EVALUATION OF DURABLE LINING FOR CONTROL OF MALARIA VECTORS IN RURAL AFRICAN HOUSES IN BUDALANGI, WESTERN KENYA

BY

ZIPPORAH KEMUNTO NYAKUNDI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN APPLIED PARASITOLOGY AND VECTOR BIOLOGY.

DEPARTMENT OF ZOOLOGY

MASENO UNIVERSITY

ABSTRACT

Malaria is one of the most serious and prevalent vector-borne parasitic diseases, affecting millions of people worldwide. Despite the control efforts being undertaken, the global burden of malaria remains enormous. Insecticide-treated nets (ITNs) have been widely promoted by World Health Organization as the main means for controlling malaria. However, achieving high net coverage and adherence to net use might be difficult to attain. Thus, there is need to develop additional tools for controlling malaria to supplement the use of ITNs. Thus, this study investigated the efficacy of insecticide-treated durable lining (DL) for control of malaria vectors in Budalangi, western Kenya. This area is a flood-prone zone, located at the shores of Lake Victoria, with no much information on efficacy of DL and transmission of malaria amongst children aged 6 months – 15 years. A total of 10 villages were selected on the basis of previous similar study on unknown vector density. Five villages were used as non-intervention while the remaining 5 villages were used as intervention villages. Additionally, some two villages were selected for indoor residual spraying, for comparison purposes. Indoor resting female adult malaria vectors were collected using pyrethrum spray collection. Members of the *An. gambiae* complex were identified by polymerase chain reaction (PCR). Blood samples were collected from children 6 months -15 years old since they are more vulnerable to the disease using, finger-prick method and parasite checked by microscopy. Anemia was monitored using battery-powered Hemocue machine. Circum-sporozoite protein in mosquitoes was determined by enzyme-linked immunosorbent assay (ELISA) technique. There was highly significant reduction in indoor resting densities between intervention and non-intervention for *An.gambiae* (85%; $P<0.0001$) and *An. funestus* (60%; $P<0.0001$). Sporozoite rate for *An. funestus* was significantly higher in non-intervention villages (4.58%; $P<0.008$) while for *An.gambiae,* there was no significant change. Overall, parasite prevalence did not differ in the intervention, non-intervention and IRS ($P=0.549$). Parasite density varied in the intervention, non-intervention and Indoor residual spraying (IRS) ($P<0.0001$) similar to anemia ($P<0.0001$). DL retained more insecticide and recorded higher mortality (>80 %), compared to IRS which recorded < 64 % reduction in mortality ($P<0.03$) for a period of 10 months. The present study has demonstrated that DL is effective in reducing malaria vector density and providing protective efficacy to the children, thus it can be used to complement the existing malaria control tools.

CHAPTER 1

1.0 INTRODUCTION

1.1 Background information

Malaria is one of the most serious and prevalent vector-borne parasitic diseases, affecting millions of people worldwide. In Africa, south of the Sahara, malaria remains the leading cause of morbidity and mortality (WHO, 2005:WHO,2011). Despite the control efforts being undertaken, the global burden of malaria remains enormous, though the process of control has accelerated since 2006 (WHO, 2008a). An estimated 655 million people die of malaria and 86% of these people are children under five years of age (WHO,2011). It is estimated that for every 40 seconds, a child dies of malaria (Sachs and Malaney, 2002), of which more than 15% of the survivors are left with severe sequalae, and brain or neurological damage that may impair the development and learning activity (WHO, 2006a). In addition to these effects, the disease takes an economic toll as well because of reduced productivity, which is responsible for an estimated average loss of 1.3% of economic growth annually in countries with intense transmission (Sach, 2001).

Of the more than 500 Millions of Africans who die of malaria each year, 70% of these are children under 5 years of age and (WHO, 2011). It is estimated that malaria kills an African child every 30 seconds, and remains one of the most important threats to the health of pregnant women and their newborns (WHO, 2005). In sub-Saharan Africa, all-cause mortality in children under five years of age is an important indicator of the burden of malaria (WHO, 2005). Opiyo *et al.* (2007) showed that the median expenditure for treating the sick children was estimated as US$ 3.1. These depict an overwhelming burden in children, thus, the elimination of the deadly *Plasmodium* that causes the disease is an issue that requires immediate attention and concerted effort.

Control of the disease relies mainly on vector control through indoor residual spraying (IRS) and insecticide-treated nets (ITNs) are key and important components of WHO global strategy (WHO, 2004). ITNs have been shown to be effective in preventing malaria morbidity and mortality and, in addition, provide protection to those not sleeping under ITNs (Binka *et al.*, 1998; Hawley *et al.*, 2003; Nevill *et al.*, 1996). However, the impact of ITNs relies heavily on achieving significant behavioral change amongst recipients to ensure that the most vulnerable sleep under the nets at the right time, and with the correct use of the ITNs. Experience with large scale community programs has shown that in many settings, achieving high levels of adherence and ITN retention and use can be very difficult, especially amongst communities which have competing priorities and little awareness of malaria (Alaii *et al.*, 2003; Majori *et al.*, 1987). Also the use of ITNs has resulted in behavior change in the feeding hours of mosquitoes (Govella *et al.*, 2010; Mbogo *et al.*, 1996; Pates and Curtis, 2005). Thus, there is an urgent need for alternative and innovative long-lasting tools and insecticides for community-based malaria prevention.

Indoor residual spraying has become one of the primary tools for national malaria control program in Kenya and elsewhere in sub-Saharan Africa. Historically, the use of insecticides has been paramount in the fight against diseases example use of insecticide Dichloro-diphenyl-trichloroethane (DDT) was generally achieved in eradicating malaria from several countries in the 1950 (Gramiccia, 1988). However, IRS programmes have various stumbling blocks. Repeat intervention is required every 6 to 12 months. The use of IRS is specifically restricted to smooth and non-porous surfaces, which means that the uniformity of coverage cannot be guaranteed (WHO, 2006d). Safe spraying equipment, personal protective gear and well-trained personnel are needed in an IRS program. In addition, issues regarding logistics, safety, acceptability and cost-effectiveness need to be considered. Thus, achieving high coverage and adherence to continuous spraying remains a major

challenge (WHO, 2006d). In addition, though the change in vector abundance due to IRS is well documented, comparison with its compliment durable lining has not been established, thus this study will shed light on latter.

The emergence of pyrethroid resistance in *Anopheles gambiae* has become a serious concern to the future success of malaria control. This has been observed in various studies (Anto *et al.*, 2009; Cuamba *et al.*, 2010; Vulule *et al.*, 1994). Despite considerable success of malaria control programs in the past, malaria still continues as a major public health problem in several countries. Vector control is an essential part for reducing malaria transmission and became less effective in recent years, due to many technical and administrative reasons, including poor or no adoption of alternative tools. Hence the quests to evaluate the effectiveness of the new alternative tools for malaria control.

The study site which was located in Budalangi, western Kenya is situated around Lake Victoria and is a flood prone zone. It is classified as an area of stable malaria having altitudes ranging from 0 to 1,300 meters (KMIS, 2010). Malaria continues to be a major health problem especially in difficult to reach areas. While plenty of scientific evidence shows effectiveness of treated nets, little is known about the effectiveness and durability of DL under field conditions. Thus, this study has generated critical information which can be used by the national malaria control program. In view of this, an evaluation of durable lining was undertaken in Budalangi to assess the impact on vector density, malaria prevalence, and durability.

1.2 Statement of the problem

Budalangi is located next to the shores of Lake Victoria, which is characterized by intense transmission throughout the year with annual entomological inoculation rates between 30 and 100 (NMS, 2009). Although many studies of malaria epidemiology, immunology, and drug resistance have been conducted at many sites in western Kenya there is little published literature describing malaria prevalence and vector density in Budalangi. Hence, accurate information about the burden of malaria infection is required both to plan local malaria control efforts and to measure the impact of such efforts.

Insecticide-treated nets (ITNs) and, more recently, long-lasting insecticide nets (LLINs) and IRS, have become the primary tools for national malaria control programs in Kenya and elsewhere in sub-Saharan Africa (WHO, 2004; WHO2006b). However, there are challenges associated with the existing tools. Material durability of LLINs is now proving to be much shorter than first expected and the method of washing and drying has effect on insecticidal power, which if not rightly applied efficacy is reduced (Atieli *et al.*, 2010). In addition, achieving high net coverage and adherence to net use remains a major challenge. IRS is effective, but short-lasting (3-6 months) and, therefore, difficult to sustain (WHO, 2006b). Due to these problems, there is the need to evaluate the new tool, durable lining, and assess its effectiveness and durability in controlling malaria.

In Kenya, at least 14,000 children are hospitalized annually for malaria, and there are an estimated 34,000 deaths among children under the age of five years, each year (NMS, 2009). Use of LLINs and ITNs has demonstrated health benefits especially among young children and pregnant women (Lengeler 2004; Gamble *et al.* 2007). However, the age groups least likely to use ITNs are school-age children (Noor *et al.* 2009b). For example, studies in Kenya indicate that school-age children are most likely to sleep under poor quality nets (Githinji *et al.* 2010; Atieli *et al.* 2011) and house-hold

sleeping arrangements, such that school-age children sleep on the floor and in areas where it is not possible to hang nets, which may affect the consistent use of nets by this age group (Alaii *et al.* 2003; Iwashita *et al.* 2010). In the absence of information regarding children in Budalangi, there is need to provide data since infection may not be the same in all settings owing to differences in the underlying intensity of malaria transmission.

1.3 Justification

Despite considerable efforts to control and treat the disease, the most recent estimates show that about 24 million Kenyans are at risk of infection each year, with the most affected being pregnant women and children (DOMC, 2010). Malaria continues to be a major health problem, especially in difficult to reach areas. The burden of malaria is still high in endemic areas located next to the shores of Lake Victoria in which Budalangi forms part of it. Budalangi, is located around Lake Victoria, western Kenya, an area prone to floods. The study aims to provide information on vector density, malaria prevalence in children and evaluate DL in controlling malaria. These data will aid the design of future control efforts and will serve as a baseline against which the results of current and future malaria control efforts can be assessed. Because malaria is a leading cause of mortality among children, the study aims to investigate the prevalence amidst them, hence provide information on the usefulness of monitoring the impact of prevention using DL and provide advice on way forward on malaria prevention.

Over the past decade, there has been a substantial increase globally in the coverage of effective intervention to reduce mortality from malaria. ITNs and IRS has been the cornerstone of such measures and there are clear signs of sustained reduction of such measures. However, further

interventions are urgently needed to capitalize on recent progress and sustain the malaria control and elimination efforts.

The research experience and results will provide guidance to the country's National Malarial Control Program (NMCP) for scaling-up the most efficacious and cost-effective malaria prevention and reduction activities, and specifically inform decisions about whether countries and malaria control programs should consider phasing out IRS or replacing or supplementing IRS programs with durable lining.

1.4 General objective

To evaluate, the effectiveness of durable lining in controlling malaria vectors, and assess its suitability and use amongst rural community in Budalangi, western Kenya.

1.4.1 Specific objectives

i. To determine and evaluate the efficacy of durable lining in controlling malaria vectors in intervention, non-intervention and indoor residual spray (IRS) villages in Budalangi.

ii. To determine the prevalence of malaria among children in the intervention, non-intervention and IRS villages in the area of the study.

iii. To compare the efficacy and longevity of IRS and DL in killing malaria vectors.

1.5 Research Questions

I. What is the efficacy of durable lining in controlling malaria vectors in Budalangi, western Kenya?

II. What is the prevalence of malaria among children in the study population in the intervention and non-intervention areas of Budalangi, western Kenya?

III. What are the comparisons between efficacy and longevity of DL and IRS on killing malaria vectors in Budalangi, western Kenya?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Malaria

Malaria is caused by blood-borne protozoan parasites of the genus *Plasmodium*. Four species of *Plasmodium (P.vivax, P.ovale, P.malariae, P.falciparum)* parasitize man, of which *P. falciparium* is the most virulent. Malaria parasites are transmitted exclusively by mosquitoes of the genus *Anopheles*, with over 400 species of mosquitoes in the genus; only 10% of the *Anopheles* species have been implicated suitable malaria vectors. In sub-Saharan Africa, where 90% of the world's malaria-infected people are found, most transmission is caused by three anopheline species, *Anopheles gambiae, A. arabiensis, and A. funestus*, with *A. gambiae* being the most important (Collins and Paskewitz, 1995; Ramirez *et al.*, 2009). These mosquitoes carry infective sporozoites in their salivary gland, which they transfer to the blood stream of man during a blood meal; within the mosquito, the parasite undergoes a complex developmental cycle that, depending on parasite species and environmental conditions, may be as short as eight days or longer than two weeks (Ramirez *et al.*, 2009).

The parasite life cycle (Figure 1) starts in the human host when *Plasmodium* sporozoites enter the bloodstream after being transmitted via a mosquito bite. From there, the sporozoites infect the liver cells and disappear from the bloodstream within approximately 30 minutes. The sporozoites mature into schizonts, which rupture and release merozoites into the blood circulation, where they infect red blood cells. They further undergo asexual multiplication: some merozoites mature again into schizonts that lead to more merozoites; others differentiate into gametocytes which, when picked up by a second mosquito during a further bite, undergo a series of transformations in this second

mosquito, leading eventually to the production of new sporozoites that can infect another human being. Two types of *Plasmodium* – *P. ovale* and *P. vivax* – can persist in the liver of an infected patient and cause relapses by invading the bloodstream weeks, or even years, later.

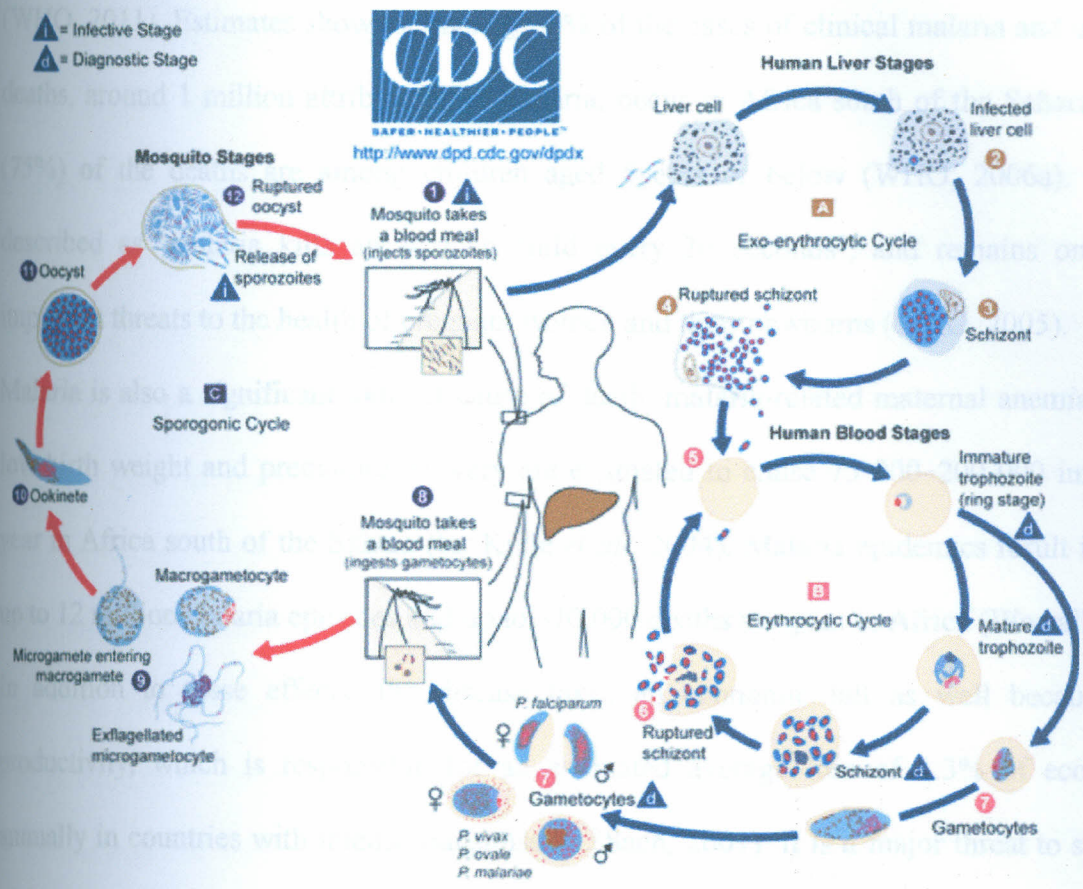


Figure 1: Life cycle of *Plasmodium* parasites (www.cdc.gov/malaria/about/biology/index/html)

2.1.1 Global burden of malaria

The global burden of malaria remains enormous, though progress in malaria control has accelerated dramatically since 2006 (WHO, 2008). According to the report (WHO, 2011), an estimated 655 million people die of malaria and majority are children under five years of age. Of these, 91per cent of the cases were from the African region (WHO, 2011). The bulk of the burden of malaria mortality

in 2007 occurred among the estimated 108 million African children exposed to stable *P. falciparum* transmission (Hay *et al.*, 2009).

Africa remains the region that has the greatest burden of malaria cases and deaths in the world (WHO, 2011). Estimates show that nearly 60% of the cases of clinical malaria and over 90% of the deaths, around 1 million attributable to malaria, occur in Africa south of the Sahara. The majority (75%) of the deaths are among children aged 5years or below (WHO, 2006a). This has been described as 'malaria kills an African child every 30 seconds', and remains one of the most important threats to the health of pregnant women and their newborns (WHO, 2005).

Malaria is also a significant indirect cause of death: malaria-related maternal anemia in pregnancy, low birth weight and premature delivery are estimated to cause 75 000–200 000 infant deaths per year in Africa south of the Sahara (ter Kuile *et al.*, 2004). Malaria epidemics result in an estimated up to 12 million malaria episodes and up to 310 000 deaths per year in Africa (Worrall *et al.*, 2004).

In addition to these effects, the disease takes an economic tall as well because of reduced productivity, which is responsible for an estimated average loss of 1.3% of economic growth annually in countries with intense transmission (Sach, 2001). It is a major threat to socio-economic development in the world and is also one of the major disease burdens in sub-Saharan Africa, where 15% of all disability life-years are lost to malaria (Chima *et al.*, 2003).

2.1.2 Malaria burden in Kenya

In Kenya, malaria is a major public health problem with its burden and transmission patterns varying across the country. Four malaria epidemiological zones have been identified on the basis of parasite prevalence (Noor *et al.*, 2009a) and climatic conditions depicting four zones (www.kemri.org) of malaria transmission risk: 1) Perennial high transmission zones (endemic areas): these are areas of

stable malaria transmission and include parts of Western, Nyanza and Coast provinces, where malaria transmission occurs all year round and community parasite prevalence often exceeds 50 per cent; 2) Malaria epidemic-prone areas: including highland districts in Western, Nyanza and the western Rift Valley provinces, and the arid and semi-arid lowlands of northern and south-eastern Kenya. Malaria transmission in these areas is seasonal with annual variations in transmission intensity. Human populations have poorly developed immunity to malaria because exposures are infrequent. All persons are vulnerable to severe clinical illness and complications from *Plasmodium* infection; 3) Arid/seasonal: including the north Rift Valley and parts of Central, Eastern, Coast and North Eastern provinces where malaria risk is generally low but transmission occurs along water bodies; 4) Low risk malaria zones: these areas include Nairobi and parts of Central and central Rift Valley provinces. In this zone the transmission is limited by temperatures, which are often too low to allow the development of malaria parasites in the vector. Although the risk of malaria transmission is low in this zone, changes in temperature, occasioned by climate change and variability, may result in transmission of *Plasmodium* parasites.

It is estimated that the disease accounts for 30% of all outpatient consultations and 19% of inpatients (NMS, 2009). At least 14,000 children are hospitalized annually for malaria, and there are an estimated 34,000 deaths among children under-five years of age each year. Annually, an estimated six thousand pregnant women suffer from malaria-associated anemia, and four thousand babies are born with low birth weight as a result of maternal anemia. Economically, it is estimated that 170 million working days are lost each year because of malaria illness (www.kemri.org).

The economic burden of malaria for households can be extremely high. Treatment costs for small-scale farmers in rural Kenya have been estimated to be as high as 7% of the monthly household expenditure (Chuma *et al.*, 2006), not considering any costs for prevention measures. Despite the

decline in pediatric admissions, coastal region of Kenya (Okiro *et al.*, 2007) and in Rusinga Island, the median expenditure for treating the sick children was estimated as US$ 3.1(Opiyo *et al.*, 2007)

Malaria risk and disease burden is inequitably distributed, not only at global and regional levels but also at household level because of poor housing, lack of education and access to healthcare services. This creates a vicious cycle of enhanced vulnerability to malaria due to increased exposure, high household medical costs, reduced ability to pay for treatment, and so on (Bates *et al.*, 2004; (Chuma *et al.*, 2007). The production loss as a percentage GDP is 2 to 6% yearly, while 9 to 18% to small scale farmers and agribusiness is lost (www.doh.gov.za/issues/malaria).

2.2 Efficacy of vector control tools

Strategies for preventing malaria involve four different approaches: reduction of human-mosquito contact; reduction of vector density; reduction of parasite reservoir; chemoprophylaxis and intervention through vaccine production (Enayati and Hemingway, 2010). The historic successful eradication of malaria in various parts of the world was achieved primarily by vector control using DDT, indicating that renewed efforts in this field, other than the current insecticide-based strategies, should be considered as a central aspect of any malaria eradication strategy. The success of malaria control measures in some highly-endemic countries, such as Rwanda, Zambia, Zanzibar, Sao Tome & Principe, The Gambia and Kenya has led to reductions in deaths from malaria of 50% or more (WHO, 2008). These developments have led to an increased focus on vector control. There are several techniques involved in vector control; however, ITNs/LLINs and IRS are the main tools currently being urged to upscale for use (WHO, 2004).

2.2.1 Insecticide-treated nets and LLINs

An insecticide-treated net is a mosquito net that repels, disables and/or kills mosquitoes coming into contact with insecticide on the netting material. There are two categories of ITNs: conventionally treated nets and long-lasting insecticidal nets. The former needs regular retreatment, a follow-up action that has proven difficult to achieve at field level. The latter is a relatively new technology that retains the efficacy for at least 3 years. Pyrethroids are the only chemical group recommended for use in ITNs (van den Berg, 2009). The fact that only one class of insecticide is used for treatmentof nets is disturbing, where there is high pyrethroid resistance, efficacy is likely to reduce (N'Guessan *et al.*, 2007).

ITNs are important component of the roll back malaria campaign to reduce malaria morbidity and mortality in Africa (Zaim *et al.*, 2000). ITNs have been shown to reduce overall mortality by at least 20% on average in regions of Africa where malaria is the leading cause of death of children under five years of age (Lengeler, 2004). (Gamble *et al.*, 2007) observed ITNs to be effective in reducing adverse effects of malaria in pregnancy. A systematic review of randomized control trials involving the use of ITNs by pregnant women found that miscarriages in Africa were reduced by a third in those women who were in their first few pregnacies. Trials conducted in western Kenya using ITNs where distribution was near complete in the area of study, resulted in 19% reduction in malaria prevalence and 90% vector reduction (Gimnig *et al.*, 2003b; ter Kuile *et al.*, 2003). In addition, ITNs have been shown to be effective in preventing malaria morbidity and mortality, in addition to providing protection to those not sleeping under ITNs (Binka *et al.*, 1998; Hawley *et al.*, 2003; Nevill *et al.*, 1996).

Novel results have been noted in trial situations where all are given nets; however, in real situation, ITNs distibution is sparse within the village and coverage is not enough to achieve a community effect (Gimnig *et al.*, 2003a). Also, ITN adoption in most malarious areas remains very low and public health agencies frequently have insufficient resoures to provide complete ITN coverage for all individuals at risk. Pyrethroid-treated nets have the capacity of exito-repellancy in mosquitoes that come into contact with treated nets; this property will restore efficacy when nets have become holed (Hill *et al.*, 2006). However, Darriet *et al.* (2000), in comparing nets against *An. gambiae* s.s., observed that there was a significant higher exiting and lower blood feeding when nets where intact than when they were holed; similar findings have also been observed by Irish *et al.* (2008).

In a historical review, (Bayoh *et al.*, 2010), showed reduction of the vector in western Kenya after long use of ITNs. This is exciting and gives hope that the reduction could occur if ITNs were applied in all regions. But these results were from a region where research has been done ever with free distribution of nets. These have been observed (Cohen, 2010) where there is free distribution of nets and usage is high. However, uptake reduces significantly even when there is 90% subsidy on the price of insecticide-treated nets.

At very high rates of ITN use, ITNs can generate large reductions in transmission intensity that could provide very large reductions in transmission intensity and effective malaria control in some areas, especially when used in combination with other control measures. At high entomological inoculation rate (EIR), ITNs will probably not substantially reduce the parasite rate, but when transmission intensity is low, reductions in vectorial capacity combine with reductions in the parasite rate to generate very large reductions in EIR (Le Menach *et al.*, 2007). Although ITNs have been included in the national malaria control programs of many countries as a vector control option, low re-treatment rates of ITNs in most countries (Dabire *et al.*, 2006) are

seriously affecting ITN programs. Hence, the use of nets pre-treated with insecticide offers solution to this problem by making the insecticide available, even after multiple washings.

The WHO Global Malaria Program is calling on national malaria control program and their partners, involved in insecticide-treated net interventions, to purchase LLINs. The LLINs are strong and can make insecticide available up to twenty washes (WHO, 2006d).Currently, many countries in malaria-endemic Africa are scaling up the coverage of long-lasting insecticidal nets (LLINs). These program, funded by President's Malaria Initiative (PMI), intend to have a lasting impact on malaria transmission (WHO, 2006a). LLINs have been observed to be effective in reducing malaria burden (Bhattarai *et al.*, 2007; Dabire *et al.*, 2006). Despite of these, it is probable that, due to many other factors, including resistance development, poor handling, and wear and tear on the nets makes them less effective. In addition to these, the material durability of LLNs is also now proving to be much shorter than first expected, where wash and drying regimen has an influence on the insecticidal activity of LLNs in that abrasive washing in the field leads to subsequent loss of insecticide (Atieli *et al.*, 2010).

2.2.2 Indoor residual spraying

IRS is the application of long-acting chemical insecticides on the walls and roofs of all houses and domestic animal shelters in a given area, in order to kill the adult vector mosquitoes that land and rest on these surfaces. The primary effects of IRS towards curtailing malaria transmission are: i) to reduce the life span of vector mosquitoes so that they can no longer transmit malaria parasites from one person to another (sporogonic cycle), and ii) to reduce the density of the vector mosquitoes. In some situations, IRS can lead to the elimination of locally important malaria vectors. Some

insecticides also repel mosquitoes and, by so doing, reduce the number of mosquitoes entering the sprayed room and, thus, human-vector contact (WHO, 2006b)

Indoor residual spraying (IRS) has previously been recommended for areas of low-to-moderate transmission; discrete, accessible communities such as islands and refugee camps, or for epidemic response (WHO, 2000). Currently, the Division of Malaria Control is using IRS as lead intervention in averting malaria epidemic in the highlands is priority for the Ministry of Health. The Division of Malaria Control has been implementing a timed and well coordinated Indoor Residual Spraying (IRS) campaigns in the 16 districts classified as Highland Epidemic Prone. The districts include Kericho, Nandi north and south, Lugari, Nyamira and others (www.kemri.org). Now, this stance is changing and the WHO now promotes wider application of IRS, including in highly endemic settings in sub-Saharan Africa (WHO, 2006c).

IRS has an advantage of being able to make use of much wider range of insecticide than ITNs for which pyrethroids are the only class used. WHOPES approves the use of twelve insecticides of four classes for IRS: pyrethroids (PY), carbamate, organophosphate (OP) and organochlorine (WHO, 2006b). The extended use of this range is important because of its benefits for the management of insecticide resistance and hence long-term sustainability of vector control (Pluess *et al.*, 2010). This gives the possibility of alternating the insecticide and switching to another insecticide incase of emerging resistance (Pluess *et al.*, 2010). But DDT has long been the cheapest insecticide and the one with the longest residual efficacy against malaria vectors (6–12 months depending on dosage and substrate). Other insecticides have relatively shorter residual effect (pyrethroids: 4–6 months; organophosphates and carbamates: 2–6 months) (WHO, 2006b). Thus, the use of DDT alternatives might require two to four spray cycles per year instead of one, depending on the length of the transmission season, with important operational and financial implications for spraying program.

IRS has played a major role in reducing malaria transmission in different areas such as Kenya (John *et al.*, 2009; Zhou, 2010;) and Equatorial Guinea (Sharp *et al.*, 2007). Despite the achievements by IRS, it requires good infrastructure and logistics, campaign, planning and timing to have any chance of success (Rowland, 1999). 80% coverage is recommended by WHO but to attain this is often difficult due to financial constrains. Residual effect on various surfaces of walls differs depending on several factors (Najera, 2001). Furthermore, the efficacy of the spray also depends on the cooperation of the inhabitants to get complete coverage of their houses and their intervention after spray by certain practices, such as mud plastering and white washing that affects the residual efficacy. Hence, to have uniform insecticide is a challenge; it is inevitable to explore new technologies to such as durable lining to achieve this.

2.2.3 Durable lining

Durable Lining (DL)/wall lining is a simple, durable and effective alternative to IRS. Insecticide-treated plastic sheeting (ITPS) and insecticide-treated wall lining are known as durable lining; they have potential as a long-lasting insecticidal surface for malaria vector control when used as lining for interior walls and ceilings inside the home. It is a lining of loosely- woven, high-density polyethylene panels which, when installed on the walls of a house, provide protection against mosquitoes and enhance the beauty of traditional rural home interiors. The insecticide is incorporated into every strand of the colored yarn. Through a controlled migration technology, the insecticide is continually refreshed at the surface of the fabric. Mosquitoes and other insects that come in contact with this treated surface acquire a lethal dose while resting or walking on the liner surface (www.zerovector.com).

Deltamethrin is incorporated into the plastic polymer during the manufacturing process and takes place under strictly controlled conditions. The controlled migration of active ingredient to the surface of the yarns prevents high insecticide concentrations on the DL surface, thereby reducing the exposure risk for homeowners. The long-lasting effect of DL eliminates the need for retreatment. Like other pyrethroids, deltamethrin causes skin irritation to sensitive persons. The effect is considered temporary and non-toxic. However, to reduce discomfort, persons expecting prolonged exposure are encouraged to use rubber gloves, avoid rubbing eyes or mucous membranes during installation, and to wash hands with soap and water immediately after exposure (www.zerovector.com).

Durable lining (DL) can be used in combination with LLINs and whereas IRS requires repeat intervention, this tool can last as long as the LLINs and does not require behavior change. It is also factory treated hence uniform dose ensured regardless of the wall texture or shape. This can be applied in rural setting with minimal supervision and can be easily monitored. (www.zerovector.com).Given the inherent problems associated with the WHO recommended strategies for malaria control, it is important to evaluate other control tools to complement these existing tools. Durable wall lining has not been widely tested for malaria control in Kenya and more specifically in Budalangi and thus this study generated will generate important information which can guide future evaluations of DL in the country.

2.3 Challenges to effective vector control

Pyrethroid resistance is emerging, despite early optimism that rapid toxicological action of this newest class of insecticides would not produce resistance (Malcolm, 1988). Resistance is not

evolving through unique new mechanisms, but rather through existing mechanisms which are being enhanced and cross- resistance is occurring (Brogdon and McAllister, 1998). A far more development is the appearance of target site resistance (also termed as knockdown resistance) to pyrethroids. This has been observed in several important vectors in multiple locations (Brogdon and McAllister, 1998).

Vectors with the knockdown resistance (kdr) gene are resistant to DDT and pyrethroid groups of insecticides, and this has serious consequences for malaria vector control, because pyrethroids and DDT are the two main groups of chemicals used. The *kdr* gene is being reported from an increasing number of countries; thus, even in countries without a history of DDT use, resistance to DDT is emerging in populations of malaria vectors (WHO, 2006b). Moreover, there are records of a change in vector behavior from indoor resting to outdoor resting in response to indoor spraying, as well as a change in daily pattern of biting and host choice in response to ITN interventions (Phillips, 2001).

In addition, many control programs are structured under Ministry of Health, without organizational links to other government ministries (e.g. environment, education, agriculture and tourism), municipal entities (e.g. engineering, sanitation and water resources) or links to stake-holders in communities (e.g. businesses, educators, community groups and NGO's). Various techniques are being used to control mosquitoes, but the programmatic approaches are generally not linked with mosquito surveillance, fundamental information about the ecology and behavior of the vector species, or heath-systems data on disease. In addition, a common problem is the lack of stable funding for mosquito control operations and the lack of initiatives to apply leverage in support of mosquito control by other government and community entities. In many respects, the IVM global strategic framework is designed to overcome some of these problems, but is proving difficult in reality (Beier *et al.*, 2008).

Apart from the political controversy associated with any use of genetically modified organisms, there are practical drawbacks. The success rate of genetic-transformation techniques is low, leading to possible inbreeding-related decreases in GMM population viability. The genetic manipulations often involve laboratory strains, whose ability to survive in the wild, let alone invade wild mosquito populations, is questionable. For most, though not all, proposed GMMs, their spread and maintenance in the wild have to be ensured by the repeated release of modified mosquitoes; strategies based on naturally invasive driving mechanisms, such as homing endonuclease genes or transposable elements, present an advantage in this respect. There is also the complication that malaria is transmitted by many mosquito species, and GMMs might have to be designed for each of them or restricted to the main culprits. Moreover, if genotype–genotype (mosquito–*Plasmodium*) interactions exist at the within-species level, modification of a given gene might affect *Plasmodium* only on a local scale. Finally, release of *Plasmodium*-resistant mosquitoes might increase transmission of other *Anopheles*-borne diseases, such as filariasis (Michalakis and Renaud, 2009; Ramirez *et al.*, 2009).

2.4 Malaria prevalence in children in Kenya

A child means every human being below the age of 18 years unless under the law applicable to the child, majority is attained earlier (UNICEF, 1989). An estimated 8.2 million cases of malaria are reported in Kenya every year, out of a total population of 30 million. In Kenya alone, malaria kills an average of 72 children less than five years of age each day (Chuma *et al.*, 2006). Young children, however, bear a considerable burden in terms of malaria morbidity and mortality. In Kenya, primary school students miss 11% of school days per year because of malaria (Leighton, 1993). The adverse effects on schooling are likely to go far beyond the number of days lost per year, as absenteeism increases failure rates, repetition of school years, and dropout rates (Sachs and Malaney, 2002).

Insecticide-treated mosquito nets (ITNs) and indoor-residual spraying (IRS) are recommended strategies for preventing malaria in children (WHO, 2008a). Large randomized trials conducted in western Kenya on use of ITNs and IRS has been observed to result in reduction of malaria (Gimnig *et al.*, 2003b; ter Kuile *et al.*, 2003; Zhou *et al.*, 2010). For instance in the coastal region of Kenya, Okiro *et al.* (2007) observed decline in pediatric admissions, while in the highlands of western Kenya, also, (Zhou *et al.*, 2010) has shown reduction in parasite prevalence in school children. However, this is not consistent across the country (Okiro *et al.*, 2010). In addition, nets delivered in 2006-2007 are already due for, and need, replacement; failure to replace could lead to a resurgence of malaria cases and deaths. Unfortunately, few know the need of replacement; despite the campaigns on the use of the nets, majority does not know, thus further interventions are needed to sustain malaria control and elimination.

More efforts have focused on children under five however the age groups least likely to use ITNs are school-age children (Noor *et al.*, 2009b). The latter group of children is still susceptible to parasite infection. According to KMIS, (2010) the prevalence in children below five years increased from 4 per cent in 2007 to 8 per cent in 2010 and children five to ten years have the highest prevalence of malaria. This group (5-10years) thus forms a large reservoir of asymptomatic infection that perpetuates the malaria transmission cycle. Hence, the study focused on prevalence across all age groups to have precise information on what occurs across the age groups.

2.5 Longevity of mosquito control methods

ITNs have been included in the national malaria control programs of many countries as a vector control option; low re-treatment rates of ITNs in most countries (Dabire *et al.*, 2006) are seriously

affecting ITN programs. Hence, the use of nets pre-treated with insecticide offers solution to this problem by making the insecticide available, even after multiple washings.

LLINs are currently preferred to conventional insecticide treated nets for use in malaria control program (Gimning *et al.*, 2003; Lengeler C, 2004). Long-lasting insecticidal nets (LLINs) reduce human–mosquito contact, which results in lower sporozoite and parasite rates. The biological activity generally lasts as long as the net itself (3–4 years for polyester nets and 4–5 years for polyethylene nets) (WHO 2005). Field evaluation of LLINs has shown remarkable bioefficacy (Lindblade *et al.*, 2005; Banek *et al.*,2010). However, the insecticidal activity of the nets is not promising for future malaria control due to loss of efficacy faster than anticipated (Lindblade *et al.*, 2005, Atieli *et al.*,2010). In addition to the LLINs, treatments of screens, curtains, canvas tents, plastic sheet, tarpaulin, with insecticides may provide a cheap and practical solution for malaria vector control. These treated materials have also been explored in various communities and have found to be effective (Diabate *et al.*, 2006; Chander *et al.*, 2010).

Indoor residual spraying (IRS) using DDT was, for many years, the mainstay of mosquito control in areas with endemic malaria, particularly during the WHO-led eradication campaign (WHO, 2006). DDT has long been the cheapest insecticide and the one with the longest residual efficacy against malaria vectors (6–12 months depending on dosage and substrate). Other insecticides have relatively shorter residual effect (pyrethroids: 4–6 months; organophosphates and carbamates: 2–6 months). Thus, the use of DDT alternatives might require two to four spray cycles per year instead of one, depending on the length of the transmission season, with important operational and financial implications for spraying program (WHO, 2006. Despite the known fact of the residual life span of the insecticide used in IRS, longevity of IRS on different surfaces differs and this poses serious challenges on sustainability of this method (Najera, 2001).

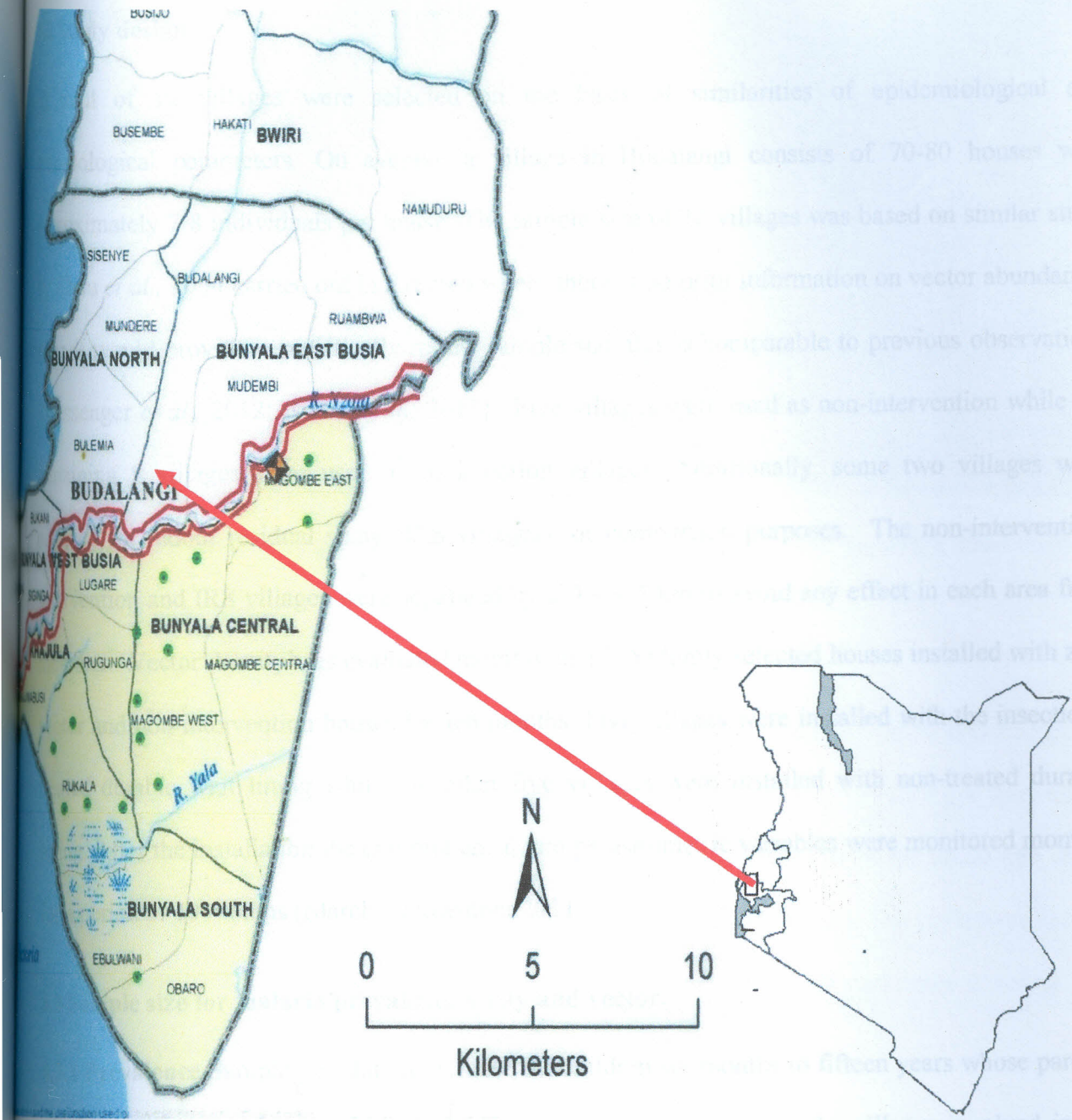
Therefore, with the existing limitations on the longevity of IRS and LLINs, there is an urgent need to investigate the new tools such as durable lining and assess its longevity in the control of malaria vectors.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 The study site

The study was conducted in Budalangi area in western Kenya; it lies at longitudes 33° 57′ and 34°00 E′ and latitudes 0°03′ S and 0°06′ S. The altitude in this region ranges between 1130 m-1275m above sea level. Budalangi is one of the most devastated floodplains in western Kenya is underlain by loose sedimentary deposits that have been deposited by floods. Approximately 28,000 people live in this floodplain. However their spatial distribution is quite uneven, with some places being completely deserted. An explanation to this is the flood- related problem associated with this floodplain (Figure 2). Rainfall pattern in Budalangi is mainly bi-modal (two rainfall seasons in a year). The major season occurs in March to June (the long-rains season) while the other season (short-rains) occurs in October to December. Malaria transmission in this area is intense and occurs year-round with peaks after the long rainy season and short rainy season. Most residents of this area are of the Luhya ethnic group and they live in scattered family compounds that consist of 1 or more houses and surrounding agricultural fields. The economic activities within the region involve small scale farming, fishing and weaving of baskets. Most houses are constructed using sticks and mud with a roof of grass thatch or corrugated metal (www.irinew.org). Like other areas in western Kenya, the most prevalent malaria parasite is *P. falciparum*. The primary malaria vectors in this region are *An. gambiae* and *An. funestus* (Mattias *et al.*, 2010).



RE 2: MAP OF STUDY SITE

3.2 Study design

A total of 10 villages were selected on the basis of similarities of epidemiological and entomological parameters. On average a village in Budalangi consists of 70-80 houses with approximately 7-8 individuals per house. The sample size of 10 villages was based on similar study by Zhou *et al.*, 2004 carried out in a region where there is no prior information on vector abundance. Also it would provide a statistically robust sample size this is comparable to previous observations (Messenger *et al.*, 2012; Burns *et al.*, 2012). Five villages were used as non-intervention while the remaining 5 villages were used as intervention villages. Additionally, some two villages were selected for indoor residual spray (IRS villages) for comparison purposes. The non-intervention, intervention and IRS villages were separated by at least 5 km to avoid any effect in each area from the other. Vector density was evaluated monthly in 10 randomly selected houses installed with zero vector and non-intervention houses for ten months. Five villages were installed with the insecticide treated durable wall lining while the other five villages were installed with non-treated durable lining. After the installation the entomological and parasitometric variables were monitored monthly for a period of 10 months (March – December 2011).

3.3 Sample size for malaria prevalence study and vector.

3.3.1 Prevalence Sample population included all children six months to fifteen years whose parents accepted to participate as the study begins. They were selected from the villages involved in the study. After recruitment till completion no child was added except those whose parents accepted on the onset of the project. The sample size was calculated using the following formula for sample size calculation for prevalence based on Whitney and Ball (2002).

N/1-q

N- Is the subject required in the final study

q- Is the proportion expected to refuse to participate, drop or move out before the study ends.

Assumption is made on q and in this case approximately 10% default

Thus N= 40/1-0.10

45

Hence in each village at least 45 children would remain till the end of the study.

3.3.2 Sample size for Mosquito Houses for mosquito surveillance were randomly selected from the intervention, non-intervention and IRS villages. A selection of ten houses was done from each village in the intervention, non-intervention and IRS. The number of houses was arrived at according to Zhou *et al.*, 2004 sample size detemination for collection of mosquitoes.

3.4 Durable lining installation

All households in ten (10) villages were installed with durable lining, 5 villages were used as intervention and 5 villages were non-intervention. The zero vectors will be installed on the walls of the houses using nails and nails caps (Figure 3).

Figure 3: Durable lining installation in the house in the study site depicting total coverage upto the eaves.

3.4.1 Indoor Residual Spray villages

Spraying of houses was done on the selected villages using Deltamethrin-K-Othrine WG 250 used in IRS. The deltamethrin WG 250 was provided in water soluble sachets suitable for one spay tank to mix with known volume of water (10L) and applied on the inside walls of the house and on the interior parts of the roof to give a required application rate (0.02-0.04 g m^2). The walls were sprayed evenly in vertical spray pattern, spraying from top to bottom of all internal walls ensuring even coverage. IRS was done in all the houses within the selected villages.

3.4.2 Bioassay procedure

Durable lining was installed in houses by nailing the DL on the using roofing nails with nail caps. Efficacy of the DL in killing the mosquitoes was assessed using WHO cone tubes (WHO, 2008b). This experiment was conducted in 20 randomly selected houses within the study area. Insecticide

susceptibility tests were carried out using the standard WHO (1996) protocol. This was done immediately after the spraying, and once every two months. The tests were done using three WHO cones which were attached to the wall lining using masking tape. Ten female non- blood fed *Anopheles gambiae* mosquitoes aged 2-5 days old were introduced into the WHO cones and held in vertical position. The mosquitoes were then exposed for 3 minutes and subsequently transferred to holding cups. Knockdown count was done for every 30 minutes and 1hour. Thereafter, the mosquitoes were provided with 10% sucrose pads awaiting mortality assessment after 24 hours holding period. The mosquito strain used was laboratory reared *Anopheles gambiae s.s* Kisumu strain which is susceptible to insecticides (Vulule *et al.*, 1994).

3.4.3Mosquito collection

Indoor resting densities of mosquitoes were collected using pyrethrum spray collection (PSC). PSC was be done according to previous studies (WHO 2002; Bayoh *et al.*,2010). This was done from 07:00 hours to 10:00 hours in the morning. The process involved the following steps: mixing of pyrethrum fifty milli-liters with ten liters of kerosene placed in a pump. Emptying the house, closing windows and white sheets were laid upon the floor and over the furniture within the house ensuring complete coverage. Spraying the house was done beginning with eaves from outside, and then thereafter, the inside of the house was sprayed. After filling the room with insecticide mist, the collector left the house and closed the door and waited for ten minutes for mosquitoes to be knocked down or killed. After these, beginning from the door and moving to interior rooms the sheets are removed carefully not to lose mosquitoes. Mosquitoes are then collected and placed in vials and kept on ice until identification and processing was done.

3.4.4 Mosquito identification and processing

3.4.4.1 Morphological identification

Morphological identification is a technique that is used to distinguish species which appear similar in structure but differ genetically. Members of anopheline species consist of species which look similar in appearance though different genetically. To distinguish these species morphological keys are often used. Mosquito were collected from the field then transported back to the laboratory and first identified using morphological with reference from previous studies by (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987). The identification focused dark spot at the upper margins of the wings which is common to all *Anopheles*. The palps are elongated and segmented into three. A pale spot on second dark area, a light spot between the two dark spots on vein 6 two dark spots on vein 6 and absence of fringes on vein 6 are features for *Anopheles funestus*. Speckles on the legs, third pre-apical dark area on vein 1 with a pale interruption and tersi 1-4 with conspicuous pale bands are features or *Anopheles gambiae* (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987).

3.4.4.2 *Molecular identification*

Polymerase chain reaction (PCR) is a simple and powerful tool for an amplification of target deoxy-ribonucleic-acid (DNA) *in vitro*. PCR allows amplification of a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence after which isolation of the target DNA fragments are separated by high resolution.

To distinguish members of *Anopheles gambiae* complex, polymerase chain reaction was done. Separation is based on the intergenic spacer of *An. gambiae* complex of ribosomal (rDNA). Legs and wings of females of *Anopheles gambiae* s.l were frozen at -20°C in labelled vials before molecular

identification by PCR into *Anopheles gambiae* s.s or *Anopheles arabiensis* (Scott *et al.*, 1993) (Appendix III).

3.4.4.3 ELISA- Assays

Enzyme-linked immunosorbent assay (ELISA) is a test that uses antibodies and color change to identify a substance. Antigens from the sample are attached to a surface. Then, a further specific antibody is applied over the surface so it can bind to the antigen. This antibody is linked to an enzyme, and, in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal; most commonly a color change in the substrate. Performing an ELISA involves at least one antibody with specificity for a particular antigen. To determine whether the mosquitoes were infected with circum-sporozoite protein an ELISA assay was done. The head and thorax of adult females were separated from the abdomen, placed in 5 ml Eppendorf tubes, macerated and the triturate used for sporozoite ELISA according to Wirtz *et al.* (1987) (Appendix IV).

3.4.5 Parasitological diagnosis of malaria

To estimate malaria prevalence from the three villages, 2-5µl of blood was obtained from the children who accepted to be involved in the study by finger pricking with the assistance of a properly trained and qualified medical laboratory technician. Thin and thick blood films were made on clean slides properly labeled for each participant. The blood collected by finger pricking was used in making thin blood films for species identification while the thick blood films were used for parasite count, densities and stages identification. The smears were allowed to air dry then fixed in methanol and stained in 4% Giemsa for 30 min. The stained smears were then examined using the magnification of × 1,000 (oil immersion) to identify and count the parasite species. A slide was regarded as negative if

the examination of thick films failed to show the presence of asexual parasites. For each study participant, auxiliary temperature was measured, and inquiry was made if they had experienced fever in the previous 24 hours. Those who exhibited fever through digital thermometers and reported fever in the last 24 hrs were further tested on the spot using malaria rapid diagnostic test kits – RDTs (Orchid Biomedical Systems, Goa, India). Those that were positive for *P. falciparum* with RDT were treated according to the national guidelines for treatment of malaria (DOMC, 2010). The number of parasites was done using tally counters record made on data sheets. Hemoglobin was measured by a portable photometer in the field (HemoCue AB, Angelholm, Sweden) at 3 month interval from fingertip capillary blood.

3.4.6 Ethical

Informed consent was sought from all participants of the study and permission was sought from the local leaders and community members to conduct the study within their communities. Permission was also sought from individuals before entering the compounds. The study was granted ethical approval from KEMRI/National Ethics review committee (SSC 1366). Informed consent was issued to those willing to participate in the language of choice (English, Kiswahili or Kinyala) before enrollment into the study. The participant was asked to sign on the consent form if the study procedures have been explained and the participant agrees to participate in the study (Appendix II).

3.5 Statistical analysis

Data was analysed using SPSS v. 12 (SPSS Inc., Chicago, IL, USA) and SAS V.9.0 (SAS Institute Inc, Cary, NC, USA. The number of fed indoor mosquitoes in intervention houses was compared with the number of fed indoor resting mosquitoes in control houses and IRS villages using Poisson regression (SAS V.9.0). Chi-square was used to compare the sporozoite infection rates amongst the intervention, non-intervention and IRS villages (SPSS v. 12). The relationship between *P. falciparum* prevalence and parasite density was determined across age, between intervention non-intervention and IRS villages. To examine the effects of age on parasite prevalence and infection density, ages were stratified into age groups: <1, 1–5, 5–10 and 11–15. Arcsine transformation was done on parasite prevalence then a Chi-square (χ^2) test was used to determine differences in prevalence among age groups and intervention and non-intervention villages (SPSS v. 12). Analysis of variance (ANOVA) with logarithm-transformed parasite density was used to examine the difference among age groups and intervention and non-intervention villages' parasite density (SAS V.9.0). Survivorship of the mosquitoes after exposure was used to determine the efficacy of DL while longevity was determined by mortality of mosquitoes (24 hrs) after exposure over time period. Kaplan-Meier survival (Log-Rank) was used to determine survivorship of mosquitoes between durable lining and IRS (SAS V.9.0). A two sided $P \leq 0.05$ was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Species Composition

A total of 3,817 mosquitoes were collected during the study period. Culicine mosquitoes accounted for 76.4% of all mosquitoes collected; *Anopheles gambiae s.l.* accounted for 18.7% of all mosquitoes collected, while the remaining 4.8% were *An. funestus.* The PCR results showed that 26.6 % were *An. gambiae s.s.;* the remainder were *An. arabiensis* 68.1 %.

4.1.1 Indoor resting densities

Indoor-resting densities were calculated as the number of female *Anopheles gambiae s.l.* and *Anopheles funestus* collected divided by the number of houses sampled/per night during collection. Overall, the indoor resting densities of *An. gambiae s.l.* were 0.80 female/house/night, 0.12 female/house/night and 0.03 female/house/night in non-intervention, intervention and IRS, respectively. For *An. funestus,* the indoor resting densities were 0.17 female/house/night, 0.07 female/house/night, 0.11 for non-intervention, intervention and IRS, respectively. Overall, the indoor resting density of *An. gambiae s.l.* and *An. funestus,* were 5 folds higher in non-intervention villages compared to intervention villages.

Indoor-resting densities of *Anopheles* in the period of study varied for the two species. For non-intervention villages the *An. gambiae* populations were relatively low during March-May, with a peak in June-September and an increase in September 2011- November 2011 (Figure 4a). For *Anopheles funestus,* there was a slight decrease in May 2011-July 2011 and September 2011 but a rise in peak in October-December 2011 in non- intervention area. The mean monthly variation of mosquitoes is shown in Figure 4b.

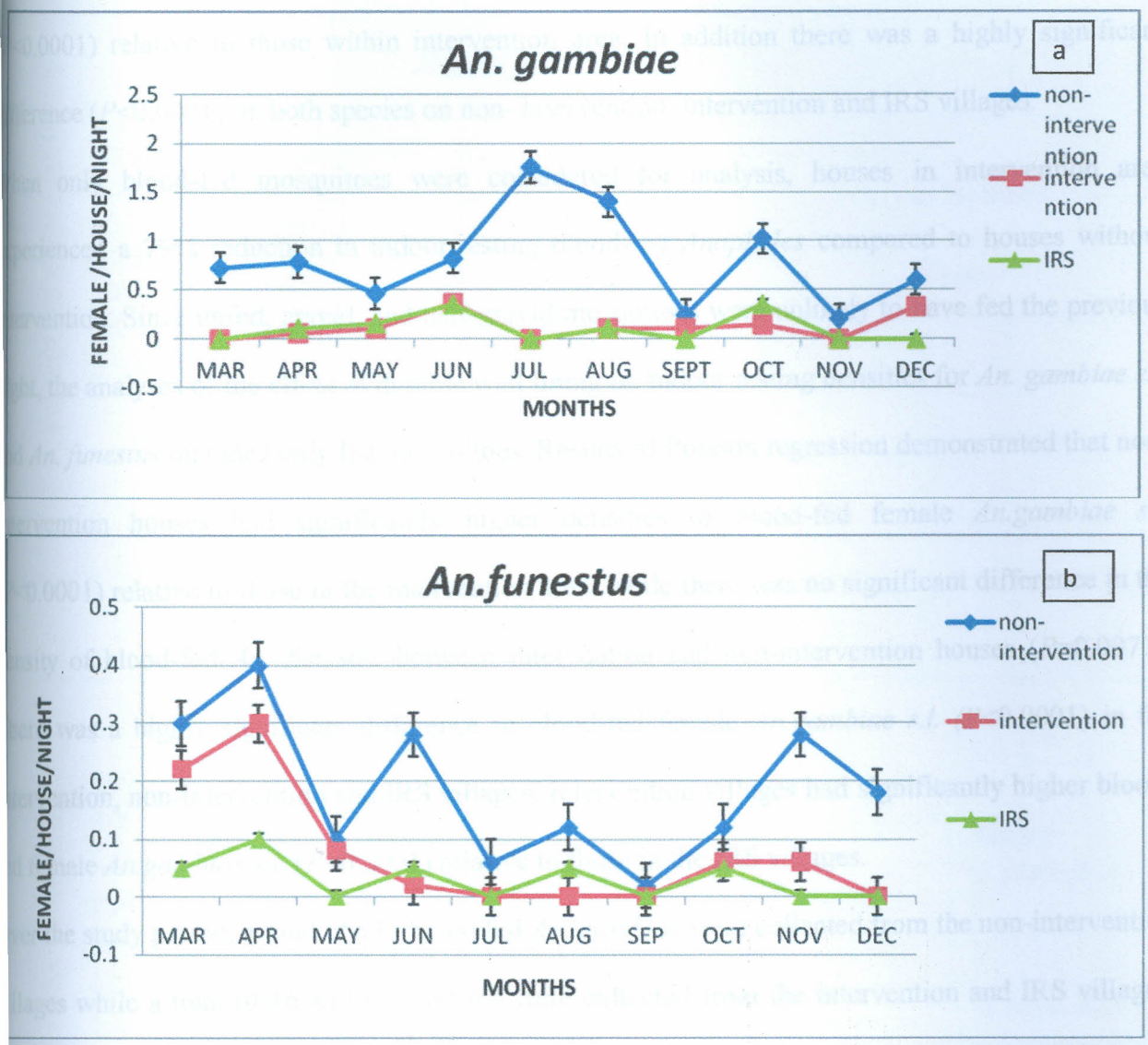


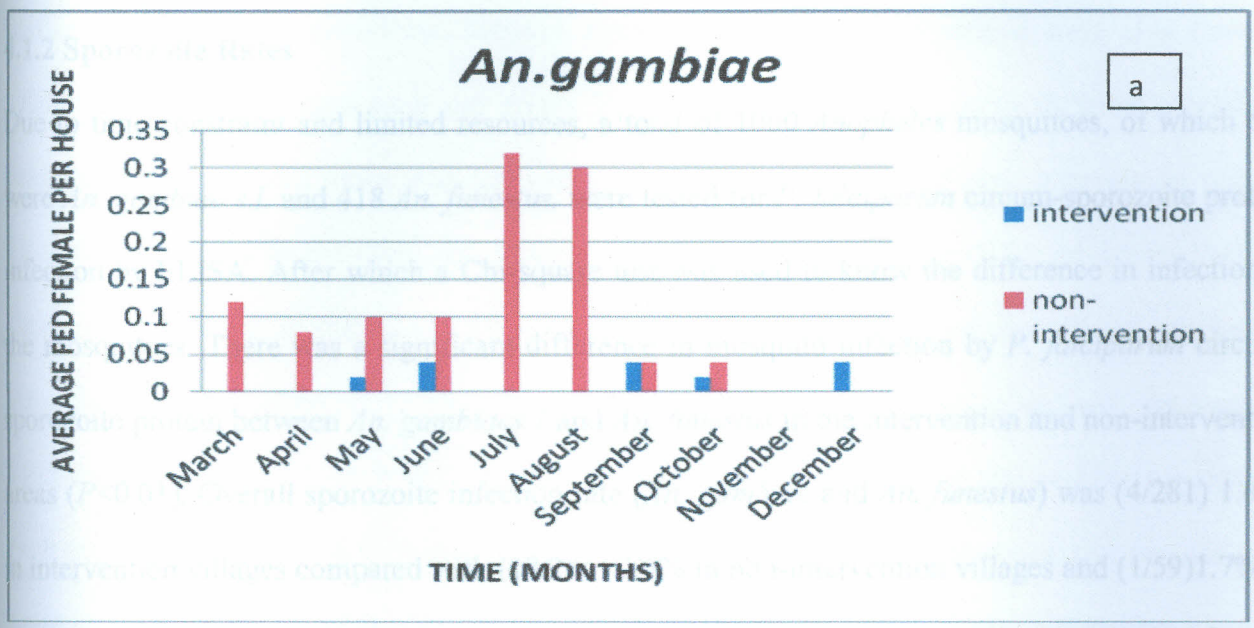
FIGURE 4a,b: Variation in *Anopheles gambiae s.l.* and *Anopheles funestus* mean numbers and standard error of density per house per night in the intervention, non-intervention and IRS.

Analysis done by Poisson regression showed there was a highly significant reduction ($P<0.0001$) when the total number of female mosquitoes, including all the gonotrophic stages were included in analysis; 85% and 60% reduction was observed for *An.gambiae s.l.* and *An. funestus* respectively, between the intervention and non-intervention areas. For both *An. gambiae s.l.* and *An. funestus,* when the total numbers of female mosquitoes, including all the gonotrophic stages were included in analysis, non-intervention houses had significantly higher densities of female *An. gambiae s.l.*

.0001) relative to those within intervention area. In addition there was a highly significant rence ($P<0.0001$) on both species on non- intervention, intervention and IRS villages.

n only blood-fed mosquitoes were considered for analysis, houses in intervention area rienced a 79% reduction in indoor-resting blood-fed *Anopheles* compared to houses without vention. Since unfed, gravid, and half-gravid mosquitoes were unlikely to have fed the previous , the analyses of the effect of durable wall lining on indoor resting densities for *An. gambiae s.l.* *An. funestus* included only fed mosquitoes. Results of Poisson regression demonstrated that non-vention houses had significantly higher densities of blood-fed female *An.gambiae s.l.* .0001) relative to those in the intervention area, while there was no significant difference in the ty of blood-fed *An. funestus* between intervention and non-intervention houses ($P=0.0977$). e was a highly significant difference in blood-fed female *An.gambiae s.l.* ($P<0.0001$) in the vention, non-intervention and IRS villages. Intervention villages had significantly higher blood-emale *An.gambiae s.l.* ($P<0.0001$) relative to those in the IRS villages.

the study period, a total of 110 blood fed *An. gambiae* were collected from the non-intervention ges while a total of 16 and 0 blood fed were collected from the intervention and IRS villages, ctively. On the other hand, a total of 27, 14 and 0 blood fed *An. funestus* was collected in the ntervention, intervention and IRS villages, respectively. The mean number of blood fed helines from March 2011 to December 2011 is shown in Figure 5a. High numbers of blood fed *gambiae* mosquitoes were collected from the non-intervention villages as compared to vention and IRS as shown in Figure 5b. Overall, a decrease in the number of blood fed uitoes in the intervention villages were recorded (Figures 5a, b).



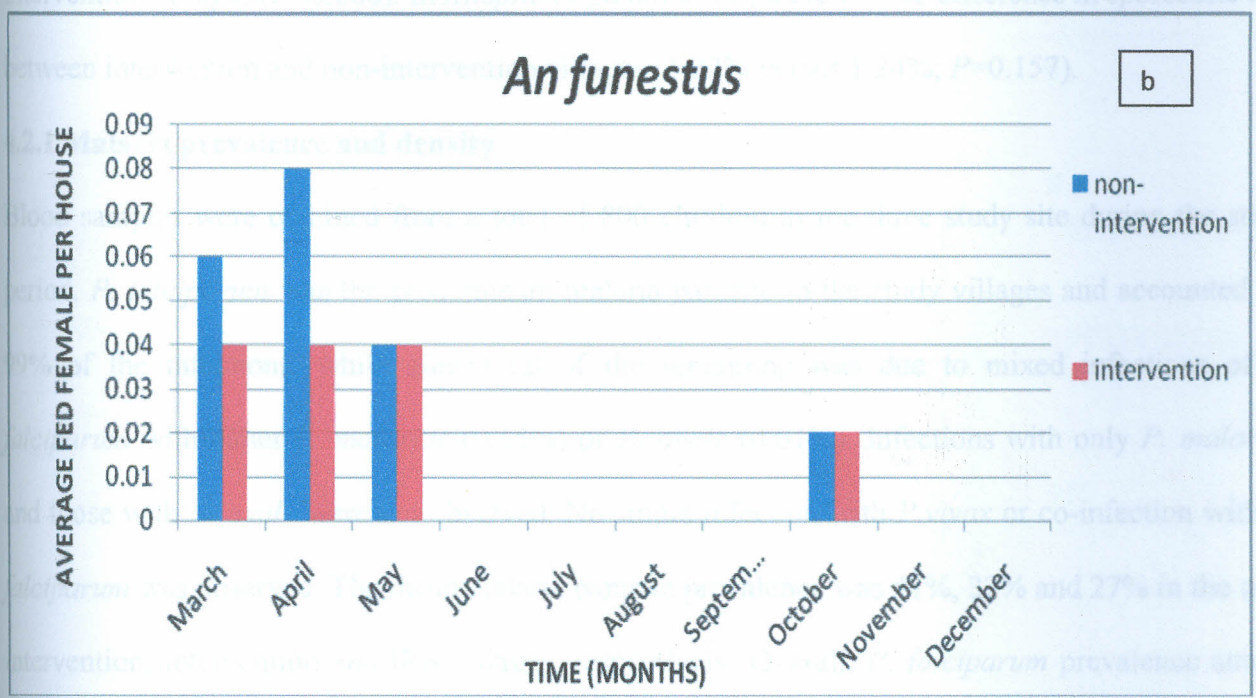


FIGURE 5a,b: Indoor resting densities of blood-fed anopheline mosquitoes across the months in the intervention and non-intervention villages.

4.1.2 Sporozoite Rates

Due to time constraint and limited resources, a total of 1060 *Anopheles* mosquitoes, of which 608 were *An. gambiae s.l.* and 418 *An. funestus,* were tested for *P. falciparum* circum-sporozoite protein infection by ELISA. After which a Chi-square test was used to know the difference in infection in the mosquitoes. There was a significant difference in mosquito infection by *P. falciparum* circum-sporozoite protein between *An. gambiaes.1* and *An. funestus* in the intervention and non-intervention areas ($P<0.01$). Overall sporozoite infection rate (*An. gambiae* and *An. funestus*) was (4/281) 1.4 % in intervention villages compared with (18/286) 2.6% in non-intervention villages and (1/59)1.7% in IRS. Sporozoite rate in *An. funestus* was higher in non- intervention villages (4.58 %) than in intervention villages ($P<0.008$). In *Anopheles gambiae s.l.*, there was no difference in sporozoite rate between intervention and non-intervention villages (1.22% versus 1.24%; $P=0.157$).

4.2.1 Malaria prevalence and density

Blood samples were obtained from a total of 800 children in the three study site during the study period. *P. falciparum* was the predominant malaria parasite in the study villages and accounted for 99% of the infections, while almost all of the remaining was due to mixed infections of *P. falciparum* with either *P. malariae* (0.15%) or *P. ovale* (0.01%). Infections with only *P. malariae* and those with *P. ovale* were not observed. No single infection with *P.vivax* or co-infection with *P. falciparum* was observed. The mean malaria parasite prevalence was 41%, 32% and 27% in the non-intervention, intervention and IRS villages, respectively. Overall, *P. falciparum* prevalence among intervention, non-intervention villages and IRS was comparable insignificant ($\chi^2 = 1.2$, df =2, $P=0.549$). Figure 6 summarizes the temporal dynamics of the *P. falciparum* prevalence (number of positive children/number of children examined) carried out from March- December 2011 in the study villages.

Difference in parasite prevalence between intervention and non-intervention were also assessed. Observations varied across each age group. Prevalence was not significantly different in children less than one year and more than ten years between intervention and non- intervention villages ($P>0.05$), whereas in the other age groups (1-5yrs, $P<0.03$; 6-10yrs, $P<0.01$; 11-15yrs, $P=0.34$), there was reduction in prevalence between intervention and non-intervention villages (Figure 7). Also there was no difference in parasite prevalence between the intervention and non –intervention villages ($\chi^2 = 0.49$, df =1, $P=0.485$). However, children in the non-intervention area had consistently high prevalence average of 41% infection in the three sites of study and the prevalence was still high across all the age groups in the intervention, non-intervention and IRS villages (Figure 7).

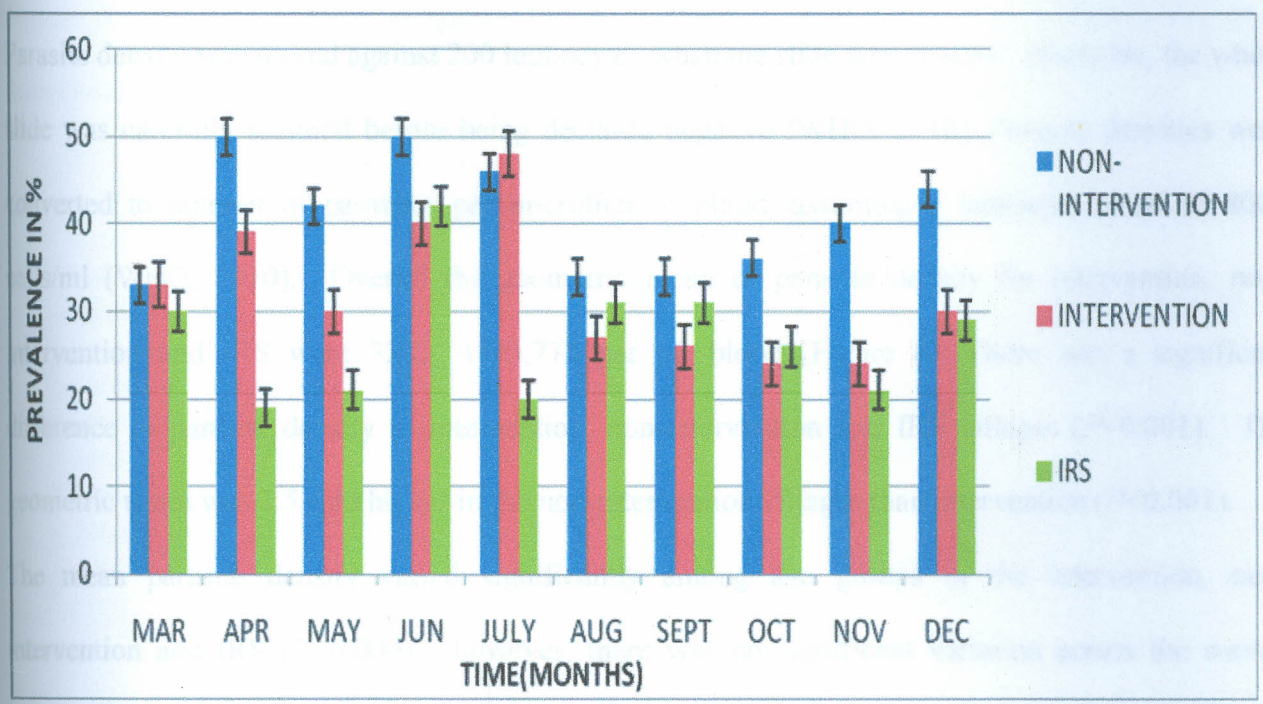


FIGURE 6: Prevalence rate of malaria in the intervention, non-intervention and IRS villages during the survey period. Error bars represent 95% confidence interval.

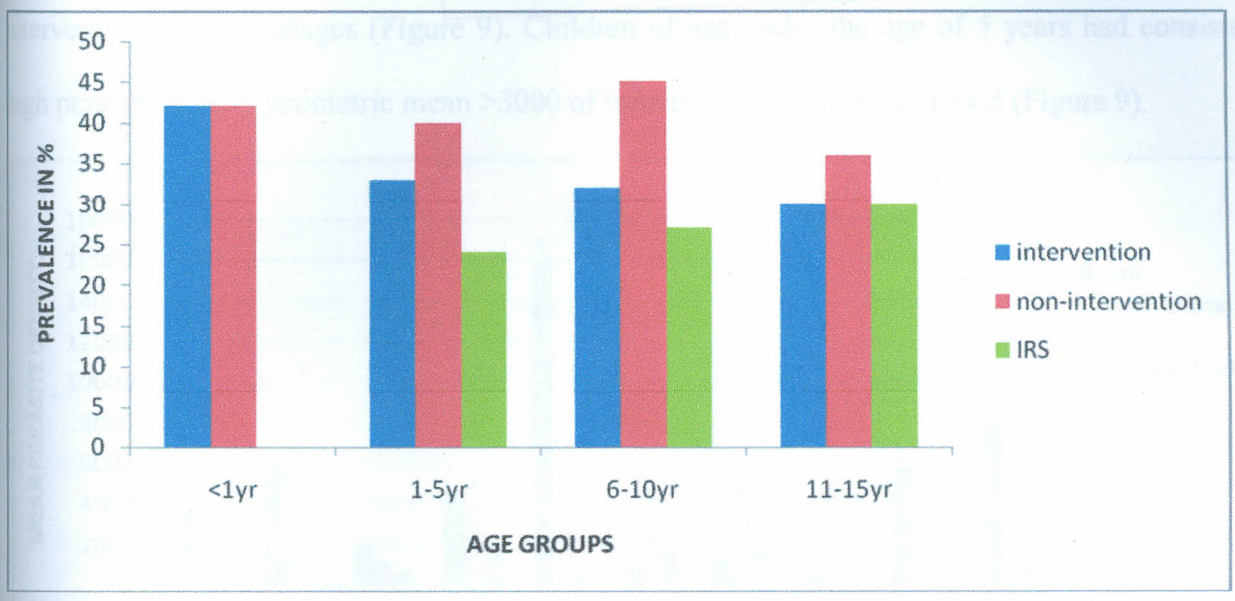


Figure 7: Age-specific prevalence of *Plasmodium falciparum* parasite in the intervention, non-intervention and IRS villages.

Parasite density was scored against 200 leukocytes when the slide was positive; otherwise, the whole slide was carefully scanned before being declared negative (WHO, 2010). Parasite densities were converted to number of parasites per microliter of blood, assuming a leukocyte count of 8000 cells/ml (WHO, 2010). Overall the geometric mean of parasite density for intervention, non-intervention and IRS were 3282, 4893,773 per µL blood (Figure 8). There was a significant difference in parasite density in intervention, non-intervention and IRS villages ($P<0.001$). The geometric mean was 1.5 fold higher in the non-intervention villages than intervention ($P<0.001$).

The mean parasite density varied significantly among age groups in the intervention, non-intervention and IRS ($P<0.0001$); however, there was no significant variation across the survey months ($P=0.07$). On the other hand, when comparison was made between the intervention and non-intervention areas, parasite density did not vary significantly among survey months ($P=0.17$), but the variation was highly significant among age groups ($P<0.0001$; Figure 9). Parasite densities declined inversely with the age of the children irrespective of the whether it was in the intervention, non-

intervention or IRS villages (Figure 9). Children of age under the age of 5 years had consisten high parasite density geometric mean >3000 of infected erythrocytes/μL blood (Figure 9).

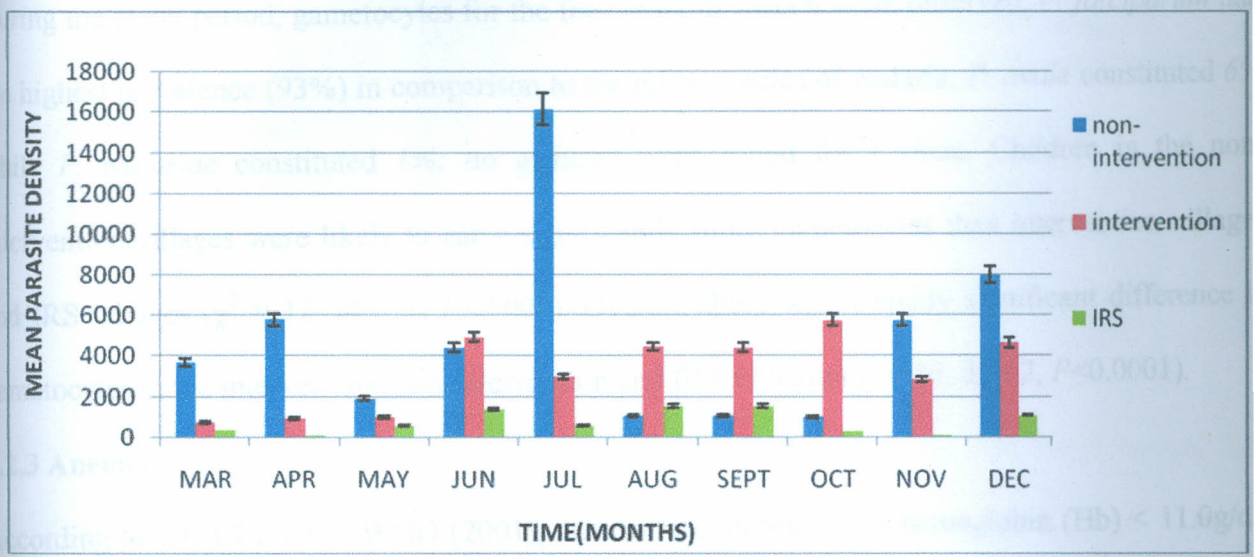


Figure 8: Geomtric mean of parasite per microliter of blood in the intervention, no interventioon and IRS villages. Error bars represent 95% confidence interval.

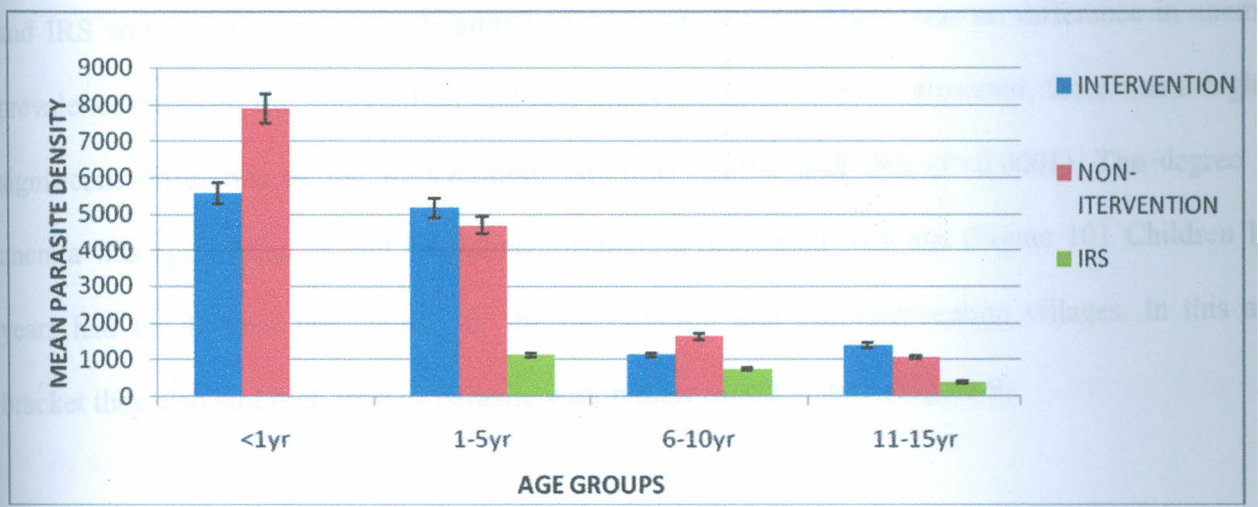


FIGURE 9: Age specific geometric mean of *P. falciparum*–infected erythrocytes per microlit of blood. Error bars represent 95% confidence interval.

4.2.2 Gametocyte prevalence

During the study period, gametocytes for the four malaria species were observed. *P. falciparum* had the highest prevalence (93%) in comparison to the other species of malaria; *P. ovale* constituted *6%* while *P. malariae* constituted *1%; no* gametes were found for *P.vivax.* Children in the non-intervention villages were likely to carry significantly more gametocytes than intervention villages and IRS villages (χ^2 = 12, df = 1, P<0.001). Overall, there was a highly significant difference in gametocytes in the intervention, non-intervention and IRS villages (χ^2 = 56, df = 2, P<0.0001).

4.2.3 Anemia

According to UNICEF/UNU/WHO (2001), anemia was defined as a hemoglobin (Hb) < 11.0g/dL and categorised as mild (Hb 9.0-10.9 g/dL), moderate (Hb 6.0-8.9g /dL) or severe (Hb< 6.0 g/dL). A total of 379 (8%) children were anemic. Of these, severe anemia (SA) comprised 0.79%, moderate anemia (MA) 15.5% and mild (MD) 83.6%. The mean Hb levels for intervention, non-intervention and IRS were 11.47g/dL,11.7g/dL and12.3g/dL, respectively. There was no difference in anemia prevalence between the intervention and non-intervention (P=0.670). However, there was a highly significant difference in the intervention, non-intervention and IRS (P<0.0001). The degree of anemia was age-related as and prevalence of anemia decreased with age (Figure 10). Children 1-5 years had the highest prevalence for the intervention and non-intervention villages. In this age bracket they also had high load of parasite within their blood system (Figure 9).

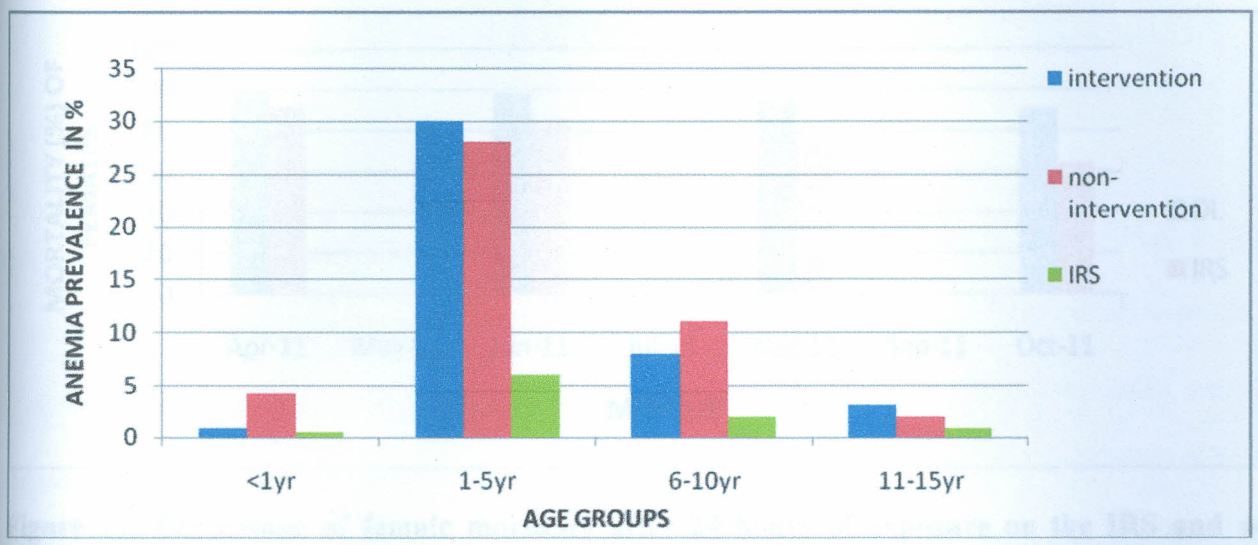


Figure: 10 Prevalence of anemia across specific age groups in the intervention, non-intervention, and IRS villages.

4.3 Durability and longevity of DL and IRS

4.3.1 WHO ASSAY

WHO susceptibility test was done on DL and IRS to evaluate their efficacy to retain their insecticidal power against *Anopheles* mosquito. Average 24-h post-exposure mortalities of *An.gambiae* exposed to DL and IRS after intervention are presented in Figure 11. DL and IRS caused more than 80% mortality in *An.gambiae* with 3-min exposure, at the onset of the assay, but the percentage of mortality gradually decreased to 64% after 8 months of use on IRS wall, while more than 80% mortality was still recorded in DL at the same period after use. To test the difference in DL and IRS survival analysis test was done.

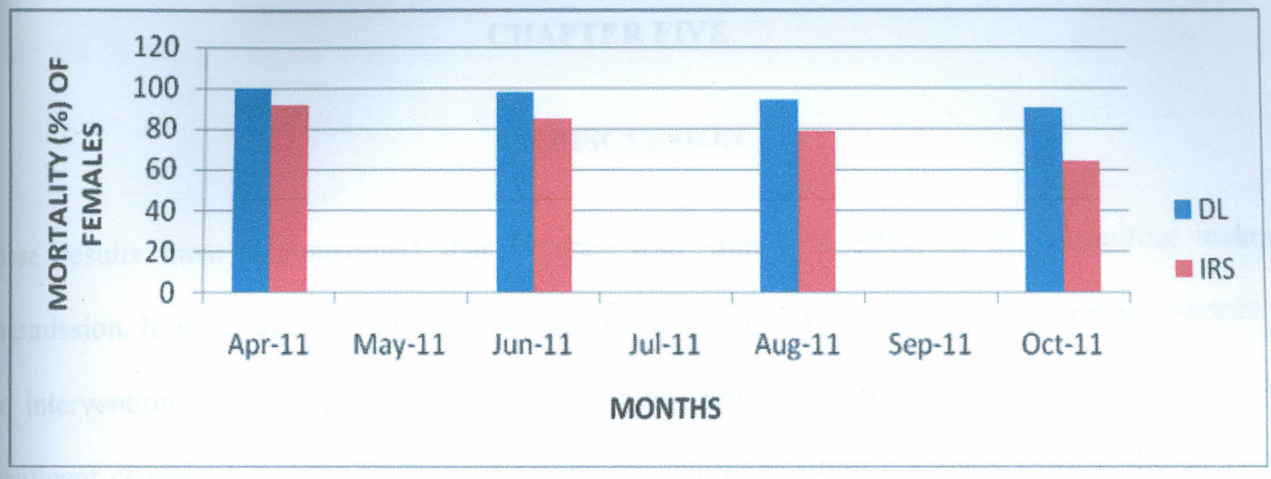


Figure 11: Percentage of female mortality after 24 hours of exposure on the IRS and wall linining villages during the period of study.

The mortality rates and the survival estimates of treatments remaining effective in killing the Kisumu susceptible train of *An. gambiae s.s.* on DL and IRS is shown in Figure 12. In IRS there was a higher survival of mosquitoes compared to DL (Log-Rank) analysis revealed significant differences (χ^2=4.92, df = 1, P=0.030).

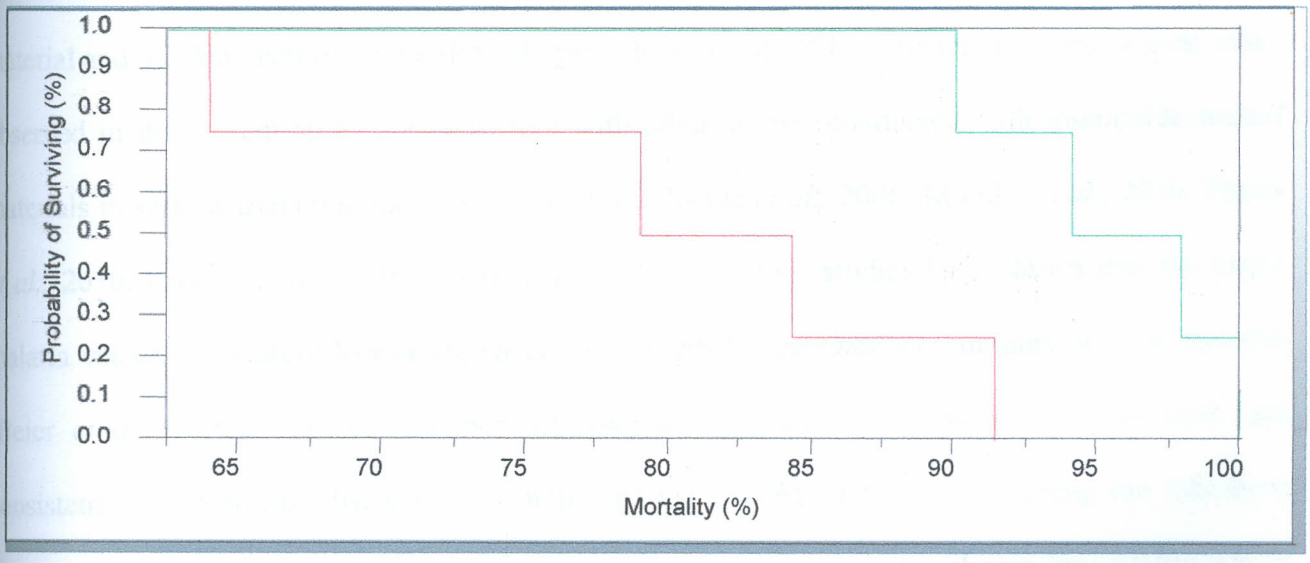


Figure: 12 Kaplan-Meier survival curves with mortality interval for each group IRS and DL. Red line represents the IRS while the green wall lining.

CHAPTER FIVE

5.0 DISCUSSION

These results have demonstrated that durable wall lining is effective in controlling malaria transmission. In general, there was a remarkable decline in the two most important malaria vectors in the intervention area compared to non-intervention and IRS villages. However, there was no significant change in malaria prevalence among the children within the study sites. Children below five years had the greatest parasite burden compared to the other older children. Durable lining retained high levels of insecticide, good performance and high mortality in WHO cone bioassays.

The current study found that there was 81% reduction in the indoor resting densities of mosquitoes in intervention areas compared with non-intervention areas. There was a significant decline in *Anopheles gambiae s.l.* and *An. funestus* by 85% and 60%, respectively. Reduction in the mosquito density could be explained by mass killing of DL or the exito-repellent effect of the insecticide in the material and the deltamethrin in the IRS villages. These results of DL impact on entomological index observed in the current study are consistent with other studies conducted with insecticide treated materials in various trials (Gimnig *et al.*, 2003b; Lindblade *et al.*, 2006; Mutuku *et al.*, 2010; Pluess *et al.*, 2010; Russell *et al.*, 2010; Zhou *et al.*, 2010). Previous studies have shown that the major malaria vectors in western Kenya are species of *Anopheles gambiae s.l.* complex and *An. funestus* (Beier *et al.*, 1990). The low numbers of *Anopheles gambiae s.s.* observed in this study are consistent with previous observations which reported decline of the species along the lakeshore (Mathias *et al.*, 2011). For *An. funestus*, the low estimate is consistent with previous studies which showed that it is highly susceptible to chemical control (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987; Gimnig *et al.*, 2003a). The proportion of culicines found was high since they are not

affected by the insecticide used for control (Lindblade *et al.*, 2006). From the results of this study, *Anopheles gambiae s.s.* constituted 26.6%, while *Anopheles arabiensis* constituted 68.1%. This is similar to the shifts in sibling species composition due to selective pressure of domestic insecticide interventions previously recorded for *A. gambiae,* relative to *A. arabiensis* in western Kenya (Bayoh *et al.*, 2010; Lindblade *et al.*, 2006). Mean density of both intervention and non-intervention houses experienced upsurges in mosquito during the month of June-august 2011 and September- November 2011; increase was much higher for the households without intervention. This is due to the effect of long and short rains during these seasons, thus increasing the breeding sites for mosquitoes. Despite these factors, there was a significant reduction of mosquitoes between the interventions and non-intervention and IRS, where there was 4 folds reduction in study villages. This indicates that DL provided a significant deterrent effect, thus suggesting its prevention capacity. Blood-fed mosquitoes were few in intervention and IRS areas. This could be due to frustration of female mosquitoes, making repeated flights on the wall, thus picking the lethal dose and also possibly the exito-repellency effect of the pyrethroid. DL decreased the proportion of blood-fed mosquitoes during the previous night. This is similar to previous observations (Gimnig *et al.*, 2003b; Mathenge *et al.*, 2001) who found reduction in blood-fed mosquitoes in intervention area. There was no difference observed for *Anopheles funestus* blood-fed mosquitoes and this may be because majority died or presumably left unfed before making contact with the host. In addition in this study, the proportion of sporozoite-infected mosquitoes (sporozoite rate) was found to be quite variable from intervention to non-intervention and IRS villages. The sporozoite rate was 1.4 % in intervention villages compared with 2.6% in non-intervention and 1.7% in IRS villages. There was a remarkable difference in sporozoite rate for *Anopheles gambiaes.l.* 1.22% in the intervention and 1.24% in the non-intervention while for *Anopheles funestus* 1.69% in the intervention and 4.58 % in the non-intervention ($P<0.01$). This

areas like Budalangi. Also the present study has shown a considerable reduction of geometric mean of parasite following distribution of DL with slight reduction in the intervention villages compared to non-intervention area. In addition reduction was also noted in the IRS villages. This reduction is probably a result of repellent and killing action of the DL and IRS on malaria vectors. Studies done in similar setting of intense perennial transmission have demonstrated reduction in parasitemia with introduction of insecticide-treated nets (Gimnig *et al.*, 2003b; Hawley *et al.*, 2003; ter Kuile *et al.*, 2003). The main burden of malaria also occurred in the first 5 years of life and decrease in burden as age increased. This suggests development of immunity due to life-long exposure (Munyekenye *et al.*, 2005). These is similar to previous studies which have shown that in areas of stable transmission, higher prevalence and parasitemia of malaria is associated with children of younger age (Lengeler, 2004). The age related burden has been is consistent with previous studies (Binka *et al.*, 1998; Burns *et al.*, 2012; Munyekenye *et al.*, 2005). Significant change in gametocytes was seen in the intervention areas. The marked difference can be partly attributed to the protective efficacy of DL which reduced vector density. *P. falciparum* gametes constituted the greatest load thus indicating that it was responsible for malaria infection in the children. However, the other species cannot be ruled out. This study showed that the numbers of children with anemia were few (8%); those with severe anemia were less than 1% in the villages under study. Consistent with other studies (Greenwood *et al.*, 1991) the study suggests severe anemia is a rare occurrence in the community. Unlike other studies which have reported that anemia a common feature of malaria infection (ter Kuile *et al.*, 2003) the finding reveals that this is not always so; it may vary depending on the environment. Majority of children (83.6%) in the study had mild anemia despite the parasitic load in the blood system. The lack of significant changes in hemoglobin levels among children in this study is likely due to the high level of access to local diet, which includes fish from the lake.

Efficacy testing of DL showed consistency in results for 10 months of use and stay on the wall. IRS efficacy tested on the wall in the houses revealed decrease in mortality which was observed after six months where bioassay activity had been conducted in the field for a period of 10 months. Unlike DL; though they have similar chemical content it retained insecticide during the bioassay period hence maintaining its biological activity for the 10 months. In the current study DL after 10 months of use was still found effective and provided mortality of more than 80% in bioassay test compared to IRS whose mortality was less than 64% after the same time. IRS results are in line with recent studies done on South Cameroon which have observed similar trend of deltamethrin on the surface of the wall (Etang *et al.*,2011). In another recent knowledge attitude and practice study in Angola and Nigeria DL has been found to be effective in killing malaria vector for one year compared to IRS(Messenger *et al.*, 2012). The use of DL as a long-lasting alternative to IRS has the potential to be widely implemented at community level with relatively limited technical or logistical infrastructure in place. Logistically DL could be cheaper than IRS since it has the ability to last for more than two years. Also DL might be more cost-effective than IRS which requires vertical organization and repeated six-monthly campaign. In addition the type of surface does not matter for DL like IRS which requires smooth and non -porous surfaces which does not guarantee uniform application. Other studies involving durable lining (Chandre *et al.*, 2010; Graham *et al.*, 2002), have reported that they could retain their killing ability for a duration of one year and have the potential of lasting longer. This study provides good quality, first baseline data on the effectiveness of DL in malaria control. In addition, these data provide the basis for assessment of the suitability of DL in diverse settings with varying environmental conditions to ascertain whether it could be used in all settings. Significant levels of vector mortality observed in the bioassay and the reduction in indoor resting *Anopheles* population for a period of ten months in the villages with treated lining compared to the villages with untreated DL,

are indications of possible impact of durable lining in curtailing malaria in the two-peak transmission seasons. Taken together, these results suggest that DL provided high efficacy against malaria vectors, including reduction in indoor-resting density of anopheline mosquitoes, and reduction of gametocytes. Moreover, DL retained high levels of insecticide, good performance and high mortality in WHO cone bioassays. In the present study, DL was monitored for only 10 months after distribution. Future studies are required to investigate the long-lasting efficacy of DL over, at least, three years under field conditions.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. DL has been found to be effective in reducing vector density in the study area.
2. Malaria prevalence was high in the children in the study villages; however parasite density was heavier in children less than five years.
3. DL has shown remarkable bio-efficacy, thus has the potential of the insecticide lasting longer and remaining more killing power compared to IRS.

6.2 Recommendations

1. The current study has demonstrated that DL is effective in reducing resting indoor densities of malaria vectors. Based on the observed results, wide-scale use of DL could complement the existing malaria control tools.
2. Prevalence of infection in children was high; this calls for increase in malaria control strategies to this vulnerable group. In addition, health talks on prevention, thus impact knowledge on malaria transmission, hence preventing further manifestations of infection.
3. DL has shown remarkable durability, hence should be explored as an alternative for IRS.

6.3 Recommendations for future research

1. DL has been found to be effective in reducing vector density in the study area. Based on this observation, further research needs to be done to determine its effectiveness in areas where pyrethroid resistance is high.

2. Prevalence of infection in children was high, thus due to natural variation in geographical, seasonal and annual malaria transmission, repeated cross-sectional household level impact evaluations are recommended to help establish trends over time. These represent key research and programmatic follow-up issues of malaria control in western Kenya in endemic areas.

3. Further research and follow-up on insecticide retention on DL, represent key research areas; its durability after years of consistent stay on the wall without interference also needs further investigation.

REFERENCES

Alaii, J.A., Hawley, W.A., Kolczak, M.S., ter Kuile, F.O., Gimnig, J.E., Vulule, J.M., Odhacha, A. Oloo, A.J., Nahlen, B.L., Phillips-Howard, P.A. (2003). Factors affecting use of permethrin treated bed nets during a randomized controlled trial in western Kenya. *Am. J.Trop. Med. Hyg.* **68**:137-141.

Anto, F., Asoala, V., Anyorigiya, T., Oduro, A., Adjuik, M., Owusu-Agyei, S., Dery, D., Bimi, L. Hodgson, A. (2009). Insecticide resistance profiles for malaria vectors in the Kassena-Nankan district of Ghana. *Malar. J.*, **8**:81.

Atieli, F.K., Munga, S.O., Ofulla, A.V., Vulule, J.M. (2010). The effect of repeated washing of long lasting insecticide-treated nets (LLINs) on the feeding success and survival rates of Anophele gambiae. *Malar. J.*, **9**:304.

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

Banek, K., Kilian,A., Allan,R.,(2010).Evaluation of Interceptor long-lasting insecticidal nets in eigh communities in Liberia. *Malar. J.*, **9**:84.

Beier, J.C., Keating, J., Githure, J.I., Macdonald, M.B., Impoinvil, D.E., Novak, R.J. (2008 Integrated vector management for malaria control. *Malar. J.*, 7 (Suppl 1): S4.

Beier, J.C., Perkins, P.V., Onyango, F.K., Gargan, T.P., Oster, C.N., Whitmore, R.E., Koech, D.K Roberts, C.R. (1990). Characterization of malaria transmission by Anopheles (Dipter Culicidae) in preparation for malaria vaccine trials. *J. Med.Entomol.*, **27**:570-577.

Bhattarai, A., Ali, A.S., Kachur, S.P., Martensson, A., Abbas, A.K., Khatib, R., Al-Mafazy, A.W., Ramsan, M., Rotllant, G., Gerstenmaier, J.F., Molteni, F., Abdulla, S., Montgomery, S.M., Kaneko, A., Bjorkman, A. (2007). Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. PLoS .Med., **4**:e309.

Binka, F.N., Indome, F., Smith, T. (1998). Impact of spatial distribution of permethrin-impregnated bed nets on child mortality in rural northern Ghana. *Am.J. Med. Trop. Hyg.*, **59:**80-85.

Brogdon, W.G., McAllister, J.C. (1998). Insecticide resistance and vector control. *Emerg. Infect. Dis.*, **4**:605-613.

Burns, M., Rowland M, N'Guessan, Carneiro, R.I., Beeche, A., Ruiz, S.S., Kamara, S., Takken, W., Carnevale, P., Allan, R. (2012). Insecticide-Treated Plastic Sheeting for Emergency Malaria Prevention and Shelter among Displaced Populations: An Observational Cohort Study in a Refugee Setting in Sierra Leone. *Am. J. Trop. Med. Hyg.*, **87**:242-250.

Chandre, F., Dabire, R.K., Hougard, J.M., Djogbenou, L.S., Irish, S.R., Rowland, M., N'Guessan, R. (2010).Field efficacy of pyrethroid treated plastic sheeting (durable lining) in combination with long lasting insecticidal nets against malaria vectors. *Parasit. Vectors.*, **3**:65.

Chima, R.I., Goodman, C.A., Mills, A. (2003). The economic impact of malaria in Africa: a critical review of the evidence. *Health policy (Amsterdam, Netherlands).*, **63:**17-36.

Chuma, J., Gilson, L., Molyneux, C. (2007). Treatment-seeking behaviour, cost burdens and coping strategies among rural and urban households in Coastal Kenya: an equity analysis. *Trop. Med. Int. Health.*, **12**:673-686.

Chuma, J.M., Thiede, M., Molyneux, C.S. (2006) Rethinking the economic costs of malaria at the household level: evidence from applying a new analytical framework in rural Kenya. *Malar. J.*, **5**:76.

Cohen, J., Dupas, P. (2010). Free distrbution or cost sharing.Evidence from a randomized malaria prevention experiment. *Quat. J. Econ.*, **125**:1-45.

Collins, F.H., Paskewitz, S.M. (1995). Malaria: current and future prospects for control. *Annu. Rev. Entomol.*, **40:**195-219.

Cuamba, N., Morgan, J.C., Irving, H., Steven, A., Wondji, C.S. (2010). High level of pyrethroid resistance in an Anopheles funestus population of the Chokwe District in Mozambique. *PLoS. One.*, **5**: e11010.

Dabire, R.K., Diabate, A., Baldet, T., Pare-Toe, L., Guiguemde, R.T., Ouedraogo, J.B., Skovmand, O. (2006). Personal protection of long lasting insecticide-treated nets in areas of Anopheles gambiae s.s. resistance to pyrethroids. *Malar. J.*,**5:**12.

Darriet, F., N'Guessan, R., Carnevale, P. (2000).[Evaluation in test huts of the protective effects of untreated mosquito nets against Anopheles gambiae s.s. bites]. *Sante.*, **10**:413-417.

DOMC, (2010). Malaria communication 2010-2013.

Enayati, A., Hemingway, J. (2010). Malaria management: past, present, and future. *Annu. Rev. Entomol.*, **55**:569-591.

Etang, J., Nwane, P., Mbida, J.A., Piameu, M., Manga, B., Souop, D., Awono-Ambene, P. (2011). Variations of insecticide residual bio-efficacy on different types of walls: results from a community-based trial in south Cameroon. *Malar. J.*, **10**:333.

Friedman, J.F., Phillips-Howard, P.A., Hawley, W.A., Terlouw, D.J., Kolczak, M.S., Barber, M., Okello, N., Vulule, J.M., Duggan, C., Nahlen, B.L., ter Kuile, F.O. (2003). Impact of permethrin-treated bed nets on growth, nutritional status, and body composition of primary school children in western Kenya. *Am. J. Trop. Med.*, **68**:78-85.

Gamble, C., Ekwaru, P.J., Garner, P., ter Kuile, F.O. (2007). Insecticide-treated nets for the prevention of malaria in pregnancy: a systematic review of randomised controlled trials. *PLoS. Med.*, **4**:e107.

Gillies, M.T., and, De Meillon, B. (1968). The Anophelinae of Africa, South of the Sahara. Johannesburg, South Africa. South African Institute of Medical Research N0 54

Gillies, T., and , Coetzee, M. (1987). Supplement of the Anopheles of Africa South of Sahara (Afro tropical Region) Johannesburg, Republic of South Africa. Publication of the South Africa Institute of Medical Research N0 55.

Gimnig, J.E., Kolczak, M.S., Hightower, A.W., Vulule, J.M., Schoute, E., Kamau, L., Phillips-Howard, P.A., ter Kuile, F.O., Nahlen, B.L., Hawley, W.A. (2003a). Effect of permethrin-treated bed nets on the spatial distribution of malaria vectors in western Kenya. *Am. J. Trop. Med. Hyg.*, **68**:115-120.

Gimnig, J.E., Vulule, J.M., Lo, T.Q., Kamau, L., Kolczak, M.S., Phillips-Howard, P.A., Mathenge, E.M., ter Kuile, F.O., Nahlen, B.L., Hightower, A.W., Hawley, W.A. (2003b). Impact of permethrin-treated bed nets on entomologic indices in an area of intense year-round malaria transmission. *Am. J. Trop. Med. Hyg.*, **68**:16-22.

Githinji, S., Herbst, S., Kistemann, T., Noor, A.M., (2010). Mosquito nets in a rural area of Western Kenya: ownership, use and quality. *Malar. J.*, **9**:250.

Govella, N.J., Okumu, F.O., Killeen, G.F. (2010). Insecticide-treated nets can reduce malaria transmission by mosquitoes which feed outdoors. *Am. J. Trop. Med. Hyg.*, **82:** 415-419.

Graham, K., Mohammad, N., Rehman, H., Nazari, A., Ahmad, M., Kamal, M., Skovmand, O., Guillet, P., Allan, R., Zaim, M., Yates, A., Lines, J., Rowland, M. (2002). Insecticide-treated plastic tarpaulins for control of malaria vectors in refugee camps. *Med.Vet. Entomol.*, **16:**404-408.

Gramiccia, G., and Beales, P. (1988). The recent history of malaria control and eradication. In Malaria: Principles and practices of malariology. (Wernsdorfer and Mc Gregor Eds) Churchill London, pp 1335-1378.

Greenwood, B., Marsh, K., Snow, R. (1991). Why do some African children develop severe malaria? *Parasitol. Today.*, (Personal ed 7:277-281.

Hawley, W.A., Phillips-Howard, P.A., ter Kuile, F.O., Terlouw, D.J., Vulule, J.M., Ombok, M., Nahlen, B.L., Gimnig, J.E., Kariuki, S.K., Kolczak, M.S., Hightower, A.W. (2003). Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in western Kenya. *Am. J. Trop. Med. Hyg.*, **68**:121-127.

Hay, S.I., Guerra, C.A., Gething, P.W., Patil, A.P., Tatem, A.J., Noor, A.M., Kabaria, C.W., Manh, B.H., Elyazar, I.R., Brooker, S., Smith, D.L., Moyeed, R.A., Snow, R.W. (2009). A world malaria map: Plasmodium falciparum endemicity in 2007. *PLoS. Med.*, **6:**e1000048.

Henry, M.C., Doannio, J.M., Darriet, F., Nzeyimana, I., Carnevale, P. (1999). [Efficacy of permethrin-impregnated Olyset Net mosquito nets in a zone with pyrethroid resistance vectors. II. Parasitic and clinical evaluation]. *Med. Trop.* (Mars)., **59:** 355-357.

Hill, J., Lines, J., Rowland, M. (2006). Insecticide-treated nets. *Adv. Parasitol.*, **61**:77-128.

Kish, S., N'Guessan, R., Boko, P., Metonnou, C., Odjo, A., Akogbeto, M., Rowland, M. (2008). Loss of protection with insecticide-treated nets against pyrethroid-resistant Culex quinquefasciatus mosquitoes once nets become holed: an experimental hut study. *Parasit. Vectors.*, **1:**17.

Kawashita, H., Dida, G., Futami, K., (2010) Sleeping arrangement and house structure affect bed net use in villages along Lake Victoria. *Malar. J.*, **9**:176.

John, C.C., Riedesel, M.A., Magak, N.G., Lindblade, K.A., Menge, D.M., Hodges, J.S. Vulule, J.M., Akhwale, W. (2009). Possible interruption of malaria transmission, highland Kenya, 2007-2008. *Emerg. Infect. Dis.*, **15:**1917-1924.

Kenya Malaria Indicator Survey (KIMS) (2010). Division of Malaria Control, Kenya National Bureau of Statistics and ICF Macro (2010). Division of Malaria Control, Nairobi, Kenya.

Le Menach, A., Takala, S., McKenzie, F.E., Perisse, A., Harris, A., Flahault, A., Smith, D.L. (2007). An elaborated feeding cycle model for reductions in vectorial capacity of night-biting mosquitoes by insecticide-treated nets. *Malar. J.*, **6:**10.

Leighton, C., and Foster, R. (1993). Economic impacts of malaria in Kenya and Nigeria. Major applied research paper HFs project.

Lengeler, C. (2004). Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database System.

Lindblade, K.A., Dotson, E., Hawley, W.A., Bayoh, N., Williamson, J., Mount, D., Olang, G., Vulule, J., Slutsker, L., Gimnig, J. (2005). Evaluation of long-lasting insecticidal nets after 2 years of household use. *Trop. Med. Int .Health.*, **10:**1141-1150.

Lindblade, K.A., Gimnig, J.E., Kamau, L., Hawley, W.A., Odhiambo, F., Olang, G., Ter Kuile, F.O., Vulule, J.M., Slutsker, L. (2006). Impact of sustained use of insecticide-treated bednets on malaria vector species distribution and culicine mosquitoes. *J. Med. Entomol.*, **43**:428-432.

Majori, G., Sabatinelli, G., Coluzzi, M. (1987). Efficacy of permethrin-impregnated curtains for malaria vector control. *Med .Vet. Entomol.*, **1:**185-192.

Malcolm, C.A. (1988). Current status of pyrethroid resistance in anophelines. *Parasitol. Today.*, **4:** S13-15.

Mathenge, E.M., Gimnig, J.E., Kolczak, M., Ombok, M., Irungu, L.W., Hawley, W.A. (2001). Effect of permethrin-impregnated nets on exiting behavior, blood feeding success, and time of feeding of malaria mosquitoes (Diptera: Culicidae) in western Kenya. *J. Med. Entomol.*, **38:** 531-536.

Mathias, D.K., Ochomo, E., Atieli, F., Ombok, M., Bayoh, N.M., Olang, G., Muhia, D., Kamau, L., Vulule, J.M., Hamel, M.J., Hawley, W.A., Walker, E.D., Gimnig, J.E. (2011). Spatial and temporal variation in the kdr allele L1014S in *Anopheles gambiae s.s.* and phenotypic variability in susceptibility to insecticides in Western Kenya. *Malar. J.*, **10**:10.

Mbogo, C.N., Baya, N.M., Ofulla, A.V., Githure, J.I., Snow, R.W. (1996). The impact of permethrin-impregnated bednets on malaria vectors of the Kenyan coast. *Med. Vet. Entomol.*, **10**: 251-259.

Messenger, L.A., Miller, N.P., Adeogun, A.O., Awolola, T.S., Rowland, M. (2012). The development of insecticide-treated durable wall lining for malaria control:insights from rural and urban populations in Angola and Nigeria. *Malar. J.*, **11:**332.

Michalakis, Y., Renaud, F. (2009). Malaria: Evolution in vector control. *Nature.*, **462**:298-300.

Munyekenye, O.G., Githeko, A.K., Zhou, G., Mushinzimana, E., Minakawa, N., Yan, G. (2005). *Plasmodium falciparum* spatial analysis, western Kenya highlands. *Emerg. Infect. Dis.*, **11:** 1571-1577.

Mutuku, F.M., Charles, H.K., Peter, M., Charles, M., Mwangangi, J., Muchiri, E.M., walker, E.D., Kitron, U. (2010). Impact of insecticide-treated bed nets on malaria transmission indices on the south coast of Kenya. *Malar.J.*, **10:**356.

N'Guessan, R., Corbel, V., Akogbeto, M., Rowland, M. (2007). Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerg. Infect. Dis.*, **13**:199-206.

Najera, J.A., and Zaim, M. (2001). Insecticides for indoor residual spraying. *World Health Organization*, Geneva.

Nevill, C.G., Some, E.S., Mung'ala, V.O., Mutemi, W., New, L., Marsh, K., Lengeler, C., Snow, R.W. (1996). Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. *Trop. Med. Int. Health.*, **1**:139-146.

NMS, (2009). National malaria stratedy. Division of malaria control.Ministry of public health and sanitation.

Noor, A.M., Gething, P.W., Alegana, V.A., Patil, A.P., Hay, S.I., Muchiri, E., Juma, E., Snow, R.W. (2009). The risks of malaria infection in Kenya in 2009. *BMC. Infect. Dis.*, **9**:180.

Noor, A.M., Kirui, V.C., Brooker, S.J., Snow, R.W., (2009b). The use of insecticide treated nets by age: implications for universal coverage in Africa. *BMC Public Health* **9**:369

Okiro, E.A., Alegana, V.A., Noor, A.M., Snow, R.W. (2010). Changing malaria intervention coverage, transmission and hospitalization in Kenya. *Malar. J.*, **9**:285.

Okiro, E.A., Hay, S.I., Gikandi, P.W., Sharif, S.K., Noor, A.M., Peshu, N., Marsh, K., Snow, R.W. (2007). The decline in paediatric malaria admissions on the coast of Kenya. *Malar. J.*, **6**:151.

Opiyo, P., Mukabana, W.R., Kiche, I., Mathenge, E., Killeen, G.F., Fillinger, U. (2007). An exploratory study of community factors relevant for participatory malaria control on Rusinga Island, western Kenya. *Malar. J.*, **6**:48.

Pates, H., Curtis, C. (2005). Mosquito behavior and vector control. *Annu. Rev .Entomol.*, **50**:53-70.

Phillips, R.S. (2001). Current status of malaria and potential for control. *Clin. Microbiol. Rev.*, **14**: 208-226.

Pluess, B., Tanser, F.C., Lengeler, C., Sharp, B.L. (2010). Indoor residual spraying for preventing malaria. Cochrane Database

Ramirez, J.L., Garver, L.S., Dimopoulos, G. (2009). Challenges and approaches for mosquito target malaria control. *Curr. Mol. Med.*, **9**:116-130.

Rowland, M. (1999). Malaria control: bednets or spraying? Malaria control in the Afghan refug camps of western Pakistan. *Trans. R .Soc. Trop. Med. Hyg.*, **93**: 458-459.

Russell, T.L., Lwetoijera, D.W., Maliti, D., Chipwaza, B., Kihonda, J., Charlwood, J.D., Smith, Lengeler, C., Mwanyangala, M.A., Nathan, R., Knols, B.G.J., Takken, W., Killeen, G (2010). Impact of promoting longer-lasting insecticide treatment of bed nets upon mala transmission in a rural Tanzanian setting with preexisting high coverage of untreated ne *Malar. J.*, **9**:187.

Sach, J.D. (2001). Macroeconomics and health:investing in health for economic development. Rep of commission on macroeconomics and health. *World Health Organization*, Geneva.

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

Scott, J.A., Brogdon, W.G., Collins, F.H. (1993). Identification of single specimens of the *Anophe gambiae* complex by the polymerase chain reaction. *Am. J. Trop.Med. Hyg.*, **49**:520-529.

Sharp, B.L., Ridl, F.C., Govender, D., Kuklinski, J., Kleinschmidt, I. (2007). Malaria vector control indoor residual insecticide spraying on the tropical island of Bioko, Equatorial Guinea. *Ma J.*, **6**:52.

ter Kuile, F.O., Parise, M.E., Verhoeff, F.H., Udhayakumar, V., Newman, R.D., van Eijk, A. Rogerson, S.J., Steketee, R.W. (2004). The burden of co-infection with hur immunodeficiency virus type 1 and malaria in pregnant women in sub-saharan Africa. *An Trop. Med. Hyg.*, **71**:41-54.

ter Kuile, F.O., Terlouw, D.J., Kariuki, S.K., Phillips-Howard, P.A., Mirel, L.B., Hawley, W Friedman, J.F., Shi, Y.P., Kolczak, M.S., Lal, A.A., Vulule, J.M., Nahlen, B.L. (2003). Im

of permethrin-treated bed nets on malaria, anemia, and growth in infants in an area of intense perennial malaria transmission in western Kenya. *Am. J. Trop. Med. Hyg.*, **68**:68-77.

UNICEF/UNU/WHO., (2001). Iron deficiency anaemia:assessment, prevention, and control. A guide for programme managers.

UNICEF, (1989). Implementation Hand Book for the Convention on the Rights of the Child. *UNICEF*, pg1.

Van Bortel, W., Delacollette, C., Barutwanayo, M., Coosemans, M. (1996). Deltamethrin impregnated bed nets as an operational tool for malaria control in a hyper-endemic region of Burundi: impact on vector population and malaria morbidity. *Trop. Med. Int. Health.*, **1**:824-835.

van den Berg, H. (2009). Global status of DDT and its alternatives for use in vector control to prevent disease. *Environ. Health. Perspect.*, **117**:1656-1663.

Vulule, J.M., Beach, R.F., Atieli, F.K., Roberts, J.M., Mount, D.L., Mwangi, R.W. (1994). Reduced susceptibility of Anopheles gambiae to permethrin associated with the use of permethrin-impregnated bednets and curtains in Kenya. *Med. Vet. Entomol.*, **8**:71-75.

Whitley, E., Ball, J. (2002). Statistics review 4: sample size calculations. *Crit. Care.*, **6**:335-341.

WHO, (2000). WHO Expert Committee on Malaria. World Health Organization. Geneva, Technical Report Series No. 892.

WHO, (2002) Malaria entomology and vector control. *World Health Organization*

WHO, (2004). Global strategic framework for integrated vector management. . *World Health Organization 10.*

WHO, (2005). World Malaria Report. *World Health Organization,* Geneva.

WHO, (2006a). Africa malaria report. *World Health Organization,*Geneva.

WHO, (2006b). Indoor Residual Spraying. Use of indoor residual spraying for scaling up global malaria control and elimination. *World Health Organization,* Geneva.

WHO, (2006c). Indoor residual spraying, position statement on indoor residual spraying. *World Health Organization,* Geneva.

WHO, (2006d). Insecticide-treated mosquito nets: WHO position statement. *World Health Organization,* Geneva.

WHO, (2008). World malaria report. *World Health Organization,* Geneva.

WHO, (2008b). Test procidures for insecticide resistance monitering in malaria vectors, Bio-efficacy and persistance of insecticides on treated surfaces.*World health organisation,Geneva.*

WHO, (2010).World Health Organization. Basic Malaria Microscopy. Part 1. Learner's Guide. 2nd ed. Geneva.

WHO, (2011). World malaria report. *World health organisation,* Geneva:

Wirtz, R.A., Zavala, F., Charoenvit, Y., Campbell, G.H., Burkot, T.R., Schneider, I., Esser, K.M., Beaudoin, R.L., Andre, R.G. (1987). Comparative testing of monoclonal antibodies against Plasmodium falciparum sporozoites for ELISA development. *Bull.World.Health.Organ.* **65:**39-45.

Worrall, E., Rietveld, A., Delacollette, C. (2004). The burden of malaria epidemics and cost-effectiveness of interventions in epidemic situations in Africa. *Am. J. Trop. Med. Hyg.*, **71**: 136-140.

www.doh.gov.za/issues/malaria 10/11/2010.

www.irinew.org/report, 20/2/2010

www.kemri.org 4/2/211.

www.zerovector.com 15/1/2010.

Zaim, M., Aitio, A., Nakashima, N. (2000). Safety of pyrethroid-treated mosquito nets. *Med.Vet. Entomol.*, **14**:1-5.

Zhou, G., Githeko, A.K., Minakawa, N., Yan, G. (2010).Community-wide benefits of targeted indoor residual spray for malaria control in the western Kenya highland. *Malar. J.*, **9**:67.