EVALUATION OF POLYTHYLENE BASED LONG LASTING TREATED BED NETS ON THE POPULATION DENSITY OF INDOOR RESTING *ANOPHELES* MOSQUITOES IN KANYABOLI, WESTERN- KENYA.

BY

ODHIAMBO M. T. OJERA (BED. Sc)

I56/13040/05

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN APPLIED MEDICAL ENTOMOLOGY IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

FEBRUARY, 2010

DECLARATION

This thesis is my original work and has not been presented for any study programme in any university.

Sign_____ _____

ODHIAMBO M. T. OJERA Date

I56/13040/05

This thesis has been submitted for examination with our approval as supervisors.

Sign_____ _____

PROF. ELIZABETH. D. KOKWARO Date

DEPT OF ZOOLOGICAL SCIENCES

KENYATTA UNIVERSITY

Sign_____ _____

Dr. JOHN M. VULULE Date

CENTRE FOR VECTOR BIOLOGY AND CONTROL RESEARCH

KENYA MEDICAL RESEARCH INSTITUTE (KEMRI), KISUMU.

.

FEBRUARY 2010

DEDICATION

I dedicate this research work to my children Val, Chelsie and Natalie. My loving wife Nicole who provided support and the sacrifices they made during the time that I was in the field carrying out this work. May God Almighty bless you.

ACKNOWLEDGEMENTS

This project was funded by Bestnet Europe Ltd and implemented by Kenya Medical Research Institute (KEMRI) Kisumu. I would like to thank the Centre Director, Dr. John M. Vulule for timely release of resources for the project activities. My sincere appreciation also goes to Prof Elizabeth D. Kokwaro of Kenyatta University for supervisory role during inception of this study and continued support until its completion. Miss Carolyne Musanda the nurse in charge at Ratuoro health center and her colleagues for their assistance in clinical data collection. I also acknowledge the people of Kanyaboli village for their excellent cooperation and endurance during data collection. I further wish to express my sincere gratitude to the entire staff of Dominion firm and Kenya Medical Research Institute (KEMRI) in Kisian, for their contribution towards the success of this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Plate **Page**

APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

AAR	Africa Air Rescue
CBS	Central Bureau of Statistics
CDC	Centre for Disease Control
ITNs	Insecticide treated bed nets
KEMRI	Kenya Medical Research Institute
KNBS	Kenya National Bureau of Statistics
KWF	Kenya Wastelands Forum
LLINs	Long lasting insecticide treated bed nets
MoH	Ministry of Health
PSC	Pyrethrum Spray Catch
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization
WHOPES	World Health Organization Pesticide testing Scheme

ABSTRACT

Insecticide treated bednets have been shown to have a profound impact on malaria transmission in experimental trials in sub-saharan Africa. Their effectiveness requires frequent re-treament, however re-treament rates have rarely risen above 20%. The need for re-impregnation and the relatively short life span led to the development of long lasting insecticide bed nets (LLINs) for longer field use. Though these LLINs have shown good efficacy on malaria mosquitoes under control conditions, no field studies have been done on these LLIN for longer term field use. This study was therefore undertaken to evaluate the impact of deltamethrin treated bednets Netprotect®, on density indoor resting of *Anopheles* mosquitoes in western Kenya. In addition the study also investigated the impact of Netprotect® on the feeding success of *Anopheles* mosquitoes' on human blood. These LLINs Netprotect® bednets were randomly distributed in 150 matched houses. Another 150 houses were selected 2km away as the control. These households received their bednets six months later. Indoor resting mosquitoes from both areas were collected using Pyrethrum spray catch (PSC) and then sorted according to their physiological status namely, unfed, fed and gravid. Blood meals were identified by a direct Enzyme linked immunosorbent assay (ELISA) using anti-host (IgG) conjugate. Malaria cases from both areas were followed passively in the nearby clinic by clinical diagnosis and rapid diagnosis test kit (Paracheque). Net efficacy was tested by exposing mosquitoes for 3 min using standard WHO test cones on selected net samples. Comparison of proportions between categorical variables was performed by chi-square test at 95% confidence level using SAS statistical software version 9. The findings revealed that the probability of finding *An. funestus* in control area was twice times more likely than in intervention area (OR=2.4,CI 1.7:3.5). There was decreased human feeding and increased cattle feeding by *An. funestus* in the intervention area. *An. arabiensis* from this area was found to be highly zoophilic. Bioassay on residual insecticidal activity indicated that Netprotect® bed nets killed 80% of the exposed laboratory colony of susceptable *An. gambiae s.s* (Kisumu strain) after one year of use. The malaria cases followed for seven months at Ratuoro health centre showed a reduced prevalence in the intervention area. These results indicate that Netprotect® bednets were effective in controlling *Anopheles* mosquitoes during one year of use and this enhanced their ability to reduce malaria prevalence in the intervention area.

CHAPTER ONE

INTRODUCTION

1.1 Background

Malaria caused by *Plasmodium falciparum* parasites remains one of the world's most significant health and development problems (Nahlen *et al.,* 2003). According to WHO (2000), more than 2.4 billion of the world's population residing in 100 countries is at risk of malaria and this results in an estimated 300-500 million clinical cases each year, 90% of which occur in Africa south of Sahara. In Kenya, over 80% of the population is at risk of contracting malaria. Pregnant women and children under 5 years of age are worst hit by the scourge, which claims approximately 34,000 of this population every year. It has been reported that 6000 primigravidae females suffer from severe anaemia and about 25,000 children are delivered with low birth weight annually due to malaria (MoH, 2006). Beside these lethal effects, malaria impacts on growth of children, reduces school attendance of children and work ability of adults (Brabin, 1993). The economic impact is therefore of very large scale ((Luke and Jeffrey, 2001).

In order to address malaria, Kenya developed the National Malaria Strategy (2001-2010) and adopted four main strategic interventions (MoH, 2006). These include vector control using Insecticide Treated Nets (ITNs), prompt treatment using effective drugs, malaria prevention in pregnancy and indoor spraying of houses with effective insecticides.

Insecticide Treated Nets (ITNs) have been used to protect people against mosquito bites and other insects for many years. The first nets were impregnated with permethrin in

1983 in Soumuoso Bukina Faso (Dariet *et al.*, 1984). Other insecticides such as alphacypermethrin, lamdbacyhalothrin and deltamethrin are now being used to treat these nets at an increasing frequency (Hawley et al., 2003). Randomized controlled experimental trials in sub-Saharan Africa have shown that insecticide-treated bed nets (ITNs) have a profound impact on malaria transmission in areas where the main vector *Anopheles gambiae* or *An. funestus* both late night biting species (Gimnig *et al.*, 2003). Entomological monitoring during trials of ITNs has demonstrated reduction of entomologic inoculation rates by 78-95% in various African settings (Gimnig *et al.*, 2003). While the ITNs cause mortality in many vector species, they also act as irritants or repellents, deterring mosquitoes from entering houses (Rozendaal, 1989). However, the effect of permethrin insecticide impregnation of bed nets may follow a dose response pattern in that at higher concentration mosquitoes are deterred from entering the houses while at lower concentration mosquitoes are more likely to enter the houses and acquire lethal doses of insecticides (Lindsay *et al.*, 1991).

Hawley *et al* (2003) attested that one of the main problematic issues affecting ITN programs are necessity to re-treat nets regularly all due to a number of reasons such as washing, wear and tear, ultra-violet light among others. The pyrethroid insecticide on mosquito netting wears off over time and has therefore to be replaced. While it is easy to socially market mosquito nets to populations of endemic countries, regular insecticide re-treatment has been found to be difficult to implement. As a result, re-treatment rates have rarely risen above 20%, except in the frame of scientific trials or in settings such as China or Vietnam where re-treatment was carried out free of charge by government services

(Armstrong-Schellenberg *et al*., 2002). An alternative solution to this problem is the development of netting that insecticide is incorporated or coated around fibre during manufacture so the biological activity lasts as long as the net itself (3-4 years for polyesters net and 4-5 years for polyethylene ones). These nets hence do not require any further insecticide treatment- so called "Long lasting insecticide net" or LLIN (Guillet *et al.,* 2001). There are two types of LLINs on the market that are fully recommended by the WHO Pesticide testing scheme (WHOPES). These are Olyset® incorporated with permethrin and PermaNet™ which is treated with Deltamethrin (Erlanger *et al.,* 2004). Second generation LLINs which are now emerging with better performance are Duranet™, incorporated with alphacypermethrin, Interceptor, coated with a high dosage of alphacypermethrin and Netprotect®, incorporated with Deltamethrin (CDC, 2007).

Netprotect® LLINs developed by Insect Intelligence Control (IIC) (*www.insectcontrol.net*) represent the latest technological development in the field of mosquito nets incorporated with insecticide. These nets have been developed to address the known shortcomings of LLINs at the time of conception and are made out of high-density polyethylene in which insecticide (deltamethrin) is incorporated directly into the fibre at the rate of 60mg/m^2. Despite the large body of experience documenting their impact under controlled conditions, some practical issues like the effective life of the insecticide in the net under field conditions remains unresolved.

This study was designed to evaluate field performance of Netprotect® long lasting treated nets on indoor resting density of malaria vectors and its effect on malaria prevalence within the rice growing area of Yala swamp, in Western Kenya.

1.2 Statement of the problem and justification of study

Though Insecticide treated bednets (ITNs) have been shown to have a profound impact on reducing malaria transmission in experimental trials in sub-Saharan Africa and were recommended for large scale operations, this tool did not become practical before the first Long Lasting Insecticide Nets (LLINs) were marketed and recommended by WHO (Dabire *et al.,* 2006). The first generation of LLINs included polyester and polyethylene based bednets. Several field studies showed that the polyethylene nets could last up to 7 years, whereas polyester nets in general, physically did not last more than 2-3years. Dabire *et al.* (2006) also indicated that though LLINs showed good efficacy on mosquitoes under controlled conditions, their effectiveness in the field conditions with respect to actual duration of insecticide protection in the field were not impressive. Initial data from Burkina Faso seemed to indicate that performance was not quite as good as anticipated (Muller *et al*, 2002). As a reaction to initial results of varying product performance, different manufacturers reviewed their production processes and reported to have significantly stabilized and improved the manufacturing process. This has led to development of second generation LLINs to improve on the negative experiences of first LLINs. Netprotect® bed nets have been developed on the advantage of the two first long lasting bednets using the fine mesh like polyester net but having the strength and incorporation technology of polyethylene net.

This study was thus designed to investigate the performance of second generation LLINs Netprotect® on the density of indoor resting *Anopheles* mosquitoes with the aim of assessing its effectiveness in the on malaria transmission.

1.3 Research questions

a) What is the effect of Netprotect® on the population density of indoor resting *Anopheles* mosquitoes in Kanyaboli?

b) What is the duration of the residual insecticidal activity of Netprotect® long term use?

c) Does Netprotect® have an impact on the human blood-feeding success of *Anopheles* mosquitoes within intervention houses in Kanyaboli?

d) What is the impact of Netprotect® on malaria prevalence within the intervention houses in Kanyaboli?

1.4 Null hypotheses

a) There is no difference in vector indoor resting population density in intervention and control areas in Kanyaboli village, Western Kenya.

b) There is no difference in duration of the residual insecticidal activity of Netprotect and untreated nets.

c) There is no difference on human blood feeding success of vector in intervention and control houses Kanyaboli.

d) There is no difference in malaria prevalence in the intervention and control areas of Kanyaboli village.

1.5 Objectives of the study

1.5.1 General objective

To investigate the effect of long lasting insecticide treated nets (LLINs) Netprotect® on density of indoor resting, periodic net bioefficacy and its impact on blood meal origin against *Anopheles* mosquitoes in rural Western Kenya.

1.5.2 Specific objectives

(a) To establish whether using of LLINs (Netprotect®) affect the density of indoor resting of adult *Anopheles* mosquitoes in Kanyaboli

(b) To establish the duration of the residual insecticidal activity of the LLINs in Kanyaboli.

(c) To investigate the effect of LLINs on the human blood feeding success of *Anopheles* mosquitoes within intervention houses in Kanyaboli.

(d) To determine the effect of LLINs on malaria prevalence in Kanyaboli village.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria situation in the world

Malaria is a vector-borne infectious disease caused by a eukaryotic protist of the genus *Plasmodium*. It is widespread in tropical and sub-tropical regions, including parts of America, Asia and Africa. Five species of the *Plasmodium* parasite can infect humans; the most serious forms of the disease are caused by *Plasmodium falciparum*. Malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* causes milder disease in humans that is not generally fatal (Snow *et al*., 2005). A fifth species, *Plasmodium knowlesi*, otherwise known to infect monkeys has also been shown to infect people in Malayasia and Indonesia (Anu *et al.*, 2008).

More than 2.4 billion of world population that resides in hundred countries is at risk of malaria. This results in an estimated 300-500 million clinical cases each year of which 90% occur in Africa South of Sahara (WHO, 2000). In these high burden areas of Africa, *An. gambiae*, the most efficient vector predominates throughout large areas of the continent. This combination of a highly dangerous pathogen carried from person to person by a highly resilient vector results in at least one million deaths among young African children each year (Nahlen *et al.*, 2003). According to Sachs and Malaney (2002), malaria exerts a heavy economic burden due to medical costs associated with it, loss of man-hours and premature death of workers. Most malarial countries are poor and have a slow economic growth (Luke and Jeffrey, 2001).

2.1.1 Burden of malaria in Kenya

In Kenya, malaria accounts for 30% of outpatient attendance and 20% of admissions to health facilities (MoH, 2006). About 20 million Kenyans annually are at risk of malaria especially pregnant women and children below the age of 5 years of age. It is the most common cause of death in children under 5 years of age and it is estimated to cause 20% of all deaths among children (MOH, 2006). Moreover, it remains a major public health problem, a challenge to poverty reduction and a contributor to poor economic development.

2.1.2 Malaria vectors distribution in Kenya

More than 430 species of *Anopheles* mosquitoes have been described but only less than one third are considered vectors and one or two species are known to be major drivers of disease transmission dynamics in a given area (CDC, 2007). The rate of disease transmission is dependent on vector distribution, abundance and lifespan, degree of host-vector-pathogen contact, susceptibility of the vector to the pathogen and the effects of the pathogen on survivorship of both the vector and the host. These factors are further dependent on local ecological factors such as local climatic conditions, topography, water table, occurrence and diversity of larval habitats and human lifestyles (Rwagoshora *et al*., 2007). The primary vectors of malaria in Kenya are *An. gambiae, An. arabiensis* and *An. funestus* (Beier *et al.*, 1990). *An. gambiae* and *An. funestus are* responsible for more than 90% of the malarial transmission while *An. arabiensis* responsible for the rest. Both *An. gambiae* and *An. funestus* bite primarily indoors during late night hours (Gimnig *et al.*, 2003). *An. funestus* occupies a wide range of ecological niches, is highly anthropophilic

and is susceptible to human malaria parasites. The vectorial capacity of *An. funestus* can often exceed that of *An. gambiae* in some localities (Fontenille *et al.*, 1997). This is attributed to the larvae of *An. funestus* developing in permanent swamps or pools along the streams and river systems, as opposed to those of *An. gambiae* species complex which prefer temporary aquatic habitats. *An. funestus* are less dependent on rains and become abundant during dry seasons when *An. gambiae* densities are low (Minakawa *et al.*, 2001). Thus *An. funestus* is considered a vector species that bridges malaria transmission during two wet seasons (Mbogo *et al.*, 2003). *An. funestus s.l.* consists of nine species that are difficult to distinguish morphologically (Gillies *et al.*, 1997). All except *An. funestus .s.s.* and to some extent *An. rivolorum* are believed to be zoophilic and vectors (Wilkes *et al*., 1996). *An. gambiae s.l* also consists of several species which are majorly *An. gambiae s.s.* and *An.arabiensis*. *An. arabiensis* is mostly zoophilic and common in the rice growing areas.

2.1.3 Approaches to malaria diagnosis

World Health Organization has previously indicates that several approaches to malaria diagnosis can be adapted (WHO,2000). Each approach presents characteristics such as costs, ease of performance and accuracy which will determine applicability to different situations. The main malaria diagnostic approaches include clinical, microscopic, nucleic acid amplification test and rapid diagnostic tests.

2.1.3.1 Clinical diagnosis

This diagnosis is most widely used approach in malaria diagnosis. Among clinical signs and symptoms associated with malaria, the most prominent is fever, which is often accompanied by chills, perspiration, anorexia, headache, vomiting and malaise (WHO, 2001). It has been the only feasible method in many situations, particularly in rural areas and at the periphery of health care system where laboratory support to clinical diagnosis does not exist. Residents of endemic areas are familiar with this combination of symptoms and frequently self diagnose malaria based on symptoms alone. While this method is inexpensive, require no special equipment and can be effective, clinicians often misdiagnose malaria infections. This is because the symptoms of malaria are non specific and overlap with those of other febrile illness like flu (WHO, 2000). Therefore, a diagnosis based on clinical grounds alone is unreliable and when possible should be confirmed with laboratory test. WHO (2000), further cites that misdiagnosis often leads to unnecessary prescription of malaria medication which is becoming increasingly expensive as drug resistance increases globally and new medicines are required for effective treatment. Thus, increasing the accuracy of malaria diagnosis is becoming more important.

2.1.3.2 Microscopic diagnosis

Microscopy is the most popular method used for detecting malaria infections and is usually available in better equipped clinics. The technique can confirm clinical diagnosis and provide important treatment information by identifying which parasite species are in circulation and what drug treatment to initiate. The procedure consists of collecting finger

prick blood samples, preparing thick or thin blood smears: staining smears with Giemsa and examining the smears through a microscope for presence of malaria parasites. However, many factors affect the quality of microscopic diagnosis of malaria. The experience and training of the microscopist along with quality of slide preparations, staining and reading are paramount to accurate diagnosis (WHO, 1999). Other important factors include quality of the equipment, availability of electricity and necessary reagents. Also due to delays in providing the microscopy results to the clinician, decision for treatment are often taken without benefits of the results (WHO, 1999).

2.1.3.3 Nucleic acid amplification tests

Several nucleic acid amplification technologies (NAATs) now exist to detect parasite DNA circulating in the bloodstream and they are very sensitive. NAATs technologies that are used include Real Time Polymerized Chain Reaction (PCR) for quantification of *Plasmodium falciparum* (Real time QT-NASBA) and Real Time Nucleic acid sequence based amplification (Mens *et al.*, 2006). These technologies are currently not widely available in malaria endemic areas because they require equipment as well as specialized training Interpreting NAAT results can be challenging due to the fact that parasite DNA can remain in the bloodstream long after infection is cleared. Thus differentiating an active infection from a recently cleared infection is difficult.

2.1.3.4 Rapid diagnostic tests

Rapid diagnostic tests (RDTs) are based on the capture of the parasite antigens from the peripheral blood using either monoclonal or polyclonal antibodies against the parasite

antigen targets. Malaria RDTs rely on the detection of parasite specific antigens (proteins) circulating in the bloodstream. The most common antigens for RDTs are *P. falciparum* histidine-rich Protein-2(pfHRP2) and *Plasmodium* spp. lactose dehydrogenase (pLDH). Tests based on the pfHRP2 antigen are specific to *P. falciparum*, the most dangerous species of malaria, and are more readily available and less expensive. Tests based on pLDH come in two varieties: pan-malarial tests which detect all malaria species or species specific tests that detect malaria species other than *P. falciparum.* Pan-malarial tests are also available which detect the aldolase antigen **(**Kakkilaya**,** 2003). This test uses finger-stick or venous blood, takes only ten to fifteen minutes. Even non clinical staff can easily learn to perform the test and interpret the results (Kakkilaya, 2003). These RDTs do not require a laboratory, electricity, or any special equipment and offer the potential to extend accurate malaria diagnosis to areas where microscopy services are not available such as remote locations or when the laboratory technicians are absent.

2.1.4 Vector feeding and resting habits

Many of the habits of adult mosquitoes are linked to their small size and poor adaptation to dry environments (WHO, 1982). Flight, host seeking and feeding generally takes place in humid environments. Species of *Anopheles* that are associated with open terrain and sunlit habitats mostly fly between the hours of dusk and dawn when the air is humid. Bockarie *et al.,* (2009) cited that *An. gambiae s.l* and *An. funestus,* the principal vectors of malaria in Africa have their peak of biting around midnight and that parous females (already having laid at least one batch of eggs) have a tendency to bite later than nulliparous (have not oviposited).

Mosquito species which enter houses to feed and rest indoors are described as endophagic and endophilic respectively as compared with exophagic and exophilic mosquitoes, which feed and rest outdoors respectively (WHO,1982). The exact feeding pattern of mosquitoes indoors varies with different species and circumstances but usually mosquitoes enter to feed in the early hours of the night (WHO, 1982). In areas where outdoor-resting sites are greatly reduced in dry seasons, houses are extensively used seasonally as resting places by mosquitoes that have fed outdoors on cattle. World Health Organization noted that resting behaviour varies with species and environment (WHO, 1995). Some species of mosquitoes enter houses, feed indoors on humans and then gorged with blood meal, rest indoors for 24-48 hours. Once the blood has been digested and ovaries contain mature eggs the gravid female mosquito typically leaves the house at dusk in search of suitable aquatic environment for egg laying.

For a mosquito to transmit an infection to humans, it must take at least two blood meals first for uptake of the pathogens and later for eventual transmission to a susceptible human. The degree of human-vector contact is, therefore, considered one of the most important components of disease transmission and is used in planning and evaluating the risk of vector-borne disease and the impact of vector control measures (Garrett-Jones *et al*., 1980).

2.1.5 Irrigation agro-system and malaria vectors

Irrigated rice agro-ecosystems are considered hotspots for mosquito breeding and worldwide more than 89 species of *Anopheles* are associated with rice cultivation. At

least 11 out 23 species occur in a variety of aquatic habitats present in African rice agro-systems (Muturi *et al.*, 2006). Also under irrigation, the number of mosquitoes usually increases and this increase sometimes leads to rise in malaria prevalence. In Burundi malaria parasite prevalence was estimated at between 24 and 69 percent in irrigated rice fields compared with 5-30% in nearby non- irrigated cotton growing areas. Similarly the prevalence in Hola irrigation scheme in Kenya where there is growing of cotton and vegetable has been reported to be 54% higher than surrounding non irrigated areas. This is due to an increased number of mosquito breeding sites (Mutero *et al.,* 2006). Studies in rice irrigation schemes in Kenya have shown that malaria prevalence is lower in the irrigated villages apparently because the predominant mosquito species *An. arabiensis* prefers to feed on cattle rather than humans (Mutero *et al.,* 2004). A study in Valle' du Kou, Bukina Faso also recorded low malaria transmission despite high density of *Anopheles* mosquitoes (Robert and Carnevale, 1991). This was due to the fact that anthropophilic index was low as was the mean physiological age of the anthropophilic fraction of mosquito population (annual parity rate of 30%). Hence the sporozoite index was small despite high vector density.

2.1.6 Vector control strategies and constraints

Options for vector control include environmental management, chemical control, biological control and personal protection. Environmental management such as, marshland alteration, house screening, filling, grading and drainage of wetlands avoids creation of vector breeding areas, changing natural habitats or improving human habitation to reduce abundance of target vector (WHO, 1982). Chemical management of

malaria vectors involves residual spraying, larviciding and space spraying. They are usually fast and effective. Biological methods consist of utilizing natural enemies to target mosquitoes and use of biological toxins to achieve vector management (Knols *et al.*, 2007). They include use of larvivorous fish inverterbrate predators, nematodes, bacteria and fungi. In some areas environmental management, such as the draining of wetland breeding grounds and better sanitation were adequate. Before DDT, malaria was successfully eradicated or controlled also in several tropical areas by removing or poisoning the breeding grounds of the mosquitoes or the aquatic habitats of the larva stages, for example by filling or applying oil to places with standing water. In the northern parts of the USA, malaria was eliminated in the early 20th century by such methods, and the use of the pesticide DDT eliminated it from the South by 1951(CDC, 2004). Bio-control using microbial bio-pesticides, such as *Bacillus thuringiensis*, has been used successful to control mosquito larval stages (Rishikesh *et al.*, 1998). By nature, larvae control has no immediate effect in reducing the number of biting vectors and may take several days or weeks before reduction in their numbers can be achieved or appreciated (Skovmand *et al*, 2009). Control of adult mosquitoes with entomopathogenic fungi has successfully been used in the field (Knols *et al.*, 2004). However, this pathogen tends to be specific on the mosquitoes species they can control and the habitat they will work in (Das and Amalraj, 1997). There are also serious problems, relating predominantly to costs and sustainability, with the management of traditional vector control, insecticide residual spraying and larviciding (WHO, 2001). Environmental management and biological control measures are not easy to implement, sometimes costly and not relevant in many areas (WHO, 1982). Environmental management

practices can be very effective and was the primary tool used to eradicate malaria from US and Europe (CDC, 2004). However, they typically demand high initial investment followed by continuous, but cheaper sustainment like in draining. Often, they are used to supplement vector control methods in order for them to be fully effective. A control measure that has received increasing attention in the last 20 years is the use of insecticide-impregnated bed nets or curtains (Faye *et al.,* 1998).

2.1.7 Types of nets

Several types of bed nets are available and they vary in size, material, and/or treatment. Untreated bed nets and conventional nets which require re-treatments have been used for personal protection against disease vectors since Second World War before the development pre-treated nets (Curtis,1991). Most pre-treated nets are of polyester, but nets are also available in cotton, polyethylene or polypropylene. Currently, only pyrethroid insecticides like permethrin, deltamethrin and cypermethrin are approved by World Health Organization (WHO) for use on ITNs. These insecticides have a low mammalian toxicity, but are highly toxic to insects and have a rapid knock down effect, even at very low doses. Pyrethroids have a high residual effect, they do not break down unless washed or exposed to sunlight (CDC, 2008).

2.1.7.1 Insect Treated Nets (ITNs)

Mosquito nets have been used to protect people against mosquitoes and other biting insects for many years. It is reported that the Egyptian female Pharaoh, Queen Cleopatra,

used bednets when travelling on the Nile River in100 BC against the mosquito plague (Brier, 2004). Previous reports by Ross advocated for the use of bed nets as a preventive measure against malaria infection (Ross,1910). Bed nets provide a protective physical barrier against adult mosquitoes and are enhanced by a chemical barrier with a repellant or insecticide. Curtis, (1991) found that application of this personal protection against disease vectors began during the Second World War. Between 1976 and 1984, several insecticides were tested to evaluate their entomological effects on impregnated tissues or nets. The first test of impregnated mosquito nets treated with permethrin was done in 1983 in experimental huts at Soumous field station in Bukina Faso (Darriet *et al.,* 1984). Since then, many studies have been implemented in different malaria endemic areas and their results have revealed safety, acceptability, feasibility and efficacy of ITNs in the control of vectors (Bermejo and Veeken, 1992). The efficacy of insecticide treated bed nets in reducing vector population, human-vector contact and thus preventing malaria-related morbidity in various epidemiological situations is well documented (Rozendal, 1989; Carnevale *et al.,* 1991; Gimnig *et al.,* 2003). In community-wide trials in several African settings, ITNs have been shown to reduce mortality by about 20-30 %. Untreated bed nets form a protective barrier around persons using them but mosquitoes can still feed on people through the holes on the nets (Genton *et al.,* 1994). Mwangi *et al.* (2003) showed that whereas untreated intact nets provided moderate protection against malaria, holed untreated nets do not. Lines *et al.* (1987) also highlighted another drawback with untreated nets that they divert extra biting mosquitoes to people in the same room who are not protected by nets. Hence, if the net user was a malaria immune adult and non user(s) was a malaria vulnerable child, the overall effect would be counterproductive.

Malaria prevention by killing adult mosquitoes is generally favoured because by moderately reducing vectors longevity, they radically suppress community level transmission (Killeen *et al.,* 2002). The use of nets treated with insecticides greatly enhances personal protective efficiency of bed nets. Moreover, the insecticide used for bed net treatment kills *Anopheles* mosquitoes and has become popular in bed net impregnation. Insecticide impregnated bed nets besides acting as physical barriers against blood questing mosquitoes also reduce mosquito densities indoors by killing them.

Randomized control trials have shown that insecticide treated nets ITNs are effective in reducing morbidity and mortality due to malaria in sub-Saharan Africa (Snow *et al*., 1988; De Alessandro *et al.,* 1995; Binka *et al*.,1997; Nevill *et al*., 1996). Entomological monitoring during trials of ITNs has also demonstrated reduction in entomological inoculation rates by 78-95% in various African settings (Magesa *et al.,* 1991; Jeanson *et al.,* 1994; Cuzin-Ouattar *et al.,* 1999; Gimnig *et al.,* 2003;). Miller (1991) cited that insecticide treated nets not only cause mortality in many vector species, but also act as irritants or repellents deterring some mosquitoes from entering the houses and causing early exit of others. Lindsay *et al.* (1991) suggested that the primary mode of action of ITNs is to discourage mosquitoes from entering houses while at lower concentration mosquitoes are more likely to enter the houses and acquire a lethal dose of insecticide.

According to Lengeler (2004), studies in Africa and Asia have demonstrated a more than 50 % protective efficacy for individual users of ITNs in reducing malaria episodes, 29 % protection against severe malarial disease and substantial protection against anaemia. Furthermore, the use of ITNs reduced child mortality by 18 % in five sites in Sub-

Saharan Africa (Lenegeler, 2004). In addition a review by this author demonstrated the efficacy of ITNs in both stable and unstable transmission areas.

Widespread use of ITNs resulted in an overall reduction in mortality of 19%, protected users against anaemia, and had a substantial impact on mild disease episodes. One large-scale rural study in Tanzania found out that ITNs and untreated nets reduced mortality of children by one month to four years, with protective efficacies of 27% and 19% respectively (Armstrong-Schellenberg *et al.,* 2002). Re-treating ITNs semi-annually or just before the annual peak in transmission is essential for effective vector control and is proving to be a major logistical and financial challenge. Fortunately, new types of nets with a long-lasting insecticidal property are now available, and re-treatment will soon cease to be an issue. The salutary impact of large-scale ITN programs has been demonstrated in China (Tang, 2000), Tanzania (Abdulla *et al.,* 2001; Armstrong-schellenberg *et al.,* 2001; WHO, 2005), and Vietnam (Hung *et al.,* 2002). Lengeler and Sharp, (2003) recommended that to scale up ITN use, more operational experience is required to inform national initiatives. However, recent encouraging reports show that Eritrea, Malawi, Togo, Zambia, and other countries in Africa are already scaling up the use of ITNS to bring about nationally high coverages.

2.17.2 Impediments to ITNs use

To maintain efficacy of ITNs, they must be retreated with pyrethroids at intervals of 6-12 months or more frequently if nets are washed. The need for re-treatment is the most difficult barrier to full implementation of ITNs use in endemic countries. The additional

cost of insecticide, and the ignorance about its importance, results in low re-treatment rates in most African countries. Cultural beliefs relating to fertility in some African communities prohibits mixing of beddings items of parents and children who have attained puberty has been cited as also major reason for rejection of ITNs especially during re-treatment (Alaii *et al.,* 2003).

In community-wide intervention trials bed nets and insecticides have been provided without cost to ensure high coverage in intervention groups and this has given very good results. In operational programs, coverage and use-rate may be lower, in areas of sub-Saharan Africa where ITNs have been made available. The cost of nets and insecticide is often cited as a major barrier to the uptake and maintenance of insecticide treated nets (Winch *et al*., 1997; Holtz *et al*., 2002; Okrah *et al*., 2002). In Gambia and Coastal Kenya retreatment rates decreased considerably when people had to pay for insecticide (Cham *et al*., 1997 and *Snow et al*., (1999). Social and cultural factors may also affect the level of ITNs use in a community (Snow *et al.,* 1999).

Incomplete coverage of ITNs could have several effects on the distribution and behaviour on *Anopheles* mosquito. The mosquitoes may be diverted from houses with ITNs to those without them, thus increasing malaria risk among people without ITNs (Lines *et al.,* 1997). Snow *et al.* (1997) suggested that this may have occurred in trials using untreated bed nets where the observed differences in malaria morbidity were due to increased infections in unprotected persons rather than a decrease in morbidity among bed net users. Alternatively the use of ITNs may act to depress the population of vector mosquitoes in large areas. If large enough, this community effect would provide some

protection to everyone living in a given region including those who do not posses ITNs (Hawley *et al.*, 2003). Evidence of a community effect in mosquito population has been observed in Kenyan Coast where households located within 400 meters from an intervention village had significantly fewer mosquitoes than those further away (Mbogo *et al.*, 1996). Studies in Tanzania and Burkina Faso suggested that ITNs or curtains reduced vector survival and that unprotected persons sleeping indoors or outdoors within intervention villages experienced fewer mosquito bites than persons sleeping in control villages (Dabire *et al.*, 2006). In the earlier mentioned studies, the community effects had a measurable impact on child mortality and morbidity. However, several studies in The Gambia have indicated no evidence of community wide effect (Lindsay *et al*., 1993; Thomson *et al.*, 1995; Quinones *et al.*, 1998.), but considering the time of these studies, dipped nets must have been used.

Recently, consequences have been taken to improve the low coverage especially among the poorest. Bed net campaigns are now often combined with measles campaigns and nets are given free to obtain a high coverage rate with low distribution cost, since much of the organizational work is done by the people paid for running the measles campaign. The success of these programs in obtaining high coverage has encouraged some countries to start net distribution that target everybody in endemic areas (Grobowsky *et al.*, 2005).

2.1.7.3 Types of insecticides used for net treatment

Pyrethroids are synthetic pesticides that are being used to impregnate the bed nets. They are similar to pyrethrins in their chemical structure and they act on the nervous system of insects by inactivating the sodium channels in the insect nervous systems (CDHS, 2005).

At present permethrin is the most widely used at target dose of 500mg/m^2 of netting; Deltamethrin, alphacypermetrhin and lambdacyhalothrin could also be used at dosage of between 25-50mg/m^2. According to Hawley *et al* (2003) insecticides such as, alphacypermethrin, Lambdacyhalothrin and deltamethrin are now being used with increasing frequency. This is due to the fact that these insecticides have higher killing activity on *Anopheles* mosquitoes and lower repellence compared with permethrin, thus the probability of divergence of mosquitoes due to these chemicals may be lower and any community effect may be higher (Gimnig *et al*., 2003).

2.1.8 Vector resistance to pyrethroids

Resistance by mosquitoes to pyrethroid insecticides used for impregnation of bed nets is also raising concern over the continuous use of them in vector control. Pyrethroid resistance arising from mutation of the sodium channel receptor (i.e. the knock down resistance gene (kdr)) has been reported in the most important African malaria vector *An. gambiae s.s* and is widespread in several West African countries (Chandre *et al.,* 1999; Diabate *et al.,* 2002). Metabolic based resistance to pyrethroids can be associated with the presence of high levels of oxidase activity and confers cross resistance to some organophosphorous and carbamate insecticides. This resistance mechanism was recently found in Kwazulu Natal and Mozambique (Etang *et al.,* 2005) in the local vector *An. funestus*. Increase in permethrin tolerance due to elevated levels of oxidase and esterases among *An. gambiae* following introduction of permethrin-impregnated bed nets was also reported in some villages near Kisumu (Vulule *et al.,* 1999). This has raised concern of resistance in vectors to these insecticide classes. Fortunately, the predominant *kdr*

mechanism apparently does not prevent the efficacy of pyrethroid treated nets (Darriet *et al.,* 2001). There is also strong evidence that even when *kdr* gene occurs at frequencies of 80-90%, ITNs still kill mosquitoes in huts and reduce the malaria in villages (Asidi *et al.,* 2004). One possible explanation is that while *kdr* mosquitoes need a higher dose to knock them down due to the resistance mechanism, they are also less irritated by pyrethroid insecticide and therefore tend to rest long enough on the nets to pick up a lethal dose of pyrethroid. Due to these developments, it is important therefore that studies are pursued to identify alternative insecticides with low mammalian toxicity and with little prospect that a gene for pyrethroid resistance would confer cross resistance to alternative insecticide, so that an effective switch could be made quickly. However, data from Cameroun, Benin and Cote d'voire (Chandre *et al*., 1999) indicates that the combination of *kdr* resistance and mixed oxidase allow the mosquitoes to bite and survive in houses with pyrethroid treated bednets. Further, it seems important to distinguish between the so called East African mutation in the gene for this sodium channel and the West African mutation. The West African mutation has a phenotypic expression that can be measured in bioassays, whereas such an effect has not been found from the East African mutation (Stump *et al.,*2004). However, Aram *et al*., (2004) found the East African mutant increased in frequency in villages with several years of ITN use indicating that it gave some fitness value, even if it is not yet identified.

2.1.9 Long lasting insecticide treated nets (LLINs)

Insecticide treated nets (ITNs) are well established tools for controlling malaria (Lengeler *et al.,* 2000). However, there is need for re-treatment for these nets at least once or twice

a year to maintain their efficacy and some malaria control programs have reported difficulties in maintaining a regular re-treatment service (Kachur *et al.,* 1999; Kroeger *et al*., 1997). A new type of wash resistant ITNs, the Long Lasting Insecticide Treated Net (LLIN) has been developed in order to make re-treatment service unnecessary (WHO, 2002). As regards LLINs insecticide is incorporated or coated around fibres of LLINs during manufacture so the biological activity lasts long as the net itself (3-4 years for polyesters net and 4-5 years for polyethylene ones) and hence the nets would not require any further insecticide treatment (Guillet *et al.,* 2001). The insecticides used in treatment of LLIN are the same as those used for ITNs, also because WHO only recommends insecticides already known to be effective. In the LLIN, permethrin is used at 2 g/m², Deltamethrin between 60 and 120 mg/m² and alphacypermetrhin between 150 and 250 mg/m². There are two types of LLINs on the market that are recommended by the WHO Pesticide testing scheme (WHOPES), Olyset® and permanent® for use in preventing malaria. The insecticide can resist multiple washes and is released over time to the surface of netting fibres during the 4-5 years lifespan of the net. Compared with nets treated by conventional dipping, LLINs have several important advantages such as no need for retreatment; reduced insecticide consumption; and minimum potential environmental impact. Furthermore, release of insecticide in natural water bodies during washing is greatly reduced (WHO, 2001).

More recently, several companies have developed second generation LLINs with better performance and three more of such nets are currently under evaluation by various research institutions. They include, Duranet™, Interceptor™ and Netprotect®(CDC,

2007). DuraNet™ is a long lasting insecticide treated net made out of 100% polyethylene and is impregnated with alphacypermethrin. Interceptor is made of polyester fibres and impregnated with alphacypermethrin. Netprotect® LLINs developed by Insect Intelligence Control (IIC) represent one of the latest technological developments in the field of mosquito nets incorporated with insecticide. These nets have been developed to address the shortcoming of LLINs known at the time of conception and are made out of high-density polyethylene in which insecticide (deltamethrin) is incorporated directly into the fibre at the rate of 60mg/m^2. Despite the large body of experience documenting their impact on controlled conditions, some practical issues like the effective life of the insecticide in the net under field conditions remains unresolved (Dabire *et al.*, 2006)

This study investigated the effect of Netprotect® on density of *Anopheles* mosquitoes resting indoors, the success of the mosquitoes to feed on human blood and its impact on malaria prevalence at Kanyaboli village.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

Kanyaboli (Figure 1) is one of the villages clustered around the Dominion farm and lies to the North shores of Lake Victoria on the Yala Swamp. It is situated about 70km west of Kisumu town in Western Kenya. The area encompasses about 18.5 km^2 and is adjacent to an ox-bow lake, known as Lake Kanyaboli. It has an estimated population of about 4000 people (CBS, 2006). Yala swamp is one of the most important flood plain wetlands around Lake Kanyaboli and indeed one of the largest swamps in Kenya. The swamp forms the mouth of two rivers Yala and Nzoia and is a freshwater deltaic wetland arising from backflow of water from Lake Victoria as well as the rivers flood water. The area covers 17500 ha and contains three freshwater lakes, Kanyaboli, Sare and Namboyo (Otieno, 2004). Part of Yala swamp covering about 2300 ha has been reclaimed for rice production by Dominion group of companies (Plate 1).

The total annual rainfall in this region averages 1400mm with first peak between March to April and second peak between November and December (Kenya National Bureau of Statistics, 2009). Most of the inhabitants of Kanyaboli Village live in traditional houses with mud walls and grass thatched roofs. The eaves of most houses are open allowing for unimpeded entry and exit of mosquitoes, which bite the unprotected humans sleeping in these huts. Family compounds, consisting of one or more houses are separated from each other by farmland. Apart from working in the rice fields under contract at the Dominion farms, the people also practice subsistence agriculture, growing crops such as maize,

millet and cassava. Fishing is carried out on small scale in the lake to be eaten as a source of protein and sold to supplement for monthly family income.

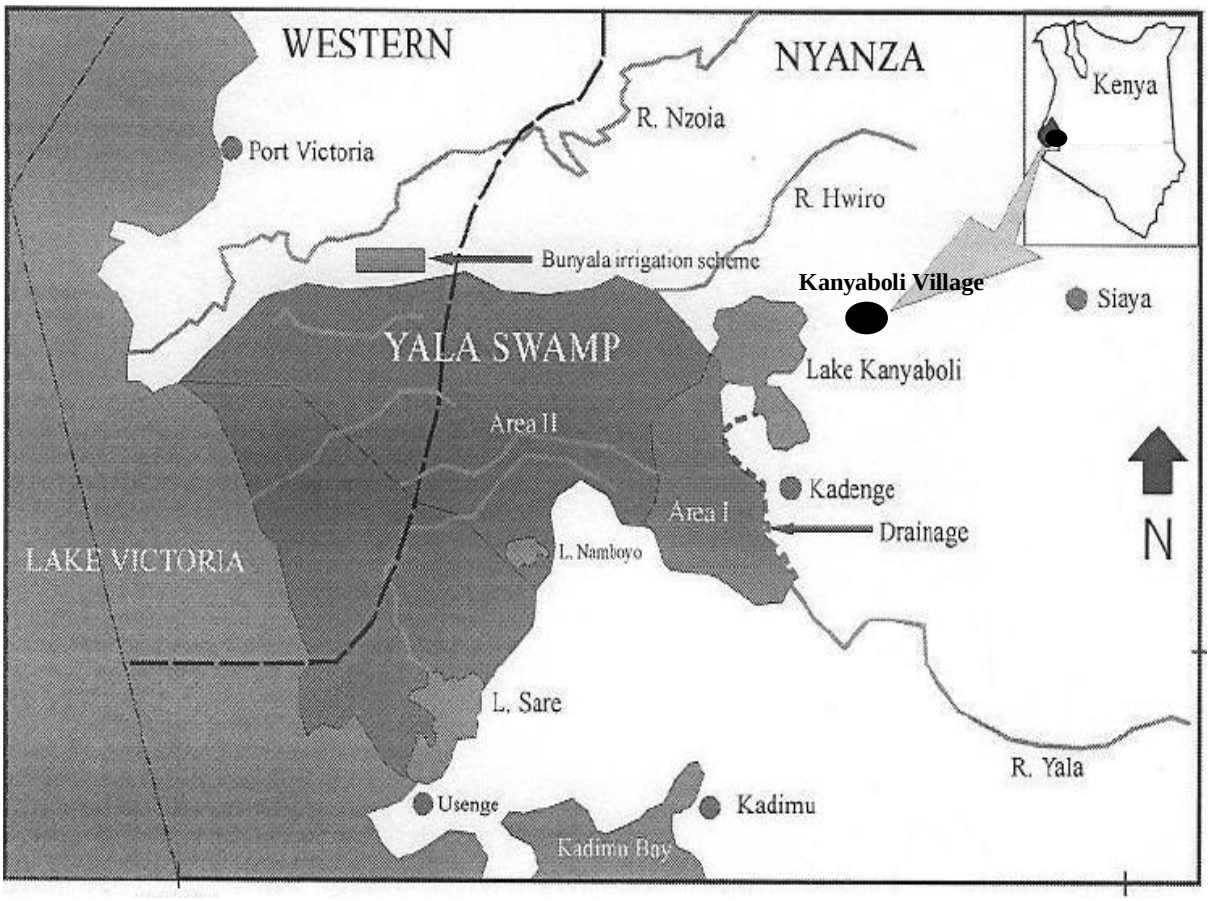


Figure 1 Map of Kanyaboli village (Abila *et al.*, 2003).

Plate 1 Part of reclaimed Yala swamp for rice production. (The arrow shows rice paddy adjacent to Kanyaboli village)

3.1.1 Study design

The study was quasi-experimental designed incorporating separate intervention and control areas. A sample size of 150 matched houses was selected through random sampling at the intervention village. Long lasting treated mosquito bed nets Netprotect® were then hanged in the selected houses. Each household received three bed nets for full coverage. In total four hundred and fifty (450) bed nets were distributed to the intervention area in December 2006. The Netprotect® bed nets were then hanged in houses by properly trained personnel from KEMRI, Kisumu, to cover all the sleeping rooms in the intervention villages (Plate 2). To cater for mosquito flight range, another group of 150 houses were also randomly recruited 2km away in the adjacent area to act as control These households were later given 450 treated bed nets in July 2007.

Plate 2. Netprotect® bed net hanged in one of the houses at Kanyaboli

3.1.2 Household identification and questionnaire adminstration

Houses were physically identified by the locally recruited field workers and assigned identification numbers. Senior members of households were then interviewed through use of a structured questionnaire (Appendix I). The information gathered included the demographic data of the household, other type of bed nets that were in use and when they were lastly re-treated with insecticide. Those found to use other types of bed nets within the intervention village were asked to keep them away and implored to use Netprotect® during the study period. Four days after distribution a random spot check was made to establish if the Netprotect® nets that were hanged by the assistants were still being used and if not the owners were asked the reasons for not using them (Appendix I).

3.1.3 Sample size determination

Population sample size was calculated using the formulae according to Quasi-Experimental Evaluation for population less than 10,000 (Human resource and skill development Canada,1998).

$n= Z^2 p (1-p) N)/ (1.96^2 p (1-p)) + (N-1)SE^2$

Z=1.96 (95% confidence level)

SE= margin of error

P= Proportion of individuals having the characteristic being measured

p-1= proportion of individual who lack it

By convention P and p-1 = 0.5

Where N is the population size and n is the sample size. Algebraically substituting this factor into the sample size equation yielded the following formulae

$n = (1.96^2 p (1-p) N)/ (1.96^2 p (1-p)) + (N-1)SE^2$

$=1.96 \times 0.25(4000)/1.96 \times 0.25 + 3999(0.0025)$

= 350 sample size.

To cater for absenteeism and uncooperative members of the village (Rea and Parker, 1992), the sample size was increased to 400 individuals from each of the villages,

3.1.4 Sampling of houses for mosquitoes and species identification

In order to establish the impact of LLINs density on indoor resting *Anopheles* mosquitoes, collections were conducted one month after distribution of the nets in the intervention villages. This was repeated twice on monthly basis between January and July

2007. Out of the 150-selected houses 30 houses were chosen randomly for mosquito collection. This was according to recommendation by WHO (2000) procedure for experimental bednets evaluation. Similarly Mugenda and Mugenda (1999) also recommend that in any experimental studies at least 10% cases are required per group. Houses for sampling were selected by two-stage cluster sampling each month upon the consent of their owners. The first stage was selection of a cluster of houses within the village (Plate 3). The second stage was random selection of fifteen (15) intervention and 15 control houses with probability proportional to size within each village. One house was randomly selected for sampling and the 14 nearest neighbours were then included in the sample.

Plate 3. Cluster of houses in Kanyaboli village

Indoor resting adult mosquitoes were monitored with a Pyrethrum Spray Catch (PSC) within the houses between 7:00 am and 10:00am. White sheets were spread on the floor and the furniture within the houses. Two collectors, one inside the house and one outside,

sprayed the selected houses with 0.025% pyrethrum emulsifiable concentrate with 0.1% piperonyl butoxide in kerosene at the same time. The collector outside sprayed around the eaves while the one inside the house sprayed the roof and the walls. The house was then closed for 10 minutes, after which knocked-down mosquitoes were collected from the white sheets and put on moist filter paper in Petri dishes. Each petri dish was labelled as per house identification number and then packed in a cool box. The collected mosquitoes were then transferred to the laboratory at KEMRI in Kisumu. All *Anopheles* mosquitoes were identified to species level using morphological keys (Gillies 1968; Gillies and Coetzee, 1987). *An. gambiae* complex was differentiated from the *An. funestus* on morphological differences on the wings, abdomen and the legs. The wing costa of *An. funestus* was identified by characteristic long dark bands in the beginning followed by pale, dark legs, with the abdomen region being smooth and shiny. The *An. gambiae* complex on the other hand has a pale band followed by dark band on the wing costa, spotted legs with the abdomen being rough and hairy. The two anophilines were then sorted out based on the abdominal status and characterized as fed, unfed, gravid or half gravid then stored in vials containing anhydrous calcium sulphate. A record sheet was completed detailing gonotrophic stages, sex and species identification.

3.1.5 Collection and preservation of blood fed mosquitoes

All blood-fed mosquitoes from each collection were preserved in labelled vials containing anhydrous calcium sulphate. Samples of blood fed mosquitoes were cut transversely between the thorax and the abdomen for each of the posterior portions containing the blood meal. The abdomen of each mosquito was ground in 50 µl of

phosphate-buffered saline (PBS) PH 7.2 with subsequent addition of 950 µl of PBS and then stored at -20°C in the refrigerator.

3.1.6 Mosquito blood meal analysis

Blood meals were identified by a direct Enzyme-linked immunosorbent assay (ELISA) at KEMRI in Kisumu, using anti-host (IgG) conjugates (Kirkegaard and Perry, Gaithersburg, MD) against human, bovine, chicken and goat described according to protocol by Beier, et *al.*, (1981). Briefly, each mosquito blood meal sample was diluted in 500 PBS and the PVC flex plates (Dynatech laboratories) were coated with 50 µl of each sample. Two negative and two positive controls were included in the microtiter plates. After overnight incubation at 4°C the plates were washed three times with PBS-Tween solution containing 0.05% tween-20 (PBS-T) and blocked with nonfat powder made up of casein (Baker No E397-07 suspended in 0.1 N NaOH, PBS, thimersol and phenol red). Plasma samples for host being tested were diluted in PBS then added in duplicate wells at rate 5 µl and allowed to react for further two hours at room temperature. Unbound antibodies were removed by washing the plates three times with PBS-Tween solution. One hundred(100) µl peroxidase substrate preparations was added to each well and the absorbance read at 414 nm after incubating for thirty minutes under room temperature. All blood meal samples were tested simultaneously against specific conjugates to identify the host.

3.1.7 Polymerized Chain Reaction for identification of *Anopheles gambiae* complex

Individual mosquito specimens from field collections were prepared for identification by removing a leg with sterile forceps and placing it into one well of a 96-well PCR tray

(P/N 951020389 Brinkmann Instruments, Inc., Westbury, New York, USA). Each well contained 100 µl of grinding buffer (0.10m Nacl, 0.20m sucrose, 0.30m Trizma base 0.01m EDTA and 100ml sterile water pH 8.0). Trays were covered securely with sterile adhesive foil and placed on water in a sonicator bath (Bransonic ultrasonic cleaner, Shelton, Connecticut, USA) at 65 C for 20-40minutes. To these trays 14ul of 8m potassium acetate was added and the mixture placed in cool ice to precipitate the protein. The mixture was then microfuged at top speed for 30 minutes and resulting supernatant which was then transferred to new tubes. 200 µl cold 95% ethanol was added to sample and placed in freezer for at least 20 minutes to precipitate the DNA. Samples were then washed with 200 µl 70% ETOH followed by 200 µl 95% ETOH and tubes inverted for complete drying (for about 1 hour). The 1 µl DNA samples and controls for *An. gambiae* and *An. arabiensis* in each row were added to wells. Blanks containing master mix in ice were also included in the wells. 14 µl master mix (Water, taq enzyme specific mosquito primers $Mgcl_2$ and10x buffer) was added to each well followed by 4 drops of heavy mineral to overlay the reaction mixture. The plates were then covered with a plate sealer and placed in gene machine. All conventional PCR reactions were performed using the Epicentre FailSafe PCR System (Epicentre Biotechnologies, Madison, Wisconsin, USA). The reaction programme had an initial step of 80°C for 1 min, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 4 min. The PCR products were separated by electrophoresis on 2% agarose TBE gels, and stained with ethidium bromide. The amplicons were visualized with an ultraviolet transillumination gel documentation system.

3.1.8 Determination of residual insecticidal activity of Netprotect®

Standard WHO bioassays were performed on random samples of Netprotect® at one-month interval to determine the killing efficacy of the residual insecticide using laboratory colony of susceptible strain of *An. gambiae s.s* (Kisumu strain). A bioassay cone was attached to one side of the net and three days old mosquitoes introduced into it. Approximately, 100 mosquitoes in replicates of 10 mosquitoes were exposed to each bed nets in 10 randomly chosen houses using standardized procedures. The tests were conducted using the standard WHO plastic cones (Plate 4) and a three minute exposure time. After exposure, females were grouped into batches of 10 for each bed net and transferred to 200 ml labelled paper cups (Plate 5). These mosquitoes were then provided with 10% sucrose solution maintained at a standard room temperature of 28°C ± 2 and relative humidity of 80% ± 10% in the laboratory. The proportion of mosquitoes knocked down at 60 minutes (KD60) was calculated and percentage mortalities were recorded after 24 hours. A similar procedure was also performed for untreated nets, which were used as a control sample. The corrected mortality was determined using Abbot's formulae (WHO, 1998) as shown below:-

$$\% \text{ (corrected mortality)} = \frac{\% \text{ MR} - \% \text{ MC X100}}{100 - \% \text{ MC}}$$

Where, MR= Mortality in replicates,

MC= Mortality in control

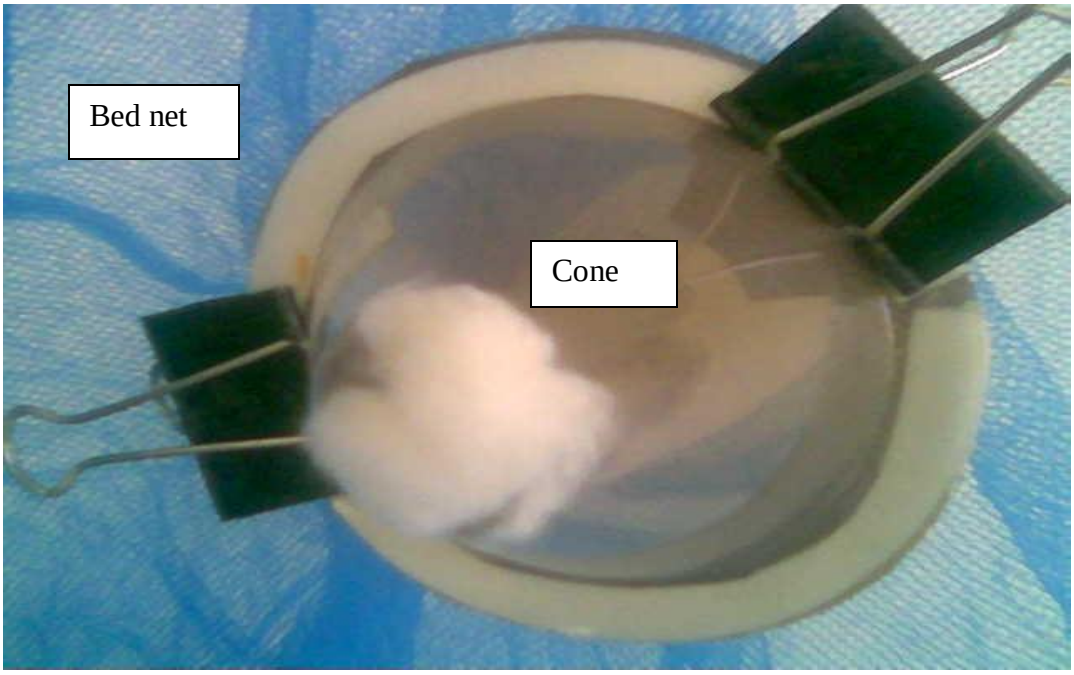


Plate 4. Standard WHO cone attached to Netprotect to test for efficacy.

Plate 5. Paper cups for holding mosquitoes 24 hours post exposure

3.1.9 Determination of malaria prevalence rate

Malaria cases were detected passively at the village clinic established by the nearby Dominion Farm (US project). People seeking treatment were compared to the list of people participating in the study either in intervention group or control group, but with status blind to the clinic personnel. Malaria parasites in suspected patients were identified using clinical manifestation and rapid diagnostic testing. Rapid diagnostic test (RDT: Paracheck PF tests kits) were carried out according to the manufacturer's instructions and individuals found to be positive by RDT treated according to MOH guidelines (in 2007 amodiaquine (10mg/day) for 3 days). Children under five years who were clinically identified as malaria positive were treated, irrespective of the results for Paracheck test as in accordance with the national guidelines. Children older than five years and adults were first examined and scored clinically, then tested with Paracheck, but only treated if a Paracheck result was positive for malaria. A questionnaire for data, on age, sex, place of residence, malaria diagnosis and treatment history for patients from both areas was administered. Malaria prevalence rate per month was determined using the following formula (WHO, 1998).

$$\text{Prevalence rate} = \frac{\text{number of positive patients}}{\text{Total number of patients}} \times 100$$

3.2.0 Data management and analysis

Comparison of proportions between categorical variables was performed by chi-square test at 95% confidence level using SAS statistical software version 9 (CDC Atlanta, Georgia USA, 2007). Repeated Poisson regression using the GENMOD procedure in SAS was used to analyze the effects of the bed nets on human feeding success on blood

meal for the *Anopheles* mosquitoes found resting indoors. For each model the number of indoor resting mosquitoes in control houses was the reference. The percentage reduction was calculated as one minus the relative risk as estimated by Poisson regression. Odds ratio was also used to determine the difference between densities of the *Anopheles* mosquito species collected in treatment and control villages.

3.2.1 Ethical considerations and clearance

The Netprotect® project was reviewed and approved by the Kenya Medical Research Institute (KEMRI) National Review Committee on Ethics and Kenyatta University graduate school board, before carrying out data collection. This study involved intrusion of privacy during indoor resting sampling and interrupting owner's daily routine, hence informed consent was sought from the individuals who participated in the study. This was done after explanation of objectives and collection methods through individual discussion and group meetings.

CHAPTER FOUR

RESULTS

4.1 Density of *Anopheles* mosquitoes resting indoor

During the six months study period, indoor resting female adult *Anopheles* mosquitoes were collected from 80 randomly sampled houses. Out of the total 807 *Anopheles* mosquitoes collected, 82.5% were collected from the control area, while 17.5% from the intervention houses (Table 1). A difference of 65% of density *Anopheles* mosquitoes resting indoors was realized. Polymerized Chain Reaction (PCR) identification of members of *An. gambiae* species complex revealed that all the 243 *An. gambiae s.l* analyzed were *An. arabiensis.* Overall two species of anopheline mosquito were identified from the 807 specimens collected, *An. funestus* being the more abundant species (69.9%), the remaining 30.1% being *An. arabiensis* (Table 1). A total of 564 *An. funestus* were collected from the houses during the six months period, 13.3% of them from the intervention area and 86.7% from the control area. These results show that the difference in densities of indoor resting *An. funestus* between the two areas was significant (χ^2 =22.63 df=1 P<0.0001, Table 2). Further the probability of finding *An. funestus* in the control area was two times higher than that of finding *An. funestus* in the intervention area (O.R=2.4, CI, 1.7-3.5). Significantly higher numbers of *An. arabiensis* were found resting indoor in the control houses compared to intervention houses (χ^2=22.6 df=1 P <0.001) and that the probability of finding *An. arabiensis* in the control area was four times more likely than in intervention area (O.R= 0.4, CI 0.3-0.4).

Table 1 Indoor resting patterns of *Anopheles* mosquitoes by species at Kanyaboli

Mosquito Species	No of mosquitoes sampled in Control (%)	No of mosquitoes sampled from LLINs (%)	% difference	Totals (%)
An.arabiensis	177 (72.8)	66 (27.2)	45.60	243 (30.1)
An. funestus	489 (86.7)	75 (13.3)	73.40	564 (69.9)
Totals	666 (82.5)	141(17.5)	65.00	807 (100)

Percentage in brackets

When seasonal abundance indoor resting density per house was plotted as a function of time, two peaks for indoor resting adult *An. arabiensis* were observed in the control villages. Highest densities appeared in April and June (Figure 2). *An. funestus* on the other hand had three peaks, in April, June and July (Figure 3). Comparing these to the intervention village, the indoor resting densities were generally low and increased slightly to about 5 mosquitoes per house in the month of April.

Table 2 Chi-square analysis of collected indoor resting *Anopheles* mosquitoes.

Mosquito species	Intervention area	Control area	P-value	χ^2- vaue	Odds ratio
An. arabiensis	117	177	<0.0001	22.64	0.4(0.3-0.6)
An. funestus	75	489	<0.0001	22.64	2.4(1.7-3.5)

χ^2 = Chi- square

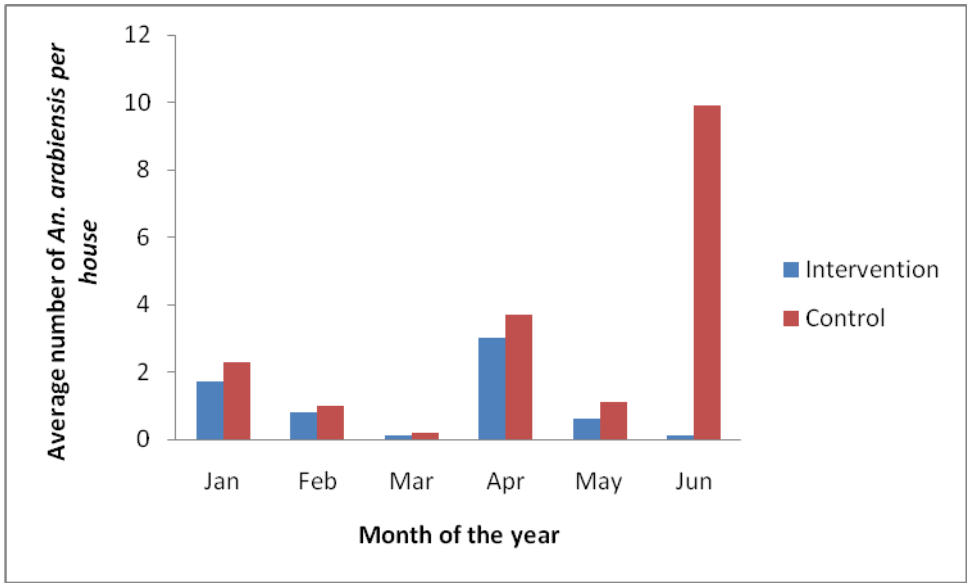


Figure 2. Average monthly indoor resting densities of *An. arabiensis* at Kanyaboli.

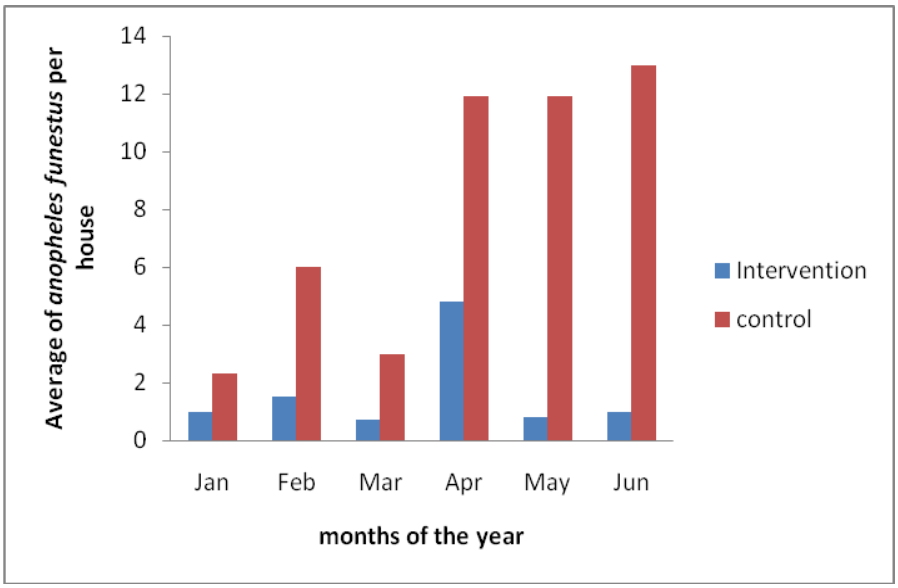


Figure 3. Average monthly indoor resting densities of *An. funestus* at Kanyaboli.

4.2 Indoor resting patterns for blood fed *Anopheles* mosquitoes at Kanyaboli

There was no significant difference in the density of indoor resting blood fed *An. arabiensis* (χ^2 =2.99, df=1, P= 0.084) collected indoor from control and intervention areas (Table 3). On the other hand, densities of indoor resting engorged *An. funestus* was significantly higher in control area compared to the intervention area (χ^2= 17.44 df=1 P<0.0001). Of the 349 *An. funestus* collected during the study, 10.17% and 76.43% were collected from the intervention and control areas respectively. However, the likelihood of finding a fed *An. funestus* in the control area was four times more than that of finding a fed *An. funestus* in the intervention area (OR=4: 95%; CI: 2.0-7.2).

Table 3 Indoor resting patterns for blood fed *Anopheles* mosquitoes

Mosquito Species	Intervention area	Control area	P-value	χ^2-value	Odds ratio
An. arabiensis	39	66	0.084	22.98	-
An. funestus	41	308	<0.0001	17.4355	4 (2.0-7.2)

4.3 Blood meal origin of indoor resting *Anopheles* mosquitoes

From the 397 blood fed *Anopheles* that were tested by ELISA technique for the origin of their blood meal, 65 % of them had their blood meals positively identified. These comprised 258 *An. funestus* and 139 *An. arabiensis* (Table 3). In the intervention area, 53% of the *An. funestus* were positive for bovine antibodies, 29% for human antibodies (IgG) and 0.02% had mixed blood meals of human and bovine. In the control area majority of the *An. funestus* were engorged on human blood meal (46%), a lesser extent

on bovine (29%) and 2.2% engorged on mixed blood meals of either chicken/human or bovine/human. Majority of *An. arabiensis* (77%) sampled from LLINs houses fed on bovine host, to a lesser extent on mixed human/bovine (5.7%) and none (0%) was positive for human blood meal. In the control villages, 42.3% of the *An. arabiensis* were positive for bovine blood meal and 4% for human blood meal, 7.7% had mixed blood meal of human/bovine, and 1% had mixed blood meal of bovine/chicken (Table 3).

Table 4 Blood meal origin of indoor collected *Anopheles* mosquitoes

			% of mosquitoes positive for vertebrate blood meals					
Mosquito Species	Sampling Area	No tested (n)	Human	Bovine	Goat	Chicken & Human	Human /Bovine	Chicken /Bovine
An. funestus	Intervention	34	29	53	0	0	0.2	0
An. arabiensis	Intervention	35	0	77	0	0	5.7	0
An. funestus	Control	224	46	12.5	0	2.2	2.2	0
An. arabiensis	Control	104	3.8	42.3	0	0	7.7	0.9

4.3.1 Feeding success of mosquitoes on host blood meals

Analysis on the effects of Netprotect on *Anopheles* mosquitoes success to feed on human blood revealed that, there was 60% more likelihood of *An. arabiensis* feeding on bovine host amongst those found resting indoors in the intervention area (Table 5). *An. funestus* on the other hand, had 54% more success in feeding on human blood in control area than in the intervention area (P=0.3458). In the control area a highly significant population of the *An. funestus* resting indoors had 21% more success in having bovine blood meal (P

<0.0001). There was no significant difference on the success of *An. funestus* having mixed blood meals of human/bovine from both the control and intervention areas (P<0.2217).

Table 5 Analysis showing success of feeding of *Anopheles* mosquitoes on different hosts

Source of	*An. Arabiensis*			*An. Funestus*		
Blood meal	**%** Change	p-value	95% CL	**%** Change	p-value	95% CL
Human	-	-	-	54	0.3458	(-0.41-1.18)
Bovine	60	0.0219	(-0.95-0.07)	21	<0.0001	(-2.31-0.78)
Human/Bovine	60	0.7446	**(-**1.71-2.34)	34	0.2217	(-22.80-0.65)

4.4 Residual insecticidal activity of Netprotect

Bioassay results on ten randomly sampled bed nets showed a knock down rate and effective mortality of 80% and 100% respectively after seven months of use. But there was a gradual decline in 24 hour mortality to 80% below recommendation by WHOPES. At least two out of ten bed nets sampled, exhibited substantial loss of residual insecticidal activity during the 12 months duration. Despite showing average knockdown rate of 70%, 4 out 10 mosquitoes exposed to the bednets were not killed. However, functional mortality was still high at 100% because these mosquitoes that survived had lost three to four of their legs. With only three or fewer legs they could no longer fly, land or bite hence they were functionally dead and would not survive for long under natural environmental conditions.

Table 6 Bioassay on Netprotect® using *Anopheles gambiae* Kisumu strain.

	Netprotect®				Untreated bed nets (control)				Corrected
Month	Sampled Nets	Mosq Exp	Kd(%) 60min	24hr Mort (%)	Sampled Nets	Mosq Exp	Kd 3min	24hr Mort	Mortality
Feb	10	10	9(90)	100	10	10	0	1(10)	100
Mar	10	10	10(100)	100	10	10	0	1(10)	100
Jun	10	10	8 (80)	100	10	10	0	0 (0)	100
July	10	10	8 (80)	80	10	10	0	0 (.5)	80
Aug	10	10	9(90)	80	10	10	0	0 (1)	80

Kd- knock down after 3 minutes exposure
Mort-mortality

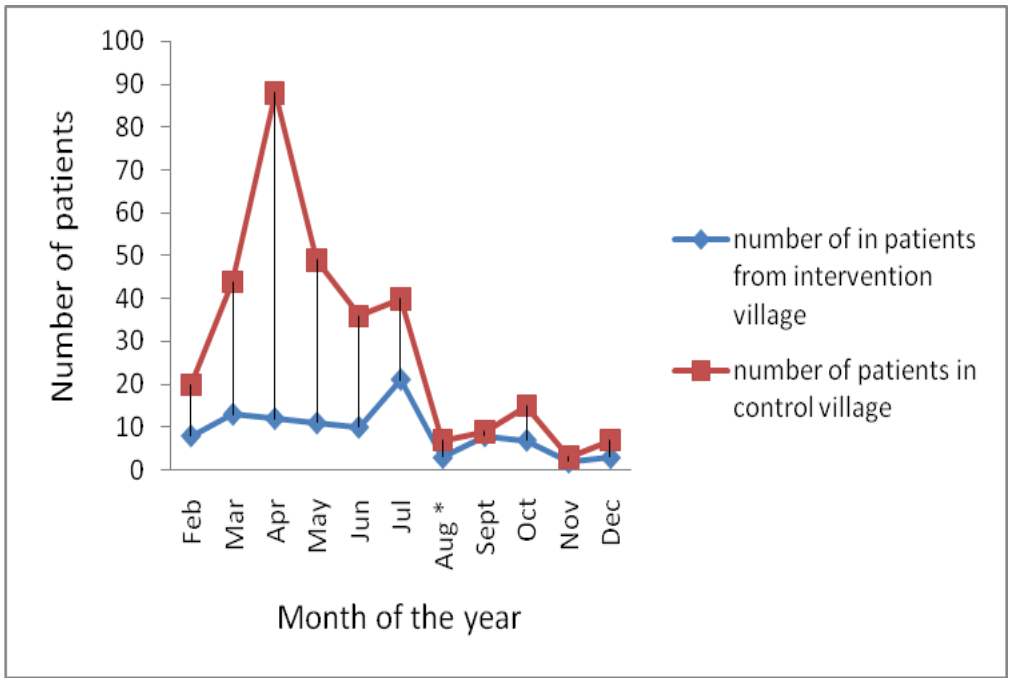
4.5 Impact of Netprotect® on Malaria prevalence

A total of 870 respondence from the study area visited the clinic between January and July in 2007. Three hundred and forty four (344) of these patients tested positive for malaria infection. Malaria cases from intervention and control were 19.5% and 80.5% area respectively (Table 7). Malaria prevalence was higher in the control than in the intervention area (Table 7). The overall effect of Netprotect® on malaria prevalence was significant between the two areas before the bednets were distributed in the control area ((χ^2=8.28, P=0.004).When bednets, were hanged in the control area in July 2007 the number of malaria cases in both areas were no longer significant (p=0.03).

Table 7 Effect of Netprotect® on malaria prevalence

	Patients positive For malaria	Total Number patients	% Prevalence rate
Intervention	67 (19.5)	220 (24.7)	30.4
Control	277 (80.52)	670 (75.3)	41.3
Total number of patients	344	890	

The malaria peak outbreak of malaria cases in the control area was recorded in April with a total 88 cases (Figure 4). However, malaria cases in intervention area remained stable and low level with a slight peak in July during the same period. When Netprotect® bednets were distributed to the control area at end of July 2007, malaria cases dropped to low levels in both areas (Figure 4).



* Month of Netprotect® hunging in control area

Figure 4. Monthly malaria cases in Kanyaboli area before and after intervention

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The use of bed nets was uncommon in Kanyaboli and the survey revealed that only 5% (n=450) households living used it to protect themselves against mosquito bites. These bed nets that were found to be in use had been given for free at the rural health centre to mothers who had young children. Ninety percent of the nets had not been re-treated.

Towards the end the study ten percent of the bed nets provided for this study were missing from the intervention houses as they had either been sold to generate supplementary income or donated to relatives from other regions. It was further revealed that some of participants were using the nets for fishing from Lake Kanyaboli since they were stronger than the fish nets.

The results showed that Netprotect® had significant impact on densities of indoor resting of both *An. arabiensis* and *An. funestus.* The number of indoor resting *Anopheles* mosquitoes was significantly lower in the intervention houses compared to control houses. The probability of finding *An. funestus* in the control area was 2.4 fold more than in the intervention area. The probability of collecting *An. arabiensis* in the intervention area however, was 2.5 times greater compared to the control area. This conforms to the results reported in Gambia, Sierra Leone and Kenyan coast studies on ITNs and curtains which demonstrated decreased indoor resting densities when Pyrethrum Spray Catch (PSC) was used as the collection method for *Anopheles* mosquitoes (Gimnig *et al.*, 2003).

However, while ITNs exhibit rapid loss of efficacy unless re-treatment is adhered to every six months this study revealed that Netprotect® efficacy on *Anopheles* mosquito was high without necessity for re-treatment during the project period. *An. funestus s.l.* was found to be the predominant vector resting indoors in this area constituting approximately 70% of the total mosquitoes collected indoors while *An. arabiensis* made the remaining 30%. Their indoor resting densities remained higher than *An. arabiensis* despite improved weather conditions favouring breeding of the *An. arabiensis* in this area. This was attributed to the abundance of larval habitats for the *An. funestus* in this region. This species is known to breed in permanent swamps or pools along the streams and river systems, as opposed to those of *An. gambiae* species complex which prefer temporary aquatic habitats (Fontanille *et al.*, 1997). Of the two species of anophilines, *An. funestus* was strongly affected by Netprotect® (reduction of 65.5% $P<0.001$) compared to *An. arabiensis*. Similarly, studies conducted by Gillies and De Meillon, (1968) demonstrated that this species was highly susceptible to chemical control measures and was slow in recolonizing an area from which it has been controlled.

These results show that there was no significant difference in densities on blood fed indoor resting *An. arabiensis* in both areas. This indicates that .*An. arabiensis* unlike *An. funestus*, was only affected by the insecticide incorporated on net fabric at close ranges and prefers to rest indoors after feeding outside on cattle.

One of the major observations noted in this study was the high degree of zoophily in houses where Netprotect® was in use. The majority of the *Anopheles* mosquitoes

sampled from intervention area did not succeed in having human blood meals and hence fed outside on cattle. ELISA blood meal analysis revealed that even in houses where some individuals were reported to have not slept under Netprotect®, *An. arabiensis* mosquitoes collected from intervention houses preferred bovine hosts for blood meal. However, a insignificant number (P= 0.346) of *An. funestus* fed on human blood were collected in these houses. This is an indication that sleeping under Netprotect® effectively provided a physical barrier between humans and adult blood questing mosquitoes thus discouraging them from feeding on humans. This conforms positively to earlier observations by Gimnig *et al*. (2003) that presence of insecticide treated nets in a house confers some protection on a person not using bed net but sleeping in the same house. Overall, *An. funestus* had 54% more success in having human blood meal in the control area than in intervention villages. This confirms that *An. funestus* were relatively more anthropohilic compared to members of the *An. arabiensis.* However, the use of the LLINs led to an increased number of *An. funestus* feeding on bovine host blood because human hosts were not easily accessible. Hence despite being known as highly anthropophilic, this species can also feed on cattle.

An. arabiensis in this area was highly zoophilic and possibly entered into the houses after feeding outside on bovine hosts. Ijumba *et al* (2002) also reported the zoophilic behavior of *An. arabiensis* in different rice growing areas in the African continent. Hence, reduced anthropophily of *Anopheles* mosquitoes in rice cultivating areas has been suggested as one of the responsible factors for low levels of malaria transmission in these areas despite the presence of high mosquito densities (Ijumba *et al*., 2002; Dolo *et al.*,

2003; Mutero *et al*., 2004). *An. funestus* was substantially more anthropophilic and endophagic than *An. arabiensis.* This observation therefore, suggests that inhabitants in the study area are more at risk of exposure to malaria transmission by *An. funestus* than the *An. arabiensis*. On the other hand *An. funestus* were more strongly affected by the presence of the bed nets than *An. arabiensis*, hence use of Netprotect® would be considered an effective intervention method of controlling mosquitoes in houses and malaria transmission in this area. In this study the design did not distinguish the bed net Netprotect® as physical barrier and as a mosquito controlling agent.

The results further indicate that the effectiveness of Netprotect® is not only determined by number people sleeping under them but also by the species of *Anopheles* mosquito present in the area. Mosquito samples tested for their source of blood meal revealed that 77% of them were from the four vertebrate hosts tested. This indicated that *Anopheles* mosquitoes in this area may have a wide host range and therefore highlights the need to include a variety of number possible hosts when testing for blood meals from the engorged anopheline mosquitoes.

Presence of indoor resting human blood fed mosquitoes could be attributed to reduced efficacy observed in some nets as observed during bioassay or low height standard of 150cm that made it difficult to tuck them in properly. In some cases sleeping arrangements in some families require that bed nets are mounted only late at night before they go to sleep. This would provide mosquitoes with ample time to bite the inhabitants. Some individuals also preferred not to sleep under the nets for citing reasons like they

feel hot or are allergic to the insecticide. These undermine the effectiveness of bed nets as they are not used regularly and appropriately.

Bioassay on Netprotect® showed a mortality of 100% during the first seven months of net use. These gradually declined to an average of 80% after one year of use. This demonstrates that the insecticidal efficacy was reduced by an average of 20% during the one year period. Amongst the bed nets tested for efficacy, 80% of the nets retained efficacy of 100% while the remaining 20% had reduced efficacy to an average of 60%. About 80% of the bednets had just been washed twice and 20% not all during the study period. Loss of efficacy on other LLINs has also been cited to be due to external factors such as, dirt and fume accumulation on the net fabric N'guessan *et al* (2008). However, functional mortality on Netprotect® was still high at 70% because the vectors disposed of the affected legs on detecting the neurological contact insecticide absorbed through their legs (www.vestergaard-frandsen.com, 2008). With only fewer legs the mosquitoes got disabled beyond the point of survival and eventually fell on the floor and would have easily been picked by predators. This suggests that insecticide treated bednets not only deterred unfed mosquitoes from entering the houses but in case of tarsal contact with treated bednets the insecticide would hurt or kill the mosquitoes. When mosquitoes were exposed for at least five minutes to bednets with reduced efficacy a high knock down rate and mortality was realized over 24 hour period. Hence blood seeking mosquitoes that landed on the impregnated bed net for long would absorb enough insecticide to kill them. This may be the reason for reduced indoor resting densities for vectors that were engorged during the study period.

Results on passive and hospital based cases showed a significance difference in malaria cases during the six months trials. This is a reflection that use of Netprotect® LLINs was effective in controlling the malaria vector and therefore reduced malaria transmission. Overall, the prevalence rate was higher in the control village compared to intervention area. Despite fewer people from the intervention going for treatment during the malaria peak season the prevalence rate was low in this area. When bed nets were introduced in both areas passive cases were no longer significant. Compared to other studies conducted in Gambia (D'Alessandro *et al.*, 1995) where epidemiological evaluation was carried out for over two year's period, ITN re-treatment was important to enhance their effectiveness in subsequent years. In response to low re-treatment rates of bednets that has been reported in many malaria endemic countries, the development of LLINs is a significant improvement.

This results showed that the second generation LLIN, Netprotect® maintained its efficacy over 2years period and hence is convenient for use in rural areas where re-treament rates are low. Further, the use of polyethylene makes them tougher and suitable for the rural conditions than polyester ones that physically deteriorate faster. A study in Cote d'Ivoire showed that especially in rural areas, the aspect of net strength is highly appreciated, whereas people in urban areas often prefer the softer polyester net (Adriana *et al.*, 2004).

5.2 Conclusions

This study demonstrated lower indoor resting density for both *An. arabiensis* and *An. funestus* mosquitoes in intervention area. There was lower human blood feeding index in intervention area compared to control area. The use of Netprotect resulted in low malaria prevalence in the intervention area despite seasonal malaria increase observed in control area.

5.3 Recommendations

1. Chemical analysis should be done on samples of bednets at different times to establish their insecticide concentration. This may aid in the refinement of the technology on the production of these bednets to enhance the duration of their insecticidal activity.

2. The length of the bednets to be increased so that they can be comfortably tacked on beds.

3. Further studies are proposed to establish difference between the effect of physical protection of Netprotect and the effect of the insecticide on the mosquito numbers.

REFERENCES

Adriana, T., Mubyazi, G., Alison, T., Hassan, M., Stephane, D., Lengeler, C. (2004). Evaluation of olyset insecticide-treated nets distributed seven years previously in Tanzania. *Malaria journal 3:19.*

Abdulla, S., Schellenberg, J.A., Nathan,R., Mukasa, O.,Marchant, T., Smith, T., Tanner, M. and Lengeler,C. (2001) Impact on malaria morbidity of a programme supplying insecticide treated bed nets in children under 2 years in Tanzania: Community cross sectional study. *Bulleting Malaria Journal 322:270-273.*

Abila, R., Marta, B. and Engelken, J.(2003). Biodiversity and sustainable management of tropical wetlands lake ecosystem. A case study of lake Kanyaboli, Kenya. WWF Publication No 65 Wagenigen, The Nertherlands.

Anu, K., Hanpeter, M., Ingrid, F., Muller, D.,Sakari, T.J. (2008). Monkey malaria in European traveller returning from Malaysia. *Emerging Infectious Diseases 14:814–6*

Aram D. S, Atieli, F.K., Vulule, J.M., Besansky.N.J. (2004). Dynamics of the pyrethroid knockdown resistance allele in western Kenyan populations of *anopheles gambiae* in response to insecticide-treated bed net trials. *American Journal for Tropical Medical Hygiene; 70(6), pp. 591-596*

Armstrong-Schellenberg, J. R.M., Minja, H., Mponda, H., Kikumbih, N., Mushi, A., Abdulla, S., Mukasa, O., Marchant, T., Tanner, M., Lengeler, C. (2002) Re-treatmentOf mosquito nets with insecticide. *Transcient Research Society Tropical Medical Hygiene;* 96:368-369.

Asidi, A.N., N'Guessan, R., Hutchinson, R.A., Traore-Lamizana, M., Camevale, P. and Curtis CF. (2004). Experimental hut comparisons of nets treated with carbamate or pyrethroid insecticides, washed or unwashed, against pyrethroid-resistant mosquitoes. *Medical Veterinary Entomology. **18**:134-40.*

Beier, J.C., Perkins, P. V., Wietz, R. A., Koros, J., Gorgan, T. P., Koech, D. K. (1988). Blood meal identification by direct enzyme-linked immunosorbent assay (ELISA) Tested on *Anopheles* (Diptera-culicidae) in Kenya. *Journal of medical entomology; 25:*9-16

Beier, J.C., Perkins,P.V., Onyango, F.K., Gargan, T.P., Oster, C.N., Whitemore, R.E.,RobertsC.R andKoech, D.K. (1990). Characterization of malaria transmission by Anopheles (Diptera: Culicidae) in preparation for malaria vaccine trials. *Journal of medical entomology; **27**:570-577.*

Bermejo, A. and Veeken, H. (1992). Insecticide-Impregnated bed nets for malaria Control review of field trials. *Bulletin of world health organization; 70:293-296*

Brier, B (2004). "Infectious diseases in ancient Egypt. *Infectious Dis. Clin. North. Am.* **18**: 17

Binka, F. N., Adongo , P. (1997). Acceptability and use of bednets in Northern Ghana. *Tropical Medical Institute of Health 2:* 499-507.

Bockarie, M., Alexander, N., Bockarie, F., Ibam, E., Barnish, G., Alpers, M. (2009). The late biting habit of parous Anopheles mosquitoes and pre-bedtime exposure of Humans to infective female mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; *90:23-25*

Brabin B.J., (1993). An analysis of malaria in pregnancy in Africa. *Bulleting of world Health Organisation 61: 1005-1016.*

California Department of Health Sciences. (2005) Safety of pesticides used to control adult Mosquitoes. *http://www.cdpr.ca.(2005).*

Centers for Disease Control. (2004). *Eradication of Malaria in the United States (1947-1951)* In *http//www.cdc.gov/malaria/history/eradication_us.htm*

Centre for Disease Control and Prevention. (2007). *Biology of Anopheles Mosquito.* In *http://www.cdc.gov/malaria/control_prevention/vector_control.htm*:

Centre for Disease Control. (2008).*Vector control .In http://www.cdc./malaria/control.*

Cham, M.K., Olaleye, B., d'Alessandro, U., Aikins, M., Cham, B., Maine, N., Williams, L.A., Mills, A. and Greenwood, B.M. (1997). The impact of charging for insecticide on the Gambian National Impregnated Net Bednet Programme. *Health Policy Plan **12**:240-247.*

Chandre, F., Darriet. F., Manguin, S., Brengues, C., Carnevale, P., Guillet, P. (1999). Pyrethroid cross resistance spectrum among populations of *Anopheles gambiae* s.s. from Côte d'Ivoire. *Journal America Mosquito Control Association* **15:**53-59.

Curtis, C.F. (1991). *Control of disease vectors in the community*. London Wolfe Publishers 1991.

Cuzin-outtarra, N., Van den Broek, A.H.A., Habluetzel, A., Sanogo-Iiboudo, E., Diallo, D.A., Cousens, S.N and Espisito,F. (1999). Wide-scale installation of insecticide-treated curtains confers high levels of protection against malaria transmission in hyperendemic area of Burkina Faso**.** *Transcient Research Society Tropical Medical Hygiene; **93**:473-479.*

D'Alessandro, U., Olaleye, B.O., McGuire, W., Langerock , P., Aikins, M.K., Thomson, MC.,(1995). Reduction in mortality and in morbidity from malaria in Gambian children following the introduction of a National Insecticide Impregnated Bednet Programme. *Lancet* 345: 479-483.

Dabire, K. R.; Abdoulaye,D., Thiery,B., Lea.P.T., Robert.T.G., Ouedraogo.J.B., Ole. S. (2006). Personal protection of long lasting insecticide treated nets in areas of *Anopheles gambiae ss* resistance to pyrethroids. *Malaria journal **15**:12*-20

Darriet, F., Robert, V., Tho Vien & Carnevale, P. (1984). Evaluation de I'fficaceit'e sur vecteurs du paludisme de la permt'ethrine en impr'egnation sur des moustiquares intacteset troue'es. World Health Organization, Geneva, Documents WHO/VBC/84.899 and WHO/MAL/84.1008.

Das, P.K and Amalraj. (1997). Biological control of malaria vectors. *Indian Journal for medical research **106:** 174-197*

Diabaté, A., Baldet, T., Chandre, F., Akogbeto, M., Guiguemdé, T.R., Darriet, F., Brengues, C., Guillet, P., Hemingway, J., Small, G. and Hougard, J.M. (2002). The role of agricultural insecticides in *Anopheles gambiae* s.l. resistance to pyrethroids in Burkina Faso, West Africa**.** *American Journal for Tropical Medical Hygiene **67:**617-622.*

Dolo, G., Briet, O.J., Dao, A., Traore, S.F., Bouare, M., Sogoba, N., Niare, O., Bagayogo, M., Sangare, D., Teuscher, T., Toure, Y.T. (2003). Malaria transmission in relation to rice cultivation in the irrigated Sahel of Mali. *Acta Tropica:**89**:147–159.*

Etang, J., Chandre, F., Guillet, P., Manga, L (2005). Reduced bio-efficacy of permethrin EC imprenated bed nets against an *Anopheles gambiae* strain with oxidase-based pyrethroid tolerance. *Malaria Journal* 3:46.

Erlanger, T. E., Enayati, A. A., Hemingway. J., Mshinda, H., Tami, A., Lengler,C. (2004). Field issues related to effectiveness of insecticide treated nets in Tanzania. *Medical Vetinary Entomology 18:* 153-60.

Fontenille, D., Lochouarn, L., Diagne. N., Sokhna, C., Lemasson, J. J., Diatta, M., Konate, L., Faye, F., Rogier, C., Trape, J. F. (1997). High annual and seasonal variations in malaria transmission by anophelines and vector species composition in Dielmo, a holoendemic area in Senegal. *American Journal for Tropical Medical Hygiene **56**: 247–253.*

Genton, B., Hii, J., Al-Yaman,F., Paru, R., Beck, H.P., Ginny, M., Dagoro,H., Lewis, D and Alpers, M. P. (1994). The use of untreated bed nets and malaria infection, morbidity and immunity *American Tropical medicine and Parasitology **88**.263-270.*

Gillies, M. T., & Coetzee, M. (1987). A supplement to the Anopheline of Africa South of The Sahara. *Publication of South African Institute of Medical Research no.55*

Gillies, M.T., & De meillon, B. (1968). *The Anophiline of Africa South of Sahara* Johannesburg, South Africa. South African Institute for Medical Research.

Gimming, J. E., Vulule, J. M., Lo, T. Q., Kamau, L., Kolezek, M. S., Phillips-Howard, P. A., Mathenge, E. M., Ter Kulle, F. O., Nahlen, B. L., Hightower, A. W.,

Hawley, W. A. (2003). Impact of permethrin treated nets on entomological indices in an area of intense year round malaria transmission. *American Journal of Tropical Medicine Hygiene **68**:16-22.*

Guillet, P., Alnwick, D., Cham, M. K., Neira, M., Zaim, M., Heymann, D., Mukelabai, K. (2001). Long-lasting treated mosquito nets: a break-through in malaria prevention. *Buletingl World Health Organization 79*: 998.

Government of Kenya. (2008). *Kenya Population Census, 1989*. Volume 1. Nairobi: Kenya National Bureau of statistic, Ministry of planning and National Development, Government Printers.

Grobowsky, M., Nobiya, T., Ahun, M., Dorma, R., Lengor, M., Zimmerman, D., Ladd, H., Hoeksta, E., Bello, A., Baffoe-Wilmot, A Amofah, G. (2005). Distributing Insecticide treated bednets during measles vaccination: a low cost means of achieving high coverage. *Bulleting of World Health Organ **83**(3):195-201.*

Holtz T.H., Muram. L. H., Mkandala. C., Chizani. N., Roberts. J.M., Machezo.A., Praise. M.E and Kachur.S.P. (2002). Insecticide-treated bed net use, anaemia and malaria parasitemia in Blantyre, District, Malawi. *Tropical Medical International Health 7:220-230*

Hawley, W.A., Philips-Howard, P.A., ter Kuile, F.O, Terlouw,D.J., Vulule, J.M., Ombok, M., Nahlen, B.L., Gimning, J.E., Kariuki, S.K., Kolczak, M.S. and HightowerA.W. (2003) Community-wide effects of permethrin bed nets on child mortality and malaria morbidity in western Kenya. ***68**(suppl 4):American Journal of Tropical Medicine Hygiene121-127*

Howard, S. C., Mambo, J., Merrill, C., Some, E. S., Donnelly, C. A. and Show, R. W. (2000). Evidence of mass Community effect of insecticide treated bednets on the incidence of malaria in Kenyan coast. *Transcient Research Society for Tropical Medical Hygiene **94**: 357-360.*

Hung, I. Q., Vries, P. J., Giao, P. T., Nam, N. V., Binh, T. Q. and Chong, M. T. (**2002).** Control of Malaria: A Successful Experience from Viet Nam. *Bulleting of the World Health Organization **80**(8): 660–66.*

Ijumba, J., Mosha, F., Lindsay, S.W. (2002). Malaria transmission risk variations derived from different agricultural practices in an irrigated area of northern Tanzania. *Medical Vetinary Entomol. **16**:28–38.*

Jaenson, T.G.T., Gomes, M.J.,Barreto dos Santos, R.C., Petrarca, V., Fortini,D., Evora, J. and Crato, J. (1994) Control of endophagic anopheles mosquito and human malaria in Guinea Bissau, West Africa by permethrin treated bed nets. *Transcient Research Society Tropical Medical Hygiene;**88**:620-624.*

Kachur, S.P., Philips-Howard, P.A., Odhacha, A.M., Ruebush, T.K., Oloo, A.J., Nahlen, B.L. (1999). Maintainance and sustained use of insecticide treated bednets and curtains three years after controlled trial in western Kenya. *Tropical Medical Institute of Health **4**:728-735.*

Knols, B.G.J., Ernst-Jan, S., Robert, A.S., Willem, T. (2004) Entomopathogenic fungi for mosquito control. *Central Journal for Insect Sscience **4**:19.*

Killeen,G.F.,Ulrike, F., Kiche, I., Gouagna, L. C. and Knols, B.G. J. (2002). Eradication of *A.gambie* from Brazil: Lessons for malaria control in Africa. *Lancet: Infectious diseases, **2**:618-627.*

Kakkilaya, B.S. (**2003)** *Rapid Diagnosis of Malaria*. Lab Medicine*; **8**(34):602-608*

Kroeger, A., Meyer. R., Mancheno. M., Gonzlez, M. and Pesse, K. (1997) Operational aspects of bed net impregnation for community-based malaria control in Nicaragua, Ecuador, Peru and Colombia. *Tropical Medicine Institute of Health **2**:589-602.*

Lengeler, C. (2000). Insecticide treated bednets and curtains for preventing malaria. *Cochrane Database syst Rev 320:CD000363*

Lengeler C. (2004). Insecticide-treated bed nets and curtains for preventing malaria. The Cochrane Database of Systematic Reviews

Lindblade, K. A., Dotson,E., Hawley,W. A., Bayoh. N., William. J., Mount. D., Olang. G., Vulule. J., Slutsker. L. and Gimnig. J. (2005). Evaluation of Long Lasting Insecticide Nets after 2 years of household use. *Tropical Medicine Institute of Health; **11**:41-50.*

Lindblade, K.A., Gimnig, J.E.,Dwight, L.M., Atieli, F.K., Sara, C. Adam, W., Hawley,A.W. and Ellen, M.D. (2005) Laboratory resistance of long lasting insecticide Nets. *Tropical Medicine Institute of Health; **10**:1022-1029.*

Lindsay, S.W., Adamiah, J.H., Miller J.E. and Armstrong, J.R.M.(1991). Pyrethroid treated bed nets effects on mosquito of the Anopheles gambiae in the Gambia complex. *Medical Veterinary Entomology; **5**:477-483.*

Lindsay, S.W., Alonso, P.L., Armstrong-schellenberg, J.R.M., Heminway, J., Adiamah, J., Shenton, F.C., Jawara, M. and Greenwood, B.M. (1993). A malaria control trial using treated bed nets and targeted chemoprophylaxis in rural in Gambia, West Africa. *American Journal of Tropical medicine and Hygiene **87** (Suppl 2): 45-51*

Lines, J., Myamba, J. and Curtis. and C.F. (1987). Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vector in Tanzania. *Medical Veterinary Entomology; **1**:37-51.*

Luke, J.G and Jeffrey, D. S. (2001). The economic burden of malaria. *American Journal of Tropical medicine and Hygiene; **64**(1-2suppl) 85-96*

Mbogo, C.N.M., Baya, N.M., Ofulla, A.V.O., Githure, J.I. and Snow, R.W., (1996). The impact permethrin impregnated bed nets on malaria vectors of Kenya Coast, *Med. Vet. Entomol **10**:251-259.*

Mbogo, C.M., Mwangangi, J.M., Nzovu, J., Gu, W., Yan, G., Gunter, J.T., Swalm, C., Keating, J., Regan, J.L., Shililu, J. L., Githure, J. I. and Beier, J. C., (2003). Spatial and temporal heterogeneity of anopheles mosquito and plasmodium falciparum transmission along Kenyan coast. *American Journal of Tropical medicine and Hygiene; **68**: 734-742.*

Magesa, S.M., Wilkes, T.J., Mnzava, A.E., Njunwa, K.J., Myamba, J., Kivuyo, M.D., Hill, N., Lines, J.D. and Curtis, C.F. (1991). Trial of pyrthroid impregnated bed nets in area of tanazania holoendemic for malaria. Part 2. Effects of malaria vector population. *Acta Tropica **49**:97-108.*

Miller, J.E., Lindsay, S.W and Armstrong, J.R.M. (1991). Experimental hut trials of bed nets impregnated with synthetic pyrethroids and organophosphate insecticide for mosquito control in the Gambia. *Med. Vet. Entomol **5**:465-476.*

Minakawa, N. Githure, J.I., Beier, J.C. and Yan, G. (2001). Anopheline mosquito survival strategies during the dry period in western Kenya. *Journal on Medical Entomology **38**:* 388–392.

Minja, H. (2001). Introducing insecticide treated mosquito nets in the kilombero valley (Tanzania):socio-cultural dimesions.PhD Thesis University of Basel,Department of Epidemiology and Public Health, Swiss Tropical Institute.

Mugenda, O. M. and Mugenda, A. G. (1999).Research methods;Qualitative and quantitative approaches. Nairobi acts press.

Müller, O., Kolé, I, and Traoré, C. (2002). Evaluation of a prototype long-lasting insecticide treated mosquito net under field conditions in rural Burkina Faso. *Transcient Research Society Tropical Medical Hygiene **96**:483–484*

Mutero, C., Kabutha ,C., Kimani, V., Kabuage, L., Gitau, G., Ssennyonga, J., Githure, J., Muthami, L., Kaida, A., Musyoka, L., Kiarie, E. and Oganda, M. (2004) A transdisciplinary perspective on the links between malaria and agro-ecosystems in Kenya. *Acta Tropica **89**:171–186.*

Muturi, J., Muriu, S., Shililu, J., Mwangangi, J., Benjamin G. J., Mbogo, C., Githure, J. and Robert, J.N. (2008). Effect of Rice Cultivation on Malaria Transmission in Central Kenya. *American Journal of Tropical medicine and Hygiene **78**(2): 270-275.*

Ministry Of Health. (2006*). Malaria treatment policy*. Government Printers, Nairobi.

Mwangi, T., Ross, A., Marsh, K. and Snow, R.(2003) The effects of untreated bednets on malaria infection and morbidity on the Kenyan coast. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. ***4:*** *369-372*

Ng'ang'a, P.N., Shililu, J., Gayathri, J., Kimani, V., Kabutha, C., Kabuage, L., Kabiru, E., Githure, J. and Mutero, C. (2008) Malaria vector control practices in an irrigated rice agroecosystem in central Kenya and implications for malaria control. *Malaria Journal. **7**: 146-148.*

Nahlen, L. B., John. P. C., David, A. (2003). *Insecticide treated bed nets. Roll Back Malaria.* Geneva: World health organization

Nguessan, R., Corbel, C., Akogbeto, M. and Rowland, M. (2007). *Reduced efficacy of insecticide treated and indoor spraying of malaria control in pyrethroid ressistance area, Benin*. MSc thesis. London school of hygiene.

Nevill, C.G., Some, E.S., Mung'gala, V.O., Mutemi, W., New, L., Marsh, K., Lengeler, C. and Snow, R.W.(1996). Insecticide- treated bed nets reduce mortality and severe morbidity from malaria among children on Kenya Coast. *Tropical Medical Institute of Health **1**:139-146.*

Otieno, M. (2004). *Mapping Landscape Characteristics of Yala Swamp, Kenya*. MSc. Thesis, Moi University.

Okrah.J.T., Pale.A., Somerfeld. J. and Muller, O. (2002) Community factors associated with malaria prevention by mosquito nets: anexploratory study in rural Bukina Faso. *Trop. Med. Int. Health **7**:240-248*

Ordnez, G. J., Kroeger, A., Avina. A. I. and Pabon. E. (2002). Wash resistant of insecticide- treated materials. *Transcient Research society tropical medical Hygiene; **96**: 370-375*.

Mens, P.F., Schoone, G.J., Kager, P.A. and Schallig, H.D.F.H. (2006). Detection and identification of human Plasmodium species with real-time quantitative nucleic acid sequence-based amplification *Malaria Journal **5(**80): 1-6*

Quasi-experimental evaluation (1998). *Determining appropriate sample size*. Human resource and skills development Canada.

Quinones, M.L., Lines, J., Thomson, M.C., Jawara, M. and Greenwood, B.M. (1998) Permethrin-treated bed nets do not have a mass killing effect on village populations of Anopheles gambiae s.l in the Gambia. *American Journal of Tropical medicine and Hygiene **92**: 373-378.*

Rishikesh, N., Wernsdorfer, W. and McGregor. **H** (**1988).** *Principles and Practices of Malariology*, Eds. Churchill Livingstone, New York 1227–1250.

Rapid test for malaria. (2008). *In http//www.path.org*.

Rozendaal, J.A. (1989). Impregnated mosquito nets and curtains for self protection and vector control. *Tropical Disease Bulleting **86***: R1-R41.

Robert and Carnvale 1991 Insecticide impregnation can restore the efficiency of torn bed nets and reduce man-vector contact in malaria endemic areas *Transactions of the Royal Society of Tropical Medicine and Hygiene, **86:** (4) 362-364*

Rwagoshora, S.I., Simonsen, P.E., Meryrowitsch, D.W., Malecela-Lazaro, M.N., Micheal, E. and Perdesen, E.N. (2007). Occurance and diversity of larval habitats and human lifestyle to human infection in an endemic community in coastal area of Tanzania *American Journal for Tropical Medicine and Hygiene **101**:51-60.*

Sachs, J. and Malaney, P., (2002). The economic and social burden of malaria. *Nature **415*:** 680-685

Scott, J.A., Brogdon, W.G. and Collins, F.H. (1993). Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal for Tropical Medicine and Hygiene **49:**520-529.*

SAS Institute. (**2007).** *SAS users guide* Version 9 First Edition. Cary., NC: SAS Institute.

Skovmand, O., Ouedraogo, D.A., Edith, S., Lea, P.T. and Thiery, B. (2009). Impact of slow release *Bacillus Sphaericus* granules on mosquito population followed in and urban environment. *Journal of medical entomology **46:** 67-76.*

Snow, R.W., McCabe, E., Mbogo, C.N.M., Molyneux, C.S., Some, E.S., Mung'ala, V.O., Nevill, CG. (1999) The effect of delivery mechanisms on the uptake of bed net re-impregnation in Kilifi District, Kenya. Health Policy Plan **14**:18–25.

Snow, R.W., Guerra, C.A., Noor, A.M., Myint, H.Y. and Hay, S.I. (2005). "The global distribution Of clinical episodes of Plasmodium falciparum malaria". *Nature **434** (7030): 214–7.*

Stump, A.D., Atieli, F.K., Vulule, J.M. and Besansky, N.J. (2004). Dynamics of the pyrethroid knockdown resistance allele in Western Kenyan populations of *Anopheles gambiae* in response to insecticide-treated bed net trials. *American Journal of Tropical Medicine and Hygiene. **70**:591–596.*

Tang L. (2000). Progress in Malaria Control in China. *Chinese Medical Journal. **113(1):** 89–92*

Thomson, M.C., Adiamah J.H., Conner. S.J., Jawara. M., Bennet. S., d'Alessandro. U., Quinones. M., Langerock, P. and Greenwood, B. M. (1995). Entomological evaluation of the Gambia National Impregnated bed net programme. *American Tropical Medicine and Parasitology **89**: 229-241*

Vulule, J.M., Beach, R.F., Ateili, F.K., McAllister, J.C., Brogdon, W.C., Roberts, J.M., Mwangui, R.W. and Hawley, W.A. (1999). Elevated oxidase and esterase levels associated with permethrin tolerance in anopheles gambiae from Kenyan villages using permethrin-impregnated nets. *Medical veterinary Entomology **13**: 239-244.*

Winch, P.J., Makemba. A.M., Makemba. V.R., Mfaume. M.S., Lynch. M.C., Premji. Z., Minjas, J.N. and Shiff. C.J.(1997). Social andcultural factors affecting rates of regular retreatment of mosquito nets with insecticide in Bagamoyo District, Tanzania. *Tropical Medical Institute of Health **2**:760-770.*

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

World health Organization. (1982). *Manual on environment management of mosquito Control with special emphasis on malaria vectors*. World Health Organization. Geneva

World health Organization. (1982). *Manual on environment management of mosquito Control with special emphasis on malaria vectors*. World Health Organization. Geneva

World Health Organization. (1998). *Insecticide treated nets and fabrics.* World Health Organization

World Health Organization. (2000). *WHO expert committee on malaria: twentieth Report. Geneva:* World health organization.

World Health Organization.(2001). *Disease fact sheet on Malaria*. World health organization

World Health Organization. (2001). *Long-lasting treated mosquito nets a break through in malaria prevention.* Bulleting of World Heath Organization V.79: 10 Geneva.

World Health Organization. (2002). *Roll back malaria infosheet 5 of 11*. Roll back malaria; WHO, Geneva

APPENDIX I

QUESTIONAIRE FOR KANYABOLI AREA IN SIAYA

Scheme 1 for interview (in the compound)

Address of compound

Name of husband or wife

Project number of house

Number of adult ( ) and children above 5 ( ), and below 5 years children in the compound

Number of pregnant women

Already use bednet
If so, for who man/men ( ), woman/women ( ), children above 5 ( ), children below 5 ( )

Permanet ( ) or Olyset ( )

Impregnated net by dipping ( )

If so, when it was last dipped
Non impregnated nets

Other tools: coil ( ) aerosol cans ( ), wall spraying ( ) 9 when last time……)

People currently under treatment for treatment for malaria man/men ( ), woman/women( )
Children above (5), children below 5 ( )

Number of Netprotect installed.

PART 2

Scheme 2 (2-4 days later)
Project number of house

Number of Netprotect found

Number of Netprotect used last night

If some nets are used, why not Netprotect®

APPENDIX II

ELISA BLOOD MEAL RESULTS ENTRY

MIM ELISA: PLATE: No…….

Blood Meal. ☐ Human ☐ Bovine. ☐ Sheep. ☐ Bird.

DATE…….

NOs…….

1	2	3	4	5	6	7	8	9	10	11
+ve	+ve									
-ve	-ve									

REMARKS…….

Appendix III

MOSQUITO COLLECTION FORM (PSC)

SPECIES

Gonotrophic Stage	*An. Gambiae*	*An. funestus*	*Culex*
Empty			
Blood fed			
Half fed			
Gravid			
Males			
Totals			

Appendix IV

Bioassay results on Netprotect followed on monthly basis

24/02/07

Net ID	No of mosq Exposed	KDR 3min	24 hr Mortality	Net ID	No of Mosq exposed	Kd 3min	24 hr mortality	% Corrected Mortality
1	10	5	10	1	10	0	0	100
2	10	5	10	2	10	0	0	100
3	10	6	10	3	10	0	2	100
4	10	4	10	4	10	0	0	100
5	10	5	10	5	10	0	3	100
6	10	6	10	6	10	0	1	100
7	10	4	10	7	10	0	0	100
8	10	4	10	8	10	0	0	100
9	10	4	10	9	10	0	2	100
10	10	5	10	10	10	0	0	100

8/03/07

Net ID	No of mosq Exposed	KDR 3min	24 hr Mortality	Net ID	No of Mosq exposed	Kd 3min	24 hr mortality	% Corrected Mortality
1	10	5	10	1	10	0	0	100
2	10	3	8	2	10	0	2	75
3	10	4	10	3	10	0	0	100
4	10	4	10	4	10	0	0	100
5	10	4	10	5	10	0	0	100
6	10	5	10	6	10	0	1	100
7	10	6	10	7	10	0	0	100
8	10	4	8	8	10	0	0	80
9	10	6	9	9	10	0	2	88
10	10	4	10	10	10	0	0	100

27/06/07

Net ID	No of mosq Exposed	KDR 3min	24 hr Mortality	Net ID	No of Mosq exposed	Kd 3min	24 hr mortality	% Corrected Mortality
1	10	5	10	1	10	0	1	100
2	10	4	10	2	10	0	0	100
3	10	3	10	3	10	0	0	100
4	10	3	10	4	10	0	0	100
5	10	2	10	5	10	0	0	100
6	10	3	10	6	10	0	0	100
7	10	4	10	7	10	0	0	100
8	10	3	10	8	10	0	2	100
9	10	2	10	9	10	0	0	100
10	10	3	10	10	10	0	1	100

10/07/07

Net ID	No of mosq Exposed	KDR 3min	24 hr Mortality	Net ID	No of Mosq exposed	Kd 3min	24 hr mortality	% Corrected mortality
1	10	5	7	1	10	0	0	70
2	10	4	10	2	10	0	0	100
3	10	4	6	3	10	0	0	60
4	10	3	3	4	10	0	0	30
5	10	0	3	5	10	0	1	22
6	10	5	10	6	10	0	0	100
7	10	4	9	7	10	0	0	90
8	10	5	10	8	10	0	0	100
9	10	5	10	9	10	0	0	100
10	10	4	8	10	10	0	0	80

17/07/07

Net ID	No of mosq Exposed	KDR 3min	24 hr Mortality	Net ID	No of Mosq exposed	Kd 3min	24 hr mortality	% Corrected mortality
1	10	7	7	1	10	0	1	67
2	10	8	10	2	10	0	0	100
3	10	5	6	3	10	0	1	56
4	10	8	3	4	10	0	0	30
5	10	0	3	5	10	0	2	13
6	10	5	10	6	10	0	1	100
7	10	6	9	7	10	0	0	90
8	10	7	10	8	10	0	1	100
9	10	6	10	9	10	0	0	100
10	10	5	8	10	10	0	0	80