstans* is most studied in the tropics (CDC, 2008). Infections by *M. perstans* while often asymptomatic can be associated with pruritus, fever, headache and arthralgias (CDC, 2008).

M. perstans is transmitted by an arthropod vector commonly called midges. *Culicoides* midges belong to the order Diptera and are the most significant genus of the family Ceratopogonidae with respect to human health (Glick, 1990). They are known as the biting midges (often referred to simply as "midges) but are also called "little mosquitoes" in Laos, "punkies" and no-see um‟s" in the U.S, "no-no‟s" in Polynesia or simply biting gnats (Braverman, 1994).

While human filarial infections such as Onchocerciasis and lymphatic filariasis have garnered international attention (Gordon *et al*., 1997) and have been the focus of global eradication efforts, mansonellosis on the other hand appears to have been neglected (Simonsen *et al*., 2011). A recent study by Simonsen *et al.,* (2011) classified it as one of the most neglected among the Neglected Tropical Diseases (NTD). Hence, up-to-date epidemiological information is essential in making differential diagnoses in endemic areas; determining the prevalence of *M. perstans* infection and the possible vector(s) involved in its transmission, and address the concerns of atrisk populations in Ghana.

1.2 Rationale

In Ghana, *M. perstans* was first reported in the Volta region (around Hohoe) by Awadzi *et al*., (1991), however, to date its prevalence remains unknown. There is inadequate information on the intensity of the disease on individuals in endemic communities. This could be accounted for by the fact that *M. perstans* prevail in poor populations (Simonsen *et al*., 2011). The infection has not yet been well characterized in Ghana and there is therefore the need for an update on the current distribution of *M. perstans* in the Asante-Akim North district and Ghana as a whole.

M. perstans is currently considered to be associated with mild pathology and individuals with *M. perstans* infection are almost always asymptomatic (Simonsen *et al*., 2011). This might be partially explained by a modulated immune response because of repeated exposure to the parasite (Klion *et al*., 1991). During an investigation into the immunopathogenesis of buruli ulcer patients in the Asante-Akim North District of Ghana, *M. perstans* nematodes were observed in the peripheral blood of study subjects (Phillips *et al*., 2014). Their findings suggested that *M. perstans* nematodes were common in Ghana and coincidentally infect patients with *Mycobacterium ulcerans* disease,

necessitating the consideration of the organisms in the management plan of buruli ulcer patients (Phillips *et al*., 2014).

Studies on *M. perstans* vectors by Sharp (1927) identified *Culicoides grahami* as a potential vector. Studies performed in Cameroon have described *C. grahami*, *C. austeni* (currently *C. milnei*), and *C. inornatipennis* as potential vectors (Sharp, 1928; Hopkins, 1952; Hopkins & Nicholas, 1952). Additional studies also conducted by Agbolade *et al*., (2006) and Wanji *et al*, (unpublished data) have incriminated *C. fulvithorax* and *C. milnei* as the vectors involved in the transmission of *M. perstans* in Nigeria and Cameroon respectively.

Identification of the vector is an essential step in the epidemiology of vector-borne diseases (Glick, 1990). Information on the abundant vector species may give a clearer indication of the potential distribution of the disease geographically, the site of danger points for high risks of contact with the vector, and to obtain alternative tools for the study of the natural cycles of parasites. Given the paucity of information regarding *M. perstans* in Ghana coupled with the existence of different strains of vector(s) involved in the transmission of *M. perstans* in Africa, it is necessary to identify the vector(s) involved in the transmission in Ghana.

1.3 Aim

The aim of this study was to elucidate the epidemiology of mansonellosis in addition to ascertaining the abundance of the various *Culicoides* species present and to determine the vector(s) of *M. perstans* in the Asante-Akim North district of Ghana.

1.4 Objectives

The specific objectives of the study were to:

i. Determine the prevalence and intensity of the disease and its distribution in the Asante-

Akim North district. ii. Identify and evaluate *Culicoides* species abundance and diversity in the Asante-Akim North district.

iii. Determine the vector(s) involved in the transmission of *M. perstans* in the selected sites and analyze the vector distribution.

iv. Identify essential factors for vector occurrence and *M. perstans* infection in the Asante-Akim North district.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Mansonella perstans* infection and epidemiology

Mansonella perstans is a tissue-dwelling nematode, which is classified among filarial worms (Cheesebrough, 1987). Early medical literature referred to it as *Acanthocheilonema perstans* or *Dipetalonema perstans* (Blacklock & Southwell, 1977; Cheesebrough, 1987). It is one of the three species (*M. perstans, M. streptocerca, M. ozzardi*) of filariasis-causing nematodes belonging to the

genus *Mansonella* (Bassene *et al*., 2015) which is responsible for causing human mansonellosis. Mansonellosis is an infection which occurs through the bite of female flies of the genus *Culicoides* (Bassene *et al*., 2015).

M. perstans is considered to be the most widespread in Africa and it is endemic in a large portion of Sub-Sahara Africa (Simonsen *et al*., 2011). It largely spreads across Africa, with the exception of the western (Eritrea, Ethiopia, Djibouti, Somalia), southern (Botswana, Swaziland, Lesotho, Namibia, South Africa), most northern (Algeria, Mauritania, Egypt, Libya, Morocco, Tunisia), and some of the island countries (Cape Verde, Madagascar, Mauritius, Comoros, Seychelles,) from which local cases have not been reported. Since the majority of individuals infected do not show major symptoms, its epidemiology has been undefined (Simonsen *et al*., 2011).

It has been estimated that more than 100 million people may be infected and as many as 600 million people in 33 countries are at risk for *M. perstans* infection in Africa alone (Simonsen *et al*., 2011). In endemic regions, the chance of infection increases with age, with prevalence reaching 100 percent in highly endemic areas. Infection of travelers is not common, but it does happen (Lipner *et al*., 2007). Non-human primates are sometimes infected, but they do not seem to be a major reservoir of infection (Lipner *et al*., 2007).

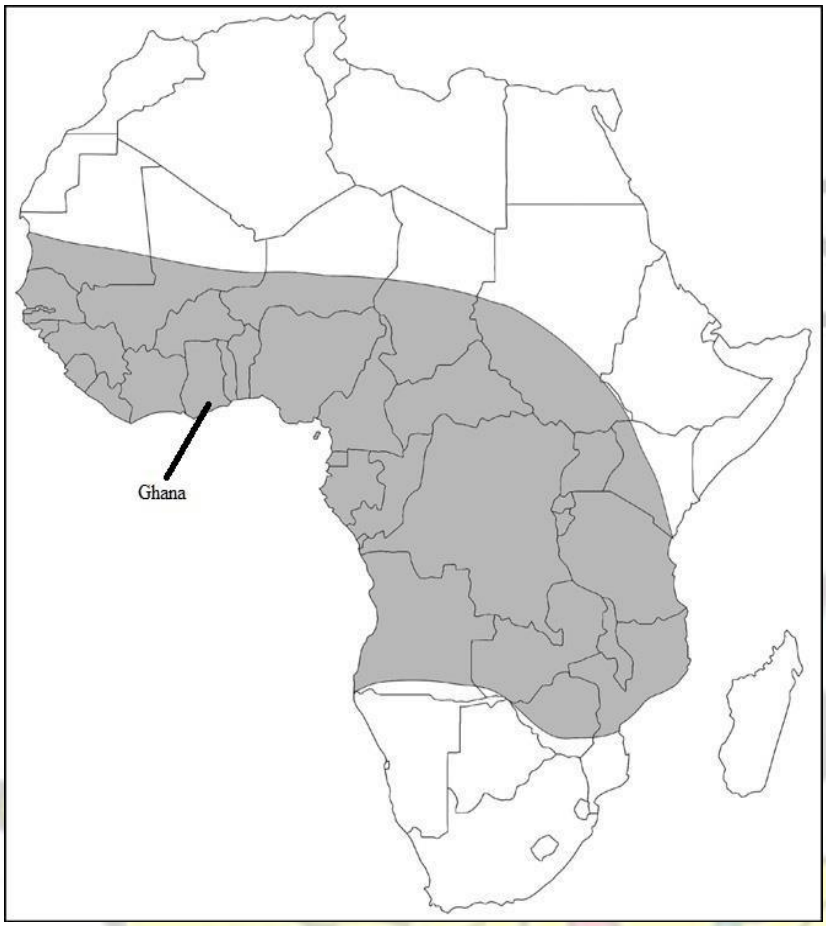


Plate 2.1: Epidemiological distribution of *Mansonella perstans* in Africa (Grey shaded areas denote regions with *M. perstans* infection) (Source: Simonsen *et al*., 2011)

M. perstans filariasis is predominantly found in rural populations and infections begin in childhood (Asio *et al*., 2009b). Prevalence of *M. perstans* varies considerably in endemic countries with regions/villages free of infections and areas where almost everybody is microfilariae (mf) positive. Sometimes different areas are found in close proximity (Simonsen *et al*., 2011). Knowledge about environmental factors that account for this differential distribution is limited although rain forest districts seem to favor *M. perstans* infections (Simonsen *et al*., 2011).

2.2 Life cycle of *M. perstans*

During a blood meal, an infected midge (genus *Culicoides*) introduces third-stage filarial larvae

(L3) onto the skin of the human host, where they penetrate into the bite wound (CDC, 2008). The L3 larvae develop into adult worms that reside in body cavities, most commonly the peritoneal cavity or pleural cavity, but less frequently in the pericardium (CDC, 2008). The size range for female worms is 70 to 80mm in length and 120µm in diameter whereas male worms measure approximately 45 mm by 60 µm. Adults produce unsheathed and sub periodic microfilariae (mf), measuring 200 by 4.5 µm that reach the blood stream (Simonsen *et al*., 2011).

The cycle continues upon the ingestion of mf by a midge during a blood meal (CDC, 2008). After ingestion, the mf migrate from the midge's mid-gut through the hemocoel to the thoracic muscles of the arthropod where they develop into first-stage larvae and subsequently into thirdstage infective larvae (CDC, 2008). The third stage infective larvae (length 750–900 µm) migrate to the midge‟s proboscis and may be seen 7–9 days after infection of the vector (Simonsen *et al*., 2011). The larvae escape from the proboscis by stretching and finally bursting the terminal membranous portion of the labrum (Sharp, 1928) and can infect another human when the midge takes a blood meal.

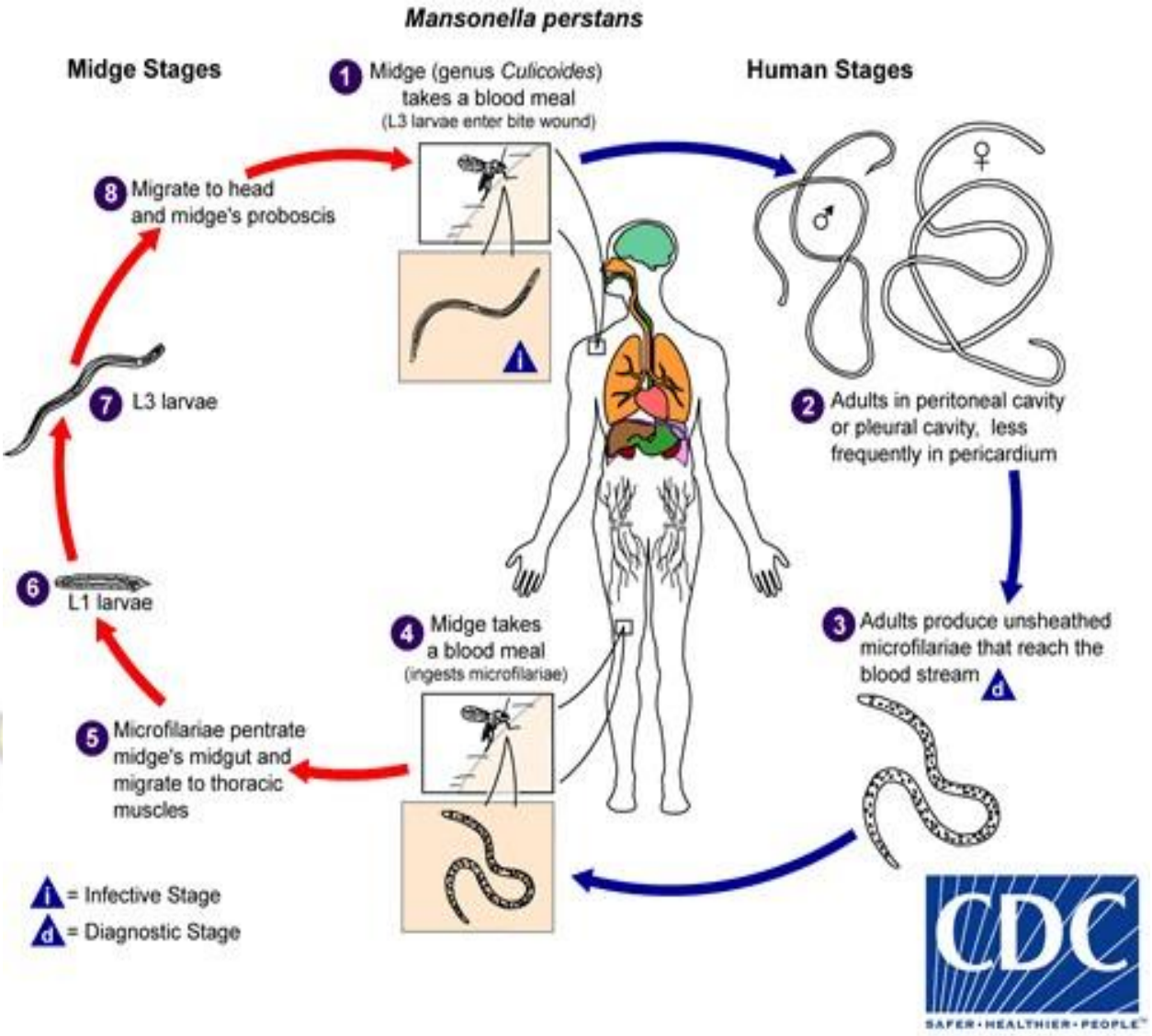


Figure 2.1: Diagrammatic presentation of the life cycle of *Mansonella perstans* infection

(Source: Centres for Disease Control (CDC), http://www.dpd.cdc.gov/dpdx)

2.3 Clinical presentation of mansonellosis

Mansonella streptocerca may cause skin manifestations such as papular eruptions, pruritus, and pigmentation changes (CDC, 2008). Eosinophilia is a common characteristic of filarial infections. *Mansonella ozzardi* can cause symptoms that include headaches, arthralgias, fever, hepatomegaly, pulmonary symptoms, adenopathy, and pruritus (CDC,

http://www.dpd.cdc.gov/dpdx).

Infections by *M. perstans* are often asymptomatic (Simonsen *et al*., 2011). The presence of *M. perstans* microfilaraemia has however been linked with disease manifestations such as joint and/or muscle ache, generalised itching of skin, fever, headaches, arthralgias, angioedema, severe pain in the abdomen and liver region, endocrinological disturbances, neurological and psychic symptoms, recurrent lymphoedema in the limbs and face resembling Calabar swellings, and nodules containing adult worms in the conjunctiva or eyelids (Agbolade *et al*., 2005; Baird *et al*., 1988; Bregani *et al*., 2006; Lansoud- Soukate *et al*., 1989).

However, symptoms of infection with *M. perstans* may include periodic dizziness, joint and back pain, pectoral and chest pains, and ocular symptoms (Anosike *et al.,* 2005b; Bregani *et al.*, 2006; Bregani *et al.,* 2007). Elsewhere, host‟s regulatory responses have been observed to be downregulated in *M. perstans* infections (Asio *et al*., 2009b).

2.4 Diagnosis and Treatment

Diagnosis and treatment vary by species. *M. streptocerca* microfilariae do not circulate in the blood, therefore it is important to take a skin snip (CDC, 2008). Blood smear for the assessment of mf is the simplest way of diagnosing *M. perstans* and *M. ozzardi* (CDC, 2008). *M. perstans* is one of the most difficult human filarial infections to treat; treatment must be specific to the infective agent (Downes & Jacobsen, 2010).

Ivermectin is effective against a broad range of filarial worms (Asio *et al*., 2009a); however, it is not effective against *M. perstans* (Downes & Jacobsen, 2010). An effective, fast acting, tolerable and easy to administer drug treatment for *M. perstans* infections still needs to be identified (Simonsen *et al*., 2011). The recent finding of *Wolbachia* endosymbionts in *M. perstans* from Mali (Kieser *et al*., 2008), and the subsequent use of doxycycline (200mg/6weeks) led to reductions in

microfilaraemia after 12 months (Coulibaly *et al*., 2009). Therefore, doxycycline appears to be a good start for the development of new drug trials.

2.5 Vector biology

Biting midges are holometabolous dipterans, progressing from egg to larva, pupa and finally the adult stages (Simonsen *et al*., 2011). Adult *Culicoides* measure 1.5-5 mm long, with a small head bearing a pair of prominent eyes and a pair of relatively long antennae with fifteen segments. The plumose antennae of the males are the auditory organs, sensitive to the female wing-beat tone, while the females have non-plumose antennae (pilose) (Boorman, 1993). The proboscis hangs down vertically from the head. In many species, the thorax is dorsally covered with black markings. The small elongated depressions, termed humeral pits, on the front dorsal part of the thorax, are typical only of the genus *Culicoides* (Boorman, 1993). The short and relatively broad wings lack scales, but in many species are covered with microscopic hairs. Typical of *Culicoides* and other Ceratopogonids is the „r – m" cross vein (Boorman, 1993).

In most species, the wings have contrasting dark and milky white spots, and when at rest the wings are placed over the abdomen like the blades of a closed pair of scissors. The legs are relatively short (Boorman, 1993). The abdomen of the females has a rounded tip, while that of the male bears a small pair of claspers. Male adults generally emerge from pupae before the females (Glick, 1990). Emerging adults usually fly only a few hundred meters from their larval habitats, but a flight range of 4.0 km has been reported for C. *variipennis* (Lillie *et al*., 1981). *Culicoides*, especially the high-flying species could be carried by air streams for hundreds of kilometers (Braverman & Linley, 1988).

A major function of the adult after emergence is mating, for which (in many species) the males form a swarm; individuals then leave the swarm to copulate in flight with individual approaching

females (Downes & Wirth, 1981). Swarming is induced by various landmarks such as a bush or the margin of a pond, including the head of an animal. Only females suck blood, from a variety of mammals and avian hosts (Downes & Wirth, 1981).

Some species have strong host preferences, while others will feed on a wider range of hosts (Linley *et al.*, 1983). Autogenous species exist, which do not need a blood meal to mature their first batch of eggs, but all require a blood meal to mature subsequent batches. Both sexes feed on naturally-occurring sugar solutions (Linley *et al.*, 1983). The biting activity of most *Culicoides* spp. is crepuscular (Braverman, 1994) and/or nocturnal, but a few species are diurnal. The length and number of ovarian cycles vary between species, and depend on the ambient conditions. The adult life span of some individuals may extend to 70 days (Lillie *et al*., 1981) while the number of generations per year depends on climate and species.

In Israel, *C. imicola* has been reported to produce eight generations between July and December, with each generation lasting approximately 23 days (Braverman & Linley, 1988). *Culicoides* lay 30-450 eggs depending on species, source of blood (host) and season (Becker, 1961). Each egg is 350-500 μm long and is dark in colour (Braverman & Linley, 1988). Eggs of most species hatch after two to nine days at favourable temperatures; for example, *C. imicola* in summer takes three days (Braverman & Linley, 1988).

Evasion of dryness by aestivation has also been recorded (Kettle, 1962). Eggs are laid in wet soil or any sort of semi-aquatic habitat, e.g. decaying plant material (Braverman *et al*., 1974). There are four larval instars and the fully-grown larva is cylindrical, whitish in colour and approximately 5-6 mm long (Kettle, 1962). This larval stage lasts longer than any other stage in the *Culicoides* development cycle. The larva has a conical head which bears a pair of small eyes, a pair of minute antennae and mandibles (Kettle, 1962). There are three thoracic and nine abdominal segments;

these lack any appendages, except that the last segment terminates in gilllike structures which have an osmoregulatory function (respiration being cutaneous) (Kettle, 1962).

Larvae move in an undulatory fashion and feed on a wide range of micro-organisms and decaying vegetable material. Many species have a narrow range of breeding-site types which can be identified, enabling control measures to be targeted for particular species (Kettle, 1962). In warm countries, development of larvae may take 14-25 days; for *C. imicola* in summer, larval development takes 13-14 days. In temperate regions, larvae of many species overwinter and therefore remain as larvae for seven months (Braverman & Linley, 1988). The pupa is 2-4 mm long, the cephalothorax bears a pair of breathing trumpets, the abdominal segments are equipped with tubercules ending in a fine hair, and the last segment bears a pair of horn-like processes (Braverman & Linley, 1988). The pupal stage lasts two to ten days; two days for *C. imicola* in summer (Braverman & Linley, 1988) and then develops into an adult (Figure 2.2).

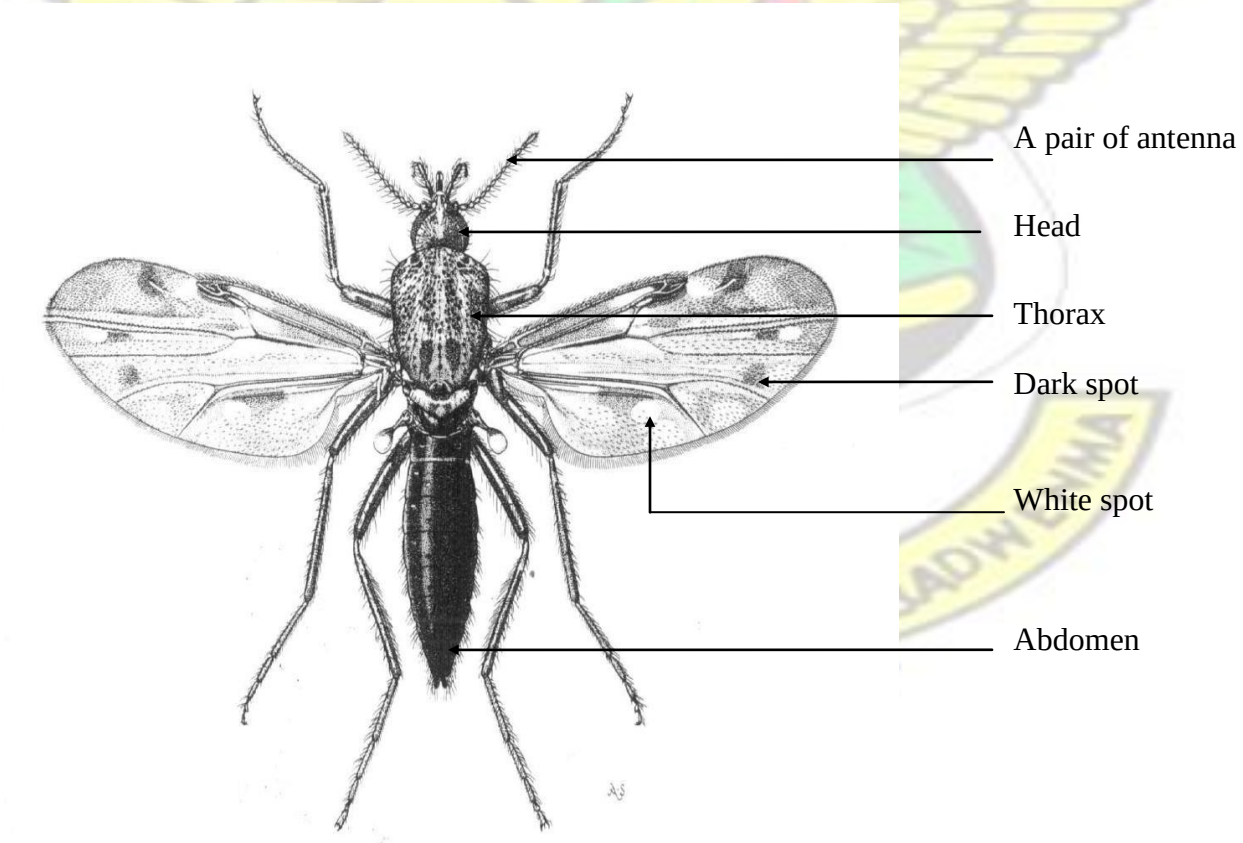


Figure 2.2: Morphology of the biting midge (Source Boorman, 1993)

2.6 Host and Parasite Dynamics

Humans are the only known reservoir for *M. perstans* (Sharp, 1927). No animal reservoirs for *M. perstans* occur as there are for *Mansonella streptocerca*, and transmission appears to occur through the bite of bloodsucking *Culicoides* flies, which are abundant in tropical ecosystems (Sharp, 1927).

Microfilariae of *M. perstans* are non-periodic and are usually present in the peripheral blood in equal amounts throughout the day and night (Manson, 1891; Low, 1903). They can also often be found in salivary gland abscesses (Mateu *et al*., 2008), an intraocular position (Cohen *et al*., 2008) and the conjunctiva (Baird *et al*., 1988).

The longevity of adult *M. perstans* in humans is unknown (Simonsen *et al*., 2011) but the microfilariae persist for approximately 4 months (Asio *et al*., 2009b). The adult worms are commonly found in the body cavities (Simonsen *et al*., 2011). Mf and adult worms with resemblance to those of *M. perstans* (named *M. vanhoofi*) have been retrieved from chimpanzees and gorillas (Nelson, 1965; Eberhard and Orihel, 1984). However, human infections are known in many areas without these great apes, and there is no proof that *M. perstans* is zoonosis (Simonsen *et al*., 2011).

Few studies have tried to incriminate the species of vectors of *M. perstans* in endemic areas (Simonsen *et al*., 2011). This issue is further complicated by the fact that the taxonomy of the tropical *Culicoides* species has not been worked on in detail (Simonsen *et al*., 2011). Most intensive studies have been carried out in Cameroon and Nigeria. Recent studies performed in Cameroon have described *C. milnei* as the vector involved in the transmission of *M. perstans* (Wanji *et al*., unpublished data). Additional studies have also been performed in different countries to search for vectors of this parasite in endemic areas, including Nigeria (Agbolade *et al*., 2006) and Congo (Noireau *et al*., 1990) and but none has been carried out in Ghana.

Biting midges have had dominant presence in rural areas; this is because the rural areas provide suitable conditions for the vector-breeding such as wet mud and leaf litter (Boorman, 1993). Various landmarks such as bushes or the margin of pond, or the head of an animal (Downes & Wirth, 1981); the abundance of plantain and banana plants, underbush and decaying plant matters are known to favour the breeding of *Culicoides* (Mercer *et al*., 2003).

2.7 Medical importance

- **Role as disease vectors**: Recent studies are steadily incriminating *Culicoides* as vectors of pathogens of man and wild animals. E.g. Bluetongue disease and African horse sickness (Kettle, 1965); they also transmit filariae (Armin *et al*., 2013) and mansonellosis in man (Noireau *et al*., 1990).
- **Role as biting pests**: These midges are a serious nuisance to humans making life almost unbearable in some areas by their annoying attack in swarm. They can occur in such numbers that they affect tourism (Linley & Davies, 1971).
- **Allergic dermatitis**: Severe and repeated biting attacks may give rise to allergic reactions and infections in humans severe enough to be a medical problem (Hase, 1934).

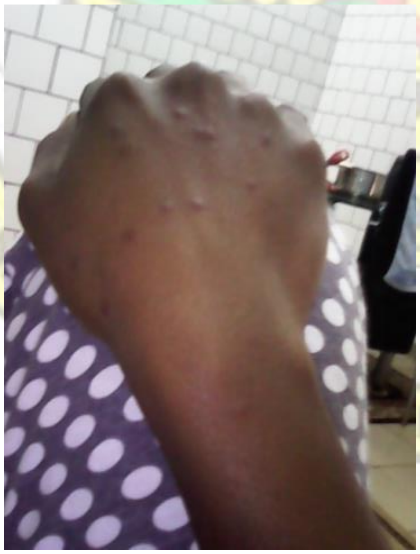


Figure 2.3: Allergic reactions on the hands of a volunteer

2.8 Control measures

"One midge is an entomological curiosity, a thousand can be hell" (Kettle, 1962). Where they occur in huge numbers, there is no doubt of the impact of these midges as biting pests and many control efforts have been directed at them (Boorman, 1993). The earliest attempts at control before modern insecticides became generally available, involved the use of pyrethroids oil applied to the breeding sites (Boorman, 1993).

Environmental management approaches have been used in Florida, Panama and in Brazil, but these methods tend to be expensive and depend on continuous support over long periods (Boorman, 1993). Difficulties arise not only in recognition of the often widely scattered breeding sites but because of windborne re-introduction of adults into treated areas (Boorman, 1993). As yet, there is no effective biological control of these biting midges (Agbolade *et al*., 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was conducted in eight neighbouring communities, in the Asante-Akim North District. The Asante-Akim North District with its capital Agogo is situated at the Eastern part of the Ashanti region. Its eastern boarder forms part of the regional boundary dividing the Ashanti and Eastern Regions. The district covers a total surface area of about 1217.7 square kilometers (472.4 sq miles) which form about five percent (5%) of the total area of the Ashanti Region, and 0.5 percent of the total area of the country. The built environment consists of 369.482 square kilometers with the natural environment forming 848.218 square kilometers of the total land area (ghanadistricts.com).

The major occupation of the inhabitants of these communities is farming, mainly plantain farming. Most of the houses in these communities are made of mud, with bamboo and palm leaves used for roofing.

This district lies in the tropical rain forest although there are some transitional zones due to farming and logging activities. The climate is tropical; temperatures vary from 20 °C to 36 °C with monthly rainfall varying from 2.0 mm in February to 400 mm in July. There are two rainy seasons; a major one extending from April to August and a minor one from October to November. Subsistence farming is the main occupation. This district is divided into four subdistricts and has a population of about 126,000 individuals (ghanadistricts.com). The District also shares common boundaries on the South with Asante-Akim South District and Kwahu South District on the West. On the South-West lies Amansie East District and on the South-East is Birim North District in the Eastern Region (ghanadistricts.com).

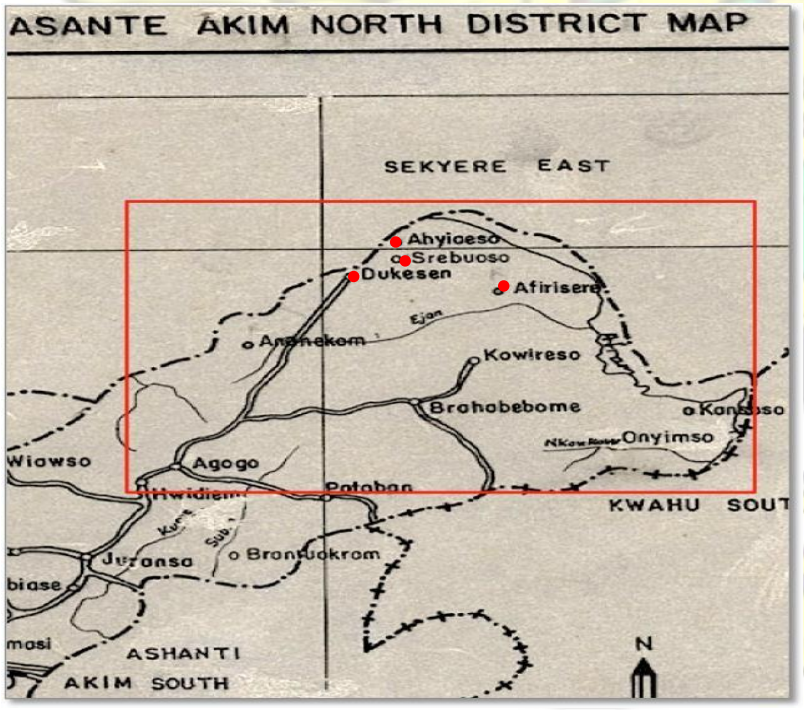


Plate 3.1 Map of Study Area: Asante-Akim North District

3.2 Ethical approval and Consent

The Committee on Human Research, Publication, and Ethics (CHRPE) of the School of Medical Sciences of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi approved the study after the review of all ethical details. Additional approval to conduct the study in the selected communities was sought from the Asante-Akim North Health District Directorate, Chiefs and Community leaders. Written informed consent was obtained from all study participants either by signing or thumb-printing.

3.3 Study design

This study was an epidemiological assessment on the disease and vector of *M. perstans*. It was conducted in these eight communities (Abutantri; Afrisere; Ananekrom; Anokye-beemu; Bebusu; Dukusen; Nhyieso and Serebuoso) in the Asante-Akim North District of Ghana. It was conducted in two major phases. The first phase of the study (i.e. parasitological survey) involved two methods, screening of all volunteers in the afore-mentioned communities for the presence of *M. perstans* filarial worm in their peripheral blood and quantifying the microfilarial load (Sedgewick count).

The second phase (i.e. entomological study) involved the collection of the arthropod vector(s) suspected to be transmitting the disease in the selected communities. This was carried out between November 2014 - February 2015 (dry season); and June – October 2015 (rainy season).

3.4 Parasitological survey

3.4.1 Screening of participants

Blood samples for parasitological examination were taken from every consenting person of 9 years and above. Using a sterile lancet, 20µl of blood was taken from the lateral upper side of the tip of

the finger, drawn into a pipette and applied onto a clean and labeled microscope slide. A clean cover slip was placed over the blood on the slide and observed under X10 objective of a light microscope. Identification of microfilariae was conducted according to keys in Learning Bench Aid Series 1-9 (Microscopy of Tropical Diseases).

Figure 3.1: Screening of participants for *M. perstans*

3.4.2 Quantification of mf load (Sedgewick count)

Nine hundred micro-litres (900 µl) of 3% acetic acid was pipetted into 1.5 ml eppendorf tubes and each tube labeled according to the participant sample identification number. Into each eppendorf tube containing the 900 µl of acetic acid, 100 µl of the corresponding participant‟s blood sample pipetted was added and mixed thoroughly using the pipette.

The solution was then poured into the Sedgewick counting chamber ensuring that there were no bubbles in the solution. It was then observed under a light microscope using X10 magnification lens. Microfilariae if present were counted in each square box including those lying across the lines separating adjacent boxes with the aid of a tally counter. Identification of microfilariae was

conducted according to keys in Learning Bench Aid Series 1-9 (Microscopy of Tropical Diseases). Results were recorded as Mf/ml.

3.5 Entomological study (Collection of *Culicoides* species)

3.5.1 Human Landing Collections (HLC)

Flies were captured daily by four fly collectors stationed near human habitations. Blood-seeking female flies were collected using locally made aspirators when they were attempting to take a blood meal on the collectors. Catches were made from 6am to 9am for the morning periods and 4pm to7pm for the evening periods (Agbolade, 2002), making a total of 6 collection hours per day. The caught flies were aspirated and stored into hourly labeled transparent plastic cups covered with fine net and blocked with cotton wool at the base. At the end of each session, flies were knocked-down by freezing for few minutes and then transferred into eppendorf tubes containing 80% ethanol and labeled appropriately. They were then transported to the laboratory for identification. The number of flies caught within each hour interval was recorded and stored in 80% ethanol for further molecular analysis.

Figure 3.2: Collection of *Culicoides* species by Human landing collection technique.

3.5.2 Light trap collection

Centres for Disease Control (CDC) light traps were mounted at proximity to both breeding sites and human habitations in four sites of the community. Attracted by UV light emitted by the trap, *Culicoides* flies were trapped in a petri plate containing 80% ethanol placed in the suspended trap. Traps collection hours were the same as for human landing collections except that the trap catches were removed only at the end of the three hours collection. Each trap was assigned a unique number with respect to its position in the field. The trapped flies were transferred into labeled 50ml falcon tubes containing 80% ethanol and placed in a cold box for transportation to the laboratory. The number of flies caught by each trap was recorded and the flies identified.

Figure 3.3: CDC light trap set at strategic position to collect *Culicoides* spp.

3.5.3 Collection of Engorged *Culicoides* using trap net on an *M. perstans* donor

3.5.3.1 Preparation of tubes for engorged collection

Tubes were prepared according to the protocol of Hopkins, (1952). Prior to collection, the tubes were filled ¼ way with Plaster of Paris. Moist filter paper strips were made and placed in the tubes. The tubes were closed with very fine mesh gauze and held with corks.

3.5.3.2 Collection of engorged Culicoides spp

Engorged *Culicoides* species were collected from the exposed legs of an *M. perstans* positive volunteer into 50ml falcon tubes using locally designed aspirators. A trap net was designed to collect engorged species on the donor in which, the net was raised. After 10 minutes, *Culicoides*

species that had landed on the donor were trapped within the net by lowering the net to cover the volunteer (Fig. 3.4a). Then, 10- 15 minutes later, the midges fully engorged were found resting on the walls of the net and were then aspirated into the designed tubes (Fig. 3.4b).

Figure 3.4: Trap net lowered to cover *M. perstans* donor (a); collector (b) aspirating engorged *Culicoides* resting on net.

3.5.3.3 Transportation of samples

Midges in the tubes were fed with 15% sugar solution and arranged in Styrofoam rack, held tightly in place and transported to the laboratory at room temperature.

Figure 3.5: Engorged *Culicoides* species in the holding tubes.

3.5.3.4. Maintenance of Engorged *Culicoides*

Each morning, 2 to 3 drops of distilled water were added using a 10ml syringe to the Plaster of Paris in the falcon tubes to keep it moist and *Culicoides* fed with 15% sugar solution. A quantity of the solution was poured into a clean bowl and a piece of cut sterile cotton gauze soaked in the solution (but not too wet) was placed on the rim of the cork for midges to feed. The cotton gauze was changed each morning during feeding to prevent fungal growth. Mortality was noted and recorded each day. This set up was kept between 23^0C - 25^0C. These midges were dissected on day 8 post-infection.

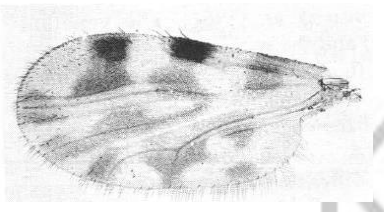
3.5.3.4.1 Preparation of 15% sugar solution

Fifteen (15) grams of sugar was weighed and dissolved in 100 ml of distilled water and stirred until completely dissolved. The solution was then sterilized by autoclaving and kept in the laboratory for daily use. Each prepared solution was not used for more than 3 days.

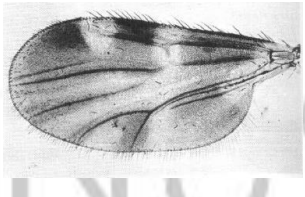
3.6 Morphological identification of *Culicoides* species

Collected vectors were taken to the laboratory, their sex determined and identified to genus level based on standard external morphological characters (wings in particular) which could only be seen when placed on a slide and viewed under a dissecting microscope. Wing pattern pictorial key for *Culicoides* was used (Glick, 1990 and Boormann, 1993).

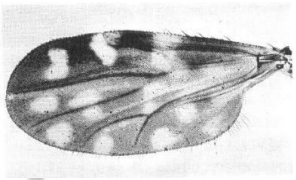
Key



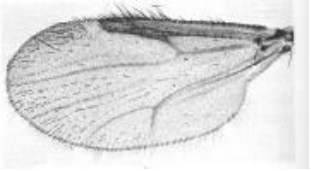
C. imicola



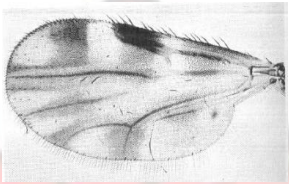
C. fulvithorax

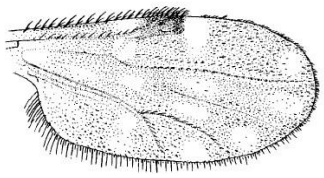


C. milnei



C. inornatipennis





C. grahami *C. neavei*

Figure 3.6: Wing Pattern pictorial key for identification of *Culicoides* spp (Boorman, 1993)

3.7 Transmission assessment

3.7.1 Dissection

Prior to dissection, the laboratory, equipment and materials used were disinfected to reduce contamination. Midges in the 50 ml falcon tubes were poured into a killing solution (washing powder + 200ml distilled water) to soften the tissues and rinsed in distilled water twice. Each fly was then placed on a slide in a sufficient volume of complete culture medium (CCM). *Culicoides* spp. were identified by morphological features (wing morphology) as described by Glick, (1990) and Boorman, (1993). Midges were then dissected (i.e. the head, thorax and abdomen of each fly was separated and placed on a slide at three different spots containing a drop of 0.9% sodium chloride {NaCl}) and examined for *M. perstans* larvae under a dissecting microscope. Larvae, if

present were classified into sausage larval stage (L1), larval stage 2 (L2) and larval stage 3 or infective larvae (L3).

3.8 Determination of entomological indices

3.8.1 Blood-fed proportion

This was done by careful examination of *Culicoides* species abdomen for the presence of blood (either partial or full) under a dissecting microscope of magnification X20 (Braverman, 1994).

Figure 3.7: An engorged *Culicoides spp* observed

3.9 Statistical Analyses

Raw data were entered using Microsoft Excel® and analyzed with Statistical Package for Social Sciences (SPSS 20.0.0). Statistical significance was set at $p < 0.005$. Chi square ($\chi 2$) test was used to assess the level of association between variables (overnight and evening catches). The Mann-Whitney test used to compare seasonal abundance of *Culicoides* species captured. The respective species abundances were established and contingency tables generated for different collection methods. Entomological indicators generated include:

Engorged proportion: Defined as the proportion of *Culicoides* species with blood in the abdomen (Partly or fully fed).

Monthly biting rate (MBR): Defined as the number of *Culicoides* visiting a collector per month and was deduced from the daily biting rates. It was a theoretical expression calculated by the formula [number of flies caught* number of days in month/ number of catching days in month] for flies collected by HLC (Agbolade *et al*., 2006).

CHAPTER FOUR RESULTS

4.1 Demographic data of study volunteers

A total of 1,230 volunteers from eight villages were recruited for the epidemiological study. All volunteers were examined for the presence of *M. perstans* filarial worms in their peripheral blood. Out of this number, 603 (49.1%) were males and 627 (50.9%) were females (Table 4.1).

From the 1,230 volunteers, 407 (33.2%) were found to be infected with *M. perstans*. Of the 407 infected, 233 (54.8%) were males while 184 (45.2%) were females. There was no significant difference between the mean ages for both sexes (p=0.188). The overall mean age was 29.2 years (Table 4.1).

Table 4.1: Demographic data of study volunteers

Gender of study volunteers	MF Status		Age (Mean ± SD) / years
	Negative	Positive	
Male	370	233	28.5 ± 12.5
Female	453	174	29.9 ± 13.4
Total	**823**	**407**	**29.2 ± 13.0**

*MF = microfilariae

4.2 *M. perstans* Prevalence rate in the Communities

Table 4.2 represents *M. perstans* prevalence assessment of the study population. *M. perstans* microfilariae were found in 407 volunteers (Table 4.1) representing an overall mf prevalence of 33.2%. The prevalence in the study communities ranged from 1.6% to 72.3% (Table 4.2). Comparing the prevalences of the communities, Ananekrom recorded the lowest prevalence of 1.6% while Anokye-beemu recorded the highest prevalence of 72.3% (Table 4.2).

Table 4.2: *M. perstans* prevalence rates in study communities

Community	Number screened	Number positive	% positive
Abutantri	132	70	53.0
Afrisere	164	62	37.8
Ananekrom	119	2	1.6
Bebuso	93	13	14.0

Anokye-beemu	210	152	72.3
Dukusen	117	46	39.3
Nhyieso	195	24	12.3
Serebuoso	200	38	19
Total	**1230**	**407**	**33.2**

*% = percentage

4.3 Comparison of age and sex of mf positive volunteers

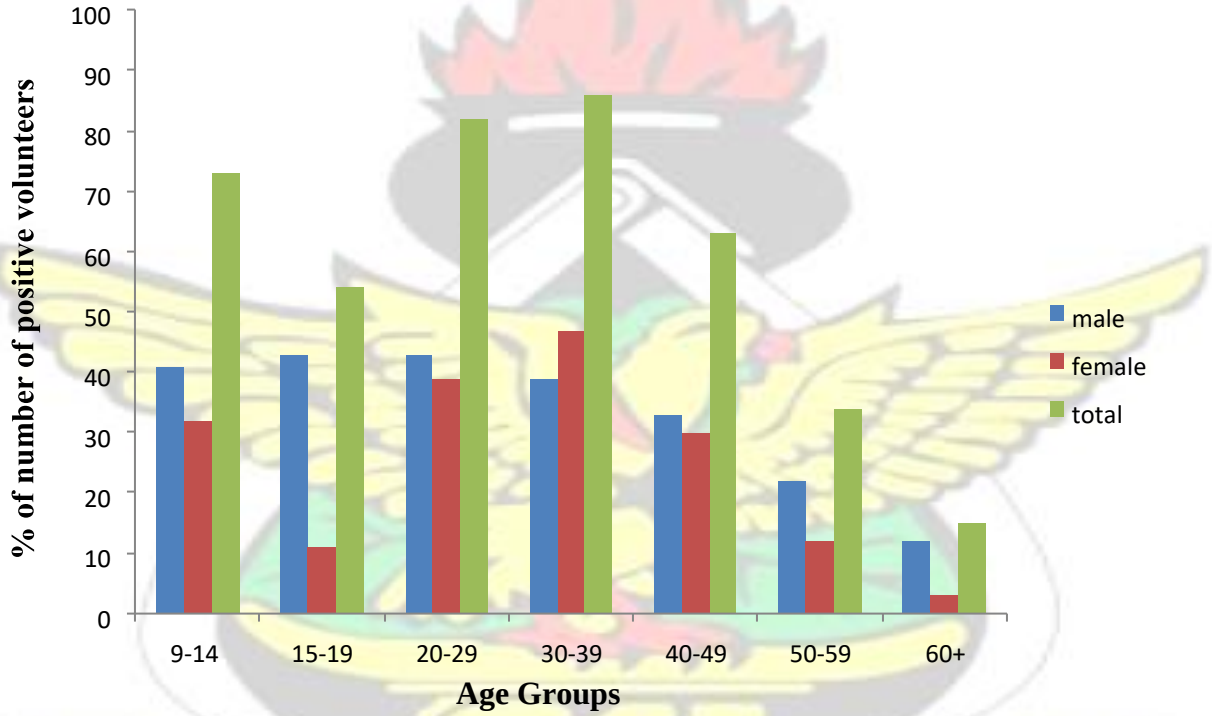


Figure 4.1: Prevalence of *M. perstans* mf in relation to age groups and gender of volunteers

Figure 4.1 shows a histogram of age groups and gender dependent assessment of *M. perstans* mf prevalence. Comparatively, the results show a higher prevalence of mf among males than females with no significant difference between them ($p=0.107$) except in age group 15 to 19 in which there is a significant difference ($p<0.001$) in the gender. It was also observed that *M. perstans* mf were

present among all selected age groups with the highest prevalence among volunteers aged 30 to 39 years.

4.4 Vector collection in study communities

A total of 2,194 *Culicoides* were captured during the survey period in the 7 study communities (Ananekrom was excluded due to the low prevalence of *M. perstans* infection [Table 4.2]). Afrisere had the lowest catch of 2.8% and Dukusen had the highest catch of 34.2% (Table 4.3).

Table 4.3: Abundance of vector at study communities

Community	Number captured	% captured
Abutantri	414	18.9
Afrisere	61	2.8
Anokye-beemu	216	9.8
Bebusu	408	18.6
Dukusen	751	34.2
Nhyieso	273	12.4
Serebuoso	71	3.2
Total	**2194**	**100**

*% = percentage

4.5 Species diversity with method of collections

A total of 1,328 and 866 *Culicoides* were captured using light traps and human landing catches (HLC) respectively. The light trap collections gave diverse species whilst HLC gave only one species in all of the study communities (Table 4.4).

Table 4.4: *Culicoides* species caught by Trap and Human Landing Catch (HLC) methods

Species	Method of collection		Total
	Trap	HLC	
Culicoides accraensis	1	0	1
C. fulvithorax	13	0	13
C. grahami	74	0	74
C. imicola	600	0	600
C. inornatipennis	0	924	924
C. milnei	67	0	67
C. neavei	322	0	322
C. schultzei	193	0	193
Total	**1328**	**866**	**2194**

*trap = CDC light trap; *HLC = human landing catch

4.6 Comparison between overnight and evening catches

The abundance of each *Culicoides* species increased significantly ($\chi^2 = 82.042$; $P < 0.001$) between the evening and overnight collections. Evening and overnight collections totals were 531 and 739 representing 41.8% and 58.2% respectively (Table 4.5). No trap collection was made for *C. inornatipennis*.

Table 4.5: Comparison of evening and overnight trap collections

Species	Time of collection		Total
	Evening	Overnight	
C. accraensis	0 (0)	1 (0.1)	1 (0.1)
C. fulvithorax	4 (0.3)	9 (0.7)	13 (1.0)
C. grahami	32 (2.5)	42 (3.3)	74 (5.8)
C. imicola	192 (15.1)	408 (32.1)	600 (47.2)
C. milnei	18 (1.4)	49 (3.9)	67 (5.3)
C. neavei	231 (18.2)	91 (7.1)	322 (25.3)
C. schultzei	54 (4.3)	139 (10.9)	193 (15.3)
Total	**531 (41.8)**	**739 (58.2)**	**1270 (100)**

*values in brackets represent percentages; ($\chi^2 = 82.042$; $p<0.001$)

4.7 Seasonal abundance of *Culicoides* species

Comparing the 2 seasons from table 4.6, rainy season had higher number of catches (58.7%) as compared to the dry season (41.3%). *C. inornatipennis* recorded the highest percentages of 40.2% and 43.5% for the dry and rainy seasons respectively. There was a significant difference ($p<0.005$) between both seasons.

Table 4.6: Comparison between rainy and dry season collections

Species	Season of collection				Total	%
	Dry	%	Rainy	%		
C. accraensis	0	0	1	0.1	1	0.05
C. fulvithorax	4	0.4	9	0.6	13	0.6
C. grahami	29	3.2	45	3.5	74	3.4
C. imicola	200	22.1	400	31.1	600	27.3
C. inornatipennis	364	40.2	560	43.5	924	42.1
C. milnei	28	3.1	39	3.1	67	3.1
C. neavei	232	25.6	90	6.9	322	14.7
C. schultzei	49	5.4	144	11.2	193	8.8
Total	**906**	**100**	**1288**	**100**	**2194**	**100**

Mann Whitney U test (p-value) between the 2 seasons p=0.0007

4.8 Entomological Indices

4.8.1 Proportion of blood-fed *Culicoides*

Out of the 2,194 *Culicoides* captured, 135 (6.2%) were found to have taken blood meal from both trap and HLC collections (Table 4.8). The source(s) of the blood meal from trap collections was unknown while that from HLC was known to be from human collectors. *C. inornatipennis* recorded the highest number of blood-fed species (Table 4.7).

Table 4.7: Proportion of blood-fed *Culicoides* species with respect to collection method

Species	Blood fed				Total	
	Yes	%	No			
	0	0	1	%		%
C. accraensis				0.04	1	0.04
C. fulvithorax	0	0	13	0.6	13	0.6
C. grahami	4	3.0	70	3.4	74	3.4
C. imicola	10	7.4	590	28.7	600	27.3
C. inornatipennis	90	66.7	834	40.5	924	42.1
C. milnei	0	0	67	3.3	67	3.1
C. neavei	25	18.5	297	14.4	322	14.7
C. schultzei	6	4.4	187	9.1	193	8.8
Total	**135**	**100**	**2059**	**100**	**2194**	**100**

*% = percentage

4.8.2 Monthly biting rate of species caught by HLC

The monthly biting rates ranged from 83 bites to 2250 per person per month. From table 4.8, Bebusu recorded the highest number of flies visiting man per month (i.e. 2250 bites) and Serebuoso (83 bites) recorded the lowest.

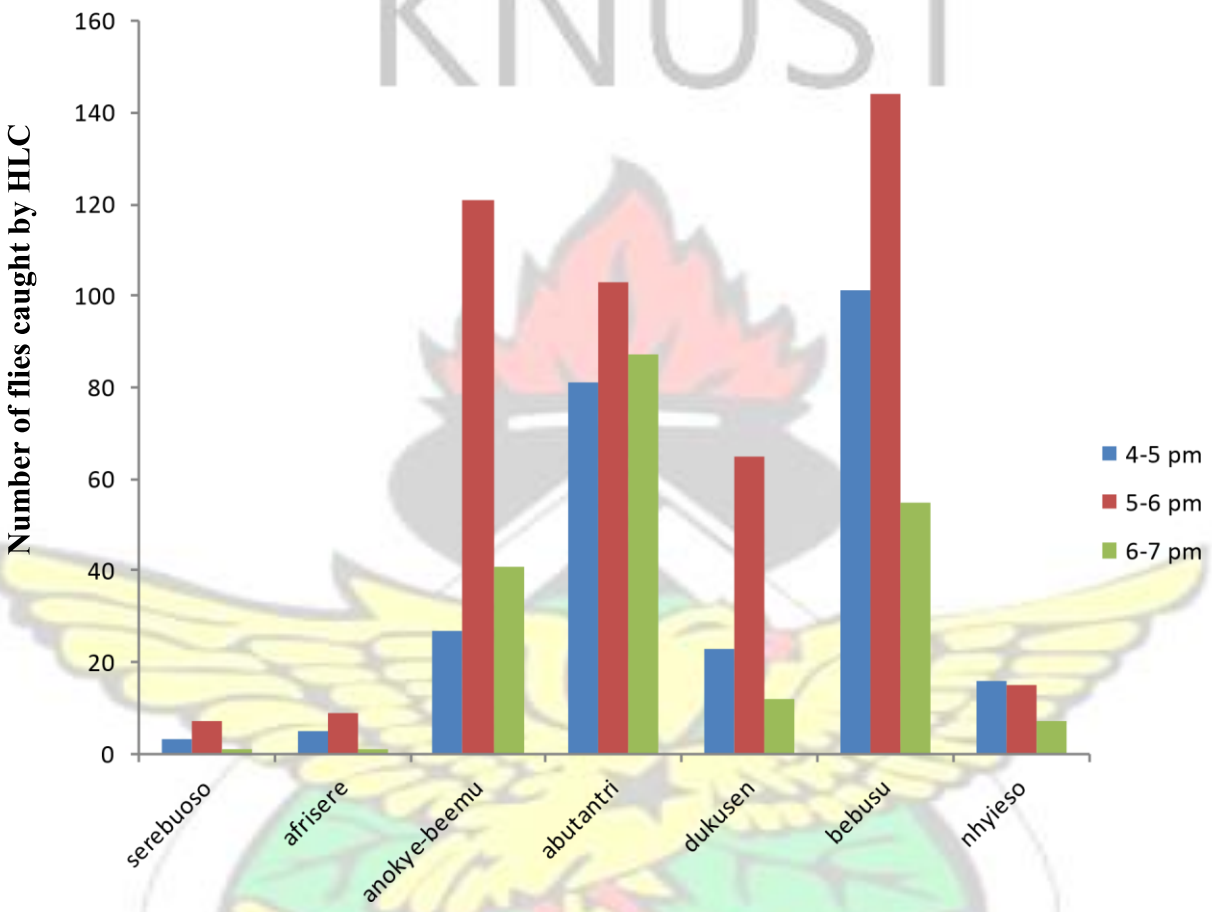
Table 4.8: Monthly biting rate of *C. inornatipennis* caught by HLC at study communities

Community	Number of female flies caught	Number of catch days	*MBR
Serebuoso	11	8	83
Afrisere	15	8	113
Anokye-beemu	189	8	1418
Abutantri	271	8	2033
Dukusen	100	8	750
Bebusu	300	8	2250
Nhyieso	38	8	285

*MBR- Monthly biting rate

The number of flies biting man per hour was observed for only *C. inornatipennis* (Table 4.8) as no other species was caught by HLC. Moreover, the peak biting activity with respect to period of

collection was between 5-6pm for all the study communities except Nhyieso where the peak biting activity was between 4-5pm. In all the study communities, 6-7pm was the period with least biting activity except at Anokye-beemu and Abutantri where it was 4-5pm (Figure 4.4).



Communities Figure 4.2: Biting pattern of *C. inornatipennis* caught at study communities

4.8.3 Infection rate

A total of 162 blood-fed *Culicoides* species were dissected and observed for *M. perstans* larvae. The infection rate was zero for all the species, as none of them was found infected with any of the larval stages (Table 4.9).

Table 4.9: Engorged *Culicoides spp* dissected

Species identified	Method of collection	Number bloodfed (engorged)	Number dissected	Number infected
C. inornatipennis	*M. perstans* +ve donor	37	27	0
C. inornatipennis	hlc	90	90	0
C. imicola	trap	10	10	0
C. neavei	trap	25	25	0
C. schultzei	trap	6	6	0
C. grahami	trap	4	4	0
Total		**172**	**162**	**0**

*+ve = positive; *hlc = human landing catch; *trap = CDC light trap

Twenty seven (27) out of the 37 engorged midges caught (72.9%) survived in the tubes. Overall, the highest mortality was observed on day 2, which decreased till day 6 and peaked again on day 7 (Figure 4.5).

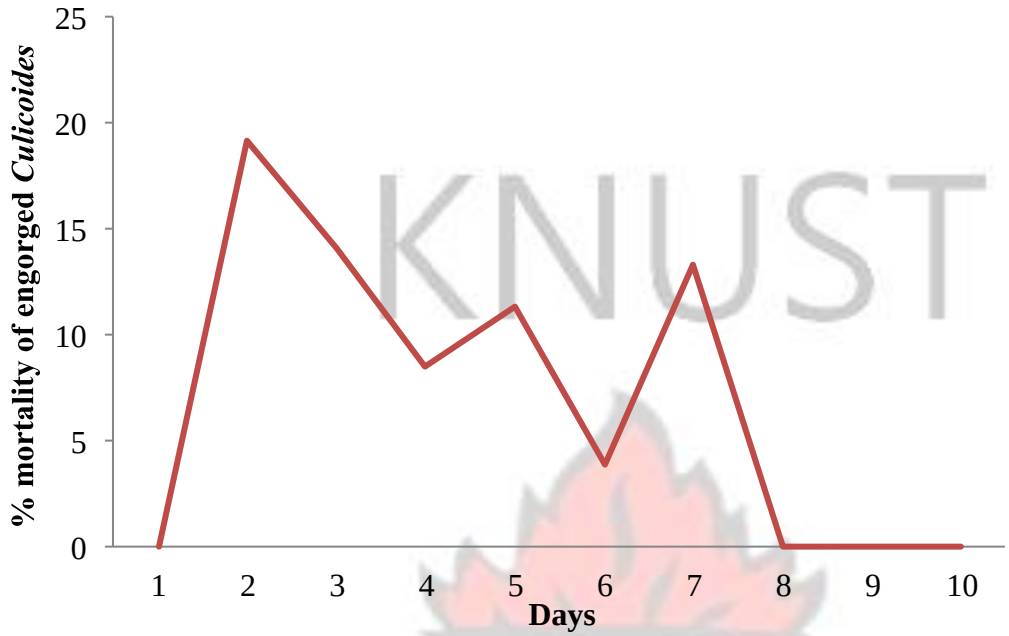


Figure 4.3: Percentage mortality of engorged *Culicoides* species

CHAPTER FIVE DISCUSSION

Mansonellosis is widespread in West African countries (Uttah *et al*., 2011). However, the respective role played by mansonellosis is difficult to assess due to the fact that it has its dominant presence in rural areas (Agbolade *et al*., 2005). Mansonellosis is not only a disease of the poor (Udoidung *et al*., 2007), rather the rural communities offer suitable conditions for the vector to breed (Boorman, 1993).

Even though mansonellosis was suspected to be in Ghana (Awadzi *et al*., 1991), it was not known until it was coincidentally found in the blood of buruli ulcer patients (Phillips *et al*., 2014). In this study, an attempt was made to establish the true level of endemicity of *M. perstans* infection in the Asante-Akim North district by determining the prevalence of microfilariae. Out of a total of 1,230 volunteers assessed for *M. perstans* microfilariae (mf) in the blood, 33.2% were found to be mf positive (Table 4.1). Among the male volunteers, the prevalence was 57.2% indicating a higher

prevalence than the females with 42.8% (Table 4.1), similar to the observations in other endemic foci in Africa (Anosike *et al*., 2004).

The prevalence of *M. perstans* in endemic areas varies greatly even with small geographical locations (Downes & Jacobsen, 2010). The analysis from this present study showed significant geographical variation in patterns of *M. perstans* prevalence ranging from as low as 1.6% to 72.3% (Table 4.2) similar to the variation shown by Onapa *et al*., 2005 in a study of school children in Uganda which found the prevalence ranging from 0.4% to 72.8%. The marked difference in prevalence rates between the various geographical areas is related to environmental factors affecting species and abundance of the vectors and their capacity to support and transmit the infection. Similar studies conducted in Cameroon and Uganda recorded low and high prevalence rates of 26.6% and 2% (Mommers *et al*., 1994; Onapa *et al*., 2001); and 55% to 100% and 96% respectively (Wanji *et al*., 2003; Fischer *et al*., 1996).

Ananekrom, which recorded the lowest prevalence rate of 1.6% (Table 4.2), was a more developed community (i.e. has a peri-urban setting) unlike the other neighbouring communities within the district. The inhabitants of this community were mostly petty traders and factory workers and as such not actively involved in farming activities. Ananekrom community lacks the availability of trees, bushes and plantain farms which are known to harbor biting midges (the arthropod vector involved in the transmission of *M. perstans*). Since the people were not actively involved in farming and the vegetation does not provide suitable habitat for the vectors; they are not exposed to the vector bites, hence the very low prevalence rate observed during the screening.

Anokye-beemu, which recorded the highest prevalence rate of 72.3% (Table 4.2), had a very typical village setting with its inhabitants being mostly farmers. The community had abundance of

trees, bushes, farms and refuse dumps which all favour the abundance of biting midges. Since the inhabitants were mostly farmers and therefore stay longer periods on their farms, they must have been exposed to the bites of these midges hence the high numbers of people that were infected with *M. perstans*. The remaining communities also had similar settlements with similar vegetations like Anokye-beemu. The inhabitants of these communities must have also been exposed to the vectoral bites hence the prevalences recorded.

The result of this epidemiological survey suggested that the prevalence of mf was higher in males than in females but there was no significant difference between them for all the age groups; except for the group 30 to 39 years where there were more infected females (54.7%) than males (Figure 4.1). The higher prevalence in the women in this category suggests that they were more exposed to the vectoral bites due to their involvement in farming and other out-door activities. The comparable prevalences in males and females as observed in this study were similar to other reports from studies conducted in Nigeria (Uttah *et al*., 2011; Useh & Ejezie, 1995). This suggested that both sexes equally engaged in out-door activities, and there were no socio-cultural outdoor restrictions.

M. perstans mf was found to affect all segments of the population (Figure 4.2), from school-age children to adults as noted in a study conducted by Simonsen *et al*., (2011). Microfilariae prevalence levels among the various age groups were not significantly different except for the 15 to 19 years age group where there was a significant difference ($p<0.001$) between the two sexes. The males in this group had a higher prevalence which can be attributed to greater exposure as a result of their out-door activities. They tend to spend a lot of time roaming through the bushes or hunting unlike their female counterparts who were required to stay home and take care of the home as the parents go to the farms; thus less exposed to vectoral bites.

It was also observed that the peak of infection was reached much earlier in males (15 to 19 years; 20 to 39 years) than in females (30 to 39 years). This confirms the findings of previous investigators on the difference in the level of filarial infection between males and females (Ripert *et al*., 1977; Noireau *et al*., 1989; Nelson *et al*., 1962). A prevalence rate of 17.9% was observed for the 19 to 14 years age group higher than those of the 15 to 19 years age group (13.3%). This might be explained by the reason that children of the former category were most often with their parents; thus most often out-doors exposing them to the bites of the vectors. This is also corroborated by the findings of Simonsen *et al*., (2011) that infections begin in childhood, which leads to maximal infection rates already in children aged 10 to 14 years (Asio *et al*., 2009b).

The wide distribution of mf could be explained by the abundance of its vector. The vegetation of the study site consisted of plantain farms, decaying plant matters and shrubs which have been identified to provide suitable habitats for *Culicoides* species (Boorman, 1993). The high numbers of *Culicoides* caught at Dukusen indicated an abundance of the vector which could be attributed to the kind of vegetation and settlements (Table 4.3). Although this community had similar vegetation like the remaining communities, it was quite peculiar in that it had very thick trees and bushes and lots of livestock which are known to provide suitable habitats for the vectors (Boorman, 1993). Afrisere, which had the lowest number of *Culicoides* collected, had very few plantain farms with very few livestock. This community had close human settlements, but farther from the natural grass vegetation, hence the low numbers of vectors caught.

The availability and type of breeding sites in an environment will influence the kind of vector species breeding in an area (Glick, 1990). Using both collection techniques (light trap and HLC), a total of 2,194 *Culicoides* species were captured. Trap collections captured more than 60% of the total catch (Table 4.4). The high numbers collected using the light trap indicated that the trap, with

its UV light incorporated, serve as a great attractant to the midges as they do not only follow the light source but also the kind of light emitted.

Trap collections gave eight different species (*Culicoides grahami, C. milnei, C. inornatipennis, C. neavei, C. imicola, C. fulvithorax, C. schultzei* and *C. accraensis*) whereas HLC gave only one species (*C. inornatipennis*). This suggests that only *C. inornatipennis* of all the species collected was preferentially anthropohilic. HLC could thus be used to describe the disease transmission dynamics of the vector. This finding is in line with those of Viennet *et al*., (2011) who also collected diverse species (15 species) using the UV light trap compared to direct aspiration i.e. HLC (6 species) from the host.

Within the trap collections, overnight catches had a significantly higher proportion of flies compared to evening collections (Table 4.5). Also it was observed that *C. milnei* which is reported as the actual vector of *M. perstans* in Cameroon (Wanji *et al*., unpublished data) was caught more in the overnight traps, confirming the findings of Hopkins (1952) that *C. milnei* is exclusively a night biting species. From this survey, no *Culicoides* were caught in the mornings (i.e. 6am to 9am). This could be attributed to the vector"s need for cool and humid conditions which were absent due to dawn breaking early and the sun rising early as well (personal observation).

The number of biting midges caught (*Culicoides*) was seasonal with the highest numbers recorded during the rains and shortly after the rains, when the temperature was high. The much available water from rainfall which kept the soil and other plant matters moist might have attributed to the higher abundance of the *Culicoides* species in the rainy season. It is generally observed that there is a higher occurrence of bush burning in the dry season in Ghana. This might have contributed to the recorded low abundance of the *Culicoides* species captured in the dry season (Table 4.6).

The reduction in the numbers of *C. neavei* in the rainy season suggested that it was more abundant in the dry season. This could further be explained by the reduction in the numbers of livestock at Dukusen, which also provided suitable habitat for the *Culicoides* vectors in agreement with Downes and Wirth, (1981) observation that the head of an animal also serves as a conducive habitat for the biting midges. Interactions with inhabitants disclosed that *Culicoides* were more abundant during the period of groundnut harvest (i.e. rainy season) in the study area. The presence of anthropophilic *C. inornatipennis* in both seasons in the study area is noteworthy.

Only 6.2% (i.e. 135 out of 2,194) of all samples collected from HLC and trap were blood-fed. The source of blood of the species caught by traps might not be humans; thus unknown. However, 66% (90 out of 135) of the blood-fed *Culicoides* caught were *C. inornatipennis* confirming that it was anthropophlic as the source of blood-meal was from human collectors. Dukusen, recorded the highest percentage (30.4%) of blood-fed *Culicoides* which is in agreement with it also recording the highest number of captured *Culicoides* (Table 4.3). This suggests that the higher the abundance of *Culicoides* species in a community, the greater the proportion of engorged (blood-fed) species caught.

HLC was used to determine the biting rate of the vectors. This is due to the reason that trap collections do not accurately estimate the biting rate of *Culicoides* species (Viennet *et al*., 2011). Bebusu recorded the highest monthly biting rate (Table 4.8) since it recorded highest number of catches made from HLC. The biting pattern of *Culicoides* showed sustained peak in the evening hours (5pm to 6pm) at all the study communities (Figure 4.2). It is also important to mention that there was no biting activity for the morning hours in all the study communities contrary to the findings of Asio *et al*., (2009a) and Uttah *et al*., (2011) who observed the highest biting peak in the mornings.

For vector incrimination, the vector should be attracted to and must bite humans. It should be able to also carry the parasite. In determining the importance of a particular vector species in disease transmission, HLC though with a lot of ethical constraints, is a very important tool in describing the disease transmission dynamics of a vector borne disease like mansonellosis. Agbolade *et al*., (2006) confirms this assertion when he used HLC methods to incriminate *C. fulvithorax* as vector of *M. perstans* in the north of Western Nigeria. Also, Viennet *et al*., (2011) who assessed vector/host contact by comparing different methods in Western France supports the argument that direct aspiration (i.e. HLC) collects exclusively host-seeking females. The exposure to the vector certainly plays an appreciable role in disease transmission (Noireau *et al*., 1990).

We were able to maintain 72.9% of the engorged *Culicoides* species that had fed on an *M. perstans* donor for ten days in the tubes described by Hopkins (1952) before they were dissected. The highest mortality was observed on days 2 and 7. The early mortality on day 2 could be attributed to the stress the midges went through from when they were aspirated and finally transported to the laboratory.

The infection rate was zero for all the blood-fed *Culicoides* dissected as none of them was found infected with ay of the larval stages of *M. perstans* (L1, L2 and L3). Also, the engorged *Culicoides* samples (collected from the *M. perstans* donor) were dissected for the presence of *M. perstans* microfilariae, but none was observed. The fact that no *C. inornatipennis* was found carrying any parasite suggests that although it is anthropophilic, it may not support the development of the parasite contrary to other findings by Duke (1965) and Linley *et al*., (1983) who incriminated *C. inornatipennis* as a vector involved in the transmission of *M. perstans*. However, more studies are needed to confirm this, specifically use PCR to determine whether they carry any mf or not.

No study in Ghana has cited biting midges as vectors of *M. perstans*, but studies in other countries have cited biting midges as vectors of *M. perstans* (Noireau *et al*., 1990; Sharp, 1928 and Agbolade & Akinboye, 2001). This study did not identify *M. perstans* in all the engorged *Culicoides* (both from *M. perstans* donor and HLC) collected in the same district where the human blood samples were studied.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

At the end of the survey, it was observed that an average of 33.2% of the people living in the study communities was infected with *M. perstans* with 57.2% being males. The biodiversity and biological activities of *Culicoides* species of the study communities has also been established. Trap collections gave diverse species whiles HLC gave only one species.

Culicoides grahami, C. milnei, C. inornatipennis, C. neavei, C. imicola, C. fulvithorax, C. schultzei were identified as the main *Culicoides* species at the study communities while *C. accraensis* was singly identified (i.e. only one caught), in addition to the above mentioned species. The most blood-fed (engorged) species were *C. inornatipennis* captured using HLC followed by *C. imicola* captured by light trap.

C. inornatipennis was the only species caught by HLC with its peak biting period between 5-6 pm in the evenings. None of these species was found infected with any of the larval stages of *M. perstans*.

6.2 Recommendations

It is recommended that

i. Since the majority of those with positive *M. perstans* microfilariae were poor socioeconomically, there is the need for public health education on the disease.

ii. Implementation of effective control strategies against *Culicoides* biting midges is essential for health promotion.

iii. More sampling of the vector should be carried out to improve the chances of finding an infected vector particularly in the night i.e. 6pm to 6am.

iv. Vector sampling should be carried out all year round to collect more diverse species.

v. The epidemiological study should be extended to other communities in Ghana to ascertain the real prevalence in the country.

vi. Even though there is no concrete drug for treating mansonellosis, doxycycline has been found to be effective in Mali, hence, similar trial should be carried out in Ghana to determine the efficacy of the drug.

6.3 Challenges

- Difficulty in getting collectors for HLC and volunteers (*M. perstans* positive)
- Bad roads leading to the communities
- Unfavourable weather (severe harmattan, strong winds, heavy rains, etc) affecting the numbers of collections made

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APPENDICES

APPENDIX 1: *CULICOIDES* SPECIES IDENTIFICATION SHEET

DATE OF COLLECTION…… SITE……..

METHOD OF COLLECTION…… COLLECTOR……..

SN	HOUR	SPECIES								FED: YES NO	AGE P N
		C. inornatipennis	*C. imicola*	*C. fulvithorax*	*C. grahami*	*C. accraensis*	*C. milnei*	*C. neavei*	*C. schultzei*		

TOTAL											

KEY:

SN: serial number; P: parous (segmented); N: nulliparous (unsegmented)

APPENDIX 2: CULICOIDES DISSECTION FORM

DATE OF COLLECTION…… SITE……..

METHOD OF COLLECTION……. COLLECTOR……

SN	HOUR	SPECIES	HEAD			THORAX			ABDOMEN			COMMENT
			L1	L2	L3	L1	L2	L3	L1	L2	L3	

TOTAL												

Dissector ……. Storage medium/condition……

APPENDIX 3: DAILY MORTALITY SHEET FOR ENGORGED *CULICOIDES*

SPECIES

DATE OF COLLECTION…… SITE ……

METHOD OF COLLECTION: …… PATIENT NAME ……

DAYS	TUBES																		FED WITH 15% SUGAR	REMARKS
	1		2		3		4		5		6		7		8		9			
	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D		
1																				
2																				

3																				
4																				
5																				
6																				
7																				
8																				
9																				
10																				

KEY: A: alive; D: dead on each day