IMPACT OF INSECTICIDE-TREATED BEDNETS AND INDOOR RESIDUAL SPRAYING ON HOST SELECTION BY *ANOPHELES GAMBIAE S.S.*, *ANOPHELES ARABIENSIS* AND *ANOPHELES FUNESTUS* IN WESTERN KENYA

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ABSTRACT

Malaria continues to be a global public health priority with unprecedented scale-up of insecticide based interventions in the sub-tropics. The World Health Organization (WHO) has approved vector control using insecticide treated nets (ITNs) and indoor residual-insecticide spraying (IRS) as the key malaria vector control strategies and has been adopted in most countries in Africa. In Kenya, despite the increase in ITN coverage and the expansion of IRS programs especially in western Kenya, mosquitoes continue to feed and transmit malaria. One question from this observation is whether the presence of these interventions have influenced the host preferences for the local vectors or whether some species, due to their feeding habit and feeding behavior, continue to proliferate and have gained dominance in this region. The purpose of this study was to investigate host preference by three main malaria vectors (*An. gambiae s.s., An. arabiensis* and *An. funestus*) in the presence of different levels of vector control interventions in western Kenya and to assess the accuracy of different mosquito sampling methods aimed at collecting vectors from the immediate presence of the host in estimating host selection for *Anopheles* vectors. The study was conducted in 4 districts; Nyando, Rarieda, Teso and Bungoma selected based on vector composition and intervention type. Mosquito samples were collected indoors (by pyrethrum spray collection and CDC light traps) and outdoors (by clay pots and CDC light traps) during the months of September to December, 2010. The samples were identified morphologically and by polymerase chain reaction (PCR), and all blood fed *Anopheles* samples were analyzed by sequencing followed by a Basic Local Alignment Search Tool (BLAST) search in the GenBank database to determine mosquito blood meal host. Results revealed no significant impact of intervention on selection for human blood meal for IRS ($p = 0.09$), both IRS and ITN ($p = 0.74$) and ITN ($p = 0.55$). The range of hosts fed on by *Anopheles* vectors in western Kenya included human (82.57%), bovine (3.63%), goat (0.65%), rats (0.28%), wild birds (0.09%) and frogs (0.09%). *An. gambiae s.s.* and *An. funestus* displayed an almost exclusive selection for human blood meal without a significant difference between the species ($p = 0.31$), while *An. arabiensis* fed on both human and cattle but with significantly higher preference for cattle blood meal ($p = 0.0002$). From the collection methods, pyrethrum spray collection (PSC) results showed 93.2% human and 6.08% cattle blood meal, light traps both indoor and outdoor had 97.1% human and 2.8% cattle blood meal while pots reported 100% human blood meal. Information generated from this study is necessary for improvement of implementation of vector control. Despite these interventions, the feeding behavior as measured by host selection was similar across all houses, indicating that interventions present in those houses did not affect host selection behavior. Two of the three malaria vector species (*An. gambiae s.s and An. funestus)* displayed strong anthropophilic trends while a third (*An. arabiensis*) fed on humans and cattle. Besides, host selection was observed to be dependent on vector behavior and was unlikely to be accurately estimated by mosquito sampling techniques aimed at collecting vectors from a specific environment of the host. The extent of human-vector contact as depicted in this study predicted continued risk of malaria infection and adequate measures are necessary to establish vector feeding behavior, prevention and control. Pyrethrum spray collections appeared to be the best tool in collection of fed and half gravid samples for blood meal analysis while at the same time provided samples for the widest range of *Anopheles* hosts.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Malaria is one of the most serious vector-borne diseases, affecting millions of people mainly in the tropics (WHO, 2010). In Kenya, malaria remains the leading cause of morbidity and mortality for the population of approximately 34 million people, accounting for about 30% of all out-patient consultations, 19% of all hospital admissions, and causing a reported 34,000 deaths annually among children under-five years of age (PMI., 2010). Prevention of malaria has overwhelmingly relied on the use of insecticides that target the adult stage of mosquitoes (Killeen *et al.*, 2002). In Africa, the primary malaria transmitting mosquitoes, the *An. gambiae s.l.* and *An. funestus* usually feed and rest in and around human habitations where targeted application of insecticides can dramatically reduce malaria transmission (Gimnig *et al.*, 2003a).

Bednets have been used traditionally to protect people from nocturnally biting insects (Lindsay and Gibson, 1988). The nets, apart from providing physical barrier, also include a formulation of synthetic pyrethroids imbedded in the fabric material (Takken, 2002). The pyrethroids are highly potent insecticides with a relatively low toxicity for vertebrates and significantly fewer environmental effects for malaria control (Takken, 2002). The insecticides used for this purpose in Insecticide Treated Bednets (ITNs) and Indoor Residual Spray (IRS) belong to the class of synthetic pyrethroids and include permethrin, deltamethrin, lambdacyhalothrin and cypermethrin (Takken, 2002). In malaria endemic countries, the ITNs and IRS are being used as effective methods for reducing malaria transmission risk (Mathenge *et al.*, 2001). Studies have shown that ITNs cause a significant reduction in malaria-attributable morbidity and mortality, especially in young children (Graves *et al.*, 1987; Alonso *et al.*, 1991). The evidence of ITN use as a successful disease control method was so great that WHO adopted this method as one of the cornerstones for its Roll Back

Malaria programme (RBM., 1999)

The ITNs have been proven to significantly alter behavioural and physiological traits and population structures of *Anopheles* mosquitoes. For example, studies in Asembo, western Kenya have shown that ITNs significantly decrease the proportion of *An. funestus* (Gimnig *et al.*, 2003b), and that of *An. gambiae* declined much lower with a competitive proportionate increase in the population of *An. arabiensis* in the area with widespread ITN use (Bayoh *et al.*, 2010). On treated nets, the pyrethroids work in three ways: they act as killing agent when the insect makes contact with the insecticide by landing on the net; pyrethroids have an irritating (exito-repellent) effect and the insect rests only briefly on the treated fabric and; the formulation in which the pyrethroid is presented contains volatiles that cause deterrence, leading to fewer mosquitoes entering a room where an ITN is present (Lindsay *et al.*, 1991; Chandre *et al.*, 2000). A study in Kenya showed no difference in house entry of *An. gambiae s.s.* and *An. arabiensis* into ITN-provided bedrooms as compared to houses with untreated nets (Mathenge *et al.*, 2001), however, the proportion of unfed and exiting mosquitoes was significantly greater in treated houses than untreated ones (Mathenge *et al.*, 2001).

Other effects of insecticide on the vector may include changes in biting behaviour expressed by outdoor biting and/or alteration of time of biting, and a change in host preference because favoured hosts can no longer be reached (Takken, 2002). However, no study has been conducted to give clear information on the impact of the insecticide-based interventions on mosquito host selection. Studies have reported a reduction in indoor biting in rooms where ITNs are installed. These however, cover endophilic mosquitoes, several of which prefer to bite humans (Mathenge *et al.*, 2001). Therefore, in areas without vector control, such mosquitoes are mostly collected in bedrooms (Takken, 2002). It is however not clear whether the reported reduction in indoor feeding translates to feeding on alternative hosts by the *Anopheles* vectors. Moreover, the exito-repellent

effect of pyrethroids causes the mosquitoes to leave rooms for the outdoors and may therefore be the reason for the observed reduction in indoor biting (Takken, 2002). A previous study (Diatta *et al.,* 1998) suggested that, *An. gambiae s.s.* can readily switch to other hosts should humans not be available and other human-biting species are similarly inclined to feed on other hosts. However, evidence of change in host preference due to intervention is lacking. Therefore, knowledge of effects of sustained intervention use on mosquito host selection with the unprecedented scale-up of insecticide-based interventions in western Kenya was necessary.

Sampling adult stages of *Anopheles* vectors of human malaria is an important and necessary process for estimating vector population density, obtaining adequate samples to measure sporozoites infection rate, and quantifying the effects of interventions (Odiere *et al.,* 2007). Different sampling techniques target differences in mosquito resting and feeding behaviour, for example, pyrethrum spray collection (PSC) method is necessarily biased towards endophilic females and such would not cover the more exophagic and exophilic vector populations (Odiere *et al.,* 2007). Besides, presence of intervention indoor may reduce indoor feeding and resting densities and instead increase outdoor resting and feeding (Mathenge *et al.,* 2001). Studies assessing the accuracy of different mosquito sampling techniques in estimating host feeding preferences are lacking. The current study was undertaken to investigate the accuracy of PSC, clay pots and CDC light traps in estimating host selection by *Anopheles* vectors.

The quantitative assessment of vector-host contact at any given time makes it possible to predict epidemiologically dangerous situations, and to take adequate measures of prevention and vector control. Success in human blood feeding by mosquitoes is a result of increased vector-human contact (Rebekah and Douglas, 2005). ITNs and IRS work principally to inhibit possible access of humans by mosquitoes within houses thus limiting chances of vectors obtaining blood meal and hence low malaria transmission (Rebekah and Douglas, 2005). This study investigated the impact

of ITNs and IRS on the host selection by *An. gambiae s.s., An. arabiensis and An. funestus* and assessed the accuracy of different collection methods in estimating the host feeding preferences by the local vectors in western Kenya. Blood-fed and half-gravid anopheles mosquitoes, both outdoor and indoor resting, were sampled to determine from the abdomen the source of blood meal.

1.2 Statement of problem

Sustained ITN use has resulted into behavioural modification and changes in vector population structures observed in *Anopheles* mosquitoes. Studies in Asembo showed that ITNs significantly decreased the proportion of *An. funestus* (Gimnig *et al.*, 2003b), and that of *An. gambiae* declined much lower with a competitive proportionate increase in the population of *An. arabiensis* in the area with widespread ITN use (Bayoh *et al.*, 2010). Decline in populations for the more anthropophagic and endophilic *An. funestus* and *An. gambiae s.s.* with the corresponding rise in the population of the more zoophagic and exophilic *An. arabiensis* (Githeko *et al.*, 1996b), should result in a remarkable decline in malaria burden in this region. However, this trend has not been observed. In addition to the observed reduced susceptibility of *An. gambiae* to permethrin associated with the use of permethrin-impregnated bednets and curtains in Kenya (Vulule *et al.*, 1994), other effects of insecticides on the vector may include changes in biting behaviour expressed by outdoor biting and/or alteration of time of biting, and a change in host preference since favoured hosts can no longer be reached (Takken, 2002). Due to the scale-up and sustained ITN use and IRS implementation in western Kenya, there are possibilities of mosquitoes developing physiological resistance to pyrethroids and having successful blood meals from individuals sleeping under ITN or in IRS-covered houses. On the other hand, the vectors may develop a behavioural resistance thus avoiding the intervention houses, in the process altering their biting time or changing to feeding on alternative hosts. As such, the current study carried out in western Kenya, investigated the extent of host selection by blood-fed and half-gravid anopheles mosquitoes, both outdoor and indoor resting, in the presence of ITNs, IRS or both interventions.

1.3 Justification of the study

The use of ITNs and IRS in vector control works to limit vector-human contact. Sustained intervention against vectors may lead to a shift to an alternative host or a change in biting time. For highly anthropophagic and endophilic vectors, a change of host may occur when the preferred host is no longer accessible, for example, a shift from human to cattle feeding may result, hence lowering their vectorial capacity. A shift to an alternative host also ensures the continued survival and reproduction of the vectors in the community with increased risk of transmission. A change in vector biting time may occur resulting into successful blood meals from humans before going under the ITNs or the vectors may rather feed on humans living in houses without intervention. It is therefore important to accurately monitor the host selection pattern in any vector control programme. This study sought to establish sources of blood meals taken by different *Anopheles* mosquitoes in western Kenya in the presence of ITNs and IRS by use of molecular techniques involving polymerase chain reaction (PCR), sequencing and BLAST search in the gene bank database to determine the vertebrate host. Information generated would be critical in the evaluation of the impact of intervention on host selection by *Anophelines*, thus leading to an improved implementation of vector control.

1.4 General Objective

To determine the impact of ITNs and IRS on the host selection by *An. gambiae s.s., An. arabiensis* and *An. funestus*.

1.4.1 Specific objectives

a) To determine the preferred host for *An. gambiae s.s., An. arabiensis* and *An. funestus* during blood meals in the presence of ITNs, IRS or both interventions in western Kenya.

b) To assess the accuracy of different sampling methods for collecting vectors from a specific environment of the host in estimating the host feeding preferences of *An. gambiae s.s., An. arabiensis* and *An. funestus* in western Kenya.

1.5 Null hypothesis

a) There is no change in host preference of *An. gambiae s.s., An. arabiensis* and *An. funestus* in the presence of ITNs, IRS or both interventions in western Kenya.

b) There is no difference in accuracy of vector sampling methods used for collecting vectors from a specific environment of the host in estimating the host feeding preferences of *An. gambiae s.s., An. arabiensis* and *An. funestus* in western Kenya.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Malaria

Infections by malaria parasite leads to increased human morbidity and mortality throughout much of the tropics and sub-tropics (Carter and Mendis, 2002). About 500 million people suffer from malaria resulting in approximately one million deaths, mostly children living in sub-Saharan Africa (Chuma *et al.,* 2010). Malaria inflicts significant costs on households and on the economy of endemic countries (Chuma *et al.,* 2010). Evidence also suggests that the economic burden is higher among the poorest in a population and that cost burdens differ significantly between wet and dry seasons (Chuma *et al.,* 2010).

In Kenya, approximately 70% of the country's surface area is prone to malaria epidemics with regular epidemics occurring in the western highlands (IEIP, 2006). The semi-arid regions in the north-east and eastern parts of the country are involved in epidemics only during intense flooding (WHO, 2009). Kenya had an estimated 15 million malaria cases in 2006 of which majority were due to *P. falciparum* (WHO, 2009). Nine million suspected malaria cases were reported in 2007, most of which were unconfirmed. The number of reported cases increased between 2001 and 2007; it is however not known whether this represents improved reporting or an increase in incidence (WHO, 2009). According to the President's Malaria Initiative report, malaria remains the leading cause of morbidity and mortality, accounting for about 30% of all outpatient consultations, 19% of all hospital admissions, and causing a reported 34,000 deaths annually among children under-five years of age (PMI., 2010). This clearly shows that the problem of malaria is a major issue and needs to be addressed with the correct measures of control and as such, has been identified as a priority across Africa.

2.2 *Anopheles* vectors

The vectors of human Plasmodium belong to the genus *Anopheles* (Service, 1993). Out of about 422 *Anopheles* species, only about 70 are malaria vectors and 40 of these are mostly vectors associated with human host (Service, 1993). In western Kenya, the main vectors are *An. gambiae s.s., An. arabiensis* and *An. funestus* (Bayoh *et al.*, 2010).

2.2.1 Identification of adult *Anopheles* mosquitoes

The adult *Anopheles* usually rest with the body at an angle to the resting surface. They have spotted wings with number, length and the arrangement of the spots differing considerably in different species. The female have long piercing proboscis and non-plumose antennae while the males have small proboscis and plumose antennae. The palps of the females are about as long as the proboscis and usually lie closely alongside (Gillies and de Meillon, 1968). *Anopheles gambiae* complex species have five pale bands on the costal margin of the wings, rough abdomen and speckled legs. On the other hand, *An. funestus* have four pale bands on the costal margin of the wings, smooth shiny abdomen and dark legs (Gillies and de Meillon, 1968).

2.2.2 Biology of adult *Anopheles gambiae* complex

The *An. gambiae s.l.* is a group of six sibling species which include *An. gambiae s.s, An. arabiensis* and *An. quadriannultus* (fresh water species), *An. merus* and *An. melas* (salt-water species) and *An. bwambae* which is a mineral water species (White, 1985). Only *An. gambiae s.s.*, and *An. Arabiensis* are present in western Kenya, however, all these species are morphologically indistinguishable but exhibit distinct and eco-ethological differences reflected in their ability to transmit malaria (Della Torre *et al.*, 2002).

2.2.2.1 Host choice

An. gambiae s.l. are largely anthropophagic, however, some studies have shown that they are more catholic in their feeding habits (Garret-Jones, 1964) since they can easily shift from one host to

another. The wide range of hosts attacked by *An. gambiae s.l.* is well established; a small proportion of females may feed on hosts such as dogs, goats and domestic fowls (Gillies and de Meillon, 1968) . In a study conducted at Ahero, Kenya, *An. arabiensis* showed a low tendency to bite man indoors or outdoors until 01.00 am (Githeko *et al.,* 1996b). The vector, however, showed increased activity in the last half of the night (Githeko *et al.,* 1996b). A previous study by the same author indicated that *An. arabiensis* population at Ahero showed a very low preference for human blood meals with human blood index (HBI) of 0.23 (Githeko *et al.,* 1994) and this appeared to be the case for the *An. arabiensis* in Rota with HBI of 0.18 (Githeko *et al.,* 1994). HBI is the proportion of freshly fed anophelines found to contain human blood. In malaria eradication programmes, this index is relevant in epidemiological assessment and modification of measures to interrupt transmission (Garret-Jones, 1964). These results suggested that the *An. arabiensis* in western Kenya have a preference for outdoor dwelling hosts other than human, hence a low vectorial capacity.

In contrast, several studies suggest that *An. arabiensis* is strongly anthropophagic. A study in Konso, southern Ethopia, comparing the catch of human- and cattle-bait using the same sampling technique, however, with different baits showed that the human-baited trap caught significantly more mosquitoes than the cattle-baited one, suggesting that *An. arabiensis* in Konso is inherently anthropophagic (Tirados *et al.,* 2006). This finding was consistent with the results which showed that placing an ox adjacent to a human landing catches did not reduce the catch of *An. arabiensis*, whereas the catch of a more zoophagic species *An. pharoensis* was reduced (Tirados *et al.,* 2006). The study in Konso made several important points about the blood feeding behaviour of *An. arabiensis*. Firstly, although the majority of the blood meal may came from cattle, the population of mosquitoes is inherently more strongly attracted to humans, such that the feeding ratio on humans is eight times higher than the expected in an area where cattle out-numbered humans by 17:1 (Tirados *et al.,* 2006). Secondly, the high proportion of cattle blood meals probably reflected a high degree

of exophagy and exophily, which compromise the efficacy of control measures targeted at the mosquito indoors (Tirados *et al.,* 2006). Thirdly, the sporozoite rates in human- and cattle-fed mosquitoes were not significantly different, hence, there was no evidence of existence of behavioural sub-populations with different host preferences (Tirados *et al.,* 2006). Fourthly, the HBI of resting mosquitoes (indoor *vs.* outdoor) was not well-matched with the distribution of host and therefore, it was imperative to conclude that mosquitoes may have moved away from the feeding host quite soon after feeding (Tirados *et al.,* 2006). Finally, the nightly biting pattern shows that humans were exposed to a significant proportion of biting mosquitoes when they were outdoors and unprotected in the early evening (Tirados *et al.,* 2006). This vector has also been shown to be about twice as likely to bite man indoors as outdoors (Githeko *et al.,* 1996b).

In western Kenya, *An. gambiae s.s.* on the other hand, are observed to be highly endophagic and anthropophagic (Githeko *et al.,* 1994; Bayoh *et al.,* 2010). A study in Sierra Leone, reported the Forest chromosomal form of *An. gambiae s.s.* to be equally endophagic and anthropophilic (Fontenille *et al.,* 1992), however, the vector was also exophilic. In situations where people are outnumbered by cattle, for example in Kanduna area in northern Nigeria, the human blood index (HIB) of *An. gambiae s.s.* was 0.28 (Service, 1970) and 0.50 (White and Rosen, 1973), the HBI for this species is usually higher (0.8 - 0.9) (White, 1974). The HBI of the *An. gambiae* complex has been showed to vary according to seasonal host changes in human and animal distributions in the Sahelian area of Senegal (Lemasson *et al.,* 1997). It has further been reported that both *An. gambiae* and *An. arabiensis* Patton bite humans sleeping outdoors (Faye *et al.,* 1997), and that such human habits may influence the biting behavior of the mosquitoes (Nobuko *et al.,* 2010).

2.2.2.2 Outdoor resting

The commonest situation in the savannas where a variety of hosts are available is the possibility of finding moderate numbers of *An. gambiae* at all abdominal stages resting outdoors (Sousa *et al.,* 2001). The natural resting sites of *An. gambiae* outdoors are much the same as those of *An.*

funestus and are seldom in exposed situations. The tendency of *An. gambiae s.l.* and more likely *An. gambiae s.s.* to leave houses while freshly fed have been observed (Githeko *et al.*, 1996c), with very few *An. gambiae s.s.* resting outdoor unlike *An. arabiensis* (Sousa *et al.*, 2001). In other studies carried out in western Kenya, it was demonstrated that *An. gambiae s.s.* has early exophily, a behaviour rarely associated with this species in East Africa (Githeko *et al.*, 1996c). The exophilic behaviour was observed to be increased by permethrin as all half-gravid females collected in the exit traps were from intervention houses.

2.2.3 Biology of adult *Anopheles funestus*

2.2.3.1 Host choice

Anopheles funestus is one of the most anthropophilic mosquitoes known, in many areas attacking man even in the presence of abundant alternative hosts such as sheep and cattle (Gillies and de Meillon, 1968). Data from Taveta Kenya showed that only 4-8% of *An. funestus* from houses had fed on cattle even though the houses in which they were caught were largely shared by cattle (Gillies and de Meillon, 1968). A study in Malagasy found that 33% of house-catches and only 3% of outdoor-catches were positive for human blood, with the remainder proportions having fed on cattle (Gillies and de Meillon, 1968), while in Lubumbashi, a number of *An. funestus* were caught in animal-baited traps (Gillies and de Meillon, 1968). Another study carried out in northern Cameroon records the presence of this species in totally inhabited areas (Gillies and de Meillon, 1968), proving that it is by no means dependent on man and domestic animals for its existence. These apparent differences in behavior between eastern and western Africa vectors may perhaps be connected to defenses in response to insecticide, *An. funestus* being rather readily eliminated by house spraying in eastern Africa, whereas in West Africa dramatic fall in numbers is the most that is achieved, even in the presence of physiological resistance. Knowledge of the possible effects of intervention on host selection by *An. funestus* in western Kenya with the increasing ITN use and distribution is therefore necessary.

2.2.3.2 Feeding indoor and outdoors

The great bulk of feeding by *funestus* takes place inside houses (Gillies and de Meillon, 1968). This is partly because it feeds in the second half of the night when most people are indoors, and partly because in some areas it shows a lesser tendency to feed outdoors than indoors (Githeko *et al.*, 1996a). With a free choice of a bait placed inside and outside houses in northern Nigeria, it was found that catches were consistently greater indoor than outdoor, the effect being partially marked in the dry season (Hanney, 1960). In comparing results from different areas, it should be noted that outside biting is more likely to be affected by windy conditions than catches indoors, so that the degree of shelter in the vicinity of the baits may have a considerable influence on the results (Gillies and de Meillon, 1968).

2.2.3.3 Resting behaviour in houses

In parallel with its man-biting habits, *funestus* show a closer adaptation to human dwellings than any other African *anophelines* (Gillies and de Meillon, 1968). In many areas, it spends the greater part of its adult life in houses, which make it the most vulnerable of species to attack with residual insecticides (Gillies and de Meillon, 1968). Estimates of the amount of movements out of houses after feeding vary to a certain extent. In East Africa, it is well established that very few leave houses the same night as they have fed (Mathenge *et al.*, 2001). In houses with mud walls and thatched roofs, greater numbers are usually found on the walls than in the roof, the upper part of the wall being more frequented than that near the floor (Smith, 1955).

2.2.3.4 Outdoor resting

Outdoor resting-sites are used less by *An. funestus* than by *An. gambiae* (Gillies and de Meillon, 1968). Unfeds, gravids and half-gravids have been caught in significant numbers in nature and in artificial shelters in Tanzania (Gillies, 1954). A study showed that outdoor resting was much more prominent during the wet season owing to the greater shade available (Gillies and de Meillon,

1968). The sites chosen by *An. funestus* are similar to those of *An. gambiae*. Thus, they do not rest on vegetation but are found mostly in shaded hollows in earth banks, tree buttresses, rock crevices, culverts, thatched fences or grain stores (Gillies and de Meillon, 1968).

2.3 Adult mosquito control measures

Malaria control policy has been emphasized on household protection against adult mosquitoes with insecticides, and improved access to medical services (Killeen *et al.*, 2002). Malaria prevention by killing adult mosquitoes was commonly superior because it fairly decreases their longevity, and can drastically curb community intensity of malaria transmission (Killeen *et al.*, 2002). Adult mosquito control measures include indoor residual spraying (IRS), space spraying and insecticide treated nets (ITNs) (Mary *et al.*, 2011).

2.3.1 Indoor residual spraying (IRS)

Indoor residual spraying (IRS) using insecticides has been the main and highly effective method for malaria vector control (WHO and UNICEF, 2005). The main purpose of IRS is to reduce the survival of malaria vector(s) entering the houses (WHO, 2007). However, it is of little use for control of malaria vectors which rest outdoor (exophilic), particularly if they also bite outdoors (exophagic) and do not enter sprayed houses (WHO, 2007). To ensure its expected outcome, all possible resting surfaces of vectors should be sprayed with appropriate insecticide, and at a prescribed amount sufficient to remain effective throughout the transmission season (Najera and Zaim, 2002). The most widely and successfully used insecticide for indoor residual spraying was dichloro-diphenyltrichloroethane (DDT) (Sadasivaiah *et al.*, 2007). It has been helpful in achieving a rapid decline in transmission during epidemics and other emergency situations, provided it was well-timed and high coverage was achieved (WHO and UNICEF, 2005). However, emergence of insecticide resistance has decreased the efficiency of IRS in malaria vector control (Coetze, 2004). DDT has already been replaced by organophosphate or carbamate insecticide such as malathion because of its negative impacts on the environment, human health, and developed resistance (Curtis,

1994). Pyrethroids such as deltamethrine and lambdacyhalothrin are effective at lower doses than DDT. Pyrethroids are much more acceptable to households because they leave no deposits on walls and can kill other nuisance insects (Curtis, 1994).

2.3.2 Space spraying

This is the application of non-residual insecticides to the outdoor environment in order to immobilize infective mosquitoes and control transmission. This is predominantly suggested for urban areas where many people assemble outdoors (IEIP, 2006). Nevertheless, this should be an urgent situation undertaken only under special circumstances and mainly during epidemics (IEIP, 2006).

2.3.3 Insecticide-treated nets (ITNs)

Bednets have been used traditionally to protect people from the nuisance caused by nocturnally biting insects (Lindsay and Gibson, 1988). The uses of mosquito nets and house protection with screening of windows, eaves and doors have long been considered useful protection methods against mosquitoes and other insects (Lindsay and Gibson, 1988). However, nets and screens when not well fitted or are torn, allow mosquitoes to penetrate or feed on the sleeper (Lines and Myamba, 1987; Alaii *et al.,* 2003). The problem of poorly used nets and screens was one of the reasons for treating them with a fast acting insecticide that will repel or kill mosquitoes before or shortly after feeding (Alaii *et al.,* 2003). Insecticide-treated bednets work as a physical barrier, preventing contact by vector mosquitoes and thus afford personal protection against malaria to the individual(s) using the nets (Rebekah and Douglas, 2005.). In malaria endemic countries, the use of ITNs is being promoted as an effective method for reducing malaria transmission risk. It has been previously demonstrated that ITNs caused a significant reduction in malaria attributable morbidity and mortality, especially in young children (Graves *et al.,* 1987; Alonso *et al.,* 1991). The evidence of ITN use as a successful disease control method was so great that WHO adopted this method as one of the cornerstones for its Roll Back Malaria programme (RBM., 1999). Studies in Ghana,

Gambia, Kenya and Tanzania have demonstrated that, the use of ITN has decreased child illness by 29-63% and childhood mortality by between 17- 63% (IEIP, 2006). The present challenge is to scale-up and sustain coverage with ITNs (Lines *et al.*, 2003) and the possibility for mosquitoes to develop resistance to pyrethroid insecticides used to impregnate bednets (Hargreaves *et al.*, 2000). ITNs need to be retreated regularly (e.g. after washing) and this presents an obstacle to its use in many parts of the world (Najera and Zaim, 2002). The solution to this problem is the use of long-lasting insecticidal net (LLINs) which need no re-treatment throughout their expected life span of 4-5 years (Najera and Zaim, 2002). The ITN contribute to reducing human-vector contact, and in households where not everyone has access to net, even non-users gain some indirect protection from biting (Najera and Zaim, 2002). Therefore, ITN should be an important part of the health program of all malaria endemic African countries.

2.3.3.1 Insecticides used in bednets for malaria control

Pyrethroids are the only group of insecticides currently recommended for use on nets to add an insecticidal effect to their mechanical protection, as mosquitoes are absolutely attracted by the odor of the sleeper inside the net (WHO, 2006). The insecticides used for this purpose belong to the class of synthetic pyrethroids and include permethrin, deltamethrin, lambdacyhalothrin and cypermethrin (Takken *et al.*, 1978). They share the property of a relatively long residual activity when kept out of daylight but break down rapidly under influence of UV-radiation (Takken *et al.*, 1978). Their mammalian toxicity is low but their effect on arthropods, including crustaceans, is generally severe (Takken *et al.*, 1978). Because of their high toxicity for mosquitoes, coupled with a long-lasting residual activity on textiles, they are considered safe for use on mosquito nets (Barlow *et al.*, 2001).

2.4 Impact of sustained use of ITNs on the malarial vector

Insecticide-treated bednets (ITNs) have been proven to significantly reduce the number of vectors

in houses with ITNs as well as to reduce the overall size of the anopheline population (Mbogo *et al.*, 1996; Gimnig *et al.*, 2003a; Gimnig *et al.*, 2003b). *An. funestus* is known to be more susceptible to insecticides than members of the *An. gambiae* complex (Gillies and de Meillon, 1968). Previous studies in Asembo showed that ITNs significantly decreased the proportion of *An. funestus* (Gimnig *et al.*, 2003b), and that of *An. gambiae* declined much lower with a competitive proportionate increase in the population of *An. arabiensis* in the area with widespread ITN use (Bayoh *et al.*, 2010). Such changes in vector numbers due to intervention would therefore suggest that the hypothesis that ITNs reduce house entry by repelling mosquitoes is not plausible. Otherwise, there should be a greater effect on the more exophilic mosquito, *An. arabiensis*, rather than the more anthropophilic and endophilic *An. gambiae* (Lindblade *et al.*, 2006). It therefore follows that, if house entry is not reduced by ITNs, their impact is likely to be through either mortality induced through contact with the ITN or increased rates of exit (Mathenge *et al.*, 2001). These reported effects of intervention on the malaria vectors observed changed population densities, increased rates of exit and reduced feeding success resulting into a shift in host preference and/or a change in the vector feeding behavior. These possible changes in mosquito feeding habit and behavior with sustained intervention use in western Kenya have not been exhaustively researched.

2.5 Insecticide-induced changes in mosquito behavior and survival

On treated nets, the pyrethroids work in three ways: first, they act as killing agents when the insect makes contact with the insecticide by landing on the net; secondly, pyrethroids have an irritating (exito-repellent) effect and the insect rests only briefly on the treated fabric and thirdly, the formulation in which the pyrethroid is presented contains volatiles that cause deterrence, leading to fewer mosquitoes entering a room where an ITN is present (Lindsay *et al.*, 1991; Chandre *et al.*, 2000). A study in Kenya showed that *An. gambiae s.s.* and *An. arabiensis* entered houses in lower numbers in ITN-provided bedrooms as compared to houses with untreated nets (Mathenge *et al.*, 2001). Moreover, the proportion of unfed and exiting mosquitoes was significantly greater in treated houses than untreated ones (Mathenge *et al.*, 2001) and may attributed to the exito-repellant effect

of the insecticide thus protecting the net users. Other effects of insecticide on the vector may include changes in biting behavior expressed by outdoor biting and/or alteration of time of biting, and a change in host preference because favored hosts can no longer be reached (Takken, 2002). However, no studies have confirmed changes in host selection due to sustained intervention use.

Studies have reported a reduction in indoor biting in rooms where ITNs are installed (Lindsay and Snow, 1988; Mathenge *et al.*, 2001). These, however, cover endophilic mosquitoes, several of which prefer to bite humans. Therefore, in areas without vector control, such mosquitoes are mostly collected in bedrooms (Takken, 2002). The exito-repellent effect of pyrethroids causes the mosquitoes to leave rooms for outdoors, hence the observed reduction in indoor biting (Takken, 2002). *An. gambiae s.s.* can readily switch to other hosts should humans not be available (Diatta *et al.*, 1998) and other human-biting species are similarly inclined to feed on other hosts (Diatta *et al.*, 1998). Most studies report some highly significant reductions in the entomological inoculation rate (EIR) which is the principal goal of ITN use (Lindsay and Snow, 1988; Mathenge *et al.*, 2001).

In studies carried out in Papua New Guinea and Kenya, a shift to outdoor biting was observed (Takken, 2002). The same trend was observed in a study in Tanzania (Megesa *et al.*, 1991). In the Papua New Guinea and Tanzania, a shift in host-feeding and time of biting were also observed. Other hosts included pigs, dogs (Papua New Guinea) and cattle (Tanzania) with biting occurring earlier in the evening, presumably because the mosquito hosts had not yet gone to bed and were easily accessible. In a previous Kenyan study (Mbogo *et al.*, 1996), mosquitoes did not switch hosts, but they began biting earlier in the evening.

The overall immediate and long-term effects of use of ITNs on mosquitoes are variable. In many cases, a reduced survival was observed as well as reduced sporozoite rates. With two exceptions, no shift to outdoor biting or non-human hosts has been recorded (Takken, 2002). In two studies,

mosquitoes started biting earlier in the evening (Githeko *et al.,* 1996b; Mbogo *et al.,* 1996). A baseline study in western Kenya (Githeko *et al.,* 1994) investigating mosquito host preference before the introduction of insecticide-based interventions, suggested possible changes in feeding behavior, such as could be induced by impregnated fabrics during and after trials. Since only few studies examined a comprehensive package of behavioural aspects related to ITN use, there is an urgent need to conduct such studies more often and in greater detail, in order to avoid long-term behavioural changes as previously noted when indoor spraying was carried out with organochlorines (Boreham *et al.,* 1978; Knols and Takken, 1998). Hence, the proposed studies investigated the extent of host selection by *Anopheles* mosquitoes in the presence of ITN or IRS or both interventions in western Kenya.

2.6 Factors influencing host selection pattern

2.6.1 Human blood index (HBI)

The proportion of mosquitoes feeds taken from human is of primary importance in malaria transmission (Burkot, 1988). The Human Blood Index (HBI), is the proportion of freshly fed *Anophelines* found to contain human blood and is estimated from a representative sample of the vector species in a particular locality at a specified time (Garret-Jones, 1964). Vector blood meals can be classified as simple (from a single meal) or mixed (from two or more meals). Mixed meals can then be classified as patent (from two or more host species) or cryptic (from two or more individuals of the same species) (Burkot, 1988).

The probability of blood meals being from two or more hosts depends on the probability of a blood meal being interrupted and the likelihood of the host being selected by the mosquito (Burkot, 1988). The proportion of cryptic mixed feeds on mother-child pairs sharing bednet range from 2.7-5.7% for members of the *An. gambiae* complex (Spencer, 1967) ranged from 13-41% for members of the

An. punctulatus complex (Hess *et al.*, 1968). The higher proportion of mixed blood meals from same host species compared with those from different host species is to be expected because of the clustered distribution of humans in houses since a mosquito interrupted while feeding on a person sleeping in a house is more likely to complete its feeding on another person than on an alternative host species (Burkot, 1988).

2.6.2 Host availability and selection

The likelihood of a mosquito feeding on human depends on its intrinsic host preference and host availability, with the latter being a function of host numbers and the ease of access to them (Burkot, 1988). Migration due to economic factors, wars and cultural patterns, can alter the relative availability of human to anopheline attack. During wars, for example, the influx and concentration of refugees and soldiers into a particular area – usually combined with simultaneous decrease in the numbers of alternative hosts – has often been associated with malarial epidemics (Russel, 1963).

2.6.3 Cultural practices

Cultural practices such as type of clothing, occupation, house construction and location, can also affect the opportunity for contact with the malarial vectors. Madras women, who are more clothed than men, have lower cases of malaria (Russel, 1963). In Sierra Leone and Nigeria, many people sleep enveloped in clothes or under fine mesh for protection against mosquitoes, while in other areas a variety of natural repellants are used, including smoky fires or a mixture of ash, cow dung and urine (MacCormack, 1984). The Sepik people of Papua New Guinea weaved large baskets in which they slept to protect themselves against mosquitoes (Burkot, 1988), while in the Gambia, large differences in the HBI in mosquitoes taken from different houses were attributed to differences in the use and condition of bednets (Port and Boreham, 1982). In some parts of Africa, 'zooprophylaxis' is a recognized measure against mosquitoes such as *An. arabiensis*; cattle are deliberately impounded near (or within) houses so that the mosquitoes feed preferentially on the cattle rather than on the people (Burkot, 1988).

2.6.4 Intrinsic preference

Host selection by mosquitoes can be 'opportunistic' or 'fixed' with selection being independent of host availability (Burkot, 1988). Among the opportunistic feeders, a wide range of the HBI can be found even within a limited geographical area as influenced by the availability or accessibility of hosts (Burkot, 1988). This appears to be the case for *An. punctulatus* in Papua New Guinea, where it was found that HBIs ranging from 7-93% among different villages within a 20km radius (Burkot, 1988). This was attributed mainly to differences in the relative availability of different hosts (Burkot, 1988).

Many workers have demonstrated a preference in *An. gambiae* for feeding on adults rather than on children (Boreham *et al.*, 1978), and in another study (Port and Boreham, 1982), it was demonstrated that the extent of feeding on each individual was related to the proportion of the total surface area or weight contributed by the individual to the group. A similar preference of adults rather than infants has been observed for *An. farauti* (Spencer, 1967). However, there is large variation in the attractiveness for different individuals, resulting into preferential feeding in some children rather than on adults by the members of the *An. punctulatus* complex (Burkot, 1988). Similar large variations in individual attractiveness can also be seen from studies of *An. albimanus* and *An. gambiae* (Burkot, 1988).

2.6.5 Host defense behavior

The host defensive behavior in response to mosquito attack can influence the choice of host species (Edman *et al.*, 1974). Moreover, the studies with *Culicines* have shown an inverse relationship between the number of mosquitoes attacking an individual host and proportion that obtain a blood meal (Burkot, 1988). The frequency of interrupted feeding depends on host defensive behavior which increases with the number of attacking mosquitoes (Edman *et al.*, 1974). Thus, as mosquito density increases, the proportion feeding on more tolerant individuals also increases (Edman *et al.*, 1974). Consistent with these findings are studies that have shown greater feeding success in

mosquitoes with groups of hosts of the same species rather than mixed species (Edman and Webber, 1975). The wide range in the proportions of cryptic mixed meals taken by Anophelines from mother-child pairs may reflect variation in the irritability and defensive behavior of different individuals (Burkot, 1988).

2.6.6 Parasite-mediated behavioural changes

Studies have shown that infected animals exhibit reduced defensive behavior and are preferentially fed upon by the mosquitoes (Day *et al.*, 1983). Changes in activity pattern associated with symptomatic infections resulted in more tolerant hosts than were prudentially fed upon during times of gametocytaemia (Burkot, 1988). Moreover, lower haematocrits in the infected animals may decrease feeding time, resulting in increased feeding success of the mosquitoes (Rossignol, 1985). Invasion of mosquito salivary glands by malaria sporozoites can induce pathology that impairs the ability of the mosquito to engorge, resulting into increased probing. During feeding, mosquitoes secrete salivary apyrase which inhibits aggregation of host platelets, thereby enhancing the mosquitoes' ability to locate blood (Ribeiro *et al.*, 1984). Hence the proposed studies will investigate the extent of host selection by *Anopheles* mosquitoes in the presence of ITN or IRS or both interventions in western Kenya.

2.7 Adult mosquito sampling techniques

Sampling adult stage of *Anopheles* vectors of human malaria is an important and necessary process for estimating vector population density, obtaining an adequate sample to measure the sporozoite infection rate, and quantify the effects of the intervention directed against the vector population (Odiere *et al.*, 2007). One of the most commonly used sampling methods for indoor resting vectors is the pyrethrum spray collection (PSC) (Service, 1993). The PSC method is biased toward endophilic female mosquitoes, especially those that are blood-fed and gravid (Service, 1993). Such sampling would under-estimate population density when vector populations are more exophagic and exophilic; and it also avoid the outdoor fraction of the population and does not yield high

numbers of males (Odiere *et al.*, 2007). Sampling the outdoor "resting" population of mosquitoes has been accomplished through hand or mechanical aspiration of vegetation and other environments offering harborage and resting sites (Service, 1993), and deployment of various resting shelters and devices such as walk-in red boxes (Meyer, 1985), diurnal resting shelters (Morris, 1981), pit shelters dug into the ground (Service, 1993), fiber pots (Komar *et al.*, 1995), and woven baskets (Harbison *et al.*, 2006). Clay pots have also been shown to be useful in sampling outdoor resting *An. gambiae, An. arabiensis, An. funestus,* and *Culex* spp. of both sexes (Odiere *et al.*, 2007). While these techniques have been used to characterize mosquitoes depending on their resting and feeding behavior, not much effort have been made to estimate host selection by different collection methods.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The study was conducted at 4 sites: Asembo, Nyando, Teso and Bungoma in western Kenya (see Appendix IA). Asembo is located in Rarieda district in Nyanza province to the west of the city of Kisumu city near Lake Victoria. Asembo has been a Centre for Disease Control and Prevention (CDC) bednet study site where permethrin-treated, conventional bednets were distributed to all of the residents by early 1999, providing nearly 100% household ownership and high rates of nightly use (Alaii *et al.*, 2003; Lindblade *et al.*, 2004). Nyando is located about 30 kilometers east of Kisumu in central Nyanza. Nyando is covered by the Kenyan Ministry of Health's Indoor Residual Spray (IRS) program with the spray done once a year using pyrethroids (PMI., 2010). Teso is located approximately 0° 26' N 34° 9' E and Bungoma 00° 34' N 34° 34' E to the north in Kenyan Western Province and both border Uganda to the west. Although, Teso and Bungoma have no intervention initiatives such as programmed ITNs distribution or IRS implementation, the residence in these districts have acquired nets due to the scale-up and promotion of distribution of ITNs by the Kenya Ministry of Health (PMI, 2010). *An. arabiensis* is the dominant vector, in Asembo and Nyando sites along the shores of L. Victoria whereas in Teso and Bungoma, there is mixed proportions of vectors with high populations of *An. gambiae s.s.* and *An. funestus.*

All these regions experience a bimodal pattern of rainfall, with the heaviest rains experienced from March through May and with a smaller peak occurring in November and December each year (Odiere *et al.*, 2007). Houses are grouped into compounds of related family members and these compounds are separated by farmland. Most inhabitants practice subsistence farming with maize (the staple crop), sorghum, millet, vegetables and a few others are keeping animals such as goats, cattle and chickens.

3.2 Study design

The study was an observational one in which sampling was performed without introducing anything new into the study population. Sampling in the four districts was done monthly for four months between September and December, 2010. During each sampling effort, indoor and outdoor collection methods were used to sample the 3 predominant vector species in the area. All collected samples were then subjected to laboratory analysis including species identification and blood meal determination.

3.3 Sample size determination

Formal sample size calculations for estimating mosquito densities was not carried out considering the nature of the study, and the fact that it was both technically difficult and misleading to estimate sample sizes priori. This task was complicated by the fact that the statistical errors associated with measuring a fixed mean is a relatively minor component of the total variation that sampling of mosquitoes was intended to capture. The vast bulk of variation associated with longitudinal entomological surveys distributed across even the smallest of geographic scales results from genuine variations in mosquito densities across space and time, which in turn are neither predictable nor quantifiable priori. The greatest challenge to accurately and precisely estimate mosquito densities and cumulative exposure of humans was therefore to obtain spatially and temporally representative samples which were large enough to capture the highly aggregated distribution of such data. Elimination of sampling error, meaning where and when samples are taken, rather than measurement error, is the predominant issue facing such surveys. Rather than rely upon existing sample size calculation procedures, none of which are designed to deal with highly aggregated data with intense underlying variation across both spatial and temporal sampling dimensions, it was considered more appropriate to base sample size considerations upon experience of existing equivalent data sets prioritizing extensive rather than intensive sampling or precise measurement (Zhou *et al.*, 2004). In which case, 20 houses were targeted during each sampling effort by pyrethrum spray collection (PSC) and pot trap and 6 houses by light traps, both indoor and outdoor.

3.4 Mosquito collection methods

Sampling of both indoor and outdoor feeding and resting mosquitoes was done by three different methods: PSC, indoor CDC light trap, outdoor CDC light trap and pot collections.

3.4.1 Clay pots

Clay pots have been shown to be useful in sampling outdoor resting *An. gambiae, An. arabiensis, An. funestus,* and *Culex* spp. of both sexes (Odiere *et al.*, 2007). Sampling was done from pots placed outdoor by the residence for purposes of trapping water and disposed cracked pots. The pots were found either near houses or in shaded area within the sampled compounds. All the sampled pots were associated with the nearest houses. Sampling was done monthly from September to December, 2010. A battery-operated backpack aspirator was used to catch adult mosquitoes resting in the pots. The aspirator is fitted with a transferable cap that was used to transfer the caught samples into adult mosquito cage for further processing. For every household sampled, details of intervention type and use and availability of domestic animals and fowl were captured by personal digital assistant (PDA). Collection and house codes were generated from the PDA and all collection details were recorded for every household sampled.

3.4.2 CDC Light trap

Two CDC light traps were used in every selected homestead. One trap was installed indoor in the sleeping area or next to a bed net and another outdoor, next to a cattle shed if present. Homesteads with intervention but difference in cattle ownership were selected in each sampled site. First, a home with ITN or IRS and more than three cattle was selected, and next were homes with intervention but one or two cattle, and finally, those homes with intervention but no cattle. The traps were suspended at around 7.00pm until 6.00am. The trapped mosquitoes were transferred from the light trap the following morning by aspirators into paper caps. Details such as collection date, compound name, collection method, collection code and method were recorded on a label attached on the paper cap. Information about presence of intervention and use the night before

collection was captured by PDA. The same details were recorded on the collection form and notebook. The collected mosquitoes were knocked down with chloroform and the paper caps were placed in a cooler box for transportation to the laboratory.

3.4.3 Pyrethrum Spray Collection (PSC)

Sampling in was performed monthly from September to December, 2010. Mosquito collections were done in 30 nearest houses to the reference compound using Pyrethroid Spray Collection (PSC) method between 6.00am and 10.00am. The PSC was conducted by spreading white sheets on the floor and over the furniture within the houses. Spraying was then done around the eaves and windows from outside and then inside the house on roof and walls using 0.025% pyrethrum emulsfiable concentrate with 0.1% pipyronyl butoxide in kerosene. The house was closed for 10-15 minutes after which the knocked down mosquitoes were collected from the sheets. All collected mosquitoes were identified morphologically as either *Anopheles* or *Culex* and transported to the laboratory on moist filter paper mounted on petri-dishes in cooler boxes for further analysis. Details of type of intervention present and availability of domesticated animals and fowl for every household sampled were captured using PDA. The number of mosquitoes collected and the number of people that slept in the house the previous night were also recorded.

3.4.4 Bednet coverage

The initiation of distribution of long-lasting, insecticide treated nets by the Kenyan Ministry of Health to pregnant mothers and children < 5 years beginning 2004 at subsidized cost has led to high rates of bednet ownership in many communities (PMI, 2010). The bednet coverage and use in Teso and Bungoma in which no controlled distribution has been performed was determined for the sampled homesteads. Using PDA, information on bednet availability, type, and nightly use was captured.

3.5 Identification of adult *Anopheles* mosquitos

The adult *Anopheles* were identified by morphological features (Gillies and de Meillon, 1968) and

through the use of polymerase chain reaction (PCR).

3.5.1 Morphological identification of *An. gambiae s.l* and *An. funestus* adults

Morphological identification was however, performed with reference to the keys as per previous studies (Gillies and de Meillon, 1968). *Anopheles gambiae* complex species have five pale bands on the costal margin of the wings, rough abdomen and speckled legs. On the other hand, *An. funestus* have four pale bands on the costal margin of the wings, smooth shiny abdomen and dark legs (Gillies and de Meillon, 1968). Relatively, *An. gambiae* appear bigger than *An. funestus*, though this may not always be the case as size may vary depending on ecological factors (Gillies and de Meillon, 1968). Additional differentiation between *An. gambiae s.s.* and *An. arabiensis* was done through PCR as described below.

3.5.2 DNA extraction

In order to extract single mosquito DNA for species identification, the legs and wings of female mosquitoes and the whole male species were used. Each mosquito was ground in 100 µl grinding buffer in a micro-centrifuge tube. The tubes were immediately placed in 65 °C water bath for 30 minutes then 14 µl of 8m Potassium Acetate added and mixed thoroughly. The mixture was then placed on cool ice water for 30 minutes and then centrifuged at 13.2 rpm for 10 minutes. The supernatant was transferred into appropriately-labeled micro-centrifuge tube and 200 µl cold 95% ethanol was added, mixed and stored at -20°C for at least 20 minutes. The samples were than centrifuged at 13.2 rpm for 20 minutes, the ethanol poured off and the tubes were rinsed with 70% and then 95% ethanol. The tubes were inverted and allowed to dry completely for at least one hour. DNA was resuspended in 100 µl TE buffer and stored at -20 °C awaiting PCR.

3.5.3 PCR identification of *Anopheles gambiae complex*

Anopheles gambiae sibling species identification was carried out according to the method

described previously (Scott *et al.*, 1993) with modifications. Three set of primers abbreviated as UN, GA, and AR designed for DNA sequence of the intergenic spacer region of *An. gambiae* complex's of ribosomal DNA (rDNA) were used for identification. The primer sequence and their expected sizes of PCR products are provided in Table 1 below.

Table 1: Oligonucleotide primer sequences, band size and boiling point for identification of *An. gambiae s.l.* sibling species.

Primer	Sequence (5' – 3') T_M(°C)	Band size (bp)
UN	GTG TGC CCC TTC CTC GAT GT	468 56
GA	CTC GTT TGG TCG GCA CGT TT	390 62
AR	AAG TGT CCT TCT CCA TCC TA	315 78

UN: Universal primer; GA: *An. gambiae s.s.* primer; AR: *An. arabiensis* primer

A PCR reaction mix of 14µl containing 7.025µl double distilled PCR water, 3µl of 5X PCR buffer, 1.5µl of 2mM dNTP mix, 0.6 µl of each of the three 1µM primers (UN, GA and AR), 0.6µl of 1mM $MgCl_2$, 0.075µl of Taq polymerase and 1.0µl of DNA template was used for every single reaction in a 96 well-plate. The reaction condition was as follows: initial denaturation at 95°C for 10 min, followed by 35 cyclic conditions consisting of 94°C for 30s, then 64°C for 30s, followed by 72°C for 45s. The cycling period was then followed by a final extension at 72°C for 5 min and a hold at 4°C. The amplified products were analyzed by gel electrophoresis in 2% agarose gel. *An. gambiae s.s.* and *An. arabiensis* were differentiated on the gel depending on the relative position of the bands with reference to controls.

3.6 Mosquito blood meal analysis

The relative amount of blood in the abdomens of blood-fed mosquitoes was scored as unfed, fed,

half-gravid and gravid. Using sterile technique, the abdomens scored as fed and half-gravid were used in the blood meal analysis.

3.6.1 DNA extraction

DNA was extracted from the abdomens using Qiagen-Dneasy Tissue Kit (Cat No. 69506), as previously described (Hamer *et al.*, 2009) with slight modifications. This commercial kit utilized single mosquito abdomen for DNA extraction.

3.6.2 Polymerase Chain Reaction

The extracted DNA served as a template for PCR using primer pairs complementary to nucleotide sequences of the vertebrate Cytochrome b (*cyt b*) gene. Each sample was tested in a reaction using mammalian primer pair (5' – CCC CTC AGA ATG ATA TTT GTC CTC A – 3') and (5' – CCA TCC AAC ATC TCA GCA TGA TGA AA – 3'). The FailSafe PCR System (Cat. No. FSP995E) was used under conditions consisting of an initial denaturation at 95°C for 3.5 minutes, followed by 36 cycles consisting of denaturation (at 95°C for 30 seconds), annealing (at 60°C for 50 seconds), extension (at 72°C for 40 seconds), and a final extension at 72°C for 5 minutes (Hamer *et al.*, 2009). Depending on the quantity needed, PCR mix was prepared in a tube or vial with every reaction of 50µL of the mix according to the recipe described in Appendix IC. A set of extraction controls containing all the extraction reagents but no samples, were also subjected to PCR to test for any possible contamination with foreign DNA. A PCR control was also introduced in the amplification to check on contamination, all these were treated as negative controls. Mammalian DNA from human and cow were used as positive controls.

3.6.3 Gel electrophoresis

A total volume of 10µl of the amplicon was visualized by electrophoresis on a 2% agarose gel stained with 0.5µg/ml of ethidium bromide. The electrophoresis was run on Tris-Base EDTA (TBE) buffer at 100V for 20 minutes and bands visualized under ultraviolet transillumination connected to a computer monitor. Negative controls were checked for any form of contamination with DNA.

Photographs of the gel images were taken for analysis. An example of the photographed image is shown in Appendix ID. The gel product was scored on the plate map for every sample, awarding values depending on the band intensity as follows.

Table 2: The gel product scores

Score of 0	No product
Score of 1	Faint product
Score of 2	Light product
Score of 3	Good Product
Score of 4	Strong product

3.6.4 PCR Products Clean up

The DNA purification was performed only in cases of successful amplification. The Qiagen – QIAquick PCR Purification Kit (Cat. No. 28106) was used in PCR product clean-up. The remaining 40µl PCR product was mixed with the buffer PB (binding buffer) and the mixture transferred to the appropriate spin column using 1000µL pipette. The mix was then centrifuged for 1 minute at 10, 400 rpm. The flow-through was discarded and the spin column placed back at the same collection tube. A volume of 700µL of buffer PE was added using a repeater and centrifuged for 1 minute at 10,400 rpm. The flow–through was then discarded and the spin column placed back at the same collecting tube and span again for 1 minute at 10, 400 rpm. The collection tubes were discarded and the spin column placed in the appropriately labeled 1.5ml tubes. Using repeater pipette, 30µL of PCR H_2O was added to the centre of membrane and let to stand for 1 minute, then centrifuged for 1 minute at 10,400 rpm. The spin column was then discarded and the purified PCR product stored at 4 °C for short storage or placed at -80 °C for long storage, prior to sequencing.

3.6.5 Sequencing

Nucleotide sequences of amplicons were obtained by direct sequencing. In preparation for

sequencing, a plate map was prepared by arranging the purified samples by column. A master-mix of H_2O and primers was prepared by mixing 500µl of H_2O and 50µl primer and loaded into appropriate wells. A volume of 2µl of the purified DNA product was loaded into the appropriate wells. The quantity of the PCR product was decreased to 1µl if the band score is 4 and the quantity compensated with water. The plate was then covered with aluminum foil and appropriate labeling done on the sides. Sequencing involved three steps consisting of, denaturation at 94°C, annealing at 54°C and extension at 60°C repeated for 35 cycles.

The sequences results were analyzed in Basic Local Alignment Search Tool (BLAST) search engine in GenBank. Using the free downloadable software, ChromasPro version 1.5, the nucleotide sequences were translated into chromatograms for sequence analysis before a BLAST search. Each chromatogram was inspected for sequence quality and presence of double nucleotide peaks, which may have indicated mixed blood meals. Samples that produced an amplicon in the reaction and satisfactorily matched by BLAST were accepted as the likely host of origin, typically with 99% sequence match. The host scientific name, common name, accession number, maximum score, total score, % identity (including numerator and denominator), and *e*-value were recorded in the database. The chromatograph was downloaded and evaluated for quality and double nucleotide peaks (Appendix IE).

3.7 Ethical Considerations

3.7.1 Consent

The study was explained to village chiefs and elders and to the individual participants for verbal consent before commencement.

3.7.2 Risk to community members

There was no risk to community members. Pyrethrum spray collection is a standard WHO approved indoor mosquito collection method (WHO, 1975). Care was taken to ensure that the spray does not get into cooked food or drinking water by ensuring these items are either covered or

removed from the house before commencement of the indoor spray. Household members were instructed to stay out of their house for 30 minutes after spraying.

3.8 Data Analysis

Analysis of variance (ANOVA) was used in comparing the means of host blood meal for different *Anopheles* species and to compare the accuracy of different collection methods in estimating the host feeding preferences. ANOVA was used in testing the similarities and variations in host selection between different species of the local malaria vectors. Proportions of intervention type and *Anopheles* host blood meal types were generated by descriptive statistics using SAS, version 9.2. To compare means per house of *An. gambiae s.l.* sampled by PSC between ITN and non-ITN houses, controlling for clustering at a household level, a longitudinal regression analysis (Diggle *et al.*, 2002) was carried out, using the generalized estimating equations procedure (GENMOD) in SAS, version 9.2. Bar graphs of proportions of *Anopheles* host blood meal by sampling methods was plotted in Microsoft Excel. Statistical significance was tested at P value of ≤ 0.05.

CHAPTER FOUR

4.0 RESULTS

4.1 General characteristics of the sampled sites

Among the 498 houses sampled in this study, 280 (56.22%) had ITNs only, 20 (4.02%) had IRS only, 30 (6.02%) had both ITNs and IRS, 71 (14.26%) untreated nets and 97 (19.48%) had no intervention (Table 3). Among the ITNs, there were 157 (76.59%) permethrin-treated nets (Olyset - net), and 48 (23.41%) deltamethrin-treated nets (PermaNet and Duranet).

Approximately 1,750 individuals spent the night in the sampled households the night before the collection. Out of these, only about 45.00% of the individuals slept in ITN provided houses while 55.00% others either did not use nets at all or had untreated bednets (Table 4).

Out of the 2,768 *Anopheles* mosquitoes sampled, both indoor and outdoor, 735 (27%) were *An. funestus*, 964 (35%) were positively identified by PCR as *An. gambiae s.s.*, 508 (18%) were *An. arabiensis* and 561 (20%) were undetermined (Table 5).

Table 3: Proportions of intervention presence in each sampled district

Intervention	Bungoma	Teso	Nyando	Rarieda	Total, n (%)
ITNs	109	138	3	30	280 (56.22%)
IRS	0	0	20	0	20 (4.02%)
ITNs and IRS	0	0	30	0	30 (6.02%)
Untreated nets	19	28	2	22	71 (14.26%)
No intervention	61	30	5	1	97 (19.48%)
Total, n (%)	189 (37.95%)	196 (39.38%)	60 (12.05%)	53 (10.64%)	498 (100%)

Table 4: Proportion of net use in the sampled population

	Number of individuals, (%)
Under ITN covered house	787 (45)
Under no net and untreated nets	963(55)
Total	**1,750**

Table 5: Proportions of *Anopheles* species

***Anopheles* species**	**N (%)**
An. funestus	735 (27)
An. gambiae s.s.	964 (35)
An. arabiensis	508 (18)
Undetermined	561 (20)
Total	**2,768 (100)**

4.2 Blood meal analysis

A total of 1,073 *Anopheles* mosquitoes' blood meals were analyzed by sequencing to identify the vertebrate host. Of the total, 835 (77.82%) human, 39 (3.63%) bovine, 7 (0.65%) goat, 3 (0.28%) rat, 1(0.09%) and 1(0.09%) frog blood meals were positively identified by a BLAST search of nucleotide sequences in the Genbank database while 185 (17.24%) failed sequencing. A summary of the numbers of host blood meal type by district and vector species is shown in Table 6.

Table 6: A summary of the numbers of host blood meal type for different *Anopheles* mosquitoes per study district

District	*Anopheles* species	Human	Bovine	Goat	Rat	Frog	Bird	Failed	Total
Bungoma	*An. gambiae s.s.*	76	0	0	0	0	0	5	**81**
	An. arabiensis	4	0	0	0	0	0	1	**5**
	An. funestus	110	0	0	0	0	1	38	**149**
	Not Amplified	41	0	0	0	0	0	0	**41**
Teso	*An. gambiae s.s.*	134	2	0	0	1	0	24	**163**
	An. arabiensis s.s.	4	0	0	0	0	0	4	**8**
	An. funestus	74	1	1	2	0	0	19	**97**
	Not Amplified	2	0	0	0	0	0	0	**2**
Nyando	*An. gambiae s.s.*	18	0	0	0	0	0	1	**19**
	An. arabiensis	126	9	1	0	0	0	31	**167**
	An. funestus	17	0	0	0	0	0	0	**17**
	Not Amplified	69	2	0	0	0	0	8	**79**
Rarieda	*An. gambiae s.s.*	6	0	0	0	0	0	0	**6**
	An. arabiensis	48	21	5	1	0	0	36	**111**
	An. funestus	72	1	0	0	0	0	1	**74**
	Not Amplified	34	3	0	0	0	0	17	**54**
Totals	**N**	**835**	**39**	**7**	**3**	**1**	**1**	**185**	**1073**
	(%)	**(77.82)**	**(3.63)**	**(0.65)**	**(0.28)**	**(0.09)**	**(0.09)**	**(17.24)**	**(100)**

High rates of human (*Homo sapiens*) feeding were observed in *An. gambiae s.s.* and *An. funestus*, with no significant difference between the two species ($P = 0.08$). *An. gambiae s.s.* and *An. funestus* had a statistically significant ($P < 0.0001$) higher proportion of human blood meal compared to *An. arabiensis,* with mean differences of 0.25 and 0.16, respectively (Table 6). Although the proportions of bovine (*Bos taurus/Bos indicus*) blood meal was observed to be low in *An. gambiae s.s.* and *An. funestus,* significantly ($P < 0.0001$) high feeding was observed in *An. arabiensis* with mean difference of 0.1 (Table 6*)*. Significant differences in the means of goat (*Capra hircus*) blood meal were only observed between *An. arabiensis* and *An. funestus* ($P = 0.05$). Other types of blood meal identified in the study were, rat (*Arvicanthis abyssinicus/Mus musculus/Rattus norvegicus*), bird (*Lanius meridionalis*) and frog (*Eupsophus migueli*) blood (Table 7).

Table 7: Comparison of the mean of hosts blood meals between different *Anopheles* mosquitoes

	Mean difference (Simultaneous 95% Confidence Interval)					
Anopheles Comparison	**Human**	**Bovid**	**Goat**	**Rat**	**Bird**	**Frog**
An. gambiae.s.s versus *An. funestus*	0.09 (-0.01- 0.19)^{NS}	0.03 (-0.02 - 0.07)^{NS}	-0.003 (-0.02 - 0.01)^{NS}	-0.01 (-0.02 - 0.01)^{NS}	-0.003 (-0.01- 0.003)^{NS}	0.004 (-0.003 - 0.01)^{NS}
An. gambiae s.s. versus *An. arabiensis*	**0.25 (0.15 - 0.35)*** **	**-0.1 (-0.14 - 0.06) *** **	**-0.02 (-0.04 - - 0.002)***	-0.003 (-0.02- 0.01)^{NS}	0 (-0.01- 0.01)^{NS}	0.004 (-0.003 - 0.01)^{NS}
An. funestus versus *An. arabiensis*	**0.16 (0.06 - 0.25)*** **	**-0.1 (-0.14 - 0.06)*** **	**-0.02 (-0.03 - - 0.00009)***	0.003 (-0.02- 0.01)^{NS}	0.003 (-0.01- 0.01)^{NS}	0 (-0.01- 0.01)^{NS}

Significant values are bolded; *** $P < 0.0001$ and * $P = 0.01$; NS – not significant.

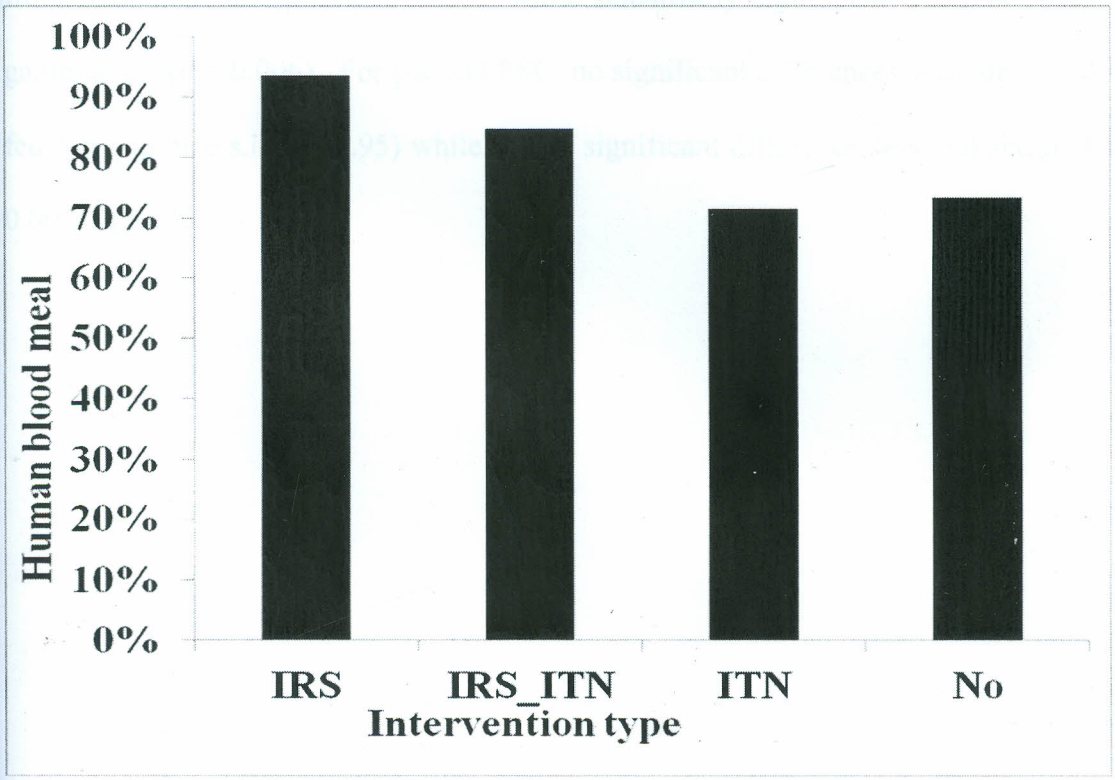
Difference in the means of host blood meals of indoor resting anophelines in the presence of ITN was performed to test for the impact of ITN on host selection. No significant differences in the means were observed between *An. funestus* and *An. gambiae s.s* for human, bovine, and goat blood meals ($P = 0.08$, $P = 0.99$, $P = 0.99$), respectively. Statistically significant differences were observed between the more zoophilic *An. arabiensis* and the more anthropophilic *An. funestus* ($P < 0.0001$, $P < 0.0001$ and $P = 0.05$) for human, bovine and goat blood meal, respectively. Similarly, significant differences were realized between *An. arabiensis* and *An. gambiae s.s.* ($P < 0.0001$, $P < 0.0001$ and $P = 0.02$) for human, bovine and goat blood type, respectively (Table 8). No significant effect of different interventions was realized on the selection for human blood meal by all the *Anopheles* vectors, and for IRS ($P = 0.09$), both IRS and ITN ($P = 0.74$) and ITN ($P = 0.55$) (Figure 1).

Table8: Comparison of means of host blood meal of Indoor *Anopheles* in the presence of ITN

Difference between means of indoor *Anopheles* in ITN houses (95% Confidence Interval)			
Anopheles Comparison	**Human**	**Bovid**	**Goat**
An. funestus versus *An. gambiae s.s.*	-0.09 (-0.18 - 0.01)^{NS}	-0.01 (-0.04 - 0.04)^{NS}	0.01 (-0.01 - 0.02)^{NS}
An. arabiensis versus *An. gambiae s.s.*	**-0.2 (-0.35 - - 0.15)*** **	**0.10 (0.06 – 0.14)*** **	**0.02 (0.01 – 0.04)***
An. arabiensis versus An. funestus	**-0.16 (-0.25 - -0.06)*** **	**0.10 (0.06 – 0.14)*** **	**0.02 (0.00 – 0.03)***

Significant values are bolded; *** $P < 0.0001$ and * $P = 0.01$; NS – not significant

Figure 1: Proportions of human blood meal by presence of intervention



4.3 Sampling techniques

Of the 2,768 *Anopheles* sampled, 1,752 (63.29%) were from PSC, 549 (19.83%) by light trap, 103 (3.72%) from pot and 364 (13.15%) by aspiration (Table 9).

In a comparison of the means of fed and unfed An. gambiae s.l. and An. funestus between different sampling techniques (PSC, CDC light trap and pot), using ANOVA, significantly higher proportions of fed An. gambiae s.l and An. funestus were collected by PSC than CDC light traps ($P < 0.0001$ and $P < 0.0001$) with a mean difference of 0.14 and 0.07 for each species, respectively. The proportion of unfed Anopheles was significantly higher in the CDC light trap than PSC ($P < 0.0001$ and $P < 0.0001$), with a mean difference of 0.41 and 0.09 for An. gambiae s.l. and An. Funestus, respectively (Table 9). While in a comparison of CDC light traps and pots, a difference 0.23 unfed An. gambiae s.l. and 0.09 unfed An. funestus was observed ($P < 0.0001$ and $P = 0.0002$) respectively. No significant differences was observed between the means of the fed An. funestus from light traps and pots ($P = 0.33$), while statistically significant difference was realized in An. gambiae s.l. ($P = 0.006$). For pot and PSC, no significant differences were observed in the means of fed An. gambiae s.l ($P = 0.95$) while highly significant difference was realized in An. funestus ($P < 0.0001$) (Table 10).

Table 9: Summary of *Anopheles* mosquitoes collected by the four different sampling techniques

		An. gambiae s.l		*An. funestus*		
Collection Method	Number of Houses	Male	Female	Male	Female	**Total N, (%)**
PSC	261	408	774	213	357	**1,752 (63.29)**
Light Trap	139	46	377	20	106	**549 (19.83)**
Pot	90	31	61	5	6	**103 (3.72)**
Back Pack	8	86	250	9	19	**364 (13.15)**
Total	498	571	1462	247	488	**2,768 (100)**

Table 10: A comparison of the means of fed and unfed *An. gambiae s.l.* and *An. funestus* between collection methods

Difference between means (Simultaneous 95% limit)				
	An. gambiae s.l.		*An. funestus*	
Collection Method	Fed	Unfed	Fed	Unfed
Light Trap versus PSC	**-0.14 (-0.20–0.08)*** **	**0.41 (0.38–0.45)*** **	**-0.08 (-0.12–0.04)*** **	**0.09 (0.06–0.12)*** **
Light Trap versus Pot Trap	**-0.17 (-0.30–0.04)** **	**0.23 (0.15–0.32)*** **	0.05 (-0.03–0.14)^{NS}	**0.09 (0.04–0.15)** **
Pot Trap veresus PSC	0.03 (-0.10– 0.15)^{NS}	**0.18 (0.10–0.26)*** **	**-0.13 (-21–0.06)*** **	0.01 (-0.05–0.05)^{NS}

Significant values are bolded; *** $P < 0.0001$ and ** $P = 0.001$; NS – not significant

Pyrethrum Spray Collections gave the highest proportions of both human and cattle blood meal in comparison with the other two sampling techniques (Figure 2). All the *Anopheles* vectors collected

from the pots were positive for human blood meals while both outdoor and indoor CDC light traps had equal numbers of human blood meal (N=34) with all samples trapped indoor being positive for human blood while only 2 vectors from outdoor light traps tested positive for cattle blood.

The indoor resting densities of fed *An. gambiae s.l* sampled by pyrethrum spray collections was observed to be statistically higher in houses without ITN than in the ITN-provided houses (χ^2 = 4.22; P = 0.04) (Figure 3).

Figure 2: Proportions of the host blood meal according to collection method

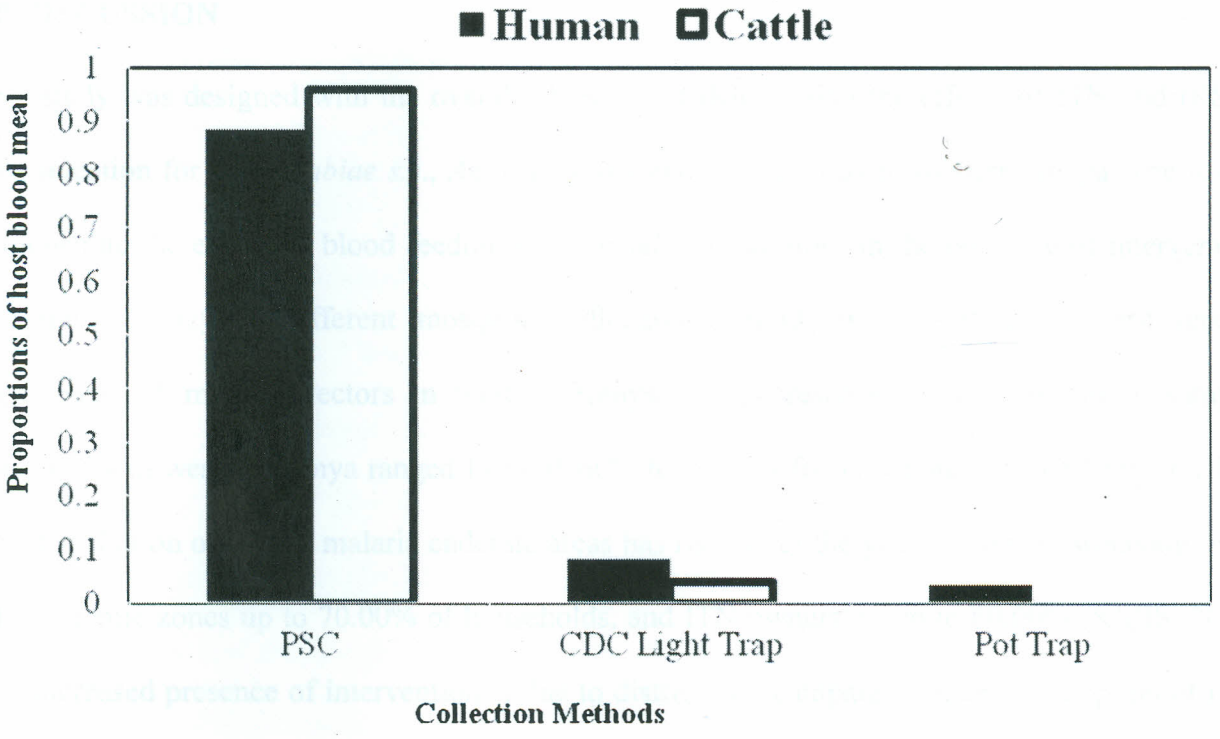
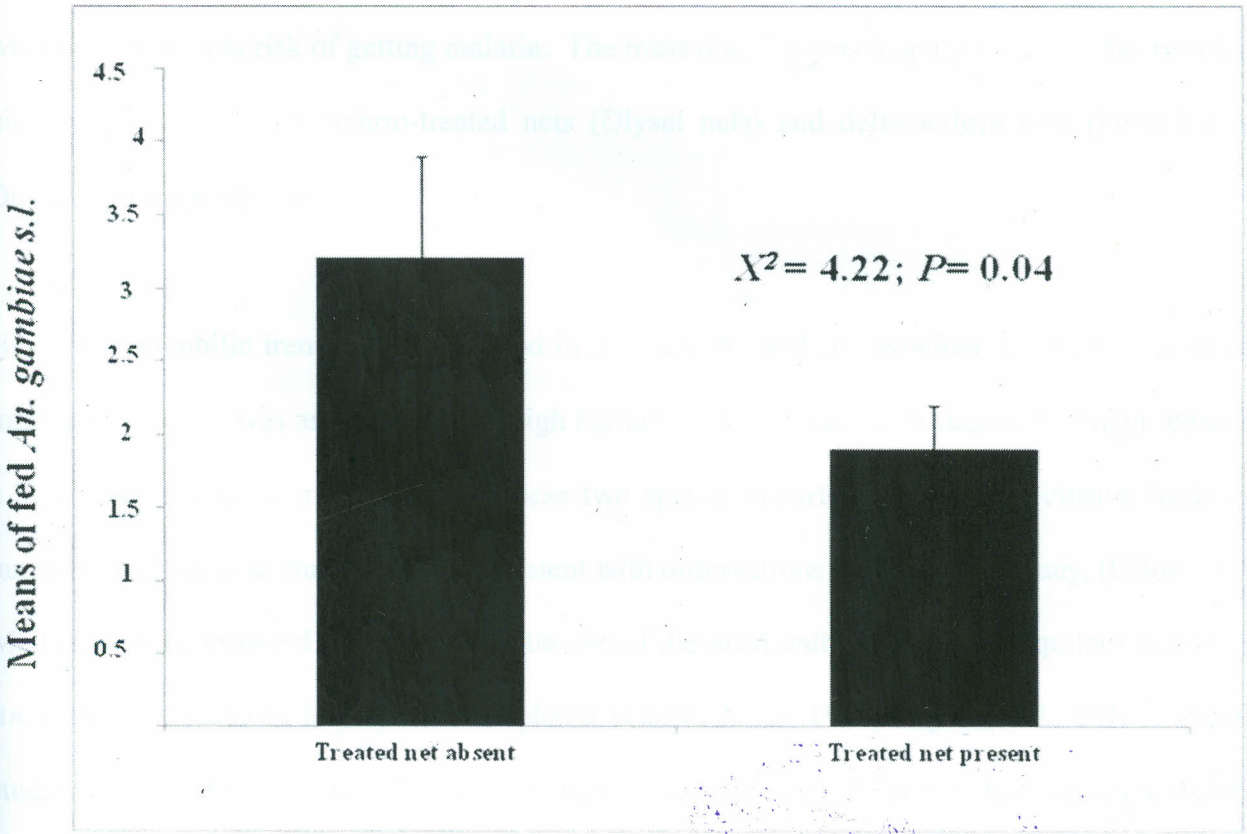


Figure 3: Mean per house of fed *An. gambiae s.l.* sampled by PSC compared between ITN and non-ITN houses



CHAPTER FIVE

5.0 DISCUSSION

This study was designed with the overall objective of determining the effects of ITN and IRS on host selection for *An. gambiae s.s.*, *An. arabiensis* and *An. funestus* in western Kenya. The results demonstrate the extent of blood feeding of *Anopheles* mosquitoes in the presence of intervention, and the accuracy of different mosquito collection methods in estimating the host feeding preferences of malaria vectors in western Kenya. The presence of intervention in sampled households in western Kenya ranged from 56.60% to 70.41% for ITN houses and 88.33% for IRS. The distribution of nets in malaria endemic areas has risen over the years with net ownership in the lake endemic zones up to 70.00% of households, and ITN ownership up to 60.00% (KMIS, 2010). The increased presence of intervention is due to distribution campaigns since the adoption of ITNs by WHO as one of the cornerstones for the Roll Back Malaria strategy (RBM, 1999). The next campaign planed for 2011/12, in which one net will be given to every two people in endemic and highland epidemic prone zones (KMIS, 2010) is expected to provide universal coverage for the whole population at risk of getting malaria. The mass distribution campaign of ITNs has resulted in high proportions of permethrin-treated nets (Olyset nets) and deltamethrin nets (PermaNet and Duranet) in the study area.

High anthropophilic trends were observed in *An. funestus* and *An. gambiae s.s.* in the presence of intervention. This was an indication of high human-vector contact with successful blood meals and a high risk of malaria transmission. These two species reported almost an exclusive feeding on humans in all the four study areas. Consistent with observations in the current study, (Gillies and de Meillon, 1968) observed *An. funestus* to be one of the most anthropophilic mosquitoes known, and *An. gambiae s.s.* to be largely anthropophagic (Garret-Jones, 1964; Bayoh *et al.*, 2010). Previous studies investigating mosquito blood meal types, have reported a trend in host selection in which the *Anopheles* mosquitoes prefer certain hosts but can easily shift to other host if their favoured

hosts is no longer reachable (Takken, 2002), possibly being under the protection of an intervention. The preference of *An. gambiae s.s.* and *An. funestus* for human blood meal was observably not affected by the presence of interventions. The vectors did not shift to feeding on alternative hosts, instead high proportions of human blood meals were observed.

The current study further identified a range of hosts attacked by *Anopheles* vectors which included frog, wild bird, rat, goat and bovine in addition to the predominant human host. These findings are consistent with studies carried out in western Kenya in which *An. gambiae s.s.* and *An. funestus* were reported to have fed almost exclusively on humans with a small proportion obtaining blood meal from alternative hosts (Githeko *et al.,* 1994). The results of this study suggest that although *An. gambiae s.s.* and *An. funestus* vectors may attack a range of alternative hosts, their selection for human host is not altered even with increased presence of intervention and their usage.

An. arabiensis on the other hand showed more selection for cattle blood meal than *An. funestus* and *An. gambiae s.s.,* an evidence of their zoophilic nature. This species have equally been reported to have a higher preference for cattle blood meal (Githeko *et al.,* 1994) than the other two anthropophagic species (*An. funestus* and *An. gambiae s.s.*). A previous study in Asembo reported *An. arabiensis* to have fed most frequently on bovines (65%) than human (13%) (Bayoh *et al.,* 2010) and another conducted at Ahero (Githeko *et al.,* 1994), observed that *An. arabiensis* had fed on a range of hosts with the most predominant one being cattle, as was reflected by the low HBI (0.23). This would suggest that in the presence of both cattle and human, more *An. arabiensis* in western Kenya would preferentially attack cattle than human unlike *An. funestus* and *An. gambiae s.s.* However, a study in Konso in southern Ethopia, comparing the catch of human- and cattle-bait using the same sampling technique and using different baits showed that the human-baited trap caught significantly more mosquitoes than the cattle-baited one, suggesting that *An. arabiensis* in Konso is inherently anthropophagic (Tirados *et al.,* 2006).

A shift in host selection has been suggested in other studies (Garret-Jones, 1964; Takken, 2002) as a product of sustained intervention use, however, the current study does not confirm this observation. Instead, high anthropophilic trends were observed in all the malaria vectors in western Kenya with the selection for human blood meals being almost exclusive in *An. funestus* and *An. gambiae s.s.* in the presence of intervention. This finding confirms earlier observations that have indicated that a shift in host selection due to bednet is unlikely (Lindsay *et al.*, 1993; Mbogo *et al.*, 1996).

In a comparison of the proportions of bovine and human blood meal in *An. arabiensis,* an increased feeding on humans with 82% human blood and 17% cattle was observed. Although being more zoophilic than *An. gambiae s.s.* and *An. funestus*, *An. arabiensis* was observed to have an increased human blood meal, a species that has been associated more with zoophilic and exophilic trends other than anthropophagy in western Kenya. The current findings were consistent with the study in Konso where it was observed that although the majority of the blood meal may have come from cattle, the *An. arabiensis* population were inherently more strongly attracted to humans, such that the feeding ratio on human was eight times higher than the expected in an area where cattle out number humans by 17:1 (Tirados *et al.*, 2006).

Under intense insecticide pressure, it is possible that endophilic and anthropophagic individuals might be greatly reduced or even be eliminated to leave genetically zoophagic and exophilic adults (Githeko *et al.*, 1994). Such changes in vector population densities and abundance as a result of sustained ITN use have been observed in parts around the lake region of western Kenya with the decline in the population of *An. funestus* (Gimnig *et al.*, 2003a) and that of *An. gambiae s.s.* with a proportionate increase in the population of *An. Arabiensis* in Asembo (Bayoh *et al.*, 2010). The population of *An. arabiensis* at Ahero, has been reported to be more zoophilic and exophilic (Githeko *et al.*, 1994). Consequently, they may easily avoid the indoor-based intervention strategies like ITNs and IRS that target indoor feeding and resting vectors. Reduction in population densities

of the more anthropophilic and endophilic *An. gambiae s.s.* and *An. funestus* in western Kenya due to sustained intervention use may have effectively reduced competition for the genetically zoophilic and exophilic species, *An. arabiensis.* The observed increased human feeding of *An. arabiensis* which remain to dominate the vector population in Nyando and Rarieda districts, could be associated with changes in species composition. Of importance to note is the fact that the greatest proportions of the samples that were subjected to blood meals study were mosquitoes caught indoor by PSC. The increased human blood meal in *An. arabiensis* is seemingly associated with increased indoor resting for a species that has been reported to be more exophilic (Githeko *et al.,* 1996b). This may subject the vector to the effects of the insecticide-based interventions as has been observed in *An. gambiae s.s.* in western Kenya.

The correct identification of hosts of malaria vectors is important for determining the frequency at which vector population feeds on human, a true measure of human-vector contact (Githeko *et al.,* 1994). ITNs and IRS aimed against adult *Anopheles* mosquitoes in malaria control principally work to reduce or limit this contact. The results presented in this study represent potentially dangerous situation in which the vectors display high selection for human host in the presence of increasing use and presence of intervention. These observations may suggest a failure in the intervention tools against malaria vectors; however, several factors may be responsible for the observed continued human feeding in the presence of intervention.

Successful human blood meal by the vectors in the presence of interventions as observed in high frequencies of human blood, is possibly a product of behavioral or physiological resistance or both. Changes in vector feeding behavior, marked by changes in biting time and outdoor feeding (Takken, 2002) may lead to successful blood meals, while the vectors effectively avoid the lethal effect of the intervention. Such behavioral modification may involve the vectors feeding early in the evening before their preferred hosts go under the protection of the interventions as observed in early

exophily of *An. gambiae s.s.* in western Kenya (Githeko *et al.,* 1996b), in order to avoid the immediate environment of the intervention.

Reduced susceptibility to pyrethroids used in treated nets and in IRS as reported in the population of *An. gambiae s.s.* in western Kenya (Vulule *et al.,* 1994) could possibly be a contributing factor to continued human blood meals by *An. gambiae s.s.* in the presence of intervention. In a previous study (Alaii *et al.,* 2003), a consistent trend of high degree of susceptibility of *An. arabiensis* to all insecticides but moderate to high resistance to pyrethroids in *An. gambiae s.s.* in all populations in western Kenya, was reported. It was, however, suggested in the same study that the high degree of susceptibility of *An. arabiensis* could be due to the behaviour of the vector which often feeds outdoors and on cattle, and may avoid the insecticide on nets (Alaii *et al.,* 2003). Insecticide resistance undermines control of vector-transmitted diseases because it increases the number of vectors that survive the insecticide treatment (Alaii *et al.,* 2003).

Other factors that could possibly explain successful human blood meals in the presence of intervention would relate to low nightly use of ITNs by individuals, and poor condition of the nets. For example, the nets may be torn giving easy passage for the vectors to access the sleeping individual. On the other hand, spraying of the walls as in IRS may not provide adequate protection to individuals without bednets. A study in India evaluating bednets treated with deltamethrin reported bednets to be superior to IRS in the control of malaria vector *An. culicifacies* (Alaii *et al.,* 2003). The insecticide that was used in Nyando district for IRS just before the study was deltamethrin; unless this prevented house entry, the vectors may have found alternative resting places within the houses on the hangings, obtained a blood meal and exited or rested on the hangings within the house safely avoiding the treated walls. It is highly likely that these factors contribute to the observed continued anthropophilic trends in malaria vectors in the presence of ITN and IRS in western Kenya.

Host preference for *Anopheles* vectors is dependent on the intrinsic vector behavior and estimation of this phenomenon by different collection methods aimed at collecting mosquitoes at a specific environment of the host seemed unlikely. While the greatest proportion of human blood meal was detected from the PSC collection, the same was true for cattle blood meal. The presence of cattle blood meal in indoor PSC-collected *An. arabiensis* was a sign of outdoor feeding and indoor resting behavior exhibited by the vector. Capture-mark-release-recapture studies in western Kenya have demonstrated that although female *An. arabiensis* are usually exophilic, they nevertheless sometimes rest in houses (Githeko *et al.*, 1994). Most interestingly is the fact that all the malaria vectors sampled from pots outdoor were positive for human blood, but not a single bovine or alterative host blood meal was observed. This could be a result of either early exophily as reported in *An. gambiae s.s.* (Githeko *et al.*, 1996c) or exiting behavior due to subtle repellency by permethrin-treated bednets (Mathenge *et al.*, 2001).

Light traps installed near cattle shed and clay pots outdoor did not report higher proportions of bovine and other alternative blood meals as would be expected but rather more of human blood meal were detected. It was therefore unlikely that the outdoor methods sampled more of exophagic *Anopheles* population and indoor on the other hand, sampled endophagic vectors. Besides, having the CDC light traps paired for every sampled households with one indoor in the sleeping area and another outdoor near a cattle shed, did not introduce any bias in the numbers of hosts detected relative to trap position. The few fed vectors found in the outdoor light traps were positive for human blood meal, except for two samples out of thirty six. These results suggested early exophily, a behavior that has been observed in *An. gambiae s.s.* in western Kenya and noted to be increased by permethrin-based interventions indoor (Githeko *et al.*, 1996c). Thus, host selection was unlikely to be accurately estimated by mosquito sampling techniques aimed at collecting vectors from a specific environment of the host. Indoor collections by PSC proved most representative for all the possible *Anopheles* hosts in western Kenya. One important observation of this study was

that PSC, an indoor mosquito collection tool is most suitable in sampling for blood meal studies aimed at determining the extent of host selection by malaria vectors in western Kenya.

The use of CDC light traps in mosquito collections was observed to be effective in trapping host-seeking vectors. A comparison of the proportions of unfed and fed *Anopheles* between different collection methods clearly indicated that the CDC light trap have the highest proportions of unfed vectors while PSC catches were mostly fed samples. The light traps appeared to provide a form of protection to the sleeping individuals as demonstrated by increased proportion of unfed *Anopheles* vectors in the traps. The host seeking vectors were possibly attracted by the light from the trap and were hence trapped before having a blood meal.

The PSC had the greatest numbers of all the anophelines compared to clay pots and CDC light traps. A study in western Kenya comparing different sampling techniques, showed that six clay pots were generally equivalent to one indoor pyrethrum spray collection in terms of the numbers of collected mosquitoes (Odiere *et al.,* 2007). The studies were, however, not comparable in the catches between clay pots and PSC because pots were not distributed but rather sampling was done from those installed by the residents for trapping water and other domestic use. These pots were possibly visited by the residence, hence, disturbing the outdoor resting mosquitoes before sampling. Besides, there was a high variation in the number of pots sampled per household. The dismal performance of pots in this study demonstrates the fact that any study involving sampling outdoor for resting mosquitoes from pots, require specially-designed pots to limit any interference with the resting vectors.

Other than the reported high rates of human feeding by malaria vectors, a clear impact of ITNs on the feeding success of *An. gambiae s.l.* was observed in the PSC collections of the indoor resting anophelines. The results showed a reduced feeding success of 4% by *An. gambiae s.l.* in the

presence of ITNs. This observation was consistent with previous studies using experimental huts in The Gambia (Lindsay *et al.,* 1991), which reported a reduction in biting by 91% by nets with high dose of permethrin compared with the control nets. Earlier studies by the same author in a field trial equally reported a reduction in biting by bednets treated with permethrin at 500mg/m^2 (Lindsay *et al.,* 1989). Another study in western Kenya also reported reduced success in blood feeding in the night before the collection (Mathenge *et al.,* 2001).

5.1 CONCLUSION

1. No shift in host selection for *An. gambiae s.s.* and *An. funestus* and *An. arabiensis* in the presence of intervention was observed. Instead continued anthropophilic trends marked by the high proportions of human blood meals were evident with the vectors showing almost exclusive selection for humans. *An. arabiensis* was observed to be the most zoophilic of all the three vectors in western Kenya.
2. Host selection was observed to dependent on the intrinsic vector behavior and was unlikely to be accurately estimated by mosquito sampling techniques aimed at collecting vectors from a specific environment of the host.

5.2 RECOMMENDATIONS

1. The observed high rates of human blood meals presents risk of malaria infection in the presence of intervention, therefore, much more effort towards universal coverage with ITN and IRS of the general public is necessary to ensure that a greater population is protected.
2. Insecticide-based vector control interventions targeting adult mosquitoes have been adopted as the main malaria control tools; however, with the observed challenges such as insecticide resistance and increased human blood meals in the presence or intervention, new control tools should be exploited. Integrated vector control strategy involving larval control and house modification should be adapted in addition to ITNs and IRS.

5.2 SUGGESTIONS FOR FURTHER STUDY

1. Physiological resistance to insecticides in *An. gambiae s.s.* observed in western Kenya is possibly a driving force towards continued successful human blood meals in the in the presence of intervention, information linking host selection to resistance situation is necessary. Also, resistance in *An. funestus* in western Kenya population is not yet investigated and this requires much attention given the observed rise in the densities of *An. funestus*.

2. Behavioral resistance of *Anopheles* mosquitoes manifested in changes in host preference, biting time and outdoor feeding are possible results of sustained intervention use. While this study observed no shift in host preference, biting time and outdoor feeding remain to be investigated in the vector population of western Kenya.

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