AN INVESTIGATION INTO IMPACT OF UNSPRAYED SURFACES ON MOSQUITO BEHAVIOUR AND MALARIA TRANSMISSION IN AN AREA UNDERGOING INDOOR RESIDUAL SPRAYING IN NORTHERN GHANA

BY

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DECLARATION

This thesis is the result of research work undertaken by Otubea Ansah Mante towards the reward of MPhil Entomology in the African Regional Postgraduate Programme in Insect Science (ARPPIS), University of Ghana, under the supervision of Dr. Maxwell Appawu and Dr. Samuel Dadzie.

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DEDICATION

This work is dedicated to my Almighty God, my family and my friends.

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LIST OF ABBREVIATIONS

AFRO	WHO African Regional Office
AGA	AngloGold Ashanti
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic Acid
EDTA	Disodium ethylene diamine tetra acetate. $2H_sO$
EIR	Entomological Inoculation Rate
ETC	Exit Trap Collections
IPT	Intermittent Preventive Treatment
IRD	Indoor Resting Densities
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Nets
LLINS	Long Lasting Insecticide Treated Nets
MAB	Monoclonal Antibody
MBR	Man Biting Rate
MICS	Multiple Indicator Cluster Survey
MPA	Mosquito/*Plasmodium* Antigen
NMCP	National Malaria Control Program
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Collections
RAPD	Random Amplified Polymorphic DNA
RDT	Rapid Diagnostic Test
RFLP	Restriction Fragment Length Polymorphism

SDDH2O	Sterile Double Distilled Water
SEARO	South East Asia Regional Office
SNP	Single Nucleotide Polymorphism
SSA	Sub-Sahara Africa
WHO	World Health Organization
WHOPES	WHO Pesticide Evaluation Scheme

ABSTRACT

Indoor Residual Spraying (IRS) is one of the malaria vector control tools used in the control of malaria and has helped to reduce malaria transmission in many areas in Sub-Saharan Africa. This involves spraying the walls of rooms with insecticides so as to kill any mosquito that rest on the insecticide treated walls after feeding. However, recent discussions have been centered on the role unsprayed surfaces such as hanging cloths play in sprayed rooms in malaria transmission. In this study, the effect of hanged clothes on mosquito resting and exiting behaviour as well as on malaria transmission was investigated using mosquitoes captured by pyrethrum spray (PSC) and window exit trap (ETC) collections. The study was undertaken in Tarikpaa, in the Savelugu-Nanton district in Northern Ghana, an area undergoing Indoor Residual Spraying. Kulaa in the Tamale district of Northern Ghana was used as the control area with no IRS. Mosquitoes were sampled from August to December 2013. *Anopheles gambiae* s.l., *Anopheles funestus, Anopheles nili* and *Anopheles rufipes* were the *Anopheles* species collected during the period of the study making up 97.1%, 2.0%, 0.3% and 0.6% of the species respectively. Both *An. coluzzi* (M form) and S molecular forms occurred in all rooms in both areas. *An. gambiae* s.l. collected in rooms with hanging clothes in the IRS community (Tarikpaa) was lower (14.68%) compared to rooms with no hanging clothing (85.32%). The same was observed for the non-IRS community (Kulaa). A total of 18 and 100 *Anopheles* mosquitoes were collected from rooms with hanging clothes and rooms without hanging clothes in Tarikpaa respectively. The mean Indoor Resting Density (IRD) of *Anopheles* collected from rooms in the IRS area without hanging clothes was higher than that of rooms with hanging clothes (2.7 mosquitoes/room vs 0.36 mosquitoes/room). However, this difference was not significant ($F_{(1, 12)} = 2.078$, $P = 0.175$). The same trend was observed for the unsprayed community, Kulaa. A Fed

to Gravid ratio of 0.9:1 and 1:1 was estimated for mosquitoes collected exiting rooms with hanging clothes and without hanging clothes respectively. The sporozoite rate for both rooms with hanging clothes and rooms without hanging clothes in Tarikpaa was zero. The only positive mosquito samples were from the rooms without hanging clothes in the non-sprayed area (1.83%). An Entomological Inoculation Rate (EIR) of 0.0043 infective bites/man/night was estimated for *An. gambiae* collected from Kulaa. The fed: gravid ratio also indicated that more blood fed mosquitoes left the rooms immediately after feeding in the presence of insecticide on the walls. The number of *Anopheles* species collected by ETC from rooms with hanging clothes in the IRS area was significantly higher than in rooms without hanging clothes. The practice of hanging clothes in the area undergoing indoor residual spraying did not affect malaria transmission. *Anopheles* mosquitoes tend to exit rooms in the morning after feeding. This has implications for the effectiveness of the IRS in the area because many of the mosquitoes will not rest on the insecticide treated walls to be killed.

CHAPTER ONE

GENERAL INTRODUCTION

1.0 Introduction

Control of malaria represents one of the world's greatest public health challenges, especially in sub-Saharan Africa where most of the disease occurs (Mabaso, 2004). Globally, an estimated 3.3 billion people were at risk of malaria in 2011, with populations living in Sub-Saharan Africa having the highest risk of acquiring malaria: approximately 80% of cases and 90% of deaths are estimated to occur in the WHO African Region, with children under five years of age and pregnant women most severely affected (World Malaria Report, 2012). Worldwide malaria led to 216 million clinical episodes, and 655,000 deaths in 2011 (World Malaria Report, 2011). In Ghana, the National Malaria Control reports over 4,154,261 cases and 3,259 deaths (NMCP Annual Report, 2011). These estimates render malaria the pre-eminent tropical parasitic disease and one of the top three killers among communicable disease (Breman, 2001, Breman *et al*., 2004).

Malaria is an acute disease caused by the protozoa, *Plasmodium species* and transmitted through the bite of the female *Anopheles* mosquito. Mainly, five species of *Plasmodium* are incriminated to cause malaria in humans: *P. vivax*, *P. malaraie*, *P. ovale*, *and P. falciparum*. *P. knowlesi* (the relatively new parasite), which until recently was diagnosed as the more benign *P. malaraie* (Cox-Singh *et al*, 2007). *P. falciparum* has been identified as the most virulent strain causing about 800,000 deaths of malaria cases every year (John, 2010). In Africa, it accounts for 90-98% of all malaria cases. Several species of *Anopheles* are responsible for transmitting malaria and this varies according

to location. Much variation can be observed between villages and also in different micro-ecological zones a few kilometers apart (Appawu *et al.*, 2004). In Central Africa and even in Ghana, it is not rare to capture two to four different vectors (e.g. *An. gambiae*, *An. funestus*) during the same night (Fontenille and Lochouarn, 1999; Appawu *et al.*, 2001). The disease is characterized mainly by fever, severe headache, joint aches, and also by respiratory distress, neurological disease and haemolysis. Malaria is an entirely preventable and treatable disease, provided the currently recommended interventions are properly implemented. Though these interventions have been proven to work in reducing malaria morbidity and mortality, they do not achieve significant progress as stand-alone projects. Most effective programs have adopted the approach of integrating a number of interventions. These include preventive interventions such as vector control through the use of Insecticide-Treated Nets (ITNs), Indoor Residual Spraying (IRS) and, in some specific settings, larval control, and chemoprevention for the most vulnerable populations, particularly pregnant women and infants. There are also treatment-based approaches such as confirmation of malaria diagnosis through microscopy or Rapid Diagnostic Tests (RDTs) for every suspected case, and timely treatment with appropriate antimalarial medicines.

Indoor Residual Spraying (IRS) is one of the primary vector control interventions for reducing and interrupting malaria transmission and remains a powerful vector control tool (World Malaria Report, 2012). IRS is the application of long-acting chemical insecticides on the walls of all houses and domestic animal shelters in a given area, in order to kill the adult vector mosquitoes that land and rest on these surfaces. In 2011, 80 countries, including 38 in the African Region, recommended IRS for malaria control. In that year, 153 million people were protected by IRS worldwide, or 5% of the global

population at risk. In the African Region, the proportion of the at-risk population that was protected rose from less than 5% in 2005 to 11% in 2010 and remained at that level in 2011, with 77 million people benefiting from the intervention (World Malaria Report, 2012).

Different countries use different insecticides based on what works and what does not work with regards to the resistance status of the insecticide in that country. Depending on the kind of insecticide, the application is sprayed once annually, such as with Deltamethrim or Fendona or twice yearly such as with Ficam a carbamate insecticide (Reddy *et al.*, 2011). The primary effects of IRS towards curtailing malaria transmission are to reduce the life span of vector mosquitoes so that they can no longer transmit malaria parasites from one person to another thus reducing transmission and also to reduce the density of the vector mosquitoes. Some insecticides used also repel mosquitoes and by so doing reduce the number of mosquitoes entering the sprayed room, and thus human-vector contact.

Scientific evidence of IRS efficacy in reducing or interrupting malaria transmission in different epidemiological settings has been available since the 1940s and 1950s (Russel, 1955). Numerous studies have shown that IRS has substantially reduced infant and child mortality. In Africa, malaria eradication pilot projects were initiated from the 1950s to the 1970s in Benin, Burkina Faso, Burundi, Cameroon, Kenya, Liberia, Madagascar, Nigeria, Rwanda, Senegal, Uganda and the United Republic of Tanzania. These projects demonstrated that malaria was highly responsive to control by IRS with significant reduction of Anopheline vector mosquitoes and malaria, although in most cases, transmission could not be interrupted (Beales, 1989). IRS is an ideal tool when majority of vector population feeds and rests inside houses, where the vectors are

susceptible to insecticide in use, where night sleeping of people is predominantly indoors, where prevailing structures are suitable for spraying and also in settlements furthest from health facilities.

In Ghana, IRS began with the AngloGold Ashanti Malaria Control Programme in Obuasi in 2005. It has been shown to reduce malaria cases from about 12,000 in 2005 to about 4,000 in 2008. The Presidents' Malaria Initiative (PMI) of the United States of America in 2008 undertook IRS in five districts of the Northern Region; Tolon-Kumbungu, Savelugu-Nanton, Karaga, Gushiegu and West Mamprusi. Ghana has two main season which affects the pattern of the malaria disease in the country; the rainy and dry seasons. In the northern Ghana, there is a prolonged rainy season. In the south, two rainy seasons occur, from April to July and from September to November.

1.1 Rationale

IRS as a tool in malaria control has been used since the 1931's in Kwazula-Natal a district in South Africa. A dramatic reduction in the number of malarial cases was observed after the first in the KwaZulu-Natal province indoor spraying with pyrethrum in 1932 (le Sueur *et al*., 1993). In Ghana, IRS in Obuasi records a decrease in malaria cases at the Edwin cade hospital in Obuasi from about 5800 in 2005 to 1000 in 2008 (AGA report, 2010).

The premise of IRS is that the malaria causing mosquitoes, in Ghana, *An. gambiae* and *An. funestus* bite indoors and rest indoors. After biting, mosquitoes tend to rest on walls which in an IRS area has been sprayed with an insecticide. While resting on the walls, the insect picks a lethal dose of the insecticide and eventually dies. The resting surfaces for indoor resting mosquitoes range from sprayable structures such as walls, roofs,

windows to unsprayable structures such as household objects (Nagpal *et al.*, 2012) including hanged clothes. It has been observed that most people tend to hang clothes and also possess other materials in their rooms which are not sprayed. These hanging clothes are usually clothes that have been worn a number of times by residents and thus contain body odour which serve as attractants for mosquitoes. Chemicals from human body odour, sweat, and breath have been identified and proven to attract a variety of mosquito species (Cork and Park 1996, Healy and Copland 2000, Bernier *et al.*, 2002, Healy *et al.*, 2002) These hanging clothes and other unsprayed surfaces may serve as alternative resting places for mosquitoes even in the presence of the insecticide on the walls, thus avoiding the insecticides on the wall.

This study aims to investigate the impact of unsprayed surfaces on mosquito behaviour and malaria transmission in an area undergoing indoor residual spraying. This investigation was done in an area undergoing Indoor Residual Spraying and an area where Indoor Residual Spraying has not been undertaken.

1.2 Objectives

To evaluate the impact of unsprayed surfaces on mosquito behaviour in an area with IRS activities

1.2.1 Specific Objectives

- To determine the species composition and distribution of *Anopheles* mosquitoes collected resting from rooms with sprayed and unsprayed surfaces
- To determine the room density of *Anopheles* mosquitoes in rooms with and without hanging clothes
- To investigate movement of mosquitoes from rooms with sprayed and unsprayed surfaces
- To determine the man biting rates of *Anopheles* mosquitoes in rooms with and without hanging clothes.
- Determine the sporozoite rates of mosquitoes from the two study areas.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

2.1 History of Malaria

Humans began to take notice of the malaria disease millions of years ago. It has been postulated to have been transferred from gorillas to humans (Lui *et al.*, 2010). Mention is made of malaria as far back as 2700 BCE when the Chinese were drawing relationships between fever and enlarged spleens. However, in 400BC, Hippocrates known later as the father of medicine was the first to describe malaria fever and draw a close to precise relationship between malaria and stagnant water. He is reported to have said 'Those who drink [stagnant water] have always large, stiff spleens and hard, thin, hot stomachs, while their shoulders, collarbones, and faces are emaciated; the fact is that their flesh dissolves to feed the spleen" (Lehrer, 1979).

Malaria became an important and dominant disease when the number of deaths attributed to malaria became more or equal to the number of people killed in a war due to death from weapons. In those days, a lot was unknown about the disease. However, enough was known about its effects and in some cases were even used as biological weapons.

2.2 Epidemiology of Malaria

Malaria epidemiology is concerned with the study of the patterns and factors that affect the distribution of malaria and also the use of these patterns to produce an effective control of the disease. Malaria is an acute disease of humans characterised by chills,

fever, headache, anaemia etc. caused by the *Plasmodium* parasite through the bite of an infective female *Anopheles* mosquito. It is prevalent between latitude 450 N and 400 S around the world and is responsible for more deaths per year than any other arthropod-transmitted disease. (Foote and Cook, 1959)

Globally, malaria is known as a dangerous disease that causes malaise, other health complications and also death amongst people of all ages, the highest number of deaths recorded in children. However, malaria has been eradicated in some parts of the world. The decline or ``natural recession'' of indigenous malaria in industrialized countries during the middle of the 19th century (Hackett, 1995) has been attributed to several factors, such as drainage of swampy areas, better animal husbandry, improved housing, greater availability of quinine, and general socioeconomic improvement. Indeed, the achievement of malaria eradication on the continent of Europe is viewed as a major success in the chequered history of global malaria eradication (Bruce-Chwatt; 1980, 1985).

As seen in Figure 1, In Africa, malaria can be found in mainly in West and East Africa, the incidence being greater than 200 cases. In South and North Africa, malaria incidence is between 0 – 4 cases. Across 107 malaria-endemic countries, estimated incidence in 2004 totalled 402 million (range 350-500 million) cases, of which around 57% occurred in World Health Organization Africa Regional Office (AFRO) and 30% in World Health Organization South East Asia Regional Office (SEARO). This includes an estimated 311 million (range 270-400 million) cases of *falciparum* malaria, of which around 72% occurred in AFRO and 19% in SEARO. These numbers are around 6-fold higher than cases globally recorded in national health information systems, or around 17-fold higher for non-African countries.

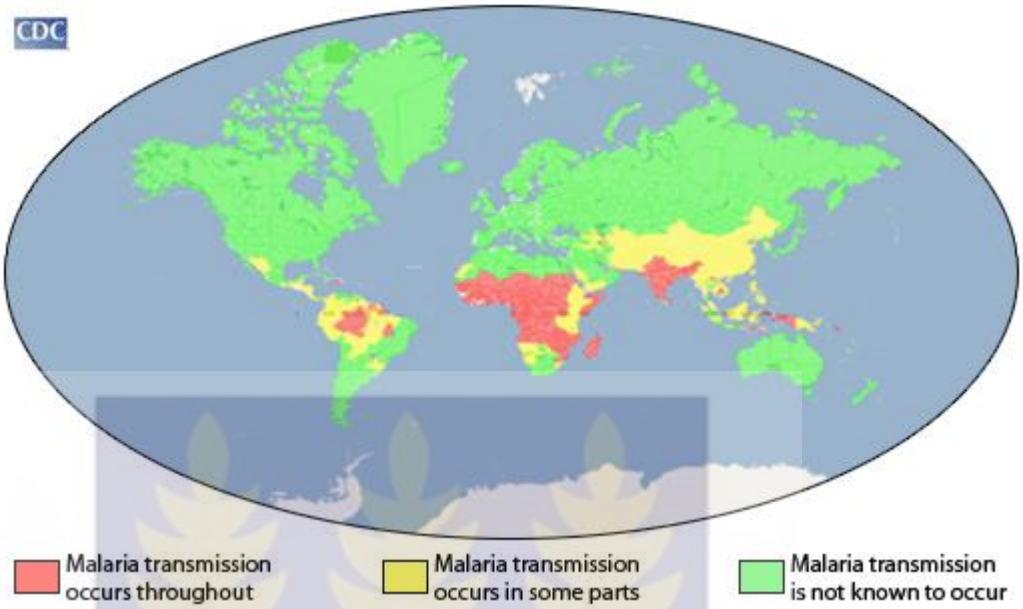


Figure 1: World distribution of malaria

Source: http://www.cdc.gov/malaria/about/distribution.html 02/06/2014

In Ghana, malaria is endemic and stable and constitutes one of the leading causes of morbidity and mortality, especially among pregnant women and children under the age of five. The Ministry of Health (MOH) estimates that 3 to 3.5 million cases of suspected malaria are reported each year in public health facilities, representing 30-40% of outpatient attendance. Of this figure, over 900,000 are children under the age of five. Malaria also accounts for about 61% of hospital admissions of children under five years and 8% of admissions of pregnant women. It is estimated that malaria accounts for 22% of under-five mortality and 9% of maternal deaths (The President's Malaria Initiative, 2007).

Ghana's efforts to control malaria date back to the pre-independence era, when various strategies were employed at different times. In 1999, the country adopted the Roll Back

Malaria initiative and has since been implementing a combination of curative and preventive interventions. Ghana subscribed to the Abuja Accord of the year 2000, by African Heads of States, which sought to achieve 60% coverage of malaria interventions by the year 2005, focusing particularly on pregnant women and children under five in need of access to suitable and affordable combinations of personal and community protective and curative measures such as insecticide-treated mosquito nets (ITNs) and prompt, effective treatment for malaria. The Abuja Accord sought to ensure that at least 60% of all pregnant women at risk of malaria, especially those in their first pregnancies, have access to appropriate chemoprophylaxis or Intermittent Preventive Treatment (IPT) (DHS, 2008).

2.3 The Parasite

Malaria is caused by the parasite, *Plasmodium* which belongs to the family Plasmodiidae, Order Haemospiridia, Class Aconoidasida, Phylum Apicomplexa, Subphylum Alveolata and Kingdom Chromoalveolata. There are several species of *Plasmodium* affecting a range of organisms including birds, lizards, monkeys and man. In man, five species of *Plasmodium* have been identified: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and in recent times, *P. knowlesi*. *P. falciparum* is known as the most virulent species; causes most of the severe cases of malaria. In Africa, *P. falciparum* is the most prevalent and the most virulent, but in most malaria endemic regions multiple sympatric species are found and co-infection within individual human hosts or the mosquito vector population is common (Gnémé *et al.*, 2013). The effect of mixed species infections on clinical outcome has been described as both beneficial (Smith *et al.*, 2001) and adverse (May *et al.*, 2000). *P. falciparum* is found mostly in Sub-Saharan Africa. Malaria episodes caused by *P. falciparum* in Ghana accounts for 90-95% of

total malaria cases (MICS, 2013). The distribution of *P. vivax* is concentrated in the Horn of Africa, covering Djibouti, Eritrea, Ethiopia, Somalia, and Sudan. It is estimated malaria cases in these areas annually can be attributed to *P. vivax* infections (Mendis *et al.*, 2001). In eastern and southern Africa, *P. vivax* represents around 10% of malaria cases but 1% of cases in western and central Africa. In Ghana and sub-Saharan Africa in general, *P. vivax* malaria infections have not yet been detected. This can be attributed to the presence of the Duffy negative trait in people of West Africa. The Duffy negative trait is an inherited red cell phenotype that lacks the receptor for invasion of the human red cell by the merozoites of *P. vivax* (Sanger *et al.*, 1955).

P. malariae is known to cause a more benign form of malaria, also known as the quartan malaria parasite because paroxysms occur every three days. *P. malariae* is rare but still exists in Central and Western Africa. It is usually seen as mixed infections with *P. falciparum*. In Ghana, infections with *P. malariae* account for less than 10% of the malaria cases annually (MICS, 2013).

P. ovale is found primarily in West Africa, though in recent years, *P. ovale* infections have been recorded in the Western Pacific and Asian mainland. In Ghana, the MICS survey reports of less than 2% of malaria infections annually are due to *P. ovale*. Initially, most *P. knowlesi* malaria infections were misdiagnosed as *P. malariae*; the early blood stages of *P. knowlesi* morphologically resemble *P. falciparum*; the mature blood stages and gametocytes are similar to those of *P. malariae (*Singh *et al.*, 2004*). P. knowlesi* has been reported from South East Asian countries such as Malaysia, Thailand, Vietnam, Myanmar and Philippines.

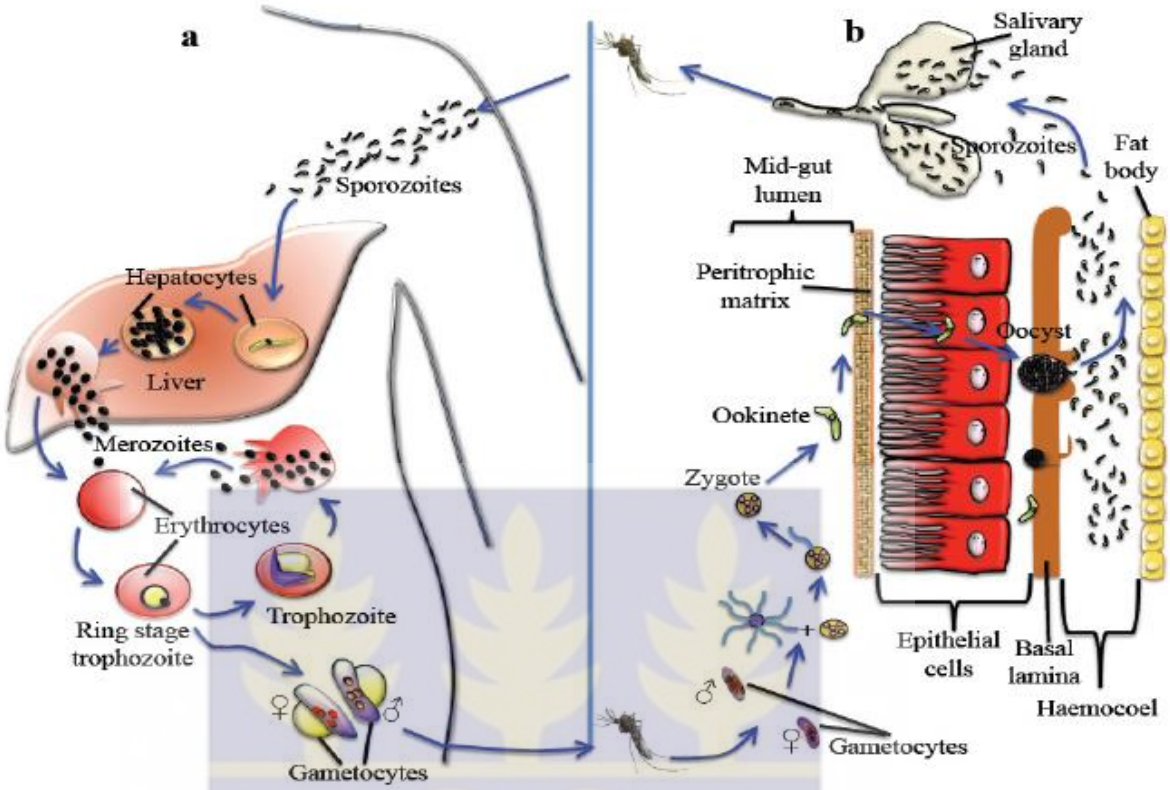


Figure 2: Life Cycle of Malaria Parasite

Source: Aboagye-Antwi, F., 2010.

Most parasites infect one or two hosts to complete its life cycle. The malaria parasite possesses an indirect or complex life cycle. The parasite infects both humans (the host) and the mosquito (the vector). The parasite changes morphologically throughout the various stages of its life cycle. When a mosquito infected with the *Plasmodium* parasites bite a human being, the parasites are introduced into man as sporozoites and invades the liver cells; hepatocytes. In the liver, the sporozoites undergo asexual reproduction, dividing into numerous cells, a process known as exo-erthrocytic shizogony. At this stage, the parasite is asymptomatic and will remain in the liver for about 7 to 10 days. In certain species, *P. vivax* and *P. ovale*, the sporozoites develop into the hypnozoites which can stay in the liver from weeks to years. The parasite now

in the form of merozoites are released into the blood stream where they invade red blood cells. The invasion of red blood cells by the merozoites is by means of specific receptors on the erythrocyte membranes and ligands on the surface membrane (Fujioka and Aikawa, 2002), a process known as erythrocytic schizogony. Merozoites develop into trophozoites then to shizonts, which rupture releasing merozoites that infect more erythrocytes. Clinical symptoms are manifested at this stage (NIH, 2007). Concomitantly, small portions of the parasites differentiate from newly invaded merozoites into sexual forms, which are macrogametocyte (female) and microgametocyte (male) (Fujioka and Aikawa, 2002). During a blood meal, the mosquito ingests macrogametocyte and microgametocyte. In the gut of the mosquito, the microgamates exflagellate, forming eight haploid motile microgametes. One microgamete fuse with a macrogamete to form the zygote. Within 18–24 hours, the non-motile zygotes transform into motile ookinetes. The ookinetes cross two barriers: the peritrophic matrix and midgut epithelium. After traversing the midgut epithelium, the ookinete reaches the extracellular space between the midgut epithelium and the overlaying basal lamina, and transforms into an oocyst. Ten to 24 days after infection, depending on the *Plasmodium* species and ambient temperature, thousands of sporozoites are released into the hemocoel and the motile sporozoites invade the salivary gland epithelium (Fujioka and Aikawa, 2002). This process inside the mosquito is known as the sporogonic cycle (CDC, 2006). When an infected mosquito bites a susceptible vertebrate host, the *Plasmodium* life cycle begins again.

2.4 The Vector

Female mosquitoes belonging to the genus *Anopheles* transmit malaria parasites. Other mosquitoes, *Aedes* and *Culex* are responsible for transmitting other diseases. There are about 490 species of which only about 60-70 have been incriminated as vectors of malaria under natural conditions; and about 30-40 species are of major importance. (Fig 3) The *Anopheles* belong to the order Diptera, *An. gambiae* s.s. a member of the homonymous species complex comprising six recognized sibling species, is the most anthropophilic malaria vector worldwide (Coluzzi, 1992). Together with its sibling species *An. arabiensis*, they are responsible for more than three-quarters of all *P. falciparum* inoculations (Coluzzi, 1984, 1994). In Ghana, the major vectors are the *An. gambiae* species complex and the *An. funestus* complex. These species generally bite late in the night, are indoor resting, and are commonly found in the rural and peri-urban areas where socio-economic activities lead to the creation of breeding sites (GHS, 2008). The *An. gambiae* complex is made up of the *An. gambiae* s.s. *An. merus*, *An. melas*, *An. quadriannulatus A.*, *An. quadriannulatus B.*, *An. arabiensis*, and *An. bwambae* (Coetzee *et al.*, 2000). In addition to the *An. gambiae* complex, large parts of Africa are also home to other dominant vector species, including *An. funestus*, *An. nili* and *An. moucheti* (Sinka *et al.*, 2010)

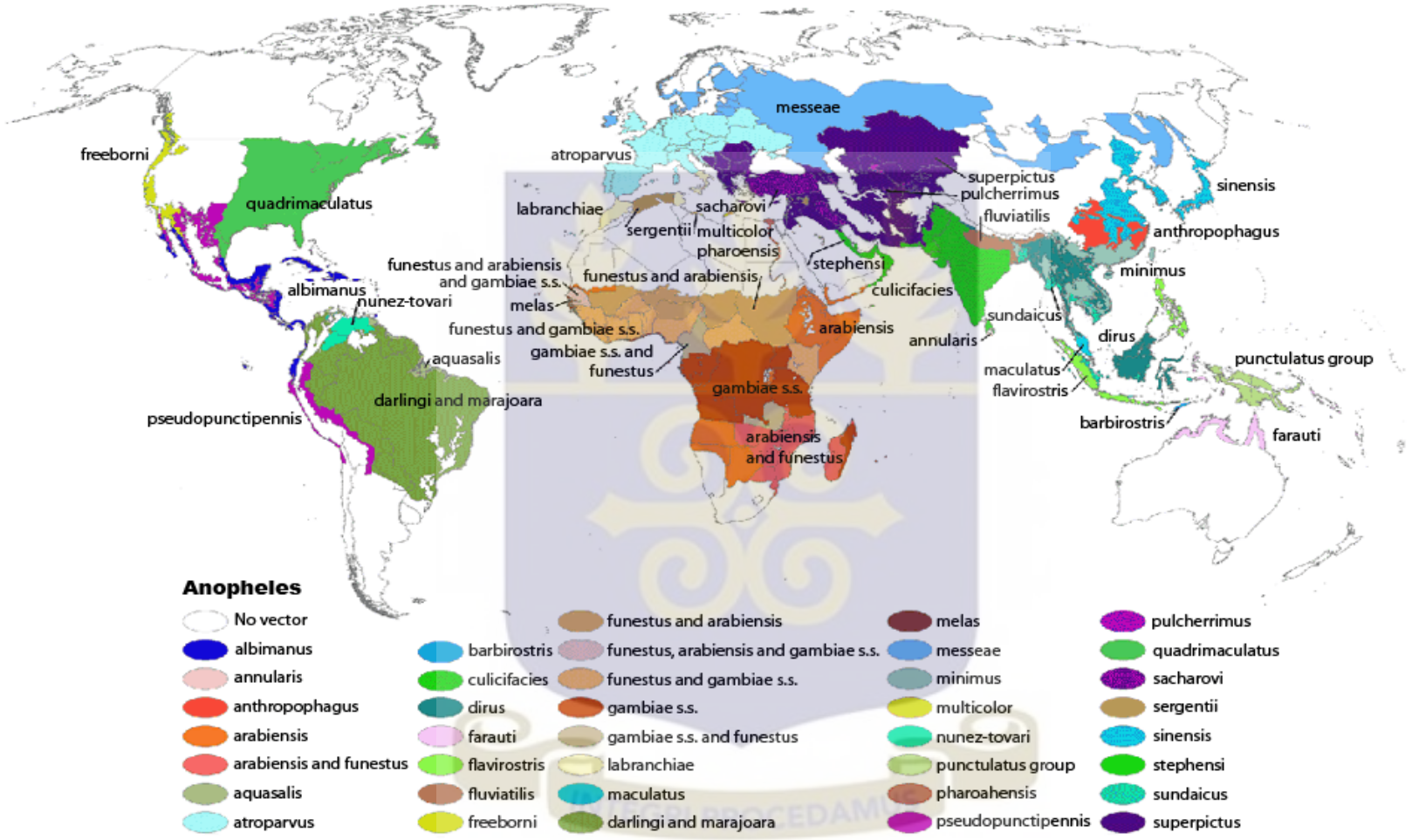


Figure 3: World distribution of *Anopheles* mosquitoes Source: Kiszewksi *et al.*, 2004.

2.4.1 *Anopheles gambiae* complex

The complex consists of seven closely related, morphologically indistinguishable sibling species originally defined by polygene chromosome analysis (Fanello *et al.*, 2002) but varying in their ecological, behavioural and vector competence (Djogbénou *et al.*, 2010). These however, can occur in sympatry with each other (Lindsay, 1998; Coluzzi *et al.*, 2002).

2.4.1.1 *Anopheles gambiae* sensu stricto (s.s.)

An. gambiae s.s. is the most efficient vector of malaria. *An. gambiae* Giles sensu stricto (s.s.) indicate the existence, in West Africa, of five chromosomal forms designated Bamako, Bissau, Forest, Mopti and Savanna (Coluzzi *et al.*, 1985). *An. gambiae* s.s. is usually found in sympatry with *An. arabiensis*, *An. gambiae* predominated in saturated environments, whilst *An. arabiensis* in sites subject to desiccation (Lindsay, 1998). Larval habitats are generally small, temporary, sunlit, clear and shallow fresh water pools (Giming, 2001).

2.4.1.2 *Anopheles arabiensis*

An. arabiensis is described as zoophilic, exophagic and exophilic (Sinka *et al.*, 2010), however the feeding and biting patterns of *An. arabiensis* varies depending on geographical location (Gilles and de Million, 1968). Larval habitats are similar to those of *An. gambiae* s.s. although *An. arabiensis* is able to utilize a greater variety of locations than *An. gambiae* s.s., including slow flowing, partially shaded streams (Giming, 2001).

2.4.1.3 *Anopheles melas/merus*

An. melas is largely anthropophilic, even in the presence of animals, this species readily feed on man (de Meillon, 1947). This species is a brackish water-breeding member of the *An. gambiae* complex that is distributed along the coast of West Africa and is a major malaria vector within its range (Deitz *et al.*, 2012), Although it withstands very high salinity, it usually found breeding in saline waters which is the same or lower than seawater (Akogbeto, 1995). *An. melas* is not an effective carrier of the malaria parasite, in endemic malaria zones, observed sporozoite indices are at least ten times lower than that of *An. gambiae* s.s. (Bryan, 1983).

2.4.1.4 *Anopheles bwambae*

This species is known only form the vicinity of Burunga hot springs in Bwamba Country, Toro District, Uganda, where it breeds in brackish water from geothermal springs and other halophilic mosquitoes such as *Aedes albocephalus, Ae. natronius* and *Culex tenagius* (White, 1985). *An. bwambae* can also be found in the Semiliki forest of Uganda, where the adult is found resting on the buttress bases of large trees, on fallen logs and sticks and loose dry leaves (White, 1985). *An. bwambae* is described largely as anthropophilic with endophilic tendencies, although in the absence of man, it tends to be zoophilic (White, 1985).

2.4.1.5 *Anopheles quadriannulatus* A/B

An. quadriannulatus species A and B, found in southern Africa and Ethiopia, respectively, are considered medically unimportant: human malaria parasites have never been detected in wild caught *An. quadriannulatus* females (Coetzee *et al.*, 2000). *An.*

quadriannulatus A and B display characteristics that are believed to have existed in ancestral forms of the complex, *i.e.* standard chromosomal arrangements, disjointed distribution and adaptation to temperate climates (Coluzzi *et al.*, 2002). *An. quadriannulatus* A and B are considered strictly zoophilic although recent laboratory and field studies report equal feeding preference for human and cattle (Pates *et al.*, 2001; 2005; 2006).

2.4.2 *Anopheles funestus*

An. funestus, in some cases, have a greater impact on malaria transmission even than *An. gambiae* (Gilles and de Million, 1968; Coetzee and Fontenille, 2004). *An. funestus* is anthropophilic; Charlwood *et al.* surmised that *An. funestus* may have been the first Anopheline species to specialize on biting humans; that their preferred larval sites (permanent water bodies in savannah-like environments) are likely to have been areas where humans first settled.

An. funestus is a member of the *funestus* Subgroup (Garrous *et al.*, 2005), which includes: *An. aruni, An. confusus, An. funestus, An. parensis* and *An. vaneedeni*. The members of this subgroup exhibit important variation in their biology and behaviour, especially in regard to malaria vectorial capacity and are only morphologically distinguishable during certain stages in their development (Gillies and de Meillon, 1968; Gillies and Coetzee, 1987; Coetzee and Fontenille, 2004). Only *An. funestus* is regarded as an important vector of malaria in this subgroup Coetzee and Fontenille, 2004), however Wilkes *et al.*, 1996 recently showed by salivary gland dissection that *An. rivulorum* (from the *An. funestus* group) from Tanzania was infected with *P. falciparum*.

2.5 Identification of members of *Anopheles* species complex

Correct species identification is the starting point for studying the epidemiological role of vectors (Bortel et al; 2001). Species recognition are scientifically valid and meaningful if they refer to taxonomic units having biological significance, that is to species defined according to the concepts developed by evolutionary biologists and generally applicable to sexually reproducing organisms (Dobzhansky, 1970; Mayr, 1963; White, 1978). Morphologically distinct organism are thus easily identified with differences in morphology – larval and pupa anatomy, wing spots, legs and abdomen. Reproductive isolation may be acquired with or without morphological divergence (unpublished, 2005), i.e. species undetectable by morphological differences. Identification is mostly achieved using morphological criteria, but this fails when sibling species and species with overlapping morphological characters are involved (Van Bortel, W., 2001).

A number of methods and markers have been established to distinguish between morphologically indistinct species including cytogenetic; crossing experiments, the study of chromosomal complement with particular reference to differential banding patterns of the polygene chromosomes from ovarian nurse cells of semi gravid of adult females (Coluzzi and Sabatini, 1967), isozyme analysis, classical genetic markers, mitochondrial DNA, ribosomal DNA (rDNA), microsatellite DNA and Random Amplified Polymorphic DNA (RAPD).

2.5.1 Morphological Identification

Preliminary identification of species in the field is still largely based on the use of morphological features. Gillies and de Meillon, (1968) and Gillies and Coetzee, (1987) provide detailed information on the morphological identification of Anopheline mosquitoes.

Unfortunately, the proliferation of species groups and complexes within many of the Anopheline taxa is rendering the use of morphological characteristics invalid for the identification of important malaria vectors (Beebe and Cooper, 2000).

2.5.1.2 Morphological Identification of *Anopheles gambiae* s. l and the *Anopheles funestus* group

Gillies and De meillon, (1968) and Gillies and Coetzee, (1987) provide detailed information on the morphological identification of Anopheline mosquitoes. *Anopheles gambiae* s.l. and *Anopheles funestus* are identified morphological by their palps, wings, legs and abdomen.

The general coloration of *Anopheles gambiae* s.l. is yellowish brown to grayish brown and could be darker depending on the area of collection. Females usually have three pale bands on palps. The apical band is broader enveloping the whole of the 5th and apex of the 4th segment. (Evans, 1938; Roberts and Janovy, Jr., 2000) The femora, tibia and 1st tarsal segment of the hind legs are speckled to variable degrees, while the 5th tarsus is all black. The wings have pale scales that are creamy white and tinged with yellow. The 3rd dark area (preapical dark spot) on vein 1 with a pale interruption, sometimes fused with preceding pale area (Gilles and Coetzee, 1987) (Fig 4) The abdomen is mainly light brown in colour with hairs, but the eighth tergite usually has scales, which may extend to the seventh.

Female *An. funestus* have three pale bands on palps with rather variable width that are generally narrow. The abdomen is usually dark brown devoid of scales. Legs are generally dark; tibia with small, apical, white spots. Wing markings of *An. funestus* species show the characteristic four pale spots on the costa (Hervey *et al.*, 1998).

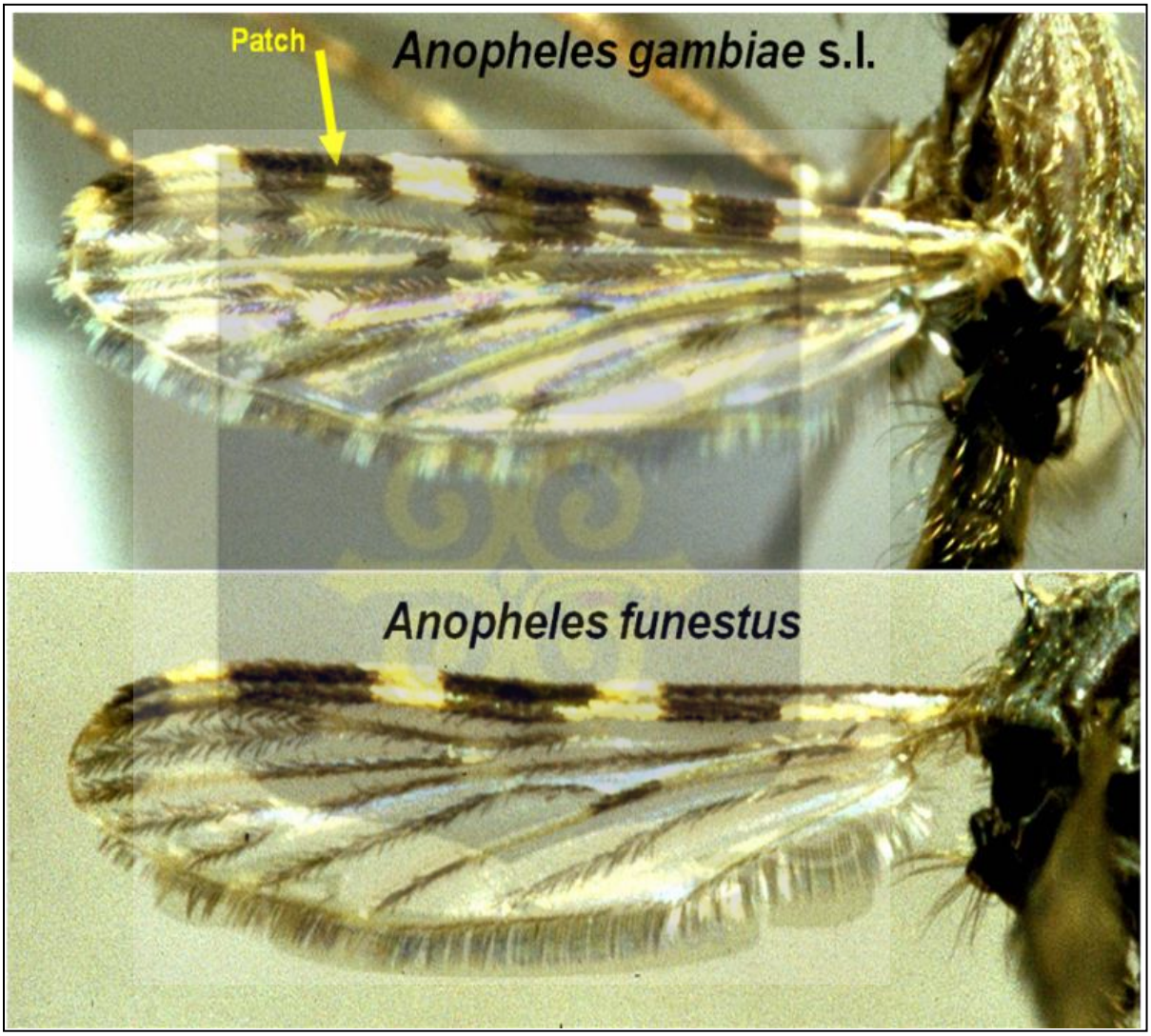


Figure 4: Differences in banding pattern between the wings of *Anopheles gambiae* complex (top) and *Anopheles funestus* complex (bottom).
Source: Professor Rolph Garms, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

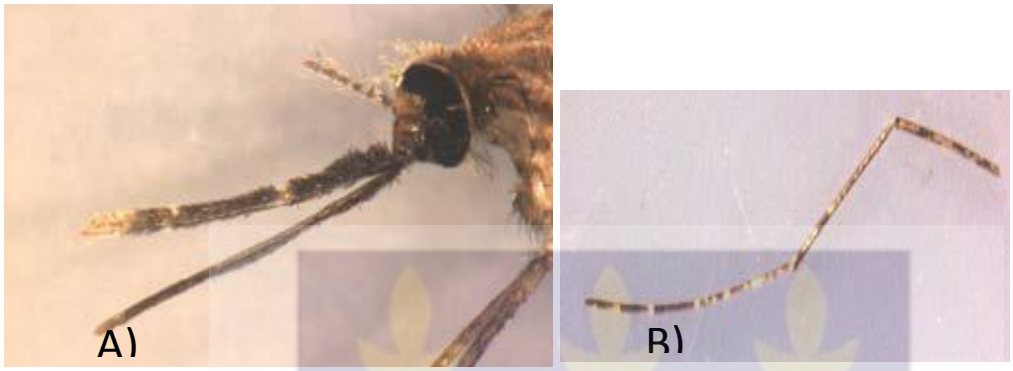


Figure 5: Diagrams of *An. gambiae* showing the A) Palps and B) legs of *An. gambiae*

2.5.2 Cuticular analysis for identification

Interests in the cuticular lipids of insects has grown steadily in recent years encouraged partly by the discovery that these substances play a part in species recognition (Howard *et al.*, 1982; Bonavita-Cougourdan *et al.*, 1987). Carlson and Service, (1980) reports of using cuticular compounds to establish a significant difference between *An. gambiae* and *An. arabiensis*.

2.5.3 Molecular Identification

This involves the use and analysis of DNA for identification. This has been found to be the most direct analysis of genetic material possible and provides an easy to use method (Post and Boakye, 1992). The use of DNA overcomes previous challenges to the identification of species. Some techniques involve specific parts or stage of the insect (karyotyping of polytene chromosomes, cytogenetic). Isozyme analysis also involves

the samples being used fresh or kept frozen until analysis. Classical genetic markers involve targeting a specific genetic fragment using techniques such as single Nucleotide Polymorphism (SNPs), Single-strand conformation polymorphism (SSCP) and restriction fragment length polymorphism (RFLP). Other analysis involves the use of mitochondrial DNA, ribosomal DNA (using the intergenic spacer and internal transcribed spacers) as is used in Polymerase Chain Reaction (PCR)-based diagnostic tools that differentiate between cryptic taxa, microsatellite DNA and random amplified polymorphic DNA (RAPD) that have been used to distinguish between *An. gambiae* and *An. arabiensis.*

2.5.4 PCR Technology

Polymerase Chain Reaction (PCR) is an in vitro technique that involves the exponential amplification of a specific DNA fragment of interest by repeating cycles of polymerase-mediated oligonucleotide primer extension (Mullis and Faloona, 1987; Saiki *et al.*, 1988; Ochman *et al.*, 1988; Innis *et al.*, 1990) The development of polymerase chain reaction (PCR) has facilitated the diagnosis of number of viral infections. It possesses several obvious advantages, such high specificity, sensitivity, rapidity, etc. (Morita, 1994). PCR is based on the ability of DNA polymerase to synthesize exponential amounts of strands of target DNA complementary to the target strand using repetitive series of cycles.

The reaction components, DNA template, DNA polymerase, oligonucleotide primers, deoxynucleotides, Taq polymerase and the reaction buffer are all included in a reaction mixture and the amplification reaction is carried out by simply cycling the temperature within the reaction tube (Saiki *et al.*, 1988)

One cycle of PCR is composed of three steps, that is, denaturing the template, cooling to allow annealing of primers to their complementary sequence and extension of the annealed oligonucleotide by the DNA polymerase when warmed.

Denaturing the template is carried out by heating the reaction mixture to a temperature, 92-95^{0}C. The temperature is reduced (to between 50-55^{0}C) to allow the primers which are oriented at their 3' ends pointing towards each other to anneal to template (White *et al.*, 1989). The temperature is raised to between 72^{0}C to allow the DNA polymerase to add nucleotides unto the annealed primers. This ends one cycle, temperature is increased again to 92-95^{0}C to repeat cycles.

The accurateness and yield is influenced by numerous components of PCR, including the buffer conditions, the PCR cycling regime (i.e., temperature and duration of each step), and DNA polymerases. (Cha and Thilly, 1993).

2.6 Life Cycle of the Vector

The mosquito's life cycle is in four stages; egg, larvae, pupae and adult. The mosquito thus undergoes a complete metamorphosis. The female mosquito needs a blood meal to develop its eggs. Prior to this, the female mates once in its lifetime. During which a store of sperms needed for further fertilisation is stored in the spermatheaca of the female's abdomen. When the eggs are developed, they are laid on the surface of water bodies. The Anopheles will oviposit in relatively clean water; hoof-prints and rain pools to streams, swamps, canals, rivers, ponds, lakes, rice fields, and sometimes even dirty water. (WHO Entomology Manual, 2013). Mosquitoes of different species and genera have different oviposition sites. A mosquito lives for 3 to 4 weeks. During these weeks, about 7 batches of eggs are laid. Each batch contains about 100 to 150 eggs. The eggs

of the *Anopheles* which are boat-shaped and about 1mm long are laid singly with floats on each side of the egg. Eggs also possess a network of outer layer of chorion that holds a film of gas and contributes to the buoyancy of the egg, provides it with a part of its coloration and serves as a plastron when the egg is submerged (Hinton, 1967). In the tropics, viable eggs will hatch in 2-3 days into larvae but in cooler temperate regions, it may take 4 to7 days or longer to hatch (Service, 1980) and much longer in colder regions.

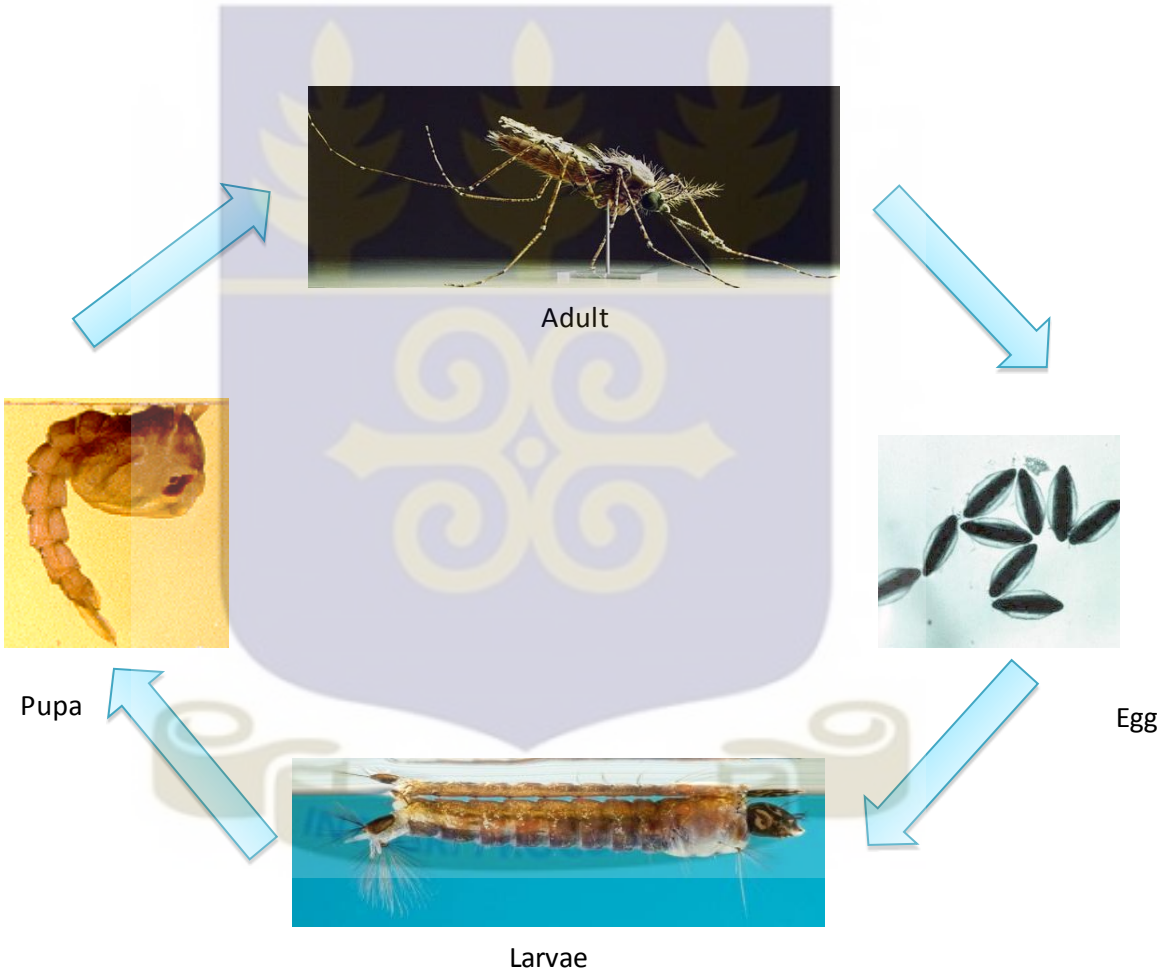


Figure 6: Life Cycle of *Anopheles* Mosquito
Source: J. Brunhes, 1998

The larvae lie just below the surface of the water and respire by lying parallel to the water surface to obtain supply of oxygen and also feed by filtering food particles from

the water. When disturbed, the larvae move below into the water but immediately move back to the surface. There are four larval stages or instars. After 1 to 2 days, the first instar sheds its skin and becomes the second instar, followed by the third and fourth instars at further intervals of about two days each (WHO Entomology Manual, 2013). The larva remains in the fourth instar stage for 3 to 4 more days before changing to become a pupa. The total time spent in the larval stage is generally 8 to10 days (WHO Entomology Manual, 2013) at mean water temperatures of 25 to 28^{0}C.

The pupa is comma shaped and bears respiratory trumpets. This stage is an inactive stage where prominent changes take place (pupa loses its larval features and begins to turn into an adult). Pupal development takes 2 to 3 days in the tropics. At the end of pupa life, the skin on the dorsal surface splits and the adult mosquito emerges. The adult waits awhile for its wings and body to dry and harden before it flies off.

2.7 Socio-Economic Impact of Malaria

Malaria affects directly and indirectly the millennium development goals. Malaria affects six out of the eight of the goals; achieve universal primary education, promote gender equality and empower women, reduce child mortality, improve maternal health, combat HIV/AIDS, malaria and other disease and to ensure environmental sustainability. Currently malaria is confined to Africa, South America and Asia, prevalent in 110 countries and territories. Ankomah Asante and Asenso-Okyere reports in 2003 that in Sub-Saharan Africa (SSA), malaria is ranked second after HIV/AIDS accounting for 10.6% of the disease burden. Many other serious diseases predominantly found in poor countries clearly are a consequence of poverty, caused by inadequate sewage treatment, unsafe drinking water, poor hygiene, or substandard housing.

Malaria, though, does not follow this pattern – its severity, and the difficulty in eradicating it, are determined mainly by climate and ecology (Gallup and Sachs, 1998). Sachs and Malaney (2002) have also observed that where malaria prospers most, human society have prospered least. Globally, malaria endemic regions, the regional and sub-regional zones, mark similar boundaries as the world's poorest countries as shown in Figure 7.

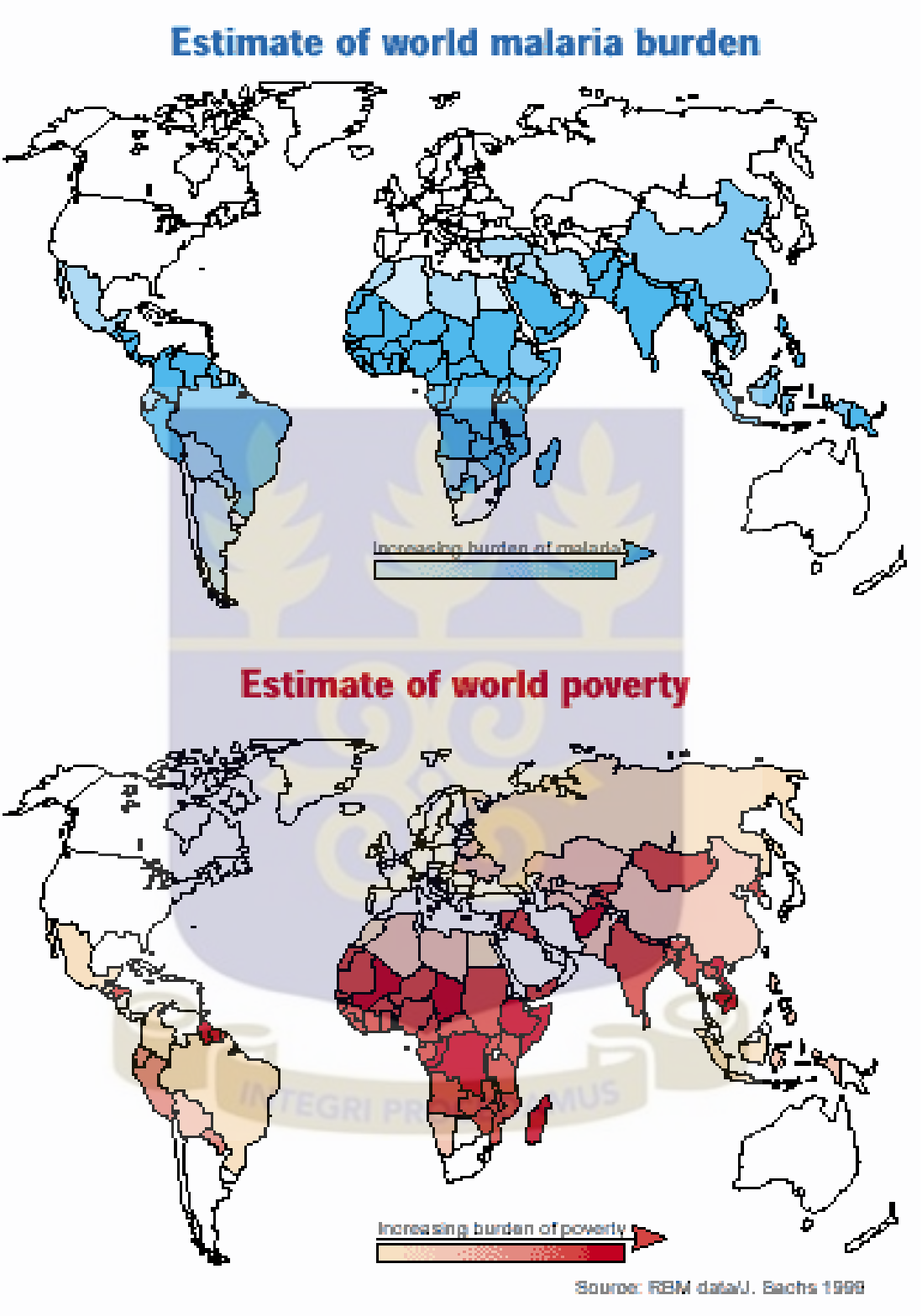


Figure 7: Map of malaria burden and poverty levels
Source: Sachs, 1999

Malarial countries are not only poor, but economic growth in malarial countries over the past quarter century has been dismal. Malaria, reduces economic growth rate by 1.3% in malaria and takes about 40% of public health expenditure in endemic countries (Gallup and Sachs, 2001). Growth of income per capita from 1965 to 1990 for countries with severe malaria has been 0.4% per year, while average growth for other countries has been 2.3%, over five times higher (Gallup and Sachs, 1998).

The cost of malaria cannot be quantified only in terms of cost spent on the illness. The costs involved are both direct (covering the cost of payment for illness) and indirect (covering the effects of illness on family and the society). It is hard to evaluate the cost of lost opportunities of household members who help out a person with malaria. Most attempts to directly measure the lost work due to malaria (which ignore these problems) find small or no impacts (Chima and Mills, 1998). Direct and indirect costs of illness for malaria were less than 10% of the household income, but still significant when combined with the costs of other illnesses (Russell, 2004). The direct and indirect costs of malaria in Africa exceed US$2 billion a year (GHS, 2008). Iron deficiency anaemia *per se* has been shown to affect the cognitive skills of children as well as their cognitive abilities in later life (Pollit *et al.*, 1989, and Lozoff *et al.*, 1991). Malaria is a known cause of anaemia especially in children, though malaria alone cannot be credited with this; *P. falciparum* infection is believed to be a major contributory factor to the etiology of severe anaemia seen in malaria endemic areas (Koram, 2000). Incidence of severe anaemia and age-specific rates of anaemia strongly correlate with the intensity of *P. falciparum* transmission (Clark and Tomlison, 1949).

The socioeconomic impact of malaria tends to be difficult to quantify, however, it cannot be overlooked. Malaria affects everyone irrespective of social or economic

standing, thus well to do individuals and investors are affected by it. As long as malaria protection is imperfect and cumbersome, well-to-do foreign investors and tourists may stay away from malarial countries (Gallup and Sachs, 2008).

In most African societies, the success of a man is measured by his ability to produce healthy children. In most communities in Northern Ghana for example, men are considered successful by the number of wives and most importantly, the number of children these wives have produced. Every year at least 30 million women in malarious areas of Africa become pregnant; most of these women live in areas of relatively stable malaria transmission (WHO, 2013). Malaria in pregnancy, however, in endemic areas of Ghana causes a myriad of problems including low birth weight, anaemia in mother and child, maternal deaths and poorer infant survival.

As reported by Asante and Asenso-Okyere 2003, malaria contributes to the loss of productive time not only to the economically active patients but also the caretakers of sick children. The value of productive time lost to the households amounted to ¢75,681.21 (US$ 8.92) per case of malaria. The indirect cost represented 56.48% of the total cost of illness to the household. About 9 workdays were lost by economically active patients while more than 5 workdays were lost by their caretakers. School children also lost about four school days on the average due to the malaria illness (Asante and Asenso-Okyere, 2003).

2.8 Malaria Control

Malaria control involves a group of curative and preventive measures employed to bring down the burden of the disease until they are no longer of a public health concern. Malaria control can be defined as reducing malaria morbidity and mortality to a locally

acceptable level through deliberate efforts using the preventive and curative tools available today. These measures are usually interrelated and multipronged. Interventions for malaria control differ from country to country depending on the epidemiology and topography of the country and largely on logistics available.

The control of malaria dates back to the period with Ronald Ross' demonstration in 1897 that mosquitoes transmit malarial parasites, efforts to control malaria were naturally focused on attacking the mosquito vector by various measures, mainly directed against the aquatic stages, for example, coating marshes with paraffin (to kill *Anopheles* mosquito larvae) and draining stagnant water. Although the results were spectacular in some areas, there was a growing realisation that effective control of malaria depended on other factors, including the availability of better drugs than quinine and a greater understanding of the epidemiology of the disease under various environmental conditions. With the discovery of Dichlorodiphenyltrichloroethane (DDT), an all-out effort was made to eradicate malaria by attacking adult mosquitoes. Eradication was achieved in a few countries such as Spain, Portugal, Yugoslavia, Hungary, Bulgaria, Romania, Poland, Italy and Netherlands. However, in most countries, eradication was not accomplished in, mainly because of inadequate health infrastructures, social and political factors. In more recent years, control efforts have been marred by drug-resistant parasites, marking the era of more effective drugs and drug combinations and the monitoring of resistance in these new drugs. Insecticide-impregnated bed nets have become the preferred method of vector control. Although the search for better tools continue today, the experience from the past emphasise the need to strengthen health systems to ensure that they are capable of delivering effective interventions and of assessing their effectiveness in controlling malaria.

Currently, tools for malaria control available (known broadly as vector control), include the use of Long lasting Insecticide-Treated Nets, Indoor Residual Spraying, and source reduction by larviciding. Intermittent preventive treatment of malaria in infants, young children and those attending school, Intermittent preventive treatment for pregnant women and prompt access to effective treatment. These strategies are deployed with effective education and communication.

2.8.1 Long lasting Insecticide-Treated Nets (LLINs)

Treating mosquito nets and other fabrics with insecticides makes them an effective method of reducing malaria attacks and deaths (Alonso *et* al., 1993; Lengeler, 1998). The premise for the effectiveness of LLIN use is that the vectors that cause malaria bite and rest indoors and also have peak biting times between dawn to dusk. Thus the use of ITNs at night serves as a barrier between the human host and the mosquito whilst the insecticide kills and repels the mosquito. LLINs available now are made from polyester, polyethylene and more currently polypropylene. The long lasting insecticide in mosquito nets are mainly pyrethroids; deltamethrin, permethrin and alpha-cypermethin.

A Cochrane review concluded that ITNs reduce overall mortality by about 20% in Africa (range 14%-29%) and that, for every 1,000 children aged 1-59 months protected by ITNs, about six lives are saved each year. The review also concluded that ITNs reduce clinical episodes of uncomplicated malaria caused by *P. falciparum* and *P. vivax* infections by 50%, as well as reducing parasitaemia.

For the effect of ITNs on malaria in pregnancy, Ter Kuile *et al.*, (1999) reported that women in their first to third pregnancies living in insecticide treated bed net villages

were significantly less likely to develop malaria parasitaemia compared to those living in control villages, and to become anaemic. The incidence of low birth weight and low birth weight combined with stillbirths, abortions and intra-uterine growth retardation were significantly reduced by 28% and 25% respectively. A study in The Gambia (D'Alessandro *et al.*, 1995) also indicates insecticide treated bed nets reduce morbidity and mortality in pregnant women. The study, carried out in an area of high malaria endemicity with seasonal transmission and parasite inoculation rate of 1-10 demonstrated that during the rainy season there were significantly fewer primigravidae with parasitaemia in villages with insecticide treated bed nets than in control villages even though there was no difference in the prevalence of severe anaemia or in the mean haemoglobin level. Thus insecticide treated bed net had an effect on malaria prevalence but not on severe anaemia which was demonstrated to be low during the dry season in women using insecticide bed nets than in control villages but no difference between the two villages during the rainy season. On the other hand, during the rainy season there were fewer preterm deliveries among women who used insecticide treated nets than women who used no nets. The mean birth weight of children born in villages with treated bed nets was 130g higher than that of children born in control villages and at the individual level. A study carried out in Ghana by Binka *et al.*, (1996) indicated that the distribution of impregnated bednets was associated with an overall reduction in all-cause mortality of 17% in children aged 6 – 59 months and reduced malaria-specific mortality by 22%. Binka *et al.*, also stated a 17% protective efficacy, although this was lower in other comparative studies carried out in other countries such as The Gambia (D'Alessandro *et al.*, 1995) and Kenya (Nevill *et al.*, 1996) which reported 25% and 33% respectively.

Recent studies have shown that such nets are a cheap and effective method of reducing

man-vector contact and child morbidity (Curtis *et al.* 1992; Bermejo & Veeken, 1992)

Though ITNs have been found to be effective, coverage must be high, it is suggested that children sleeping without nets may even receive fewer infective bites in a village where bed net coverage is high (Clarke *et al.*, 2001) and nets used every night to ensure this effectiveness. Thus net distribution campaigns and projects should be followed by extensive, effective communicative messages.

2.8.2 Larviciding

In recent years, larviciding has received more attention and focus as a response to growing concern with drug and insecticide resistance. ITNs and IRS are currently the most effective vector control options, however, these tools will not be optimally effective in areas where mosquitoes exhibit outdoor resting and biting behaviours, or where the widespread use of ITNs and IRS has controlled endophilic mosquitoes, but left a smaller, more intractable population of exophilic and exophagic mosquitoes (Bayoh *et al.*, 2010, Lindblade *et al.*, 2006). The sustainability of IRS and ITNs is further threatened by the appearance of pyrethroid resistance in some mosquito populations (Read, 2009).

Larviciding forms a part of interventions on the breeding sites of malaria vectors by larval source management. Larval source reduction involves the regular application of chemical or biological agents to kill mosquito larvae in their aquatic habitats (WHO, 2012). Larviciding is usually used as a part of integrated vector control approaches and not as a stand-alone intervention. Although, if used alone has the potential to affect both indoor and outdoor resting mosquitoes as compared to IRS or ITNs (WHO, 2011), but larvicides, unlike IRS and ITNs, act on a single, non-transmitting stage in the mosquito

lifecycle and can only impact disease by reducing vector abundance (Kouznetsov, 1977). Larvicides used range from contact poisons, stomach poisons to hormones, insect growth regulators and other biological agents. Currently, the recommended agent/insecticide for larviciding include *Bacillus thuringiensis isrealensis* (Bti), Chlorpyrifos, Diflubenzuron, Novaluron, Pyriproxyfen, Fenthion, Pirimiphos-methyl, Temephos, and Spinosad (WHOPES, 2013). These insecticides are available in different formulations. The appropriate agent/insecticide, right formulation, timing and quantity are based largely on the biology of the targeted species and the ecology of the water body. Larvicides can be applied from either the ground by truck, boat, and hand held devices or by aerial application. Larviciding is recommended to be used as a compliment to the other core interventions such as the use of ITNs or IRS and should never be used as a substitute for ITNs or IRS in areas with significant malaria risk. Also its use is normally restricted to areas where breeding sites are few, fixed and findable especially in urban communities.

2.8.3 Indoor Residual Spraying (IRS)

Indoor Residual Spraying (IRS) is one of the primary vector control interventions for reducing and interrupting malaria transmission (World Malaria Report, 2012). Historically, IRS was largely responsible for the tremendous accomplishments of malaria programmes in Europe, Asia and the Americas that resulted in hundreds of millions of lives being saved between the 1940s and the 1980s. In recent years, pilot projects have demonstrated that malaria was highly responsive to control by IRS with insecticides (mainly DDT). Significant reductions in anopheline vectors and malaria were recorded but transmission could not be interrupted (Kouznetsov, 1977).

IRS is a standardised, well-controlled method of regulating vectors of malaria. Indoor Residual Spraying (IRS) is the application of a long-lasting, residual insecticide to potential malaria vector resting surfaces such as internal walls, eaves, and ceilings of all houses or structures (including domestic animal shelters) where such malaria vectors might come into contact with the insecticide (WHO, 2013). IRS reduces mosquito life span, reduces mosquito density, indirectly reduces the number of mosquitoes biting and thus reduces malaria transmission.

In 2011, 80 countries, including 38 in the African Region, recommended IRS for malaria control. In the same year, 153 million people were protected by IRS worldwide, or 5% of the global population at risk. In the African Region, the proportion of the at-risk population that was protected rose from less than 5% in 2005 to 11% in 2010 and remained at that level in 2011, with 77 million people benefiting from the intervention (World Malaria Report, 2012).

IRS works on the premise that the *Anopheles* mosquitoes that come indoors to feed at night, after feeding on humans, is more likely to rest on a sprayed surface and pick up a lethal dose of insecticide, and die eventually. This prevents further bites by the infective mosquito and thus breaks transmission. However, effective IRS requires good coverage of all sprayable surfaces, epidemiological and entomological monitoring, and bioassays for insecticide susceptibility of the local vector populations. It also includes the selection and use of appropriate insecticides. Selection of insecticide should be made after information on the local transmission, ecology and epidemiology, e.g. length of transmission season, levels of transmission, nature of sprayable surfaces, susceptibility or otherwise of mosquitoes to insecticide obtained and analysed. The insecticide should be safe, efficacious, cost-effective, available and with acceptable levels of residual

effect. Insecticides approved by the World Health Organization for use in IRS can be found in Appendix I.

The spraying technique involves the use of pressure pumps fitted with nozzles to deliver the insecticide, at the recommended dosage, with the spray lance kept at 45 cm away from the wall surface, spraying should be done from roof to the floor using a downward motion with a spray discharge rate of 740 to 860 ml per minute.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study Area

The study was carried out in Tarikpaa and Kulaa in the Savelugu-Nanton and Tamale districts of Northern region respectively (Figure 8). The Northern Region is the largest region in Ghana in terms of land mass, occupying an area of 70, 384 square kilometers with a population of 2,479,461. It is bordered on the north by the Upper East and Upper West Regions, on the south by the Volta and Brong Ahafo Region, the Republic of Togo to the east and Cote d'Ivoire to the west.

The topography of the Region is mainly flat except for areas such as Gambaga escarpment where the land rises to about a height of 400 to 450 meters above sea level. The vegetation cover is savannah with grass and sparsely distributed drought resistant trees such as the baobab and the Shea tree. The region has a tropical climate. Temperatures range from 14°C (59°F) at night to 40°C (104°F) during the day. The rainy season lasts for about 5 months from June to October. The Northern Region is also characterized by a cold dry season from November to February and a hot dry season from March to May.

Tarikpaa is a rural community, located: (9°35′11.30″N, 0°54′11.39″W), which has been a beneficiary community of the PMI IRS program since 2008. The community was sprayed for four years with alphacypermethrin (a pyrethroid insecticide) from 2008 to 2011. In 2012 however the community was sprayed with an organophosphate insecticide (pirimiphos methyl CS formulation) due to the declining susceptibility of the predominant vector species in the Savelugu-Nanton district to pyrethroid insecticides.

Kulaa, at (9°26′56.32″N, 0°43′41.37″W), on the other-hand has no history of indoor residual spraying as a malaria control intervention, and so served as control site for comparison. Though it is located within the Tamale municipality, Kulaa is a rural community with fairly similar characteristics as Tarikpaa.

Figure 9 shows the average rainfall pattern of Savelugu-Nanton and Tamale districts for the year 2013. It also indicates peak rainy period between July and November. The average annual rainfall is 71mm and 76mm for Savelugu-Nanton and Tamale districts respectively. The study sites experience stable, endemic malaria transmission (MICS, 2013). A peak in transmission often follows the long rains. The communities have huts made of mud and roofs of thatch or roofing sheets. The huts selected for this study had roofs of thatch and no eaves. The compounds are made of several huts, joined into a wall that fully encloses the compound, usually representing one family. The huts are mostly round and sometimes square. Huts are smoothed over with a water-proof mixture. The people in the study site are mostly farmers.

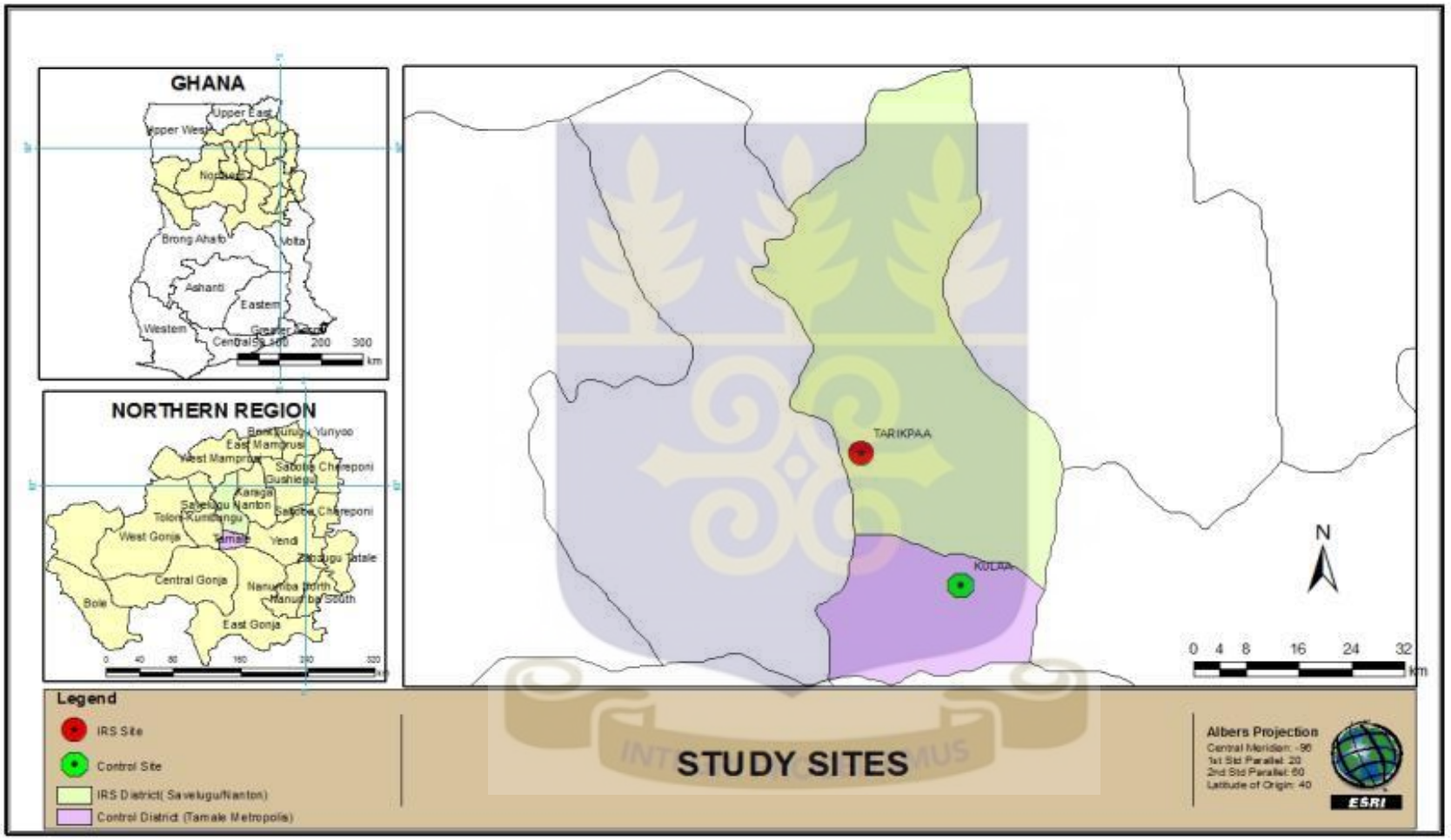


Figure 8: Map showing areas of mosquito collection

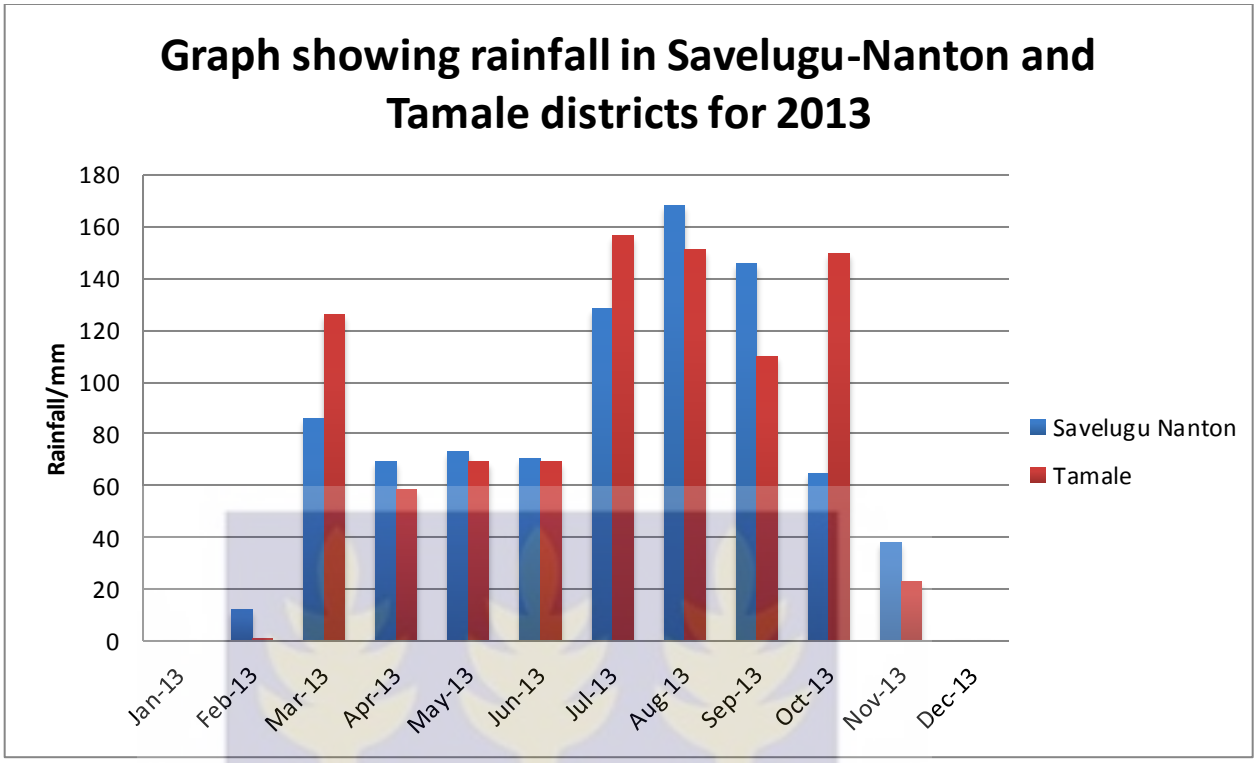


Figure 9: Rainfall pattern for study districts

3.2 Mosquito Collections

Mosquito collections were done using two methods for mosquito sampling: Pyrethrum Spray Collections (PSC) and Exit Trap Collections (ETC). PSC was used to collect indoor resting mosquitoes whilst ETC was used to collect mosquitoes that exit the rooms during the night. PSC can be used to determine directly the indoor resting density (the number of mosquitoes resting indoors during the day), the human-biting density (indirectly) and the seasonal changes in indoor resting densities. Exit Trap Collections can be used to determine the species that rest outdoors, the relative numbers of mosquitoes resting outdoors, seasonal changes in outdoor resting habits and any alteration in the relative numbers of mosquitoes resting outdoors following the application of insecticides in houses and other buildings. Collections were undertaken between August and December 2013.

In each community, two (2) compounds were selected randomly. The distance between compounds were about 150-200m. In each compound, two rooms were used, one of the rooms were provided with a wardrobe into which all hanging clothes that would have otherwise been hanged on lines in the room were kept enclosed in the wardrobe throughout the period of the collections. The other room was left with clothes hanging as is normally done in the hut. Thus in each community, two compounds were chosen and two rooms in each compound were used. (Figure 13). The same design was used in the other community (Figure 13).

In every month, two sessions of collections were carried out. In each session, four collections were made using both the PSC and ETs. This was done simultaneously for the two communities

3.2.1 Pyrethrum Spray Collections

Pyrethrum Spray Collections (PSC) involves using a pyrethrum space spray to knock down mosquitoes resting inside a house and collecting them on white sheets spread on the floor and other surfaces in the house.

Using PSC, it is possible to collect all mosquitoes from a well-enclosed room. The collection allows for quantitative studies including measurement such as indoor resting densities and human biting densities (WHO, 2013).

PSCs were done within the hours of 6.00 and 8.00 in the morning. After the inhabitants have left the room, the floor of the room was covered with white sheets. The doors were closed and the room sprayed with Raid® Insecticide containing pyrethroids (prallethrin and sumithrin). The rooms were vacated after spraying. After 10 minutes, the white sheets were carefully lifted and contents were all placed on one sheet. This sheet was then held at its four corners and brought outside. The mosquitoes from the sheets were picked with forceps and placed in petri dishes and sent to the laboratory. (Figure 10) These petri dishes were lined with damp cotton wool and filter paper to prevent the mosquitoes from drying up. The number of people who slept in the room the previous night was also recorded.

Pyrethrum Spray Catches were carried out in all of the settings described (Figure 13). Collections made were stored in paper cups, labelled appropriately according to the date, time, method and place of collection and taken to the laboratory. In the laboratory, the number of catches were noted and species identified.

Figure 10*:* Mosquitoes being collected from white sheet after spraying rooms

3.2.2 Exit Trap Catches

Some mosquitoes tend to be endophagic but exophilic. Exit or window traps are commonly used to capture mosquitoes leaving houses (WHO, 2013). Thus mosquitoes obtained from traps are used to determine the species that bite indoors but rest outdoors, the effect of control measures such as indoor residual spraying on movement and feeding habits of the mosquitoes and effect of insecticides as shown by the numbers of dead mosquitoes collected and by mortality rate of those caught alive (WHO, 2013).

The exit traps cages were fixed on the window in each of the rooms; with hanging clothes or without hanging clothes. Cages were fixed on the windows (Figure 11) in the evening between the hours of 1800 and 1900 prior to the PSC the next morning. Mosquitoes trapped in cages were removed with an aspirator in the morning and placed into paper cups covered with mesh and labelled appropriately (Figure 12). The paper cups were subsequently placed carefully in boxes and transported to the laboratory for processing. The cups were observed within a 24 hour period and mortalities were recorded.

Figure 11: An exit trap fixed in a window

Figure 12. Mosquitoes being picked from exit traps with aspirator into paper cups

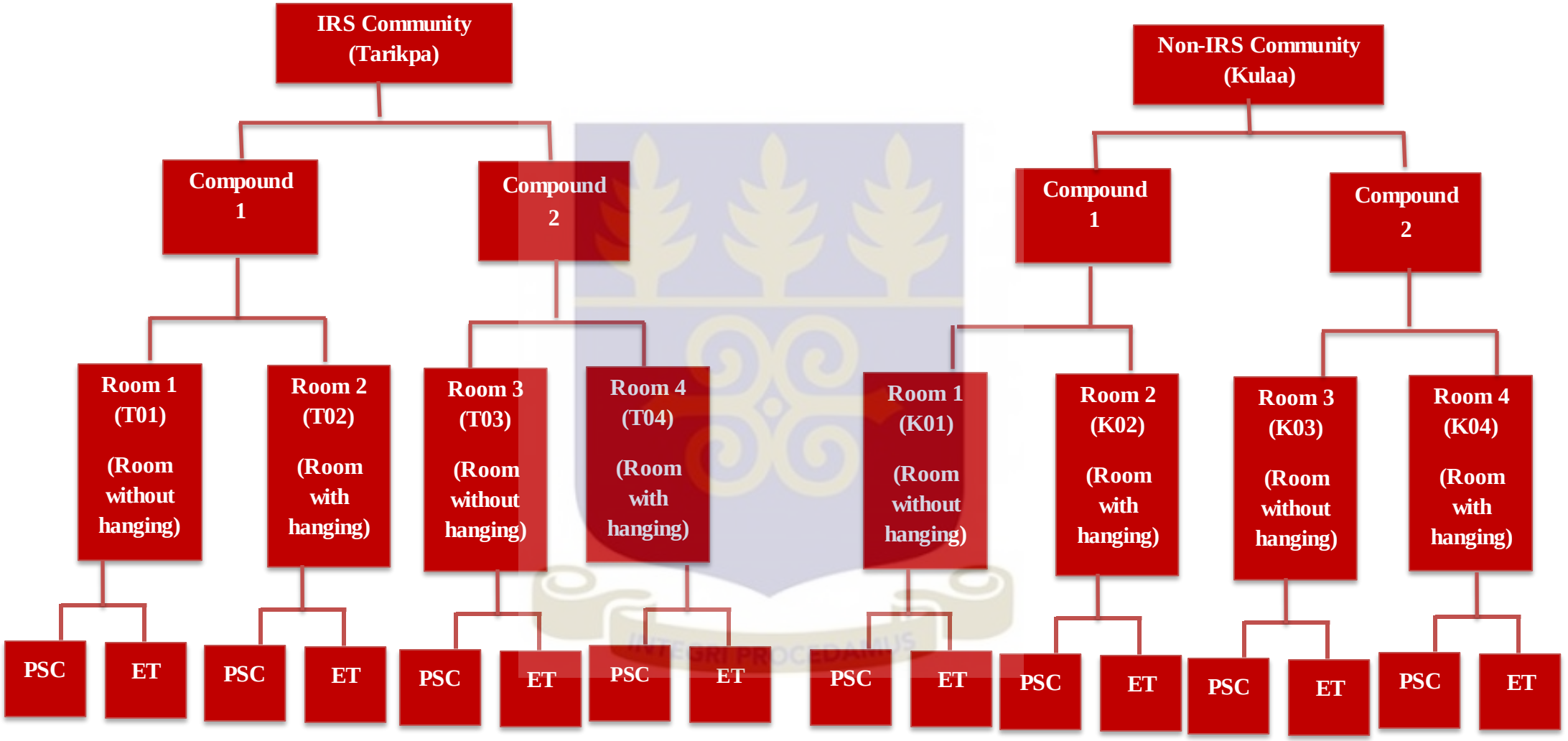


Figure 13: Illustration of the study design

3.3 Processing of Mosquitoes

Mosquitoes obtained from Pyrethrum Spray Catches and those captured with Exit Traps were sorted out morphologically into various genera (Gillies and De Meillon, 1968). The physiological states of the abdomen were classified as unfed, blood fed, half gravid and gravid. Unfed females in which the abdomen was completely empty (without blood), narrow and collapsed. Freshly-fed females in which at least the first six abdominal segments, viewed ventrally, were filled with red blood. Half gravid females in which the first three to five abdominal segments were filled with dark-coloured blood at various stages of digestion.

Gravid females in which the blood was completely digested or remained narrow blackish line in the first abdominal segment and the abdomen was distended with eggs. The mosquitoes after morphological identification were placed in 1.5µl microcentrifuge tubes and appropriately labelled with species of mosquitoes, place, date, and method of collection. These tubes were kept in zip lock bags containing silica gel for further analysis.

3.3.1 Morphological Identification of mosquitoes

With the aid of a light microscope and identification keys, (Gillies and Coetzee, 1987; Gillies and de Mellion, 1968) the mosquitoes were morphologically identified.

Culex species was identified by its brownish, dull coloured appearance, short palps and the absence of patches or spots on legs and wings. *Aedes* species possess distinctly black and white rings, on legs and abdomen. *An. gambiae* had pale patches, 3 bands on palps and wings with 3rd main dark area of vein 1 with pale interruption, sometimes fused with

preceding pale spots. *An. funestus* was identified as mosquitoes with no pale interruption on 3rd main dark area of vein 1 on wings, palps with less than 4 bands and pale at apex and legs without pale patches. *An. rufipes* were identified as mosquitoes with hind tarsal segments 4 and 5 entirely white; 3rd main dark area on 1st vein of wings with a pale interruption, or with a short extension of the subcostal pale spot into the dark area on first vein. *An. nilli* had palps with pale apex and no other pale band on palp.

3.3.2 Molecular Identification of Mosquitoes

3.3.2.1 Polymerase Chain Reaction

3.3.2.1.1 DNA Extraction

Using Cochran's formula (Bartlett *et* al., 2001) for sample size determination, mosquitoes were selected to be processed for DNA extraction. The legs of the selected desiccated mosquitoes were removed into 1.5µl microcentrifuge tubes. 15µl of double distilled water (ddH_2O) was placed in a tube per leg. The legs were ground with a plastic pestle and homogenate placed in a water bath at 90^0C for 15min. After 15min, homogenate was removed, centrifuged for 5min at 16000g and used immediately or stored at -40^0C.

3.3.2.1.2 Amplification of Ribosomal DNA

The amplification followed the protocol used by Scott *et al.*, (1993) and oligonucleotide primers universal (UN), gambiae (GA), merus/melas (ME) and arabiensis (AR) Table 1 shows the oligonucleotide sequences in the 5'-3' direction and their melting temperatures. The PCR reaction mix of 20µl contained 1X PCR buffer, 2µl (Invitrogen, USA), 1.5 mM $MgCl_2$ (Promega, USA), 0.25 mM each of the 4 deoxyribonucleotide triphosphates (dATP,

dTTP, dGTP and dCTP), 0.15µM of oligonucleotide primers, 0.5µ of *Taq* polymerase enzyme (Promega, USA) and 3µl of the extracted DNA supernatant. Sterile double distilled water (sddH_2O) was added to make up the volume to 20µl.

Table 1: Ribosomal DNA (rDNA) intergenic spacer species-diagnostic primers, their melting temperatures (Tm) and species represented (Scott *et al.*, 1993).

Primer	Sequence (5'-3')	Melting Temp (Tm°C)	Size (bp)	Species
UN	GTG TGC CCC TTC CTC GAT	58.3	468	-
GA	CTG GTT TGG TCG GCA CGT TT	59.3	390	*An. gambiae s.s.*
ME	TGA CCA ACC CAC TCC CTT GA	57.2	464	*An. melas/merus*
AR	AAG TGT CCT TCT CCA TCC TA	47.4	315	*An. arabiensis*

The amplification reaction was carried out with a PTC 100 thermo cycler (MJ Research Inc, USA). The PCR was carried out with a program of initial denaturation at 94°C for 3min, followed by 33 cycles of, 94°C for 30 sec, annealing at 50°C for 30 sec, and extension at 72°C for 30 sec. Thermo cycler was also set to store at 4°C until removed.

7µl of each PCR product was then mixed with 1µl Orange-G (5x) gel loading dye and electrophoresed through a 2% agarose Tris-borate-EDTA gel containing 0.5µg/ml of Ethidium bromide in a 1x TAE buffer. The amplified fragments were visualized with short wave ultraviolet light. The results were photographed with a UV illuminator Sony Toyobo

FASAII. The size of each sample was estimated by comparison with a standard 100bp molecular weight ladder (Promega, USA) and results recorded.

3.3.2.2 Identification of *An. gambiae* M and S Forms

The PCR amplification of a 1.3 kb rDNA fragment is followed by digestion with restriction enzymes Hha I and a specific pattern is obtained for each form (Favia *et al.*, 1997). This was done using the protocol of Fanello *et al.*, 2002 and on the principle that the restriction site for the enzyme Hha1 lies within the *An. gambiae*-specific fragment (Scott *et al.*, 1993).

After the amplification, a master mix of 1.5µl of 10x enzyme buffer, 0.15µl of 100x Bovine Serum Albumin (BSA), 0.1µl of Hha1 (10µ/µl) and sddH_2O to make up a volume of 10µl. 5µl of the master mix was added directly to the PCR product, centrifuges and incubated at a temperature of 37°C for 5 hours.

Table 2: Alignment from the 3' to 5' end of the 561- 600 fragment of the IGS regions in M and S of the *Anopheles gambiae* s.s. complex. The arrow indicates the restriction site of the Hha I enzyme in *An. gambiae s* form.

Molecular Forms	Sequence
An. gambiae S form	GTGCCCCTTCCTCGATGGC**GC**AACGAACCATCTTGGTCTG ↑
An. gambiae M form	GTGCCCCTTCCTCGATGGC**AT**AACGAACCATCTTGGTCTG ↑

After digestion, fragments were run through a 2% Ethidium bromide agarose gel and photographed under ultraviolet light illumination. The *An. gambiae* S-form digestion profile was characterized by two fragments, 257and110bp long, which are due to the presence of the Hha I restriction site. The *An. gambiae* M form does not have this restriction site and thus is characterized by a single 367bp fragment (Fanello *et al.*, 2002).

3.4 Sporozoite Detection by Enzyme Linked Immunosorbent Assay (ELISA)

The head and thorax of each sample were placed into a 1.5µl microcentrifuge tube and appropriately labeled. The tube was filled with 50µl of Grinding Solution. The sample is ground with a plastic pestle to obtain a homogenous solution. Grinding solution was used to wash pestle to obtain a 200µl of the Mosquito/Plasmodium Antigen (MPA) suspension. This was stored at -40^{0}C until ready to use. A plate map bearing Identification number of samples were recorded on a plate map as indicated in Figure 14.

Using the protocol of Wirtz *et al.*, 1987, the 96 well polyvinyl chloride, u-shaped micro titration plate was coated with a capture monoclonal antibody (MAb) at a coat rate of 0.1µg/50µl/well. Plate left overnight at 4^{0}C. The following solutions were prepared for the ELISA: Phosphate Buffered Saline (PBS), Grinding solution, blocking buffer and washing buffer. The protocols for these are attached as Appendix II. Plate was flipped empty and banged on paper towels. The remaining sites for protein binding on the micro titer plate was saturated with blocking buffer 200µl per well and incubated for an hour at room temperature. After an hour, plates were flipped empty, banged and washed twice with the washing buffer (PBS/Tw-20). Mosquito extracts and controls were loaded and incubated at

50µl/well at room temperature for two hours. Plates were flipped and washed with washing buffer three times. The second labeled antibody, MAb-peroxidase conjugate (0.05µg/50µl/well) in blocking buffer was incubated for an hour in darkness. Plates were flipped empty, washed and banged three times. 100µl/well of substrate was incubated at room temperature for 50min. Plates were read between 30 to 60 min. Plates with change in color were recorded on plate map as positive whilst the others were recorded as negative.

A	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam
B	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam
C	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam
D	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam
E	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam
F	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam	+ve	+ve
G	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam	BLK	BLK
H	sam	sam	sam	sam	sam	sam	sam	sam	sam	sam	-ve	-ve
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

Figure 14: Illustration of 96 well micro titer plate

sam-sample

+ve – positive control

-ve – negative control

BLK - block

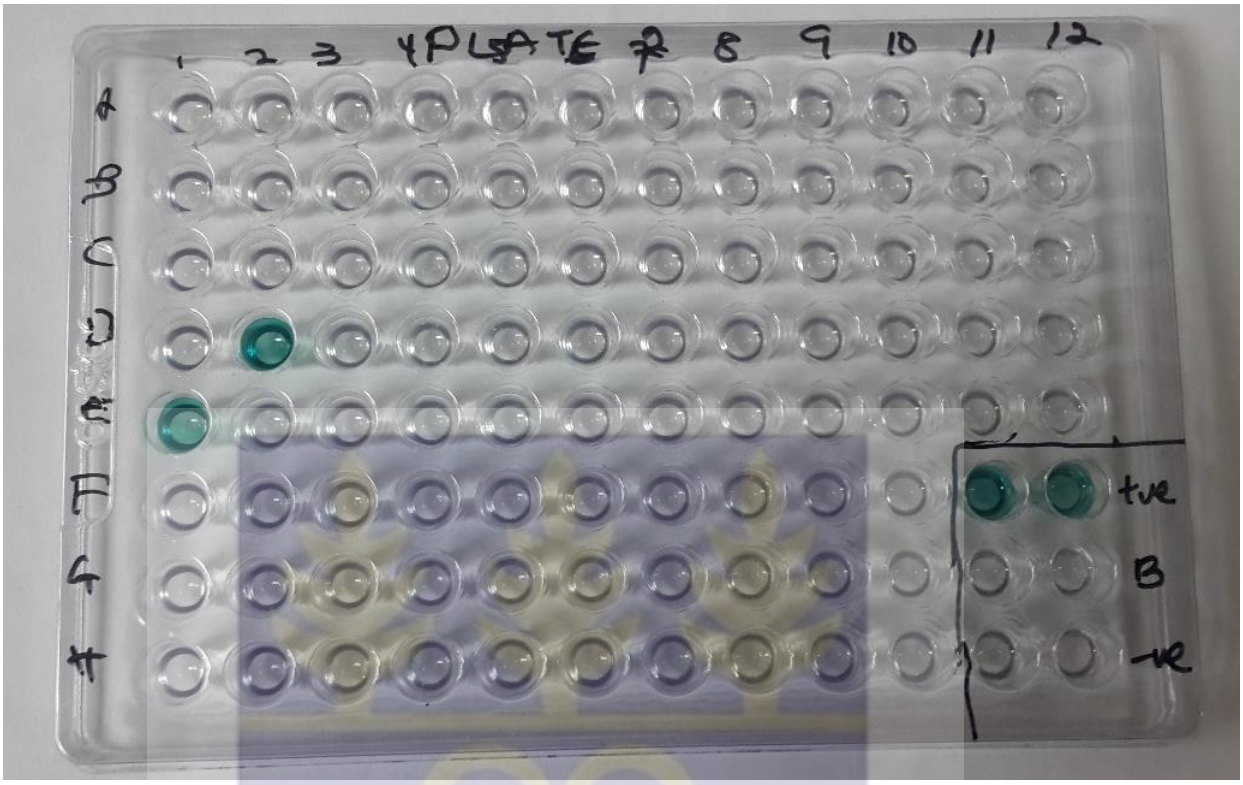


Figure 15: Microtiter plate showing positive colour change and negative and positive controls

3.5 Data Analysis

Completed forms were organized into date, site and capture method. These forms were entered into database fields using Ms Excel datasheets for use in statistical analysis. Data was transported into IBM SPSS (Version 12) Analysis of Variance will be used to test for significant differences between densities of mosquitoes from PSC obtained in rooms without hanging clothes and rooms with hanging clothes. Statistically significant differences were based on comparisons that yielded P values <0.05 and 95% confidence interval.

The Man Biting Rate (MBR) was obtained from an index of the indoor resting blood fed females per inhabitant. The Entomological Inoculation Rate (EIR) is a measure of the intensity of malaria transmission, it is an indirect measure of the number of infective bites of the mosquito per man per night. This was determined by the multiplication of the sporozoite rate and the MBR. The assumption made here is that the vector is predominantly endophagic and endophilic in behaviour (Garrett-Jones and Shidrawi, 1969). The sporozoite rate was calculated as the number of mosquito samples positives for *P. falciparum* obtained from ELISA tests divided by the total number of mosquito samples examined.

CHAPTER FOUR

RESULTS

4.0 Vector Species Composition and Distribution

Anopheles gambiae s.l., *An. funestus, An. nili* and *An. rufipes* were the *Anopheles* mosquito species collected during the period of the study using both the Pyrethrum Spray Collection (PSC) and Exit trap Collections (ETC) methods. *An. gambiae* s.l. was the predominant vector species collected from all the sites making up about (642/661) 97.1% of the total number of *Anopheles* species (Table 3). *An. funestus, An. nili* and *An. rufipes* made up (13/661) 2.0%, (2/661) 0.3% and (4/661) 0.6% respectively of the total *Anopheles* species. The majority of the mosquitoes collected in all the study areas were obtained from Exit Trap Collections (569/661) 86.1% compared to PSC (92/661) 13.9% Table 3.

The largest number of all *Anopheles* species collected were from the non-IRS area, 82% (543/661) compared to IRS area, 23.7% (118/661) Table 4. *An. gambiae* s.l. was collected in high numbers in all the months of the study period whilst *An. funestus* was collected towards the end of the rainy season, October and November.

An. gambiae s.l. collected in rooms with hanging clothes in the IRS community (Tarikpaa) was lower, 14.68% compared to rooms with no hanging clothing, 85.32% (Table 4). The same was observed for the non-IRS community (Kulaa) where *An. gambiae* from rooms with hanging clothes was 18.3% compared to 81.61% for rooms with no hanging clothes. The number of *Anopheles* species collected by ETC from rooms without hanging clothes in the IRS area was significantly higher ($P<0.05$) 15, (79.2%) than in rooms with hanging clothes, 57, (20.8%) Table 5. The same trend was observed in the non-IRS area, although much higher numbers were collected than non-IRS area (Table 5). The number of indoor

resting *Anopheles* collected by PSC also indicated that less mosquitoes were resting in rooms with hanging clothes, 5 (11.1%) compared to rooms without hanging clothes, 40 (88.9%) in the IRS area (Table 6). However, in the non-IRS area more mosquitoes were collected in rooms with hanging clothes than from rooms without hanging clothes although the difference was not significant. The mean number of *An. gambiae* obtained from Tarikpaa for rooms with no hanging clothes was not statistically different from rooms with hanging clothes. ($F_{(1, 12)}$ =3.981; p= 0.072). In Kulaa, also, difference between the mean numbers obtained from rooms with no hanging clothes was not statistically different from those from rooms with hanging clothes.

Table 3: Species composition of *Anopheles* mosquitoes collected by the Pyrethrum Spray and Exit Trap methods from Tarikpaa and Kulaa in the Savelugu-Nanton and Tamale districts of Northern Ghana.

Anopheles **species**	**Pyrethrum Spray Collections**			**Exit Trap Collections**			**Total No. of *Anopheles* collected by PSC & ET**
	Tarikpaa (IRS)	**Kulaa (Non IRS)**	**Total No. (%)**	**Tarikpaa (IRS)**	**Kulaa (Non IRS)**	**Total No. (%)**	
***An. gambiae* s.l.**	43	42	85.0 *(92.4%)*	66	491	557.0 *(97.9%)*	**642.0** *(97.1%)*
An. funestus	2	1	3.0 *(3.3%)*	6	4	10.0 *(1.8%)*	**13.0** *(2.0%)*
An. nili	1	1	2.0 *(2.2%)*	-	-		**2.0** *(0.3%)*
An. rufipes	-	2	2.0 *(2.2%)*	-	2	2.0 *(0.4%)*	**4.0** *(0.6%)*
Total	46	46	92 (13.9%)	72	497	569.0 (86.1%)	**661.0**

Table 4: Species composition and abundance of *Anopheles* species collected by PSC and ETC in rooms with and without hanging clothes in an IRS (Tarikpaa) and a Non-IRS (Kulaa) areas in the Savelugu-Nanton and Tamale districts of Northern Ghana.

Species of *Anopheles*	IRS Area (Tarikpaa)			Non-IRS Area (Kulaa)			Total No. of Mosquitoes collected
	Rms WH[1] No. (%)	Rms NoH[2] No. (%)	Total	Rms WH[1] No. (%)	Rms NoH[2] No. (%)	Total	
An. gambiae	16 *(14.68)*	93 (85.32)	109	98 (18.38)	435 (81.61)	533	642
An. funestus	2 (33.3)	6 (75.0)	8	2 (40)	3 (60)	5	13
An. nili	0	1 (100)	1	0	1 (100)	1	2
An. rufipes	0	0	0	4 (100)	0	4	4
Total	18	100	118	104	439	543	661

Rms WH[1] *= Rooms with hanging,* ***Rms NoH[2]*** *= Rooms with no hanging,* ***No.*** **=** *Number*

Table 5: Species composition and abundance of *Anopheles* species collected from ETC in rooms with and without hanging clothes in an IRS (Tarikpaa) and a Non-IRS (Kulaa) areas in the Savelugu-Nanton and Tamale districts of Northern Ghana.

Species of *Anopheles*	IRS Area (Tarikpaa)			Non-IRS Area (Kulaa)			Total No. of Mosquitoes collected
	Rms WH[1] No. (%)	Rms NoH[2] No. (%)	Total	Rms WH[1] No. (%)	Rms NoH[2] No. (%)	Total	
An. gambiae	11 *(16.67)*	55 (83.33)	66	76 (15.48)	415 (84.52)	491	557
An. funestus	4 (66.67)	2 (33.33)	6	3 (75.00)	1 (25.00)	4	10
An. nili	0	0	0	0	0	0	0
An. rufipes	0	0	0	2 (100)	0	2	2
Total	15 (20.8%)	57 (79.2%)	72	104 (20.9%)	439 (88.3%)	497	661

Rms WH[1] *= Rooms with hanging,* ***Rms NoH[2]*** *= Rooms with no hanging,* ***No.*** *= Number*

Table 6: Species composition and abundance of indoor resting *Anopheles* species collected by PSC in rooms with and without hanging clothes in an IRS (Tarikpaa) and a Non-IRS (Kulaa) areas in the Savelugu-Nanton and Tamale districts of Northern Ghana.

Species of *Anopheles*	IRS Area (Tarikpaa)			Non-IRS Area (Kulaa)			Total No. of Mosquitoes collected
	Rms WH[1] No. (%)	Rms NoH[2] No. (%)	Total	Rms WH[1] No. (%)	Rms NoH[2] No. (%)	Total	
An. gambiae	5 *(11.63)*	38 (88.37)	43	22 (52.38)	20 (47.62)	42	85
An. funestus	0	2 (100)	2	1 (100)	0	1	3
An. nili	0	0	0	0	0	0	0
An. rufipes	0	0	0	2 (100)	0	2	2
Total	5 (11.1%)	40 (88.9%)	45	25 (55.5%)	20 (44.4%)	45	90

Rms WH[1] *= Rooms with hanging,* ***Rms NoH[2]*** *= Rooms with no hanging,* ***No.*** *= Number*

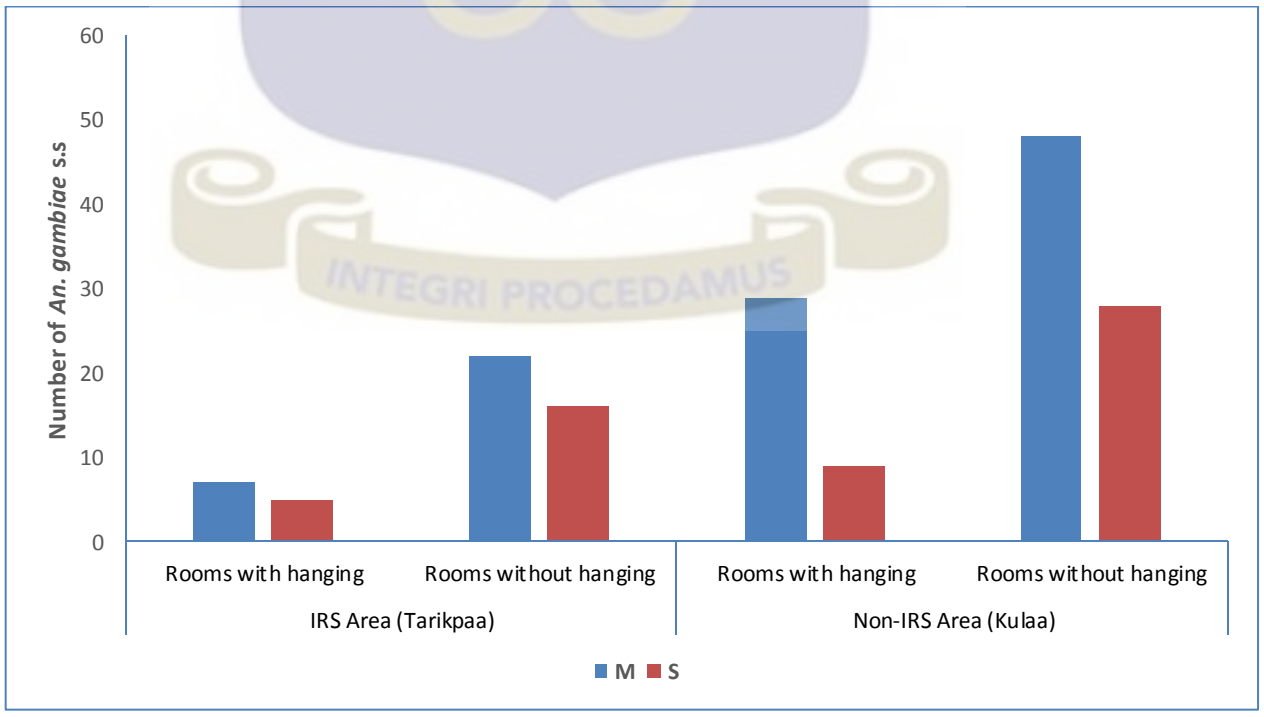
4.1 Identification of Sibling Species and Molecular Forms of *An. gambiae*

A total number of 242 of *Anopheles gambiae* s.l., (83, Tarikpaa; 159, Kulaa) were analyzed. In all the study areas, *An. gambiae* s.s. was the only sibling species identified.

A total of 65 samples from IRS area were successfully amplified by PCR and out of this, 20% (13/65) of *An. gambiae* s.s were from rooms with hanging clothes whilst 80% (52/65) *An. gambiae* s.s were from the rooms with no hanging clothes. The same trend was observed in the non-IRS area.

Out of a total of 164 *An. gambiae* s.s analyzed, *An. coluzzi* (M form) was the predominant species found in both the IRS and non-IRS areas. The same trend was observed in rooms with hanging clothes and rooms without hanging clothes in all the study areas (Figure 16)

Figure 16: Molecular identification of *An. gambiae* s.s. from rooms with and without hanging clothes in Tarikpaa (IRS) and Kulaa (non IRS) areas



4.2 Indoor Resting Densities of *Anopheles*

Table 7 provides a summary of Indoor Resting Densities (IRD) of *An. gambiae* s.s. and *An. funestus* with their gonotrophic stages. The mean IRD for Tarikpaa (1.54 mosquitoes/room) and Kulaa/unsprayed community (1.50 mosquitoes/room) were not statistically different ($F_{(1, 12)}$ =0.001; p= 0.973).

The mean IRD of *Anopheles* collected from rooms in the IRS area without hanging clothes was higher than that of rooms with hanging clothes (2.7 mosquitoes/room vs. 0.36 mosquitoes/room). However, this difference was not significant ($F_{(1, 12)}$ = 2.078, P = 0.175). There was also no significant differences in IRDs between rooms in the unsprayed community, Kulaa with hanging clothes and rooms without hanging clothes (1.42 mosquitoes/room vs. 1.57mosquitoes/room) ($F_{(1,12)}$ = 0.011, P = 0.917)

A blood fed to gravid ratio of 4.5:1(Table 7) was estimated for *Anopheles* collected from rooms with no hanging clothes in the IRS area. In the rooms with hanging in the same area, a blood fed to gravid ratio of 5:1 was also estimated for *Anopheles* mosquitoes. A similar observation was made in Kulaa where mosquitoes from rooms with no hanging clothes had the blood fed to gravid ratio of 16:1(Table 7), indicating that they are more likely to leave the room after feeding. The rooms with hanging clothes in the non-IRS area however had a blood fed to gravid ratio of 1.2:1.

Table 7: Indoor resting density and gonotrophic stages of *An. gambiae* s.s. and *An. funestus* collected from IRS (Tarikpaa) and

Study Site	*IRDs*[1] *(sd)*	Abdominal Condition				BF: G Ratio
		UF	BF	HG+G	Total	
IRS Area (Tarikpaa)						
R_{WH}[2]	0.36 *(± 0.63)*	0	5	1	6	5:1
R_{NoH}[3]	2.71 *(± 4.28)*	5	27	6	38	4.5:1
Non IRS Area (Kulaa)						
R_{WH}[2]	1.57 *(± 3.11)*	0	12	10	22	1.2:1
R_{NoH}[3]	1.43 *(± 1.67)*	3	16	1	20	16:1

Non-*IRS (Kulaa*) areas in Nothern Ghana.

IRDs[1] *= Indoor resting densities,* R_{WH}[2] *= Rooms with hanging,* R_{NoH}[3] *= Rooms with no hanging,* ***UF****=Unfed,* ***BF****=Blood Fed* ***HG****=Half Gravid,* ***G****=Gravid*

4.3 Movement of *Anopheles* Mosquitoes

The results from the Exit trap collections (ETC) are presented in Table 8. The abdominal composition of mosquitoes collected were analyzed to assess their post-feeding behavior on the basis of the relative proportions of blood fed and gravid individuals.

Table 8 shows that for the IRS Area (Tarikpaa), mosquitoes collected after exiting the rooms during the night from rooms with hanging and no hanging showed almost the same F to G ratio of. (0.9:1 and 1:1). This suggests an equal tendency for mosquitoes to rest indoors or move outside after feeding for both rooms.

In the non-IRS area, Kulaa, rooms with no hanging showed F: G ratio of 0.09:1. This indicates that the mosquitoes stayed in rooms and develop their eggs post feeding than before attempting to go out for oviposition. A similar behaviour was observed in the mosquitoes exiting from rooms with hanging with F: G ratio of 0.24:1. This indicates that the mosquitoes complete their gonotrophic cycles before exiting the rooms. This shows that most of the mosquitoes will rest indoors before exiting the rooms.

Table 8: The abundance and gonotrophic stages of *An. gambiae* s .l. collected by the Exit trap from IRS (Tarikpaa) and Non-IRS Areas (Kulaa) in the Northern Ghana.

Study Site	Abdominal Condition				BF: G Ratio
	UF	*BF*	*HG+G*	*Total*	
IRS Area (Tarikpaa)					
R_{WH}[1]	1	5	5	11	1:1
R_{NoH}[2]	11	21	23	55	0.91:1
Non IRS Area (Kulaa)					
R_{WH}[1]	39	9	28	76	0.24:1
R_{NoH}[2]	273	12	130	415	0.09:1

R_{WH}[1] = *Rooms with hanging,* R_{NoH}[2] = *Rooms with no hanging,* ***UF****=Unfed,* ***BF****=Blood Fed* ***HG****=Half Gravid,* ***G****=Gravid*

4.4 Mortality of *Anopheles* Mosquitoes in Exit Traps

The mortalities of *An. gambiae* s.l. that were caught exiting rooms is shown in Table 9. A total of 55 exiting mosquitoes were collected from rooms with no hanging clothes and 11 from the rooms with hanging clothes in the IRS area (Tarikpaa). The immediate mortality of *Anopheles* collected from rooms with hanging clothes (3.63%) was significantly lower than in rooms without hanging clothes (23.6%) Table 9. The reverse was the case in the non-IRS area. The delayed mortality (mortality after 24hrs) of *Anopheles* collected in rooms with hanging clothes was higher (22.2%) than in rooms without hanging clothes, 9.5% in the IRS area. The actual mortalities of the species were similar in both environments (36.3% and 30.9%) respectively.

Table 9: Mortality rates for exiting of *An. gambiae* s .l. from rooms in IRS (Tarikpaa) and non-IRS areas (Kulaa) in Northern Ghana

Study Site	Total No. Exiting Mosquitoes	Immediate Mortality (%)	Delayed Mortalities (%)	Actual Mortalities (%)
IRS Area (Tarikpaa)				
R_{WH}^{1}	11	3.63	22.22	36.36
R_{NoH}^{2}	55	23.63	9.52	30.90
Non IRS Area (Kulaa)				
R_{WH}^{1}	76	28.57	10.71	34.21
R_{NoH}^{2}	415	0.96	9.58	10.36

R_{WH}^{1} = *Rooms with hanging*, R_{NoH}^{2} = *Rooms with no hanging*, **No.** = *Number*

4.5 Entomological Parameters

The Man Biting Rates (MBR), Sporozoite Rates (SR) and Entomological Inoculation Rates (EIR) of both *An. gambiae* s.l. and *An. funestus* is shown in Table 10. It was observed that when the rooms in Tarikpaa were compared, a person is likely to be bitten 13.57 times in a 100 days in rooms without hanging clothes whilst in rooms with hanging clothes, a person is more likely to be bitten 1.6 times in 100 days. The non-IRS area, Kulaa recorded the reverse of the results found in the IRS area, Tarikpaa. A person is more likely to be bitten 39.3 times in the room with hanging clothes whilst in the rooms without hanging clothes, a person is more likely to be bitten 23 times. The mosquitoes obtained from Kulaa were more than that obtained from Tarikpaa thus as the results indicate, there is a greater likelihood of being bitten in Kulaa than in Tarikpaa.

The sporozoite rate was calculated as the number of positives obtained from ELISA tests divided by the total number examined. The rooms with hanging clothes and without hanging clothes in the spray area, Tarikpaa both had a sporozoite rate of zero. There were no mosquito samples from these two rooms that tested positive for *P. falciparum*. Thus the rate of infectivity in rooms with hanging clothes and rooms without hanging clothes in the IRS area, Tarikpaa are the same. The non-spray area, however, had a sporozoite rate of 1.83 and 0 from rooms without hanging clothes and rooms with hanging clothes respectively.

The EIR is an indirect measure of how many bites of the mosquito will be infective with the *Plasmodium* parasite per man per night. It was observed that the EIR estimated for rooms with hanging clothes and without hanging clothes in the spray area was zero (Table 10). However, in the non-IRS area, EIR of 0.0043 infective bites per man-night was

estimated for *Anopheles* in rooms without hanging clothes translating to an annual EIR of 1.57 (Table 10).

Table 10: Entomological parameters for *An. gambiae* and *An. funestus* collected from the IRS (Tarikpaa) and Non-IRS (Kulaa) areas

Entomological Parameters	***MBR**** ***(bite/man/night)***	***SR (%)***	***EIR (Infect. bites/man/night)***
IRS Area (Tarikpaa)			
$^{1}R_{WH}$	0.0163	0	0
$^{2}R_{NoH}$	0.1357	0	0
IRS Area (Kulaa)			
$^{1}R_{WH}$	0.393	0	0
$^{2}R_{NoH}$	0.2380	1.83	0.0043

MBR* = Man Biting Rate, **SR** = Sporozoite Rate, **EIR** = Entomological Inoculation Rate, *Biting rate was calculated from PSCs, $^{1}R_{WH}$ = Rooms with hanging, $^{2}R_{NoH}$ = Rooms with no hanging, **No.** = Number*

CHAPTER FIVE

DISCUSSION

5.0 Discussion

Over the past decade, there has been a massive scale-up of antimalarial interventions including insecticide-treated nets, Artemisinin-Combination Treatments (ACTs), rapid diagnostic tests kits and in selected areas, indoor residual spraying (Mueller *et al.*, 2011). The impact of these scaling up campaigns is gradually being observed. The WHO World Malaria Report, 2013 reports that between 2000 and 2012, the scale-up of interventions helped to reduce malaria incidence rates by 25% globally, and by 31% in the WHO African Region. Effective malaria control involves the integration of a number of control tools against the parasite and the vector. Vector control is a cornerstone of malaria control and it remains the most generally effective measure to prevent malaria transmission (WHO, 2013).

Indoor Residual Spraying remains one of the most useful vector control measures. Data from a meta-regression analysis of 13 studies suggest indoor residual spraying reduced malaria prevalence by 62% in the developing world (Kim *et al.*, 2012). It is suspected that when an intervention is introduced in an area to control mosquito vectors of malaria, the behaviour of the mosquito may be altered after a period of time in aversion to the control method. Shifts in biting cycle from the early hours of the morning to the evening having been shown following DDT indoor spraying in the Solomon Islands for *An. gambiae* s.l. (Taylor, 1975) and for *An. minimus* in forested terrain in northern Thailand (Ismail *et* al., 1978). In addition, Nagpal 2012 reports of *An. stephensi* changing its behaviour from

resting on sprayed walls to resting on unsprayed surfaces such as utensils, hanged clothes, and stored clothes some years post spraying.

This study was carried out to investigate the impact of unsprayed surfaces on the resting and exiting behaviour of *Anopheles* mosquito in rooms which have had indoor residual spraying (IRS).

An. gambiae s.l. was found as the dominant vector collected from all study sites and in all collection methods employed: pyrethrum spray and exit trap catches. Other species collected were *An. funestus*, *An. nili* and *An. rufipes* which were however in much smaller numbers. This is consistent with findings from a similar study carried out in Northern Ghana, where *An. gambiae* was the most predominant species found in that area (Appawu *et al.*, 2004). Majority of the *Anopheles* mosquitoes obtained were collected between September and October, two out of four of the months in which rainfall was at its highest for that particular year.

In this study, *An. gambiae* s.s. was the only sibling species of the *An. gambiae* complex recorded. Studies carried in similar settings, (Appawu *et al.*, 2004, Taylor *et al.*, 1993) recorded *An. gambiae* s.s. and *An. arabiensis* as the only species recorded with *An. gambiae* s.s. being the dominant species (Appawu *et al.*, 2004). In another study, *An. arabiensis* was found as the dominant sibling species (Taylor *et al.*, 1993). *An. coluzzi* (M form) and S forms of *An. gambiae* s.s. were both found in the study area, contrary to studies by Yawson *et al.*, 2004 which indicates that the M form was the only species found in northern Ghana. The proportions of *An. coluzzi* were high in both rooms with or without hanging clothes in both IRS and non-IRS area. This implies that the occurrence of

molecular form of *An. gambiae* s.s. were not dependent on the presence or absence of hanging clothes in the rooms.

The number of indoor resting *Anopheles* mosquitoes captured indicated that less mosquitoes were resting in rooms with hanging clothes compared to rooms without hanging clothes in the IRS area. It is expected that in the rooms with hanging clothes, mosquitoes will use the clothes as alternative resting places since the walls have been sprayed and thus more mosquitoes should be found there. The fed: gravid ratio also indicated that more blood fed mosquitoes left the rooms immediately after feeding in the presence of insecticide on the walls. This is because the insecticide on the walls may have exerted some excito-repellency effect on the mosquitoes in the rooms and so many leave the rooms after feeding. Also as expected, the IRD of *Anopheles* mosquitoes were significantly higher in the non-IRS than IRS area. The number of *Anopheles* species collected by ETC from rooms without hanging clothes in the IRS area was significantly higher than in rooms with hanging clothes. It is however, expected that the number of Anopheles species collected from rooms without hanging clothes will be higher since the resting surfaces for the mosquito will be reduced with the absence of hanging clothes compared to rooms without hanging clothes. This will corroborate studies by Nagpal *et* al., 2012 which showed that household objects like cupboards, furniture, hanging clothes, goods stacked on loft, stored clothes, cobwebs and floors can be dominant resting sites in area sprayed with insecticide, although, in this study, the focus was on the hanging clothes because in the study area, the practice of hanging clothes in the rooms was very common. And also studies by Mutinga *et al.*, 1995 which suggested that the storage of clothes in bedroom uncovered could serve as favoured resting sites from where mosquitoes would

freely feed on the inhabitants of the house. Results from this study is however, indicates that, mosquitoes after feeding left the room in the presence of the insecticide on the wall, in spite of the presence of hanging clothes.

The low delayed mortality of *An. gambiae* and *An. funestus* in exit traps from rooms with hanging clothes showed that many of the mosquitoes do not rest on the insecticide treated walls before exiting the rooms. However, this findings need to be further investigated because in the non-IRS area, the immediate and delayed mortality of *Anopheles* was higher than in the IRS area. This means that the mortalities observed may not be due the effect of the insecticide but environmental conditions that prevailed outside the rooms where the traps were fixed.

In this study, *P. falciparum* was tested because it constitutes over 98% of malaria parasite infections in the study area (Binka *et al.*, 2004). Adungo *et al.*, (1991) and Fontenille *et al.*, (2001) have also justified the use of CS-ELISA for measuring sporozoite rates in mosquitoes after comparing with salivary gland dissections. The *P. falciparum* sporozoite infection was only found for the non-sprayed area for rooms without hangings. In the spray area, infectivity rate was found to be zero for both rooms.

The intensity of malaria transmission as normally expressed as the entomological inoculation rate (EIR) was recorded for only *Anopheles* collected from the non-IRS area. This is not surprising because, this area has not been sprayed for the past six years and therefore malaria transmission is likely to be higher than the non-IRS area. This has been

corroborated by a study in the same area where malaria transmission in the non-IRS area was significantly higher than the IRS area (PMI report, 2012).

CHAPTER SIX

CONCLUSION

6.0 Conclusion

- The study identified *An. gambiae s.s.* as the most prevalent vector in the all the study areas with *An. coluzzi* being the most predominant molecular form
- A study showed that *An. stephensi* shifted their behavior by avoiding sprayable surfaces and rested on unsprayable surfaces during all its movement rhythms. It was concluded that the changed behaviour of resting of *An. stephensi* on unsprayable objects in sprayed rooms largely accounted for failure of malaria control in India (Nagpal et al, 2012). However, data from this study indicated that the practice of hanging clothes in the area undergoing indoor residual spraying did not affect malaria transmission, although a more longitudinal study is needed to confirm the findings from this study.

  The study also showed that in both the IRS and non-IRS area, *Anopheles* mosquitoes tend to exit rooms in the morning after feeding. This has implications for the effectiveness of the IRS in the area because many of the mosquitoes will not rest on the insecticide treated walls to be killed.

6.1 Limitations of the Study

The limitations of the study were that:

- The sample size of mosquitoes were relatively low and these may have affected the findings of the study

- The study was done only in the rainy season and therefore the seasonal effect on interaction between hanging clothes, IRS and malaria transmission was not available

6.2 Recommendations

- A more longitudinal study is recommended to increase sampling times and size of mosquito samples that were collected and analyzed
- It is recommended that future studies will also focus on other unsprayable surfaces such as tables, chairs, cobwebs, stored clothes, utensils etc. since all these can serve as resting surfaces for *Anopheles* inside rooms
- Since IRS is known to target endophilic mosquito species, the seemingly exophilic tendencies of *Anopheles* shown in this study needs to be investigated further to ensure the effectiveness of IRS in the area. The exophily behavior may be due to the excito-repellency of the insecticides being used for IRS in the area

REFERENCES

Aboagye-Antwi, F. (2010). Environmental stress and fitness costs of *Plasmodium falciparum* infection in the malaria mosquito *Anopheles gambiae* s.s. A thesis submitted to the graduate school, Keele University, Newcastle-under-Lyme, United Kingdom, in consideration for the degree of doctor of philosophy. Pp 30.

Adungo, N. I., Mahadevan, S., Mulaya, N. L., Situbi, A. P., and Githure, J. I. (1991). Comparative determination of *Plasmodium falciparum* sporozoite rate in Afrotropical *Anopheles* from Kenya by dissection and ELISA. *Annals of Tropical Medicine and Parasitology*, **85**(4):387-394

Ahmed, K. (1989). Epidemiology of malaria in Ghana. *Ghana Medical Journal*, **23**: 190–196.

Ahorlu, C. K., Dunyo, K. S., Afari, A. E., Koram, A. K., and Nkrumah, K. F. (1997). Malaria-related beliefs and behaviour in southern Ghana: implications for treatment, prevention and control. *Tropical and International Health*, **2** (5):488-499

Akogbeto, M. (1995) Entomological study on the malaria transmission in coastal and lagoon areas: the case of a village built on a brackish lake. *Annales de la Societe Belge de Medicine* **75:** 219–227

Alonso, P. L., Lindsay S. W., Armstrong Schellenberg, J. R. M., Keita, K., Gomez, P., Shenton, F.C., Hill, A. G., David, P. H., Fegan, G., Cham, K and Greenwood, B. M. (1993). A malaria control trial using insecticide-treated bed-nets and

targeted chemoprophylaxis in a rural area of the Gambia, West Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**:37-44.

Appawu, M. A. (2004). Genetic diversity of *Anopheles* (Diptera: Culicidae) Vectors and Transmission of Malaria in Kassena-Nankana district, Northern Ghana. A thesis submitted to the graduate school, University of Ghana, Legon, Ghana, in consideration for the degree of doctor of philosophy. Pp 226.

Appawu, M. A., Baffoe-Wilmot, A., Afari, A. E., Dunyo, K. S., Koram, A. K., and Nkrumah, K. F. (2001). Malaria Vector studies in two ecological zones in southern Ghana. *African Entomology*, **9** (1): 59 -65

Appawu, M. A., Owusu-Agyei, S., Dadzie, S., Asoala, V., Anto, F., Koram, K., Rodgers, W., Nkurumah, F., Fryauff, D. J. (2004). Malaria transmission dynamics at a site in northern Ghana proposed for testing malaria vaccines. *Tropical Medicine and International Health*, **9** (1): 164-170.

Asante, A. F. and Asenso-Okyere, K. (2003). Economic Burden of Malaria in Ghana. A technical report submitted to the World Health Organization (WHO), Africa Regional Office (AFRO)

Bayoh, M. N., Mathias, D. K., Odiere, M. R., Mutuku, F. M., Kamau, L., Gimnig, J. E., Vulule, J. M., Hawley, W. A., Hamel, M. J., Walker, E. D., (2010). *Anopheles gambiae:* historical population decline associated with regional distribution of insecticide-treated bed nets in western Nyanza Province, Kenya. *Malaria Journal*, **9**:62.

Bartlett, J.E., Kotrlik, J.W., Higgins, C. (2001). Organizational Research: Determining Appropriate Sample Size in Survey Research. *Information Technology, Learning, and Performance Journal*, **19** (1): 43-50

Beales, P. F., Orlov, V. S., Kouznetsov, R. L. (1989). Malaria planning for its control in Tropical Africa. WHO and Moscow, USSR.

Beebe, N. and Cooper, R. D. (2000). Systematics of malaria vectors with particular reference to the *Anopheles punctulatus* group. *International Journal for Parasitology*, **30**: 1-17.

Bemier, U. R., Kline, Schreck, C.E., Yost, R.A., and Barnard, D.R. (2002). Chemical analysis of human skin emanations: Comparison of volatiles from humans that differ in attraction of *Aedes aegypti* (Diptera: Culicidae). *Journal of American Mosquito Control Association*, **18**: 186-195.

Bermejo, A. and Veeken H. (1992). Insecticide-impregnated bed nets for malaria control: a review of the field trials. *Bulletin of World Health Organization*, **70** (3): 293-296

Binka, F. N., Kubaje, A., Adjuik, M., Williams, L. A., Lengeler, C., Maude. G. H., Armah, E. E., Kajihara, B., Adiamah J. H. and Smith, P. G. (1996). Impact of permethrin impregnated bed nets on child mortality in Kassena-Nankana district, Ghana: a randomized controlled trial. *Tropical Medicine and International Health*. ***1*** (2): 147-154

Bonavita-Cougourdan, A., Clement, L. and Lange, C. (1987). Nestmate recognition: the role of cuticular hydrocarbons in the ant *Camponotus vagus*. *Scop. Journal of Entomology Science*. 22: 1-10

Bortel, W. Van., Trung, H. D., Roelants P., Harbach, R. E., Backeljau' T. ,and Coosemans, M. (2001). Molecular identification of *Anopheles minimus s.l.* beyond distinguishing the members of the species complex. *Insect Molecular Biology,* **9**:335–340.

Breman, J. G. (2001). The ears of Hippopotamus: manifestations, determinants and estimates of malaria burden. *The American Journal of Tropical Medicine and Hygiene,* **64**:1-11.**Breman, J. G., Alilio, M. S. and Mills A. (2004).** Conquering the intolerable burden of malaria: what's new, what's needed: A summary. *American Journal of Tropical Medicine and Hygiene,* **71**: 1-15.

Bruce-Chwatt, L. J., de Zulueta, J. (1980). The rise and fall of malaria in Europe: a historical epidemiological study. Oxford, Oxford University Press.

Bruce-Chwatt, L. J. (1985). Essential Malariology. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **79** (6): 811

Brunhes, J. (1998). Les anopheles de la région afro-tropicale, *Logiciel ORSTOM* Ed., 2.

Bryan, J. H. (1983). *Anopheles gambiae* and *An. melas* at Brefet. The *gambiae* and their role in transmission. *American Journal of Tropical Medicine and Hygiene.* **77** (1): 1-12.

Burkot, T. R., Williams, J. L. and Schneider, I. (1984). Identification of *Plasmodium falciparum*-infected mosquitoes by double antibody enzyme-linked immunosorbent assay. *American Journal of Tropical Medicine and Hygiene,* **33:** 783-788.

Carlson, D. A. and Service, M. W. (1980). Identification of Mosquitoes of *Anopheles gambiae* Species Complex A and B by Analysis of Cuticular Components.

Entomology Papers from Other Sources. **207**: 1089 http://digitalcommons.unl.edu/entomologyother/13.

Centers for Disease Control (CDC). (2006). "Schema of the life cycle of malaria." <http://www.cdc.gov/malaria/biology/life_cycle.htm>. Accessed 2007 Feb 9.

Cha, R. S. and Thilly, W.G. (1993) Specificity, efficiency, and fidelity of PCR. PCR Meth. Appl., **3:**18-29

Charlwood, J. D., Smith, T., Kihonda, J., Heiz, B., Billingsley, P. F., Takken, W. (1995). Density- independent feeding success of malaria vectors (Diptera: Culicidae) in Tanzania. *Bulletin of Entomology*, **85**:29-35.

Chima, Ikechukwu, R. and Mills, A. (1998). Estimating the Economic Impact of Malaria in Sub-Saharan Africa: A Review of the Evidence, mimeo, London School of Hygiene and Tropical Medicine, June.

Clark, H. C., Tomlison, W. J. (1949). The pathologic anatomy of malaria. (In Boyd MF. ed.) *Malariology*, pp. 874-903.

Clarke, S. E., Bogh C., Brown R. C., Pinder M., Walraven G. E. L., & Lindsay S. W., (2001). Do untreated nets protect against malaria? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **95**:457-462.

Coetzee, M., Craig, M., le Sueur, D. (2000). Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitology Today*, **16**: 74–77.

Coetzee, M., Fontenille, D. (2004). Advances in the study of *Anopheles funestus*, a major vector of malaria in Africa. Insect Biochemistry and Molecular Biology, **34**:599-605.

Coetzee, M., M. Craig, and Le Sueur, D. (2000). Distribution of African malaria mosquitoes belong to the *Anopheles gambiae* complex. *Parasitol*ogy, **13**: 149-151.

Coluzzi, M., and Sabatini, A. (1967). Cytogenetic observations on species A and B of the *Anopheles gambiae* complex, *Parasitologia*, **9**: 73.

Coluzzi, M. (1984). Heterogeneities of the malaria vectorial system in tropical Africa and their significance in malaria epidemiology and control. *Bull WHO* **62** (Suppl.): 107-113.

Coluzzi, M., Petrarca, V. and Di Deco, M. A. (1985). Chromosomal inversion intergradation and incipient speciation in *Anopheles gambiae*. *Boll. Zool.* **52**, 45-63

Coluzzi, M. (1992). Malaria vector analysis and control. *Parasitology Today* **4**:113-118

Coluzzi, M. (1994). Malaria and the Afro-tropical ecosystems impact of man-made environmental changes. *Parasitologia,* **36**, 223-227

Coluzzi, M., Sabatini, A., della Torre, A., Di Deco, M. A. and Petrarca, V. (2002). A polytene chromosome analysis of the *Anopheles gambiae* species complex. *Science,* **298**, 1415-1418

Cork, A., and Park K. C. (1996). Identification of electro physiologically active compounds for the malaria mosquito, *Anopheles gambiae*, in human sweat extracts. *Journal of Medical and Veterinary Entomology,* **10**: 269-276.

Cox-Singh, J., Singh, B. (2007). Knowlesi malaria: new, emergent and of public health importance? *Trends in Parasitology,* **24**: 9.

Curtis, C. F., Myamba, J. and Wilkes, T. J. (1992). Various pyrethroids on Bednets and Curtains. *Mem. Inst. Oswald Cruz.* **87**: (363-370)

D'Alessandro, U., Olaleye, B., McGuire, W., Langerock, P., Bennett S., Aikins, M., Thomson, M., Cham, M., Cham, B., Greenwood, B. (1995). 'Mortality and Morbidity from Malaria in Gambian Children after Introduction of Impregnated Bed net Programme', *The Lancet*, **1**:499-502.

De Meillon, B. (1947). The Anophelini of the Ethiopian Geographical Region. *Publ. South Africa Institute forMedical Research*, no. **49**.

Deitz, K. C. , Athrey, G., Reddy, M. R., Overgaard, H. J., Matias, A., Jawara, M., Della Torre, A., Petrarca, V., Pinto, J., Kiszewski, A. E., Kengne, P., Costantini, C., Caccone, A., Slotman, M. A. (2012). Genetic isolation within the malaria mosquito *Anopheles melas*. *Erratum in Molecular Ecology*, **22**:3878.

Demographic Health Survey, 2008 Ghana Statistical Service (GSS), Ghana Health Service (GHS), and ICF Macro. 2009. Ghana**.**

Djogbénou, L., Pasteur, N., Bio-bangana, S. and Baldet, T. (2010). Malaria vectors in the Republic of Benin: Distribution of species and molecular forms of the *Anopheles gambiae* complex. *Acta Tropica*, **114**: 116–122.

Dobzhansky, T. (1970). Genetics and Origin of species, 3rd ed., Columbia University Press, New York. 364p.

Donnelly, M.J., Pinto, J., Girod, R., Besansky, N.J. & Lehmann, T. (2004) Revisiting the role of introgression vs shared ancestral polymorphisms as key processes shaping genetic diversity in the recently separated sibling species of the *Anopheles gambiae* complex. *Heredity*, **92:** 61-68.

Evans, A. M. (1938). Mosquitoes of the Ethiopian Region II. Anophelini. London: British Museum of Natural History.

Fanello C., Santolamazza F., della Torre A. (2002). Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCR-RFLP. *Medical and Veterinary Entomology* **16:** 461-464.

Favia, G., della Torre, A., Bagayoko, M., Lanfrancotti, A., Sagnon, N. F., Toure, T., Coluzzi, M. (1997). Molecular identification of sympatric chromosomal forms of *Anopheles gambiae* and further evidence of their reproductiove isolation. *Insect Molecular Biology*, 6(4): 377-383

Fontenille, D. and Lochouarn L. (1999). The complexity of the malaria vectorial system in Africa. *Parasitologia*, **41**: 267-271.

Fontenille, D., Meunier, J. Y., Nkondjio, C. A., Tchuinkam, T. (2001). Use of circumsporozoite protein enzyme-linked immunosorbent assay compared with microscopic examination of salivary glands for calculation of malaria infectivity rates in mosquitoes (Diptera: Culicidae) from Cameroun. *Journal of Medical Entomology*, **38** (3): 451-454

Foote, R. H. and Cook, D. R. (1959). Mosquitoes of Medical Importance US Department of Agriculture, Agriculture Research Service.

Fujioka, H. and Aikawa, M. (2002). Structure and Life cycle, Malaria Immunology. *Chemical Immunology*, Basel, Karger. **80:** 1-26

Gallup, L and Sachs, D. (1998). The economic burden of malaria. *American Journal of Tropical Medicine and Hygiene*, **64**:85-96**.**

Gallup, J. L. and Sachs D. J. (2001). The economic burden of malaria. *American Journal of Tropical Medical Hygiene* **64**(1, 2) S: 1-11.

Garrett-Jones, C. and Shidrawi, G. R. (1969). Malaria vectorial capacity of a population of *Anopheles gambiae*, An exercise in epidemiological entomology. *Bulletin of World Health Organization*, **40**:531-545**.**

Garros, C., Harbach, R. E., Manguin, S. (2005). Morphological assessment and molecular phylogenetics of the *Funestus* and Minimus groups of Anopheles (Cellia). *Journal of Medical Entomology*, **42**:522-536.

Ghana Health Service (GHS). (2008). *Training Manual for Health Facilities*. Ghana Health Service, Ministry of Health.

Ghana Health Service (GHS). (2008) *Strategic Plan for Malaria Control in Ghana 2008-2015)*. Ghana Health Service, Ministry of Health.

Gillies and Coetzee. (1987). A Supplement To The Anophelinae Of Africa South Of The Sahara (Afrotropical Region). *Publication of the South African Institute for Medical Research*.

Gillies M. T., de Meillon, B. (1968). The Anophelinae of Africa South of the Sahara (Ethiopian zoogeographical region). 2 edition. The South African Institute for Medical Research Johannesburg, South Africa 343pp.

Gillies, M. T. and Coetzee, M. (1987). A supplement to the Anopheline of Africa south of the Sahara (Afrotropical Region) (publication no. 55). Johannesburg, South Africa: South African Institute for Medical Research, 1-143.

Gimnig, J. E., Ombok, M., Kamau, L. and Hawley W. A. (2001). Characteristics of larval anopheline (Diptera: Culicidae) habitats in Western Kenya. *Journal of Medical Entomology*, **38**:282-288.

Hackett, L.W. (1955). Malaria in Europe. London, Oxford University Press.

Hamon, J., Mouchet, J., Brengues, J., Chauvet, G. (1970). Problems facing *Anopheline* vector control : Vector ecology and behaviour before and after application of control measures. *Entomology Society of America*, **7***:* 28–44.

Healy, T. E., Copland, M. J. W., Cork, A., Przyborowska, A., and Halket, J. M. (2002). Landing responses of *Anopheles gambiae* elicited by oxocarboxylic acids. *Journal of Medical Vertinary Entomology*, **16**: 126-132.

Healy, T. P. and Copland M. J. W. (2000). Human sweat and oxopentanoic acid elicit a landing response from *Anopheles gambiae*. *Journal of Medical and Veterinary Entomology*, **14**: 195-200.

Hervey, J. P., Le Goff, G., Geoffrey, B., Hervé, J. P., Manga, L. and Brunhes, J. (1998). Les *Anopheles* de la région afro-tropicale. Software for Windows TM ORSTOM in collaboration with O.C.E.A.C. (Yaoundé, Cameroon).

Hinton H. E. (1967). WHO Entomology Manual, Observations on the biology and taxonomy of the eggs of *Anopheles* mosquitos Department of Zoology, University of Bristol.

Howard, R. W., McDaniel, C. W. and Blomquist, G. J., Cuticular hydrocarbons of the eastern subterranean termite, *Reticuliternes flavipes* (Kollar) (Isoptera: Rhinotermitidae). *Journal of Chemical Ecology* **4**: 233-245

Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White T. J. (1990). Eds. PCR Protocols: A guide to methods and applications. Academic Press, San Diego. Pp 54-59.

Ismail, I. A. H., Phinichpongse, S., Boonrasri, P. (1978). Responses of *Anopheles minimus* to DDT residual spraying in a cleared forested foothill area in central Thailand. *Acta Tropica*. **35**: 69-82.

Kamini, M., Barbara, J., Sina, Paola, M., Richard C. (2001). The neglected burden of *Plasmodium vivax* malaria. *American Journal of Tropical Medicine and Hygiene.* **64**: 97–106 **.**

Kim, D. (2012). Indoor Residual Spraying effectively reduced malaria prevalence. *American Journal of Tropical Medicine*, **87**: 117-124

Koram, K. A., Owusu-Agyei, S., Utz, G., Binka, F., Baird K., Hoffman, S. and Nkrumah, K. F., (2000) Severe anemia in young children after high and low malaria transmission seasons in the Kassena-Nankana district of Northern Ghana. *American Society if Tropical Medicine and Hygiene.* **62** (6): 670-674

Kouznetsov, R. L. (1977). Malaria control by application of indoor residual insecticides in tropical Africa and its impact on community health. *Tropical Doctor*. **7:** 81-91.

Le Sueur, D., Sharp, B. L., Fraser, C., and Ngxongo, S. (1993). Assessment of the residual efficacy of lambdacyhalothrin; A laboratory study using *Anopheles arabiensis* and *Cimex lectularius*, on treated daub wall substrates from Natal, South Africa. *Journal of American Mosquito Control Association,* **9**: 408-413**.**

Lehrer, S., (1979). Explorers of the Body. Garden City, New York: Doubleday and Co. Ltd.

Lehmann, T., Licht, M., Elissa, N. (2003). Population structure of *Anopheles gambiae*. Africa. *Journal of Heredity*, **94**: 133-147.

Lengeler, C. (1998). Insecticide treated bed nets and curtains for malaria control *(Cochrane Review*). In: The Cochrane Library 1998; 3. Oxford: Update Software.

Lindblade, K. A., Gimnig, J. E., Kamau, L., Hawley, W. A., Odhiambo, F., Olang, G., Ter Kuile, F. O., Vulule, J. M., and Slutsker, L. (2006). Impact of sustained use

of insecticide-treated bed nets on malaria vector species distribution and Culicine mosquitoes. *Journal of Medical Entomology*, **43:**428-432.

Lindsay, S.W., Parson, L. and Thomas, C. J. (1998). Mapping the ranges and relative abundance of the two principal African malaria vectors, *Anopheles gambiae sensu stricto* and *An. Arabiensis*, using climate data. *Proceedings of the Royal Society of London. Series B. Biological sciences*, **265:**847 – 854.

Liu, W. 1., Li, Y., Learn, G. H., Rudicell, R. S., Robertson, J. D., Keele, B.F., Ndjango, J. B., Sanz, C.M., Morgan, D.B., Locatelli, S., Gonder, M. K., Kranzusch, P. J., Walsh, P. D., Delaporte, E., Mpoudi-Ngole, E., Georgiev, A. V., Muller, M. N., Shaw, G. M., Peeters, M., Sharp, P. M., Rayner, J. C., Hahn, B. H. (2010). Origin of the human malaria parasite *Plasmodium falciparum* in gorillas. *Nature*, **467** (7314), 420–425.

Lozoff, B., Jimenez, E. & Wolf, A. W. (1991) Long-term developmental outcome of infants with iron deficiency. *N. Engl. J. Med.* **325**: 687–694.

Mabaso, M. L. H., Sharp, B., Lengeler, C. (2004). Historical review of malarial control in southern African with emphasis on the use of indoor residual house- spraying. *Tropical Medicine and International Health*, **9**: 846-856.

May, J., Falusi, A. G., Mockenhaupt, F. P., Ademowo, O. G., Olumese, P. E., Bienzle, U., Meyer, C. G. (2000). Impact of subpatent multi-species and multi-clonal plasmodial infections on anaemia in children from Nigeria. *Transactions of the Royal Society Tropical Medicine and Hygiene*, **94:**399-403.

Mayr, E. (1963). Animal species and evolution. Harvard University Printing Press, Cambridge, Massachusetts. P 797.

McGregor, W. I. (1988). Malaria, Principles and practice of malariology. Edinburgh, Churchill, Livingstone, 1±59.

Morita, K. (1994). Principle of PCR and Its Application for the Diagnosis of Dengue and Japanese Encephalitis. *Journal of Tropical Medicine and Hygiene*, **36**: 228-234.

Mueller I, Slutsker L, Tanner M (2011) Estimating the Burden of Malaria: The Need for Improved Surveillance. *PLoS Med* **8**(12): e1001144.

Mullis, K. B., Faloona, F. (1987). Specific synthesis of DNA in vitro via a polymerase catalyzed chain reaction. *Methods in Enzymology*, **133**: 355-350.

Multiple Indicator Cluster Survey (2013). Ghana Statistical Service.

Mutinga, M. J., Odhiambo, T. R., Kamau, C. C., Odunlaja, A., Amimo, F. A. and Wachira, D. W. (1995). Choice of resting by *Anopheles gambiae* (Diptera: Culicl) in Mwea Rice Irrigation Scheme, Kirinyaga District, Kenya. *East African Medical Journal*. **72**: 170 – 175

Nagpal, B. N., Srivastava, A., Dash, A. P. (2012). Resting behaviour of *Anopheles stephansi* type form to assess its amenability to control malaria through indoor residual spray. *Journal for Vector Borne Diseases*, **49**: 175-180.

National Institutes of Health (NIH). (2007). "Understanding malaria: fighting an ancient scourge." U.S. Department of Health and Human Services. <http://www.niaid.nih.gov/publications/malaria/pdf/malaria.pdf>. Accessed 2007 May 3.

National Malaria Control Programme (2011). Annual Report. National Malaria Control Programme, Accra, Ghana.

Nevill, C. G., Some, E. S., Mung'ala, V. O. (1996). Insecticide-treated bed nets reduce mortality and severe morbidity from malaria among children on Kenya coast. *Tropical Medicine and International Health*, **1**: 139-146.

Ochman, H., Gerber, A. S. and Hartl, D. L. (1988). Genetic application of an inverse polymerase chain reaction. *Genetics*, **120**: 621-623.

Pates, H. V., Takken, W., Curtis, C. F., Huisman, P. W., Akinpelu O. (2001). Unexpected anthropophagic behaviour in *Anopheles quadriannulatus*. *Medical Vertinary Entomology*, **15**: 293–298.

Pates, H. V., Curtis, C. F. (2005). Mosquito behavior and vector control. *Annual Review of Entomology*, **50**: 53–70.

Pates, H. V., Takken, W., Curtis, C. F, Jamet, H. (2006). Zoophilic *Anopheles quadriannulatus* species B found in a human habitation in Ethiopia. *Annals of Tropical and Medical Parasitology*, **100**: 177–179.

Perlmann. P, Troye-Blomberg, M. (2002). Malaria Immunology. *Chemical Immunology*. Basel, Karger. **80:** 1–26.

Pollitt, E., Hathirat, P., Kotchabhakdi, N. J., Missell, L. & Valyasevi, A. (1989) Iron deficiency and educational achievement in Thailand. *American Journal of Clinical Nutrition*. **50**: 687–697

Post, R. J. and Boakye, D. A. (1992). Vector taxonomy and the control of human onchocerciasis in West Africa. *Proceedings of the section experimental and Applied Entomology of the Netherlands Entomological Society*, **3**:105-109.

President's Malaria Initiative (2007). 1st Annual Report. March, 2007

President's Malaria Initiative (2013). 6th Annual Report to Congress. April, 2012

Read, A. F., Lynch, P. A., Thomas, M. B. (2009). How to make evolution-proof insecticides for malaria control. *PLoS Biology*, **7:**158.

Reddy, M. R., Overgaad, H. J., Abaga, S., Reddy, V. P., Caccone, A., Kiszewski, A. E., Slotman, M. A. (2011). Outdoor host seeking behaviour of *Anopheles gambiae* mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. *Malaria Journal*, **10**: 184.

Report of a WHO meeting on best practices and lessons learned from implementing malaria control in complex emergencies in Africa 2000–2004, WHO/RBM, and November 2004.

Report of the Malaria Response in Emergencies Workshop, Tools, Capacity, and Coordination: Are We Reaching the Most Vulnerable Populations? USAID, Washington DC, 21–22 April 2004.

Report of AngloGold Ashanti Malaria Control Program (AGA MAL) at the Malaria Vector Control Oversight Committee Meeting (MaVCOC) Sensitization meeting for stakeholders on Insecticide Resistance and Vector Control, January 2010

Roberts, L., Janovy, Jr. J., (2000). Foundations of Parasitology sixth edition. United States: McGraw-Hill Companies.

Rowland, M., Durrani, N., Hewitt, S., Mohammed, N., Bouma, M., Carneiro, I., Rozendaal, J. and Schapira, A. (1999). Permethrin-treated chaddars and top-sheets: appropriate technology for protection against malaria in Afghanistan and other complex emergencies. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93:** 465-472.

Russell, P. F. (1955). Man's Mastery of Malaria. *London, Oxford University Press*, p 138.

Russell, S. (2004). The Economic Burden of Illness for Households in Developing Countries: A Review of Studies Focusing On Malaria, Tuberculosis, and Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome *American. Journal of Tropical and Medical Hygiene,* **71**:147–155.

Sachs, J. and Malaney, P. (2002). The economic and social burden of malaria. *Nature* **415**: 680-85.

Saiki R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G.T., Mullis K. B. and Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**: 487-49.

Sanger, R., Race, R. R., Jack, J. (1955). The Duffy blood groups of New York Negroes: the phenotype fy (a- b-). *British Journal of Haematology, **1**:* 370–374.

Scott, J. A., Brodgon, W. H. and Collins, F. H. (1993). Identification of single specimen of *Anopheles gambiae* complex by polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene,* **49**: 629-646.

Service, M. W. (1980). A guide to Medical Entomology. Macmillan Press, London pp 24-52.

Service, M. W. (1993). Mosquito ecology. Field sampling methods, 2nd ed. Elsevier, Essex, United Kingdom

Singh, B., Kim Sung, L., Matusop A., Radhakrishnan A., Shamsul S. S., Cox-Singh, J. (2004). A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. *Lancet*, **363**:15834-15836**.**

Sinka, M. E., Bangs, M. J., Manguin, S., Coetzee, M., Mbogo, C. M., Hemingway, J., Anand, P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Okara, R. M.,

Van Boeckel T., Godfray, C. J., Harbach, R. E., Simon, I., Hay, S. I. (2010). The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. *Parasites & Vectors*, **3**:117 http://www.parasitesandvectors.com/content/3/1/117**.**

Smith, T., Genton B., Baea K., Gibson N., Narara A., Alpers. (2001). Prospective risk of morbidity in relation to malaria infection in an area of high endemicity of multiple species of *Plasmodium*. *American Journal of Tropical Medicine and Hygiene*, **64**: 262-267.

Takken, W. (2002). Do insecticide-treated bed nets have an effect on malaria vectors? *Tropical Medicine in International Health*, **7**:1022–1030.

Taylor, E. C., Toure, Y. T., Coluzzi, M. and Petrarca, V. (1993). Effective population size and persistence of *Anopheles arabiensis* during the dry season in West Africa. *Medical and Veterinary Entomology*, **7**: 351-357

Taylor, B. (1975). Changes in the feeding behaviour of a malaria vector, *Anopheles farauti* following use of DDT as a residual spray in houses in the British Solomon Islands Protectorate. *Transactions of the Royal Entomological Society of London.* **127** (3): 277-292

Ter Kuile, F. O., Terlouw D. J., Phillips-Hopward P. A., Hawley W. A., Friedman J. F., Kariuki S., Ya Ping Shi, Oloo A. J., Nahlen B. J. (1999). Permethrin- treated bed nets reduce malaria in pregnancy in an area of intense perennial malaria transmission in western Kenya. *Abstracts of the 48th Annual Meeting of the American Society of Tropical Medicine and Hygiene*, Nov 28- Dec 2 1999, Abstract 11**.**

Tour, T. Y., Oduola, M. J. A. and Morel C. IVI. (2004). The Anopheles gambiae genome: next steps for malaria vector control. *Trends in Parasitology*, **20**: 3

Van Bortel, W., Harbach E. R., Trung, D. H., Roelants, P., Backeljau, T. and Coosemans M. (2001). Confirmation of *Anopheles varuna* in Vietnam, previously misidentified and mistargeted as the malaria vector *Anopheles minimus*. *American Journal of Tropical Medicines and Hygiene* **65** (6): 720 – 732

Wilkes, T.J., Matola, Y.G. & Charlwood, J.D. (1996) *Anopheles rivulorum*, a vector of human malaria in Africa. *Medical and Veterinary Entomology* **10**: 108-110.

White, M. J. D. (1978). Modes of speciation. San Francisco. W.H Freeman and Co.

White, T. J., Arnheim, N. and Erlich, H. A. (1989). The polymerase chain reaction. *Trends in Genetics* **5**: 185-189.

William, A. H., Feiko, O. Ter kuile, Steketee, S. R., Nahlen, B. I., Terlouw D. J., Gimnig, E. J., Ping Shi, Y., Vulule, M. J., Alaii A. J., Hightower W. A., Kolczak, S. M., Kariuki, K. S., and Phillips-Howard A. P. (2003). 'Implications of the Western Kenya Permethrin-treated Bed Net Study for Policy, Program Implementation, and Future Research', *American Journal of Tropical Medicine and Hygiene*, **68** 168–73.

Wirtz, R. A., Zavala, F., Charoenvit, Y., Campbell, G. H. and Burkot T. R. (1987). Comparative testing of *Plasmodium falciparum* sporozoite monoclonal antibodies for ELISA development. *Bulletin of World Health Organization*, **65**: 39-45.

World Health Organization (2011). World Malaria Report. World Health Organization, Geneva, Switzerland.

World Health Organization (2012). The role of larviciding for malaria in Sub-Saharan Africa. World Health Organization, Geneva Switzerland.

World Health Organization (2012). World Malaria Report. World Health Organization, Geneva, Switzerland.

World Health Organization (2012). World Malaria Report. World Health Organization, Geneva, Switzerland.

World Health Organization (2013). World Malaria Report. World Health Organization, Geneva, Switzerland.

World Health Organization (2013). Pesticide Evaluation Scheme. World Health Organization, Geneva Switzerland. http://www.who.int/whopes/Mosquito_Larvicides_25_Oct_2013.pdf

World Health Organization (2013). Malaria Entomology and Vector Control. Training module on Malaria Control. World Health Organization, Geneva, Switzerland.

World Health Organization Pesticide Evaluation Scheme (2013). Recommended compounds and formulations for control of mosquito larvae. World Health Organization, Geneva, Switzerland.

Yawson, A. E., McCall, J. P., Wilson, M. D., and Donnelly, M. J. (2004). Species abundance and insecticide resistance of *Anopheles gambiae* in selected areas of Ghana and Burkina Faso. *Journal of Medical and Veterinary Entomology*, **18**: 372-377.

APPENDICES

Appendix I

Updated: 25 October 2013

WHO recommended insecticides for indoor residual spraying against malaria vectors

Insecticide compounds and formulations[1]	Class group[2]	Dosage (g a.i./m^2)	Mode of action	Duration of effective action (months)
DDT WP	OC	1-2	contact	>6
Malathion WP	OP	2	contact	2–3
Fenitrothion WP	OP	2	contact & airborne	3–6
Pirimiphos-methyl WP & EC	OP	1-2	contact & airborne	2–3
Pirimiphos-methyl CS	OP	1	contact & airborne	4–6
Bendiocarb WP	C	0.1–0.4	contact & airborne	2–6
Propoxur WP	C	1–2	contact & airborne	3–6
Alpha-cypermethrin WP & SC	PY	0.02–0.03	contact	4–6
Bifenthrin WP	PY	0.025–0.05	contact	3–6
Cyfluthrin WP	PY	0.02–0.05	contact	3–6
Deltamethrin SC-PE	PY	0.02–0.025	contact	6
Deltamethrin WP, WG	PY	0.02–0.025	contact	3–6
Etofenprox WP	PY	0.1–0.3	contact	3–6
Lambda-cyhalothrin WP, CS	PY	0.02–0.03	contact	3–6

Chlorfenapyr 240 SC: The current assessment of Chlorfenapyr SC (class group: pyrrole) is available in the report of the 16th WHOPES Working Group meeting, 22–30 July 2013 at: http://www.who.int/whopes/recommendations/en/

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control. WHO specifications for public health pesticides are available on the WHO homepage on the Internet at http://www.who.int/whopes/quality/en/.

[1] CS = capsule suspension; EC = emulsifiable concentrate; SC = suspension concentrate; SC-PE = polymer enhanced suspension concentrate; WG = water dispersible granule; WP = wettable powder.

[2] OC = organochlorines; OP = organophosphates; C = carbamates; PY = pyrethroids.

Appendix II

PREPARATION OF SOLUTIONS FOR ELISA TESTS

Protocol used for detecting circumsporozoite antigen in *Anopheles* mosquitoes

PHOSPHATE BUFFERED SALINE

a) Calibration of Flask

1. Pour 1, 2 or 3 litres of tap water into the flask.
2. Gently slide a stirring rod to be used along the side into the flask.
3. Mark the final volume of water in the flask.

b) Preparation of buffer

1. Discard the water in (a) above and rinse the flask with distilled water
2. Pour about 750ml of distilled water into calibrated flask
3. Rinse stirring rod with distilled water and slide into flask
4. Place flask on electronic magnetic stirrer and stir
5. Empty one bottle of Dulbecco's Phosphate Buffered Saline (DPBS) powder (for one litre) into flask while content is stirring
6. Rinse DPBS bottle thoroughly and add to contents of flask
7. Adjust pH to manufacturer's specifications if necessary
8. Add more distilled water to the content of the flask to the eve of the calibrated mark.

Store at 4^0C; Shelf life – 2 weeks

WASHING BUFFER

Washing Buffer = PBS plus 0.05% Tween 20

1. Follow the procedure for preparation of PBS above to the stage of pH adjustment
2. Add 0.5ml Tween 20 (to 1 litre PBS) while stirring
3. Add more distilled water to the level of the calibrated mark

BLOCKING BUFFER

Reagent	Amount in	
	0.50 litre	1.00 litre
Casein	2.50g	5.00g
0.1N NaOH	50.00ml	100.00ml
PBS ph 7.4	450.00ml	900.00ml

1. Suspend casein in 0.1N NaOH and bring to boil
2. After casein is dissolved, slowly add the PBS, allow to cool and adjust the pH 7.4 with HCl

SUBSTRATE SOLUTION

1. Mix KP & L ABTS (Solution A) and hydrogen peroxide (Solution B) 1:1 Immediately before use (incubate at 100µl/well)

Preparation of solution for coating one micro-titre plate (summary)

1. Stock capture MAb=0.5µg/µl
2. Coat rate is 0.1µg/50µl/well
3. One micro-titre plate contains 96*4 (100 wells). Provision against spillage
4. 10 (100x0.1)µg MAb is needed to coat one plate
5. From 1 above 10µg MAb is contained in 10 x 1/0.5 = 20µl solution
6. From 2,5000 (50 x 100) µl of MAb solution is needed to coat one micro-titre plate
7. Add 4,980 µl PBS to 20µl (10µMAb) solution
8. Coat immediately, 4^0C overnight or store frozen until use. Coat at 50µl (0.1µg MAb)/well.

PLASMODIUM CIRCUMSPOROZOITE ELISA TEST (PROTOCOL)

1. Coat MAb [50µl/well (96-well plate) 30min, Room Temperature (RT) or 4^0C overnight at a coat rate of 0.1µg/50µl/well
2. Flip plate empty and bang on tissue paper
3. Fill wells with blocking buffer (BB); 200µl/well, 1hr, RT
4. Flip plate empty, bang, wash and incubate with the test mosquito extracts and controls (50µl/well; RT, 2hrs)
5. Wash plate twice with washing buffer (PBS/Tw-20), flip empty and bang
6. Incubate MAb-peroxidase conjugate (0.05µg/50µl/well) in BB; 1hr; in darkness
7. Flip plate empty, wash and bang 3 times

8. Incubate substrate [100µl/well: (30-60mm)] RT. 30-60min: Start reading plate after 30min and finish by 60^{th} minute