"Sampling African Malaria Vectors Using Limburger Cheese and 'Milk cream' as Odour Baits"

By:

Eunice Anyango Owino

B.Sc., Hons (University of Nairobi).

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology (Medical Entomology) of the University of Nairobi.

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DECLARATION

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

Signed:..... Date:...August 11, 2006

Eunice Anyango Owino.

This thesis has been submitted with our approval as University supervisors:

SCHOOL OF BIOLOGICAL SCIENCES
UNIVERSITY OF NAIROBI
P.O. Box 30197
NAIROBI

1) Signed: ..... Date:...August 11, 2006

Wolfgang Richard Mukabana, Ph.D.

(Senior Lecturer, School of Biological Sciences, University of Nairobi, Kenya)

SCHOOL OF BIOLOGICAL SCIENCES
UNIVERSITY OF NAIROBI
P.O. Box 30197
NAIROBI

2) Signed:..... Date:...August 14, 2006.

Paul Ndegwa, Ph.D.

(Senior Lecturer, School of Biological Sciences, University of Nairobi, Kenya).

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DEDICATION

I dedicate this work to my parents Mr. Moses Owino Aduwo and Mrs. Anna Owino and to my grandfather Zaddock Aduwo who made sure that I joined school early enough and supported me to grow in my academic career.

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ABSTRACT

The efficacy of Limburger cheese as an odour bait for sampling African malaria vectors was evaluated. Studies were done in Suba district, Western Kenya at Lwanda Nyamasare village and in a screenhouse set up at the Thomas Odhiambo campus of the International Center of Insect Physiology and Ecology (ICIPE). Preliminary investigations compared mosquitoes caught by a Counter Flow Geometry (CFG) trap baited with Limburger cheese, a standard centres for disease control (CDC) light trap, an Entry trap and Man Landing Catches (MLC). Comparative studies on the efficacy of Limburger cheese and 'Milk cream' as odour baits for sampling African malaria vectors were done using CFG traps and CDC light traps. The efficacy of Limburger cheese in combination with heat and moisture in sampling *Anopheles gambiae s.s* in a screen-house was done using a prototype odour baited trap. In the Preliminary investigations there was no significant difference in the total number of *An. funestus* caught by the CDC light trap, the CFG trap baited with Limburger cheese and the MLC. In the comparative studies using CFG traps and the CDC light traps there were no significant differences in the number of mosquitoes collected in the traps baited with Limburger cheese and those baited with 'Milk cream'. The combination of heat, moisture and Limburger cheese gave a rather good attractant effect for *An. gambiae s.s* mosquitoes in the screen house. These results indicate that Limburger cheese baited traps can effectively sample wild populations of African malaria vectors, more so *An. funestus.* The results also indicate that 'Milk cream' when used as an odour bait can attract African malaria vectors will equal power as Limburger cheese. It is therefore recommendable to use 'Milk cream' as an odour bait for sampling African malaria vectors instead of Limburger cheese because, unlike Limburger cheese, 'Milk cream'is locally available in poor African villages where malaria is endemic.

1.0. INTRODUCTION

Malaria is the most serious infectious disease of humans next to HIV/AIDS and tuberculosis. Malaria threatens 40% of the world's population (Snow *et al.*, 2005). The disease is re-emerging in areas where it had been eradicated (Baird, 2000) and mortality rates related to it are increasing (Trape, 2001). This alarming situation is mainly caused by resistance of malaria parasites to drugs (Zucker *et al.*, 2003) and resistance of mosquito vectors to insecticides (Akogbeto and Yakoubou, 1999).

In 2005 the World Health Organization (WHO) selected malaria as one of the priority diseases to be combated by 2015 and laid down the goals and objectives of this ambitious goal in the Roll Back Malaria (RBM) programme, now known as the Global Malaria programme (WH0, 2005). The Global malaria programme focuses on an integrated approach for combating malaria in children under five years old and pregnant women living in malaria endemic areas in Africa. Here, Insecticide Treated Nets (ITNs), immunization, use of artemisinin based drugs as a first line treatment for malaria, and iron supplementation of young children in areas where malaria transmission is intense play a central role (WHO, 2005).

Epidemiological studies of malaria often require to measure the degree of man-vector contact and the impact of control interventions on mosquito/parasite populations (Macdonald, 1957; Bruce-Chawtt, 1985). Traditionally, this information has been inferred by assessing insects collected using human biting catches (HBC) (Garrett - Jones and Shidrawi, 1969). HBC is believed to be the most direct and reliable method in sampling and monitoring the human biting mosquito population(s) relevant to malaria transmission and control. This is because in the HBC female mosquitoes are caught when attempting to feed on the human bait (Service, 1977).

There are ethical and logistical shortcomings associated with the HBC. For example, all night collections off human bait are labour intensive and unreliable because of possible variations in skill and attractiveness of the human collectors (Lindsay *et al.*, 1993; Knols *et al.*, 1995). Such collections may also expose mosquito catchers and/or bait to potentially infective mosquito bites, thus increasing the chance of contracting malaria (Service, 1977). Human biting catches also require high levels of organization and are expensive in terms of time and manpower (Davis, 1995).

A variety of sampling methods have been tried as alternatives to HBC e.g. the CDC light trap, but it has been found that whereas this sampling method eliminates the occupational exposure to infective vectors and reduce the element of human error in capturing biting mosquitoes, light traps underestimate the relative abundance of host seeking mosquitoes (Rubio–Palis and Curtis, 1992; Service, 1993). Instead, light traps are biased towards catching parous and sporozoite-infected mosquitoes (Mbogo *et al.*, 1993; Davis, 1995). A more reliable sampling tool is needed when estimating malaria transmission intensity (Costantini *et al.*, 2002; Mathenge *et al.*, 2002).

It could provide a solution if traps baited with host odours would lure mosquitoes as strongly as normal healthy humans (Takken & Knols, 1999). However, although Carbon dioxide (CO_2), lactic acid, carboxylic acids and octenol are well known mosquito attractants and have a synergistic effect in luring mosquitoes (Kline *et al.*, 1990; Geier *et al.*, 1999; Steib *et al.*, 2001; Smallegange *et al.*, 2005), they are non-specific, and accruing evidence suggests that other host odour cues are involved in the host seeking behavior of many anthropophilic mosquitoes (Costantini *et al.*,1996; Mboera *et al.*, 1997; Geier *et al.*, 1999; Steib *et al.*, 2001). Moreover, CO_2 cannot be used conveniently in the African villages where malaria is endemic because it has to

be provided in gas cylinders or in the form of dry ice. Carbon dioxide in a gas cylinder is costly and bulky. High emission rates of carbon dioxide from the gas cylinders may also deter mosquitoes from entering the traps due to higher concentrations. Carbon dioxide in the form of dry ice lowers the temperature around the trap thus deterring mosquito attraction and its rate of release cannot be controlled as desired (Service, 1977).

Current research is concentrating on discovering specific human kairomones that lead mosquitoes to their human host. De Jong and Knols (1995) demonstrated that *An. gambiae* Gilles *sensu stricto* prefer biting the feet of an upright seated man, a behaviour that is mediated by odours from the feet. Subsequently, in 1996, Knols and De Jong discovered that Limburger cheese, which to the human nose has a smell reminiscent to foot odour, was a strong attractant to *Anopheles gambiae* Giles *sensu stricto*. Limburger cheese was also found to be attractive to *Aedes aegypti* in wind tunnel bioassays (Kline, 1998). These discoveries together with other studies by Van den Hurk *et al* in 1997 which showed that it is possible to sample an appropriate fraction of mosquitoes with a non-suction trap baited with human odour are important steps forward in the development of novel sampling, monitoring and possibly alternative methods for controlling malaria vectors and malarial illness in Africa.

The main goal of the studies reported in this thesis was to (i) test the efficacy of Limburger cheese and 'Milk cream' as odour bait sources for sampling wild populations of host seeking African malaria vectors and (ii) develop a simple odour baited trap that can be used in sampling, monitoring and controlling African malaria vectors.

2.0. LITERATURE REVIEW

2.1. Species identification and distribution of African malaria vectors

Information on identification and distribution of malaria vectors especially those that belong to species complexes that contain vector and non-vector species is important for strategic planning of malaria control programmes (Coetzee *et al.,* 2000). In tropical Africa there are two main mosquito species complexes which contain species considered to be important malaria vectors. These include the *Anopheles gambiae* and the *Anopheles funestus* complexes. The important mosquito species in these complexes that contribute to malaria distribution in most parts of Africa are *An. gambiae s.s*, *An. arabiensis* and *An. funestus* (Coetzee *et al.,* 2000).

The *An. gambiae* complex consists of at least seven closely related and morphologically identical mosquito species that vary in their ability to transmit malaria (White, 1974; Hunt, 1998). These species include: *An. gambiae, An. arabiensis, An. quadriannulatus*, *An. bwambae, An. merus*, and *An. melas*, and the latest addition *An. quadriannulatus* species B from Ethiopia. The species found in West Africa are *An. gambiae*, *An. melas* and *An. arabiensis* (Coluzzi, 1968; Toure, 1994). In East Africa *An. gambiae* and *An. arabiensis* are the major vectors responsible for the spread of malaria. *An. merus* has been found in eastern and southern Africa. *An. quadriannulatus* species B is specifically found in the Ethiopian highlands while *An. quadriannulatus* species A is restricted to southern Africa, south of Zambezi River (Coetzee, 2004).

An. gambiae and *An. arabiensis* are broadly distributed and the most efficient vectors of malaria in sub-Saharan Africa (White, 1974; Coetzee *et al.*, 2000). Their relative abundance and range of distribution appears to be strongly influenced by climatological factors, especially total annual precipitation (Lindsay, 1998). *Anopheles gambiae* is dominant in humid areas whereas *An. arabiensis* tends to predominate in semi-arid savannas (White, 1974; Lindsay, 1998; Coetzee *et al.*, 2000). Where the two species are sympatric, large changes in species composition occur seasonally, with *An. arabiensis* predominating the dry season and *An. gambiae* becoming abundant during the rainy season (Lindsay, 1998). However, *An. gambiae* may sometimes be more abundant than *An. arabiensis* in the dry season or *vice versa* (Service, 1970). The distribution of the *Anopheles gambiae* complex mosquito species is shown in figure 1.

The *An. funestus* complex is equally widespread although it is found further south into South Africa than the *An. gambiae* complex (Gillies & Coetzee, 1987). It consists of nine sibling species which include *Anopheles funestus s.s* Giles, *Anopheles leesoni* Evans, *Anopheles rivulorum* Leeson, *Anopheles vaneedeni* Gillies and Coetzee, *Anopheles parensis* Gillies, *Anopheles confusus* Evans and leeson, *Anopheles aruni* Sobti, *Anopheles fuscivenosus* Leeson, and *Anopheles brucei* Service (Cohuet *et al.*, 2003; Garros *et al.*, 2004). *An. funestus s.s, An. rivulorum*, and *An. leesoni* have a wide geographic distribution, extending throughout sub–Saharan Africa. The other members of the complex are more localized in their distribution with *An. parensis* and *An. confusus* found mainly in eastern Africa, *An. vaneedeni* in the northern areas of South Africa, *An. aruni* in Zanzibar, *An. fuscivenous* in Zimbabwe, and *An. brucei* in Nigeria (Gillies & De Meillon, 1968).

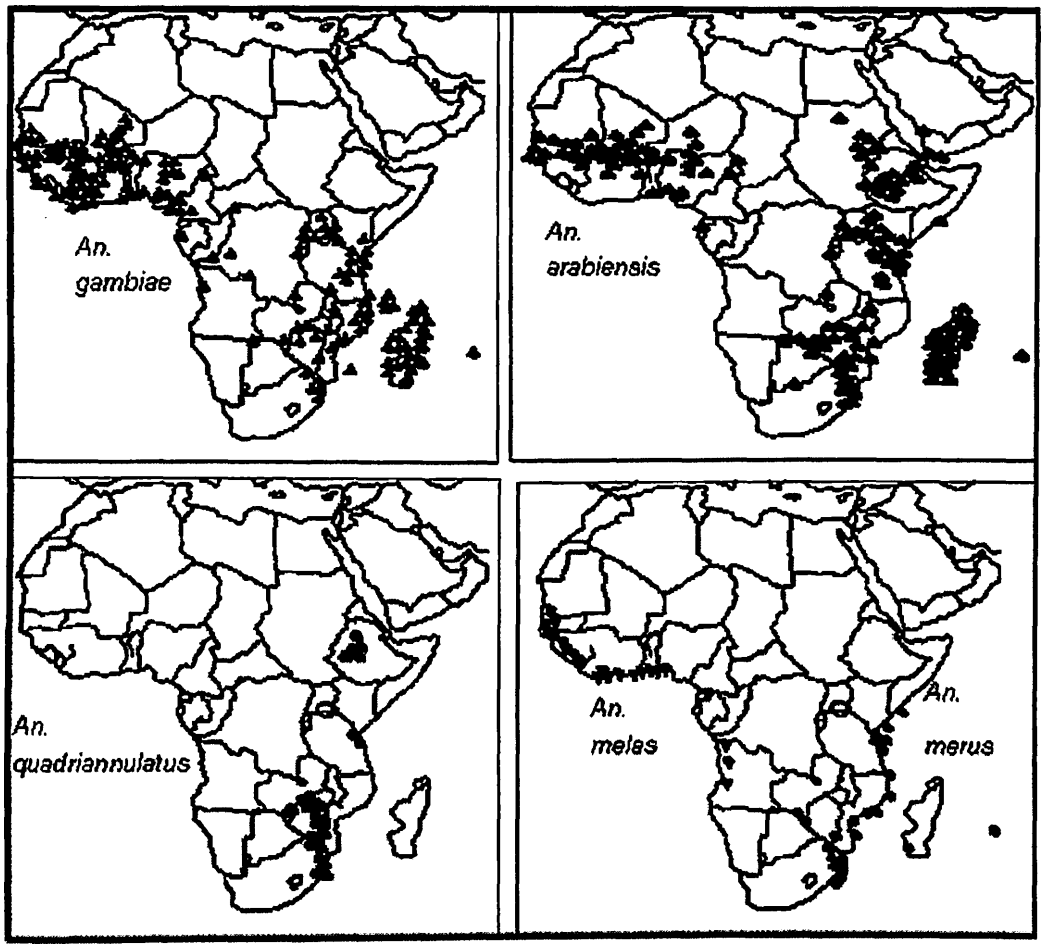

Figure 1. Maps of Africa showing the distribution of *An. gambiae* complex mosquitoes. *An. melas* and *An. merus* appear on the West and East coasts, respectively (modified after Coetzee *et al.*, 2000).

An. funestus s.s is considered to be one of the major vectors of malaria in Africa because of its highly endophillic and anthropophilic behaviour. *An. rivolurum* is primarily Zoophillic but was found infected with *Plasmodium falciparum* in Tanzania and can transmit human malaria. *An. vaneedeni* has been infected in the laboratory with *P. falciparum*, but its vectorial role has never been demonstrated in a natural environment. The other members of the complex are mainly zoophillic and do not seem to be involved in malaria transmission (Cohuet *et al.*, 2003)

Even though *An. gambiae* and *An. funestus* complex mosquitoes are the most important in transmitting malaria in Africa, there are other anopheline species that are not widely distributed but have been found to spread malaria. These species include *Anopheles pharoensis* and *Anopheles nili* among others. *Anopheles pharoensis* has been found to be involved in the transmission of *Plasmodium falciparum* malaria in the Senegal River delta (Coluzzi, 1993) and also in Ethiopia (Seyoum *et al.*, 2002). *Anopheles nili* Theobald is the main malaria vector found in the forest zone of south Cameroon (Carnevale *et al.*, 1992). Both *An. pharoensis* and *An. nili* have also been found to transmit *P. falciparum* malaria in Kilifi district in the Kenyan coast (Mbogo *et al.*, 1995)

2.2. Factors important in developing an odour baited device for sampling African malaria vectors

2.2.1. Feeding habits

Studies on feeding habits of African malaria vectors are important in developing an effective sampling device. Traditionally, studies on feeding habits of malaria vectors

is done based on the identification of the blood meal origin of field collected mosquitoes and expressing the proportion of feeds off humans in the form of the Human Blood Index (HBI) (Garrett – Jones, 1964).

Anopheles quadriannulatus that is thought to be the ancestral species of the *An. gambiae* complex feeds mostly on animals and is not considered a vector (Garrett–Jones & Boreham, 1980). The other species of the complex are all vectors, of which *An. gambiae s.s.* is the most efficient because of its highly anthropophilic character, accompanied by a preference for feeding (endophagy) and resting indoors (endophily) and high susceptibility to infection with *Plasmodium* parasites (Githeko *et al.*, 1996).

Anopheless arabiensis is an opportunistic feeder (Costantini *et al.,* 1999). Its feeding habits are known to vary from being anthropophilic to largely zoophilic depending on the geographic location. For example, the East African populations are generally less anthropophilic than West African ones, while in Madagascar mainly zoophilic populations of *An. arabiensis* occur independently of the human-bovid ratio (Duchemin *et al.*, 2001). The feeding habits of *An. arabiensis* also depends so much on the availability of human hosts relative to alternative hosts – in villages with few cattle, the Human Blood Index (HBI) of this species is usually high, but in villages where cattle are abundant the proportion usually drops in favour of bovid blood (Coluzzi *et al.*, 1979; Gillies & Coetzee, 1987).

Anopheles funestus is also anthropophilic, endophagic, and endophilic. However, it is less susceptible to infection with *Plasmodium spp* than *An. gambiae*, but because of the high densities in which it may occur, it can be a very important vector in Africa.

Combination of anthropophily and endophily puts both *An. gambiae* and *An. funestus* in the frontline among malaria vectors. Coupled with a relatively high survival rate, this behaviour ensures that across Africa both species are responsible for most of the malaria transmission (Coluzzi *et al.*, 1979; Gillies & Coetzee, 1987).

2.2.2. Host location

Host-seeking mosquitoes are exposed to a wide variety of visual, olfactory, gustatory and physical stimuli (Takken, 1991). Factors such as skin temperature, skin color, sex, age, body odour, and moisture have been demonstrated to be involved in the attraction of host seeking mosquitoes to their human host. Any one or a combination of these stimuli could potentially act as cues for host identification and location (Wright, 1975; Sutcliffe, 1987; Takken, 1991; Cork, 1996).

Therefore, information on the roles of various factors involved in the attraction of malaria vectors to their hosts is essential for the development of odour baited traps for surveillance and/control program. This is because some host attractants have synergistic effects, a potential that can be explored in developing effective sampling devices (Braks *et al.*, 2001; Smallegange *et al.*, 2005; Qui, 2005).

Host seeking mosquitoes land on their hosts in a process involving a chain of events that increase the probability of the mosquito encountering and contacting the host. The first host cues to reach the mosquito are usually volatile chemicals emanating from the skin and breathe of a potential host carried by air currents (Sutcliffe, 1987; Takken, 1991). The probability and intensity of response by the host seeking mosquito to these cues depends on the strength of the host-derived stimuli, the

strength of competing external stimuli (e.g. odours from other sources and prohibitive wind speeds), the internal state of the mosquito (e.g. circadian phase, gonotrophic nutritional status, and hunger) and the mosquitoes' genotype (i.e. the genetic component of the mosquito responsiveness to a given stimuli (Murlis *et al.*, 1981; Murlis *et al.*, 1992; Costantini *et al.*, 1999).

When a mosquito responds to the host odours and flies towards it, there is increased availability of additional host stimuli such as visual cues, heat, moisture and volatile chemical cues, which may induce the mosquito to land on the host. Once on the host, probing and biting are initiated by the quality and quantity of stimuli such as heat and phagostimulants (Murlis *et al.*, 1981; Murlis *et al.*, 1992; Takken, 1991; Costantini *et al.*, 1999).

This sequence, however, is not a rigid chain of reactions, but it is rather a flexible continuation of responses modulated by the interaction and integration in the central nervous system (CNS) of internal and external components as the mosquito experiences them (Murlis *et al.*, 1981; Murlis, *et al.*, 1992; Takken, 1991).

2.2.2.1. Responses of host seeking mosquitoes to physical stimuli

Physical stimuli play a very important role in the attraction of host seeking mosquitoes to their hosts. In 1975, Wright described mosquito attraction, as a response to warmth and humidity but doubted the need to search for a particular skin emanation as a mosquito attractant. Furthermore, he suggested that an optimum combination of warmth and humidity is more attractive than the most attractive arm or hand. Schreck *et al* (1990) reported that heat increases the attraction response of

Aedes aegypti to substances collected from human skin and placed on glass Petri-dishes and to recently handled dishes for *Anopheles quadrimaculatus.* Unhandled heated control dishes did not evoke this response. Kline and Lemire (1995) observed that the presence of heat in an odour trap baited with CO_2 and octenol enhanced the ability of the trap to attract mosquitoes as there was a significant increase in the collection size of *Aedes taeniorhynchus, Anopheles atroparvus* and *Culex nigripalpus.* Therefore, physical stimuli like heat and moisture should be put into consideration when developing an odour baited device for sampling African malaria vectors because they can have a synergistic effect with different odours in attracting mosquitoes.

2. 2. 2. 2. Response of host seeking mosquitoes to host odours

Ample evidence has shown that blood seeking female *Anopheles* mosquitoes locate their hosts mostly by olfaction. The most important malaria vector in Africa, *Anopheles gambiae* Giles *sensu stricto* is a highly anthropophilic mosquito species and the host-seeking behaviour of the females is guided by volatiles of human origin (Sutcliffe, 1987; Takken, 1991; Takken & Knols, 1999). A house occupied by humans attracted significantly more *An. gambiae* and *An. funestus* than unoccupied houses, and there was a positive correlation between the number of occupants and the mosquito catch (Haddow, 1942).

Knols *et al* (1994) and De Jong & Knols (1995a, 1998) showed that mosquitoes are guided mainly by human odours when selecting biting sites on human hosts and that the odours used in this process differ between species. It had been assumed that host seeking mosquitoes mainly depend on humidity and temperature of the different

body regions of the host in selecting biting sites but in their study De Jong and Knols (1995a) found *Anopheles atroparvus* and *Anopheles albimanus* to prefer biting around the nose of an upright seated human whereas *An. gambiae* showed a preference for biting the feet and ankles. The results were ascribed to known differences in host preference and response to odours from the preferred biting sites.

Moreover, there are considerable reports that have suggested that some host seeking mosquitoes get more attracted to some individuals than to others. This has been attributed to differing odour profiles from different people (Knols *et al.*, 1995; Geier *et al.*, 1999; Mukabana *et al.*, 2002). Schreck *et al* (1990) in their experiment with *Aedes aegypti* observed that there was a significant difference in the attraction responses to different volunteers at room temperature and the difference became more significant when the Petri dishes that contained the substances from the hands were warmed.

Lindsay *et al* (1993) found a significant difference in attractiveness between five men sleeping in artificial huts in The Gambia. Knols *et al* (1995) showed in Tanzania that the difference in attraction between human hosts was entirely odour mediated. Further studies in Tanzania revealed that the attraction between humans was different for various anopheline species; one person was significantly more attractive to *An. funestus* than any of the five others, while this person was at the same time the least attractive to *Anopheles squamosus* (Knols *et al.*, 1995). In Burkina Faso, attractiveness of different men to anophelines was also found to be odour based (Brady *et al.*, 1997).

It has also been observed in various studies that adults are significantly more

attractive to *An. gambiae* than children (Spencer, 1967; Muirhead–Thompson, 1951). This has been attributed to the amount of exhaled breath and volatile substances released from the skin, which is directly related to size (Carnevale *et al.*, 1978), surface area and weight (Port *et al.*, 1980). In other studies men have been found to be more attractive to *An. gambiae* than women (Muirhead–Thompson, 1951). This has been attributed to the fact that mammalian semiochemicals are complex and include indicators of sex, health, age reproductive status and diet (Brown, 1979). All of these studies show that human odours are greatly involved in the attraction of host seeking malaria vectors to their hosts (Knols *et al.*, 1995; Brady *et al.*, 1997: Mukabana, 2002).

Subsequently, efforts to identify the human kairomones that guide mosquitoes to their human hosts have been going on for a long time and carbon dioxide is the best-known kairomone. It is present in the expired breath of vertebrates and thus reliably signifies the presence of a potential vertebrate host (Krotoszynki, 1977; Mboera *et al.*, 1997b).

2.2.2.2.1. Response of host seeking mosquitoes to carbon dioxide

Carbon dioxide has the potential of attracting any host-seeking mosquito (Kline *et al.*, 1990). Indeed, most sampling tools for mosquitoes are being supplemented with CO_2 as an attractant to enhance the visual cues provided by the trap (Mboera *et al.*, 1997ab, Mboera *et al.*, 2000a). During host seeking carbon dioxide exerts its effects in two distinct ways. Firstly, it acts as an attractant stimulating take off so that mosquitoes orientate themselves towards the host and it also plays a role in sustaining the flight (Gillies, 1980; Healy & Copland, 1995). Secondly, carbon dioxide

has a combined action with warm moist convection currents at close range and has synergistic effects with other attractants which would otherwise have not been attractive to the mosquito on their own like, lactic acid, octenol and butanone in attracting *Aedes* and *Anopheles* mosquitoes (Gillies., 1980; Eiras and Jepson., 1991;). For example, carbon dioxide was shown to have a strong synergistic effect with Lactic acid in attracting anthropophilic host seeking *Aedes aegypti* as addition of carbon dioxide to lactic acid attracted 80% of the mosquitoes in the experiments done by Geier *et al* (1999) as compared to 20% that was attracted by the lactic acid alone.

Although carbon dioxide is a universal attractant for host seeking mosquitoes the proportion of reliance to it varies according to the species. Mosquitoes with catholic behaviours like *Mansonia uniformis*, *Anopheles pharoensis* and *Anopheles quadriannulatus* rely largely on carbon dioxide released by a certain host to get attracted to the host while species with highly specialized feeding habits like *An. gambiae, An. funestus* and *An. arabiensis* are believed to prefer odours produced by the host but not carbon dioxide *per se* (Gillies, 1969; Knols *et al.*, 1994; Costantini *et al.*, 1996). Mboera *et al* (1999) in Tanzania concluded that in indoor situations, human odour as a whole and not carbon dioxide on its own is the principle cue to which the malaria vectors *An. gambiae*, *An. funestus* and *An. arabiensis* are attracted. Carbon dioxide when used as a kairomone on its own accounts for only a minor part of the overall attractiveness of man, particularly to *An. gambiae s.l.* They actually found that human odour was the single most important factor determining the size of catches since the physical presence of a human male in addition to his odour did not increase catches significantly.

2.2.2.2.2. Responses of host seeking mosquitoes to sweat and its components

It has been found that *An. gambiae* is attracted to human sweat and more specifically to ammonia, lactic acid and carboxylic fatty acids (Knols *et al.*, 1997; Braks, 1999; Smallegange *et al.*, 2005). Mixtures of these compounds have produced high and consistent behavioral responses of the mosquitoes in the laboratory and in the field (Braks *et al.*, 1999; Smallegange *et al.*, 2005; Qui, 2005). Also, De Jong and Knols (1995a) correlated landing sites with estimated eccrine sweat gland density in *An. atroparvus* and *An. gambiae*.

2.2.2.2.2.1. Response of host seeking mosquitoes to Lactic acid

As a single stimulus lactic acid is only a weak attractant, but it acts as a powerful synergist together with other compounds from human skin odour (Geier *et al.*, 1996: Dekker *et al.*, 2002; Steib *et al.*, 2001). Earlier studies on responses of *A. aegypti* to lactic acid (Acree *et al.*, 1968; Smith *et al.*, 1970) together with recent studies on *Anopheles gambiae s.s.* (Dekker *et al.*, 2002) indicate that there might be a relationship between the attractiveness of humans and the quantity of lactic acid on their skin. Geier *et al* (1999) revealed that the difference in attraction of different individuals to host seeking *Aedes aegypti* was due to the difference in the quantity of lactic acid on their skin with individuals having higher amounts of lactic acid on their skin being more attractive. Adding lactic acid to the skin rubbings of individuals who were less attractive made them to become more attractive than their counterparts.

Lactic acid is also associated with the anthropophily of host seeking mosquitoes. This was demonstrated by Geier *et al* (1999) whereby female *Aedes aegypti* showed

no attraction to animal odour but when lactic acid was added to the animal odour extracts they showed high attractiveness with 70% of the mosquitoes being attracted. The lack of response of female *A. aegypti* to animal odour without lactic acid is consistent with the results determined by Geier *et al* (1996) whereby lactate was removed from human samples and it was found that none of the other components which contribute to the attractiveness of human skin are effective without addition of lactic acid. The large quantities of lactate on the skin, excreted by eccrine sweat glands, can be regarded as characteristic for humans and could be a factor that, in conjunction with other host odour components, guides anthropophilic mosquitoes to their hosts.

Lactic acid as well, has a high synergistic activity with ammonia and carbon dioxide. Ammonia on its own is not attractive to *Aedes aegypti* but in combination with lactic acid at 7nmol\170ppb it became attractive doubling the percentage of mosquitoes attracted. In the same study the addition of carbon dioxide to Lactic acid attracted 80% of the mosquitoes (Steib *et al.*, 2001). Interestingly, even though lactic acid acts synergistically with other components of human skin, the degree of attractiveness of animal odours combined with lactic acid demonstrates that other components, which are required for host finding, are not necessarily human specific. However, despite the fact that Lactic acid is an essential kairomone for the yellow fever mosquito *Aedes aegypti*, the selective removal of lactic acid from sweat by Braks *et al* (2001) did not affect the reaction of malaria mosquitoes. It was therefore concluded that lactic acid is not an essential component of attractive odour blends for malaria mosquitoes.

2.2.2.2.2.2. Response of host seeking mosquitoes to ammonia

Ammonia in contrast to lactic acid is an attractant to *An. gambiae s.s.* In a wind tunnel olfactometer bioassay, Braks *et al* (2001) showed that sweat was attractive to the anthropophilic *Anopheles gambiae s.s* mosquito which is the main malaria vector in tropical Africa. After incubating the sweat and selectively separating the sweat components which are mainly lactic acid, ammonia and urea and testing them in wind tunnel bioassays, they realized that only ammonia amongst the three major components was significantly attractive to the malaria mosquitoes. However, the fact that attraction was sometimes found to the fresh sweat by Braks *et al* (2001) with a rather low concentration of ammonia indicates that components other than ammonia also play a role in the host-seeking behaviour of malaria mosquitoes. The identity of these components needs further exploration.

Further experiments done to test the role played by the sweat components of ammonia, lactic acid and carboxylic acids in attracting host seeking *Anopheles gambiae* Giles *sensu stricto* by Smallegange *et al* (2005) revealed that ammonia is an attractant on its own as odourous air from sample bags containing 13.6, 136, 1,364 or 13,637 p.p.m. of ammonia attracted significantly more mosquitoes than clean air. However, the mosquitoes preferred the clean air to the highest concentration of ammonia tested, (136,371 p.p.m.) indicating that this concentration repels *An. gambiae*. Lactic acid was not attractive to the host seeking *Anopheles gambiae* Giles *sensu stricto* at any concentrations tested and none of the concentrations of lactic acid that were added to ammonia increased the attractiveness of ammonia, contrary to what was found by Braks *et al* (2001) and Smallegange *et al* (2002). However, this finding corresponded to the finding of

Dekker *et al* (2002) whereby the concentration of 0.0001g/ml of lactic acid combined with ammonia was repellent but the combination of ammonia and lactic acid attracted significantly more mosquitoes than clean air.

The synthetic mixture of 12 carboxylic acids was repellent when tested alone and in combination with lactic acid but the combination of the carboxylic acid mixture with ammonia was significantly more attractive than clean air. Therefore ammonia with carboxylic acids has a synergistic effect as the addition of ammonia to the carboxylic acid mixture made it attractive to *An. gambiae* mosquitoes (Smallegange *et al.*, 2005).

Combining ammonia with either lactic acid or the carboxylic acids did not enhance the attractiveness of ammonia. However, a synergistic effect was found when ammonia, lactic acid and the carboxylic acids were applied as a blend. In contrast Knols *et al* (1997) found that the synthetic mixture of the 12 aliphatic carboxylic acids was attractive to females of *An. gambiae* in a dual-port olfactometer. Impurities in the (commercially) obtained carboxylic acids may be given as one of the explanations for the differences in the results (Smallegange *et al.*, 2005).

These findings show that a synthetic blend of volatiles, all naturally present in human odour (Bernier *et al.*, 2000; Healy and Copland, 2000; Braks *et al.*, 2001), provides a synergistic effect on olfaction-based trap-entry responses of *An. gambiae* mosquitoes. It is clear from field studies that *An. gambiae* is strongly attracted to natural human odours (Haddow, 1942; Costantini *et al.*, 1996; Mboera *et al.*, 1997a), and the development of traps baited with highly attractive synthetic blends simulating human odours appears realistic.

2.2.2.2.3. Response of host seeking mosquitoes to octen - 3 - ol

Octenol (1-octen-3-ol) is an ingredient in "synthetic ox-odour". It has been used successfully as odour bait in tsetse fly traps (Vale, 1993). Octenol has been shown to attract more *Aedes taeniorynchus*, *Anopheles spp* and *Wyeomyia mitchellii* mosquitoes when used together with carbon dioxide (CO_2), though each attractant is capable of attracting mosquitoes on its own (Takken & Kline 1989). Van Essen *et al* (1994) evaluated the effectiveness of octenol as an attractant in EVS (encephalitis vectors surveillance) traps in Queensland, Australia. They found that *Aedes vigilax* catches increased significantly with CO_2 + light alone, but *Culex annulirostris* and *Culex sitiens* catches did not increase significantly. They concluded that octenol supplements to CO_2 significantly increased collections of *Aedes* but not *Culex.*

2.3. Limburger cheese

Cheese is a solid food made from the curdled milk of cows, goats, sheep, buffalo or other mammals. It is made by adding rennet (an enzyme traditionally obtained from the stomach lining of young cattle, but now also laboratory produced) or rennet substitutes and bacteria culture to milk. Rennet helps in curdling up the milk while bacteria acidify the milk and play a role in defining the texture and flavor of most cheese. Some cheeses also feature molds, either on the outer rind or throughout (Cogan & Daly, 1987; McGee, 2004).

Limburger cheese is a type of cheese that has a smell reminiscent to human foot odour to the human nose. It has a rind that is somewhat mottled in appearance, the

color ranging from white and yellow to brown, and a paler, softer interior. It is often eaten with other strong flavored food like onions, dark breads and dark bear. Limburger cheese originated in Limburg, Belgium but most of the cheese is made in Germany and some in the United States (McGee, 2004). It is made from pasteurized cow milk and is usually aged for three months for bacterial fermentation to take place. The bacteria used to ferment Limburger cheese is *Brevibacterium linens* (Cogan & Daly, 1987). The same bacteria are found on human skin and are partially responsible for human body odor. A likely reason for this is that the monks of Limburg who created the cheese would originally mix the milk and curds into cheese by stomping it with their feet.

Research done by De Jong and Knols in 1995(a), showed that host seeking *An. gambiae* prefers biting the feet and ankles of an upright-seated human while more generalist species like *Anopheles atroparvus* showed a preference for biting the head region. This behaviour was shown to be mediated by odours emanating from the feet (De Jong & Knols, 1995b). Subsequently, it was observed that the odour of Limburger cheese, which to humans has a smell that is strongly reminiscent of human foot odour, was highly attractive to *An. gambiae s.s* in wind tunnel bioassays (Knols and De Jong, 1996). Indeed, chemical analyses showed a strong similarity in the composition of foot odour and Limburger cheese. A synthetic mixture of 12 of the more abundant aliphatic fatty acids, present in the headspace of Limburger cheese as well as in human foot odour, have been implicated as being attractive to *An. gambiae* (Knols *et al.*, 1997; Takken and Knols., 1999).

Attraction of the highly anthropophilic *An. gambiae* to a non-human odour source (Limburger cheese) has demonstrated that human biting catches HBC, an ethically

unacceptable method of sampling malaria vectors, can potentially be replaced by a synthetic odour–baited trap as a safe and reliable surveillance and control system.

2.4. Perspectives of olfaction in malaria control

The study of olfaction and its role in mosquito host preferences and host seeking behaviour has the potential of creating new developments in the control of vectors and vector borne diseases (Costantini *et al.*, 1999). There has been interest in developing odour-baited traps to improve the quality and quantity of samples for epidemiological purposes and also to monitor vector inoculation rates in control programmes without recurring to the ethically doubtful and impractical collections on human baits (Costantini *et al.,* 1993). It has been clearly shown that the African malaria vectors *An. gambiae s s.* and *An. funestus* can be lured into mosquito traps by odour cues alone (Costantini *et al.*, 1996; Mboera *et al.*, 1997). These mosquito species are highly anthropophilic and were shown to respond positively to human body emanations and less to breath (Mboera *et al.*, 1997).

The successful demonstration of the attraction of anthropophilic malaria mosquitoes to human odour in the absence of the living human host (Braks, 1999; Smallegange *et al.*, 2005) together with the realization by van den Hurk *et al* (1997) that it is possible to sample an appropriate fraction of the mosquito population with a non suction trap baited with human odour has demonstrated the potential for the replacement of the human biting catch, an ethically unacceptable method of sampling malaria mosquitoes, by a synthetic odour-baited trap as a safe and reliable mosquito surveillance system. Semiochemicals can actually be used as lures in mass-trapping devices or decoys deviating attacking mosquitoes away from humans (Day & Sjogren,

1994). Such a system has already been developed for the surveillance and control of tsetse flies, the vectors of African trypanosomiasis (Vale, 1993).

However, the usefulness of semiochemicals in mass-trapping devices for malaria control purposes as compared to the widespread use of insecticide-impregnated bed nets is yet to be demonstrated. If the attractiveness of lures to the relevant *Anopheles* vector species will approach or exceed that of humans, then deployment of these lures in sufficient numbers might be a valuable additional tool in integrated vector management efforts like they have been in the control of tsetse flies in certain parts of Africa (Vale, 1993). For tsetse flies, it took many years of testing of hundreds of compounds and combinations thereof before an effective blend was identified. This is the stage where research with malaria vector *An. gambiae* has reached and intensive field evaluation of laboratory-identified human volatiles is the next and only logical step towards the development of odour baited entry traps for sampling and monitoring African malaria vectors.

Another area that should benefit from deeper understanding of olfaction and behaviour is that concerned with the development of insect repellants. This is because, the reduction of human vector contact plays a remarkably significant role in malaria control, and as such the use of cheap, safe, efficient persistent and cosmetically acceptable repellents could contribute greatly to widen its scope.

2.5. Mosquito sampling

Sampling mosquitoes is a prerequisite to most epidemiological studies in malaria transmission and control. Depending on the objective of the exercise a variety of sampling methods can be used (Service, 1977). Many sampling methods have been

evaluated as alternatives to human landing catch with varying degrees of success. In estimating malaria transmission intensity it is imperative that the sampling methods used are calibrated against the human biting catch (HBC) since the latter translates directly into human biting rate, which serve as an essential parameter in the estimation of both the entomological Inoculation Rate (EIR) and vectorial capacity (Odetoyinbo, 1969; Garrett-Jones and Magayuka, 1975).

The use of HBC during epidemiological studies of malaria transmission and control is considered the most representative method for determining human biting activity since female mosquitoes are collected in precisely the act of biting a person (Service, 1977). However, this method has several shortcomings. For example, the method is labour intensive (Davis, 1995) and unreliable because of variation in attractiveness of the human collectors (Lindsay *et al.,* 1993). Furthermore, the practice is unethical because the human bait/mosquito catchers are exposed to potentially infected mosquitoes (Service, 1977).

Light traps do not pose these problems and many workers have used light traps with varying success. Garrett-Jones & Magayuka (1975) in Tanzania and Costantini *et al* (1998) in Burkina Faso showed that CDC light traps used inside houses, in combination with human - occupied bednets yielded results comparable to standard human-biting collections and may therefore be used to estimate mosquito abundance.

However, several studies have showed that light traps underestimate the density of host seeking man-biting mosquitoes (Rubio–Palis & Curtis, 1992; Service, 1993). The light traps are also biased as they catch greater proportions of parous and sporozoite-infected mosquitoes (Mbogo *et al.*, 1993; Davis, 1995). This method is also relatively

expensive in terms of initial cost and running. The CDC light trap may therefore not be a very suitable device for estimating the Entomological Inoculation Rate (EIR).

Resting behaviour of many mosquitoes – is often assessed using pyrethrum spray-sheet collections (PSC) in houses or hand catch of adult mosquitoes resting on the walls, ceilings, roofs, underneath beds and amongst household objects using aspirators. However, these two methods have certain disadvantages. For example, hand catches are human biased, cumbersome, time consuming and interfere with the privacy of the occupants of the houses. The number of mosquitoes collected by either hand catch or pyrethrum spray sheet collections are also usually lower than the number of mosquitoes resting in that house since most houses have spaces around the eaves, windows and doors from which mosquitoes might fly out during spraying or hand picking or some mosquitoes might hide in crevices in the house. Also, not all mosquitoes knocked down by pyrethrum spray drop onto the sheet spread on the floor and therefore they can not be accounted for. Moreover, it has been realized that even the species of mosquitoes like *An. gambiae*, *An. stephens*i and *An. culicifacies* which have been regarded to be highly endophilic have a good proportion that rest outside houses like on the eaves, man made shelters or natural shelters.

Tools for sampling out-door host seeking anthropophilic mosquitoes in Africa are few. Most of these operate on the principle of an active air current sucking mosquitoes into collection bags (Service, 1993), or have an active odour-laden air current pumped out of traps guiding mosquitoes towards them (Costantini *et al.*, 1993). There are two major shortcomings of these methods. Firstly, the proportion of approaching mosquitoes that enter the trap upon arrival at its entrance is unknown, and, secondly, being non-directional sampling devices, they cannot be used to study mosquito flight behaviour.

Electric nets, which were initially developed as sampling tools for tsetse flies (Vale, 1993) have been adopted to study flight behaviour of mosquitoes in relation to prevailing wind directions and the influence of wind on the flight speed of West African mosquito species. They have also been useful in sampling outdoor mosquito populations (Knols *et al.*, 1998).

Traps based on mosquito attractants have also been used in sampling mosquitoes successfully. For example, Counterflow Geometry (CFG) traps baited with carbon dioxide have been used successfully to sample outdoor mosquito populations in Tanzania (Mboera *et al.*, 2000a). The CFG traps have also been used with synthetic oviposition pheromones to successfully sample outdoor mosquito populations of gravid *Culex quinquefasciatus* in Muheza Tanzania (Mboera *et al.*, 2000b). Costantini *et al* (1993, 1996) used an odour-baited entry trap (OBET) that released an air stream containing cues collected from a bait successfully to collect West African mosquito species, most of them important vectors of malaria, such as *Anopheles gambiae s.l.* and *An. funestus.* Dia *et al* (2005) conducted a comparative study between man landing catches and odour baited entry traps (OBETS) and found out that both methods were effective in sampling host seeking mosquitoes of the four malaria vectors in Senegal which were *An. gambiae s.s*, *An. arabiensis*, *An. funestus*, and *An. nili.* Mosquito age structure and infectivity rates did not differ between the two sampling methods.

Therefore, traps based on host attractants are likely to provide an objective monitoring tool for the host-seeking fraction of malaria and filariasis vectors. Such traps can be used to study the biology of these vectors as well as the epidemiology of the diseases they spread, knowledge of which is vital for planning and evaluating the outcome of

intervention strategies. In addition, simple odour-baited trapping devices could supplement other methods, such as bednets, in reducing challenge particularly in poor African villages where malaria is endemic without resorting to insecticides. Odour baited traps could eventually become an integral part of primary healthcare systems (Day & Sjogren, 1994; Mboera *et al.*, 2000a).

2.6. Justification and significance of the research

In tropical Africa malaria is one of the most serious impediments to development (Baird, 2000; Snow *et al.*, 2005). It represents 9% of the disease burden and results in over one million deaths annually, mainly of young children (WHO, 2005). Also widespread resistance to chloroquine, Sulfadoxine Pyrimedoxine (SP) drugs and general drug failure, forces governments to adopt artemisinin based drugs which are more expensive as first-line treatment for malaria (WHO, 2005).

Although vector control would be the most desirable and indeed effective means of malaria intervention, vector control *per se* has been shown to be fraught with difficulties for technical and financial reasons, as well as from inadequate understanding of the vector ecology. There are also biological constraints faced by many vector control projects and problems of program acceptance, affordability, and sustainability by the affected communities also come into play (WHO, 2005). Studies on insect behaviour have been exploited in designing control strategies for disease vectors and other pests. Light traps set beside occupied bed nets have been realised to provide a reliable method of monitoring populations of *An. gambiae s.s* (Garret Jones and Magayuka 1975; Mbogo *et al.*, 1993). However, light traps have been found not to be as attractive as the human host. Ideally, traps baited with a 'standard' host odour would lure mosquitoes as strongly as would a human host (Takken and Knols, 1999).

Other vector behaviours such as biting habits have also been exploited in designing control strategies (Githeko *et al.*, 1996) and although many studies have been done on the response of mosquitoes to olfactory (Knols & De Jong, 1996; Steib *et al.*, 2001; Dekker *et al.*, 2002) and physical attractants (Garrett-Jones and Magayuka,

1975; Mbogo *et al.*, 1993) none has come up with an effective method to assess the mosquito density. However, observations on the biting behaviour *of Anopheles gambiae s.s.* on humans have now resulted in the discovery of Limburger cheese as a strong attractant for this important malaria vector (Knols and De Jong, 1996). It had been realized that even though carbon dioxide is a good attractant to host seeking mosquitoes (Krotozynki, 1977) it is not convenient for use in African villages as it has to be either in a gas cylinder or in the form of dry ice. However, Limburger cheese acts as an attractant independently of carbon dioxide. Therefore, its discovery as an attractant to host seeking *An. gambiae s.s* by Knols and De Jong (1996) is an important step forward in the development of effective/alternative devices for monitoring malaria vectors in Africa, and possibly also for controlling them.

It is thought that the discovery of Limburger cheese and other compounds like Lactic acid, ammonia and carboxylic acids as attractants to the host seeking malaria vectors (Braks *et al.*, 1999; Smallegange *et al.*, 2005; Qui, 2005) will accelerate the development of odour-baited traps for malaria surveillance and control in sub-Saharan Africa where it has been realized that epidemiological studies of many mosquito-borne diseases are seriously hampered by the scarcity of objective sampling methods for mosquito populations.

One might even foresee the development of a Limburger cheese baited sampling device that might be used sampling of African malaria vectors during epidemiological studies of malaria transmission and control and could be used *en masse* to reduce the vector population in a village or in an individual bedroom to divert mosquitoes away from the occupants. Therefore, in an effort to make this vision come true, this thesis concentrates on testing the efficacy of Limburger cheese baited traps in sampling African malaria vectors under field and semi field environments.

2.7. Hypothesis

1. A Limburger cheese baited CFG trap is not as effective as the Man Landing catch (MLC), the Centers for Disease Control (CDC) light trap and the entry trap in sampling African malaria vectors.

2. 'Milk cream' is not as effective as Limburger cheese when used as an odour bait source in the CFG and the CDC light trap for sampling wild populations of African malaria vectors.

3. A simply developed odour baited trap is not effective in sampling African malaria vectors in poor African villages.

2.7.1. Overall objective

To evaluate the efficacy of Limburger cheese and 'Milk cream' as odour baits for sampling host seeking African malaria vectors.

2.7.2. Specific Objectives

1. To evaluate the efficacy of a Limburger cheese baited CFG trap in sampling Wild populations of African malaria vectors by comparing it with the Man landing catches, the CDC light trap and the entry trap.

2. To compare the efficacy of 'Milk cream' and Limburger cheese as odour baits in the CFG and CDC light traps in sampling wild populations of African malaria vectors. .

3. To develop a simple odour baited trap for sampling African malaria vectors in poor African villages where malaria is endemic.

3.0. MATERIALS AND METHODS

3.1. Study area

The studies reported in this thesis were conducted under field and semi-field conditions. Field studies were undertaken at Lwanda Nyamasari village while semi-field studies were undertaken at the Thomas Odhiambo Campus of the International Centre of Insect Physiology and Ecology (ICIPE). Both study sites are located along the shores of Lake Victoria in Suba District, Nyanza Province, western Kenya (figure 2). The main inhabitants of Suba District are ethnic *Luos* most of whom practise fishing as the main economic activity and carry out subsistence farming on small scale.

Suba district lies at an altitude of 1100 - 1300 metres above sea level and experiences high temperatures (17 - 34°C) throughout the year. The district experiences two rain seasons: the long rains occur from March to June and the short ones from September to November. However, the rainy seasons are not well defined with some years being characterized by more or less continuous rainfall and others by prolonged drought. The annual rainfall ranges between 700 - 1200 mm (Minakawa *et al.*, 1999).

Malaria is holoendemic in Suba district and is the leading cause of morbidity, childhood mortality and hospital admissions (Mutero *et al.*, 1998).

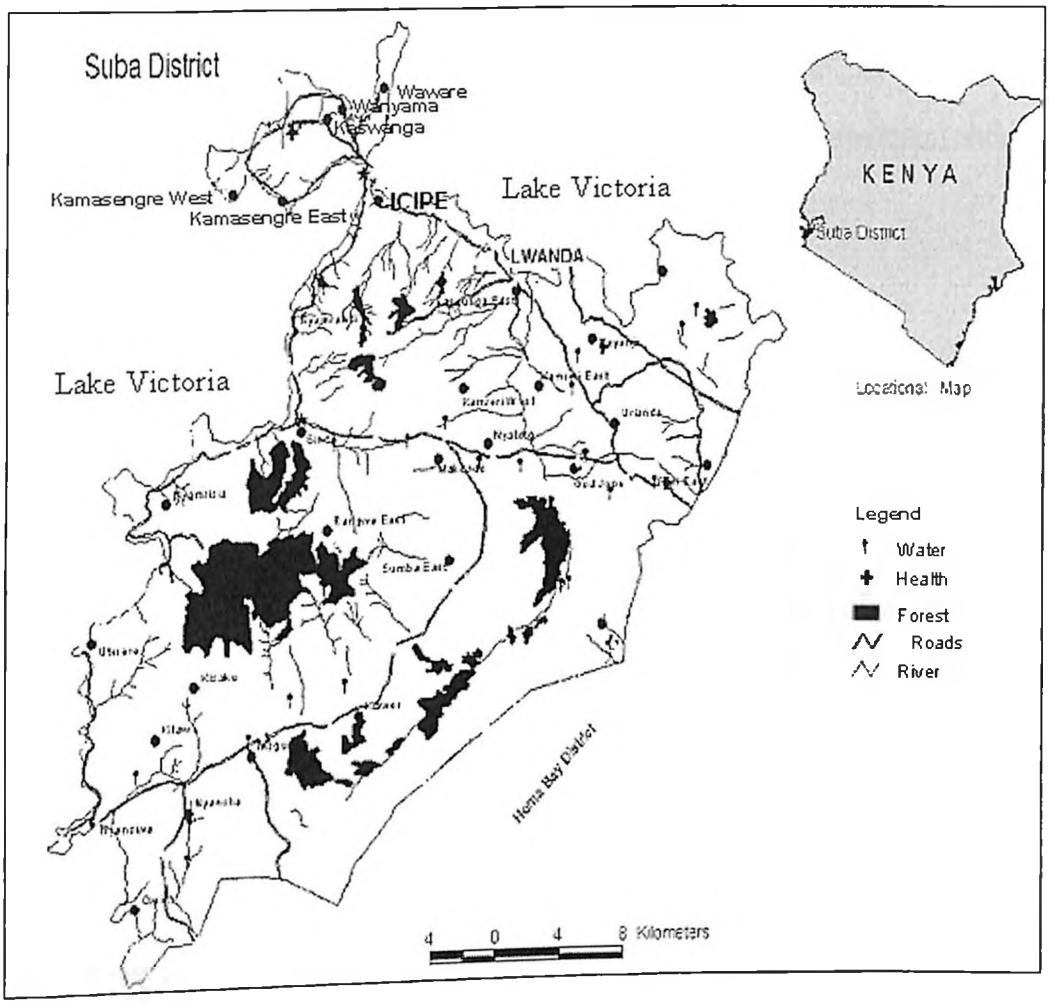

Figure 2. Map of Suba District, Nyanza Province, Western Kenya showing Lwanda Nyamasare village (Lwanda) and the Thomas Odhiambo Campus of the International Centre of Insect Physiology and Ecology (ICIPE) where field and semi field investigations were conducted, respectively.

Transmission of the disease is maintained by three main vectors: *An. gambiae, An. arabiensis* and *An. funestus* which breed in natural and artificial larval habitats (Mutero *et al.*, 1998; Minakawa *et al.*, 1999). Malaria transmission fluctuates throughout the year and reaches its climax in the rainy seasons.

3.2. Field investigations

The main aim of these investigations was to evaluate the efficacy of Limburger cheese as an odour bait source for sampling the African malaria vectors *Anopheles gambiae*, *An. arabiensis* and *An. funestus* under field conditions. These studies are based on the observations that although Limburger cheese has been shown to be a strong attractant for host seeking *An. gambiae s.s.* under laboratory conditions (Knols & De Jong., 1996), subsequent field application has been unsuccessful (Murphy *et al.*, 2001).

3.2.1. Preliminary investigations

Preliminary exploratory studies were set up to investigate whether Limburger cheese can serve as an odour bait source for attracting wild populations of three African malaria vectors *An. gambiae*, *An. arabiensis* and *An. funestus*. To do this a 4×4 Latin square experimental design (Kothari, 2004) utilizing four trapping methods was formulated (Table 1). Mosquito trapping was done using (i) a Counter Flow Geometry (CFG) trap baited with Limburger cheese; (ii) a standard Centres for Disease Control (CDC) light trap; (iii) an Entry trap; and (iv) Man Landing Catches (MLC). Each method of trapping was used separately in one of four houses per night. Trapping methods were rotated between the houses until each method had been applied four times per house.

House owners were informed about the study rationale and methodology *apriori*. The four houses used in the study were selected upon receiving consent from the household heads. The houses were designated as (i) house 1, a grass thatched house with mud walls located on dry land; (ii) house 2, an iron sheet roofed house with mud walls located on dry land; (iii) house 3, an iron sheet roofed house with mud walls located on dry land, and (iv) house 4, a grass thatched house with mud walls located next to a swamp. The owners of these houses cooked their meals outside except those of house 3. The eaves of all houses were open, a factor that allows for free ingress and egress of mosquitoes. All occupants of the experimental houses were provided with untreated mosquito bednets and asked to sleep under them. Man landing catches were done by one trained mosquito collector (a Kenyan male aged 24 years old) throughout the study. The mosquito catcher also acted as bait.

Table 1. A 4 × 4 Latin square experimental design showing how four different mosquito sampling methods were assigned to four experimental houses (1 – 4). Mosquitoes were sampled using a Counterflow Geometry (CFG) trap baited with Limburger cheese (CFG trap + LC), a standard Centres for Disease Control (CDC) light trap (CDC light trap), an Entry trap, and Man Landing Catches (MLC).

Day / Site	House 1	House 2	House 3	House 4
Day 1	CFG trap + LC	Entry Trap	CDC light trap	MLC
Day 2	Entry trap	CDC light trap	MLC	CFG trap + LC
Day 3	CDC light trap	MLC	CFG trap+LC	Entry trap
Day 4	MLC	CFG trap +LC	Entry trap	CDC light trap

3.2.1.1. Mosquito sampling

Mosquitoes were sampled as described in sections 3.2.1.1.1 to 3.2.1.1.4

3.2.1.1.1. Sampling mosquitoes with a CFG trap baited with Limburger cheese

About 0.6 grams of Limburger cheese was wrapped in a small piece of mosquito netting material and suspended inside the thin inner PVC pipe of a Counterflow Geometry (CFG) trap so that odours of the cheese could be pumped to the exterior when the fan was operated (figure 3). The trap was then suspended 20 cm above ground level on a wooden pole in the sitting room of the experimental house. Grease was applied on the string used to suspend the trap so as to prevent ants from entering the trap and devouring the mosquitoes caught. The trap was powered by

eight dry cells providing a total of 12 Volts. The dry cells were replaced daily. The trap was operated from 22.00 to 06.00 hours each experimental night. The trap and the mosquitoes collected therein were transported to the ICIPE - Mbita laboratories for processing (see section 3.2.1.2).

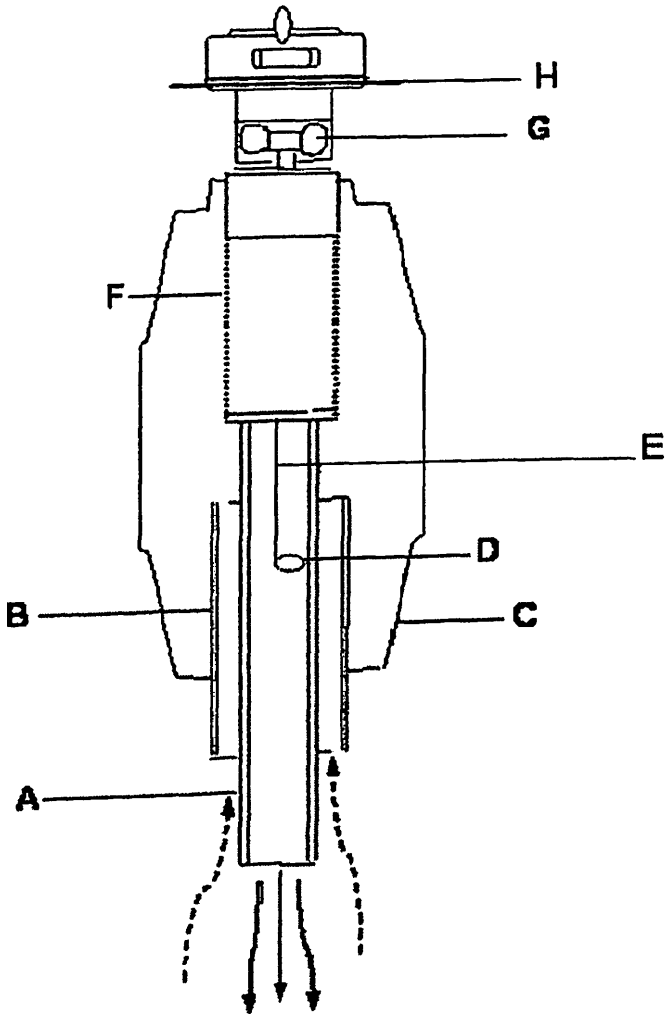

Figure 3. Counterflow Geometry (CFG) trap. The solid and broken arrows show the direction of movement of odour laden air and mosquitoes, respectively. A, central tube; B, collection tube; C, plastic transparent container; D, Limburger cheese suspended on a wire loop E; F, filter; G fan pumping air into the central tube; H, rain shield.

3. 2.1.1. 2. Sampling mosquitoes with a CDC light trap

A standard Centres for Disease Control (CDC) light trap with a 100 Milliamps (Ma) incandescent bulb (figure 4) was hang with its lower end 20 cm above the ground on a pole in the sitting room of the experimental houses. Grease was applied on the string suspending the trap to prevent ants from entering the trap and devouring trapped mosquitoes. The trap was powered by four dry cells that provided a total of 6 volts of electrical energy. The dry cells were changed daily. The trap was operated from 22.00 to 06.00 hours on each experimental night. Trapped mosquitoes were transported to the ICIPE-Mbita laboratories for processing (see section 3.2.1.2).

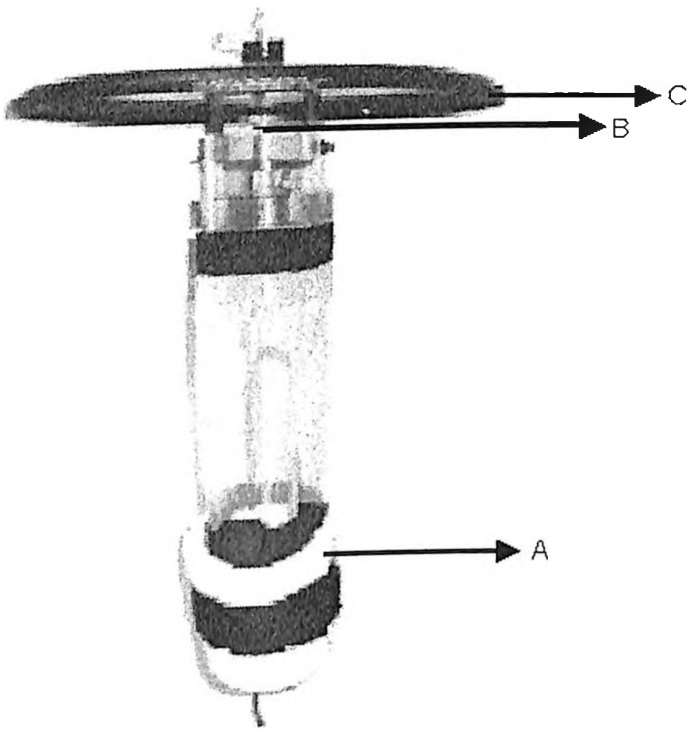

Figure 4. Standard Centers for Disease Control (CDC) light trap. A, cup into which trapped mosquitoes are collected; B, source of light; C, rain shield.

3.2.1.1.3. Sampling mosquitoes with an Entry Trap

An entry trap measuring 0.3 × 0.3 × 0.3 m with a circular aperture of 5 cm in diameter at its center was fitted onto an open window inside the sitting rooms of the experimental houses (figure 5). The trap covered the entire window space. The aperture present at the center of the trap was left open from 22.00 – 06.00 hours after which it was sealed with cotton wool to prevent mosquitoes from escaping. Mosquitoes captured were extracted from inside the trap using an oral aspirator, placed in paper cups and then transported to the ICIPE-Mbita laboratories for processing (see section 3.2.1.2).

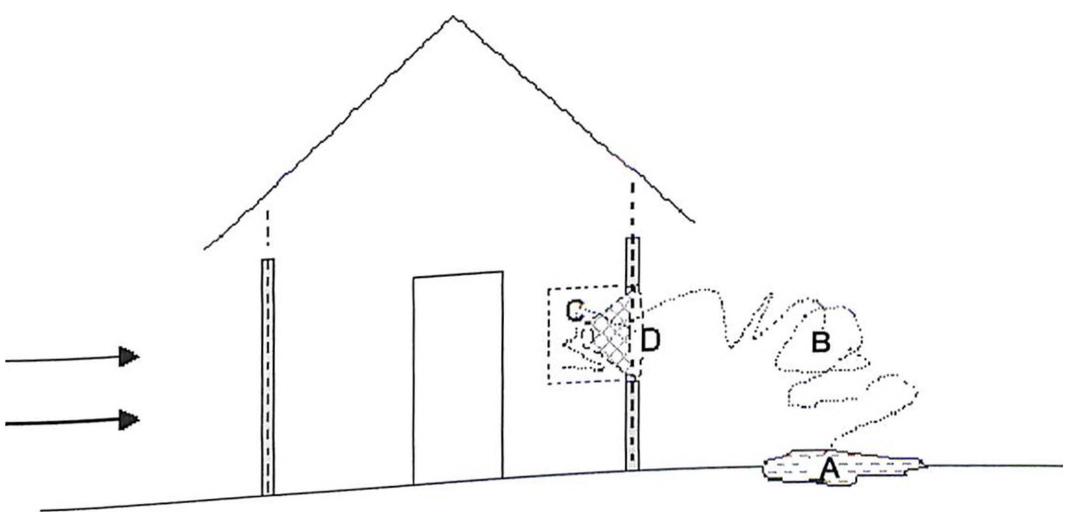

Figure 5. Illustration showing the path (B) taken by host-seeking mosquitoes emerging from a pool of water (A) and entering an entry trap (C) fitted onto a window (D). The direction of prevailing winds is depicted by the arrows

3.2.1.1.4. Sampling Mosquitoes with the Man Landing Catch technique

A man who acted as both bait and collector sat upright in the sitting room of the experimental houses and exposed his legs from 22.00 to 06.00 hours. He caught every mosquito that landed on his legs just before they could bite him using an oral aspirator and put them into a paper cup (figure 6). The mosquitoes in the paper cup were transported the next morning to the ICIPE Mbita laboratories for processing (see section 3.2.1.2).

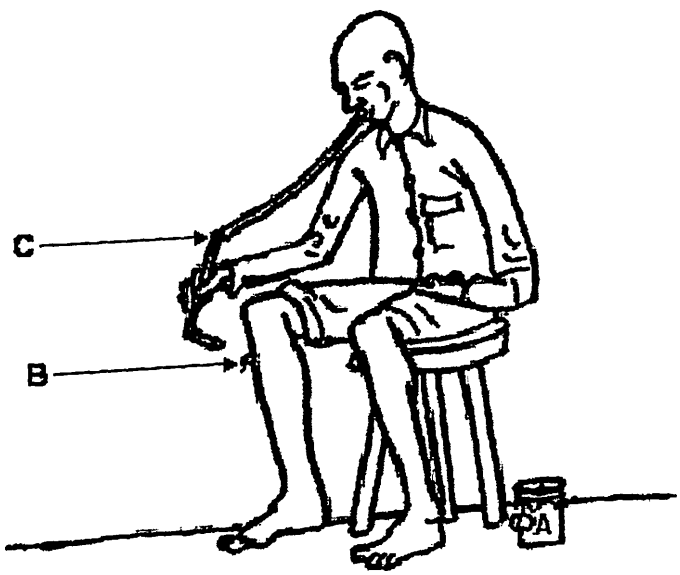

Figure 6. An upright seated man with exposed legs collecting host-seeking mosquitoes. A, paper cup; B, a mosquito that has landed on the man's leg; C, an oral aspirator.

3.2.1.2. Processing of Mosquitoes

All trapped mosquitoes were processed (see section 3.2.1.2.1 - 3.2.1.2.4) at the Thomas Odhiambo Campus of the International Centre of Insect Physiology and Ecology (ICIPE), located in Mbita Point town council, Suba District, western Kenya. The mosquitoes were killed using chloroform and all non-target insects were discarded.

3.2.1.2.1. Mosquito Identification

All the mosquitoes collected were identified into anophelines and culicines.

3.2.1.2.1.1. Identification of mosquitoes into species by morphological features

The mosquitoes were separated into anopheline (Gillies & De Meillon, 1968; Gillies & Coetzee, 1987) and culicine (Kettle, 1990) subgroups. The anophelines had spotted dark wings, with pale scales arranged in blocks on the veins. Wings of the culicine mosquitoes did not have spots. The palps of male and female anopheline mosquitoes were as long as the proboscis, with those of male mosquitoes swollen apically (figures 7 and 8, respectively). Antennae of male anopheline mosquitoes were segmented and feathered (plumose) (figure 7) whereas those of females were segmented but sparsely feathered (pilose) (figure 8). Palps of female culicine mosquitoes were much shorter than the proboscis; those of their male counterparts were as long as the proboscis but not swollen apically (figures 7 and 8, respectively). The antennae of male culicine mosquitoes were plumose while those of the females were pilose.

After morphological identification, the numbers of *An. gambiae s.l*, and *An. funestus* mosquitoes was recorded while all culicine mosquitoes were discarded because the interest in this study was to evaluate the efficiency of Limburger cheese in sampling Afrotropical malaria vectors only.

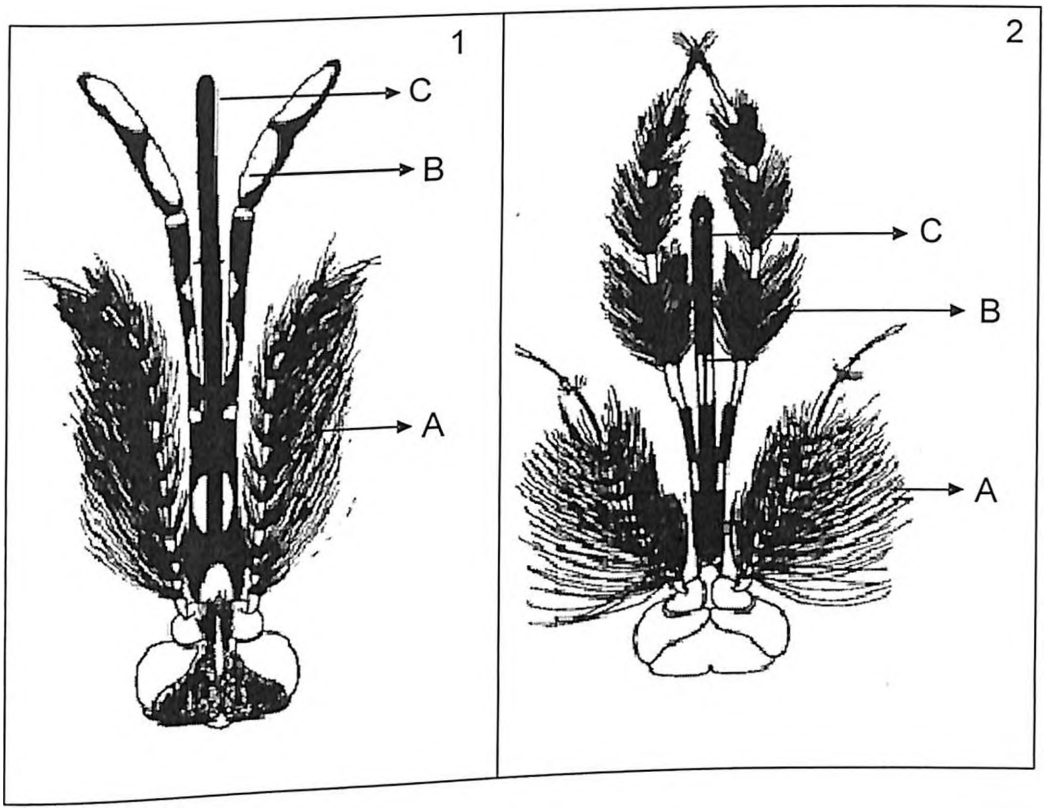

Figure 7. Heads of male anopheline (panel 1) and culicine (panel 2) mosquitoes both with plumose (heavily feathered) antennae, A; B, palps; C, proboscis. The palp of the male anopheline mosquitoes are long as the proboscis and swollen apically while those of the male culicine mosquitoes are long as the proboscis but not swollen apically.

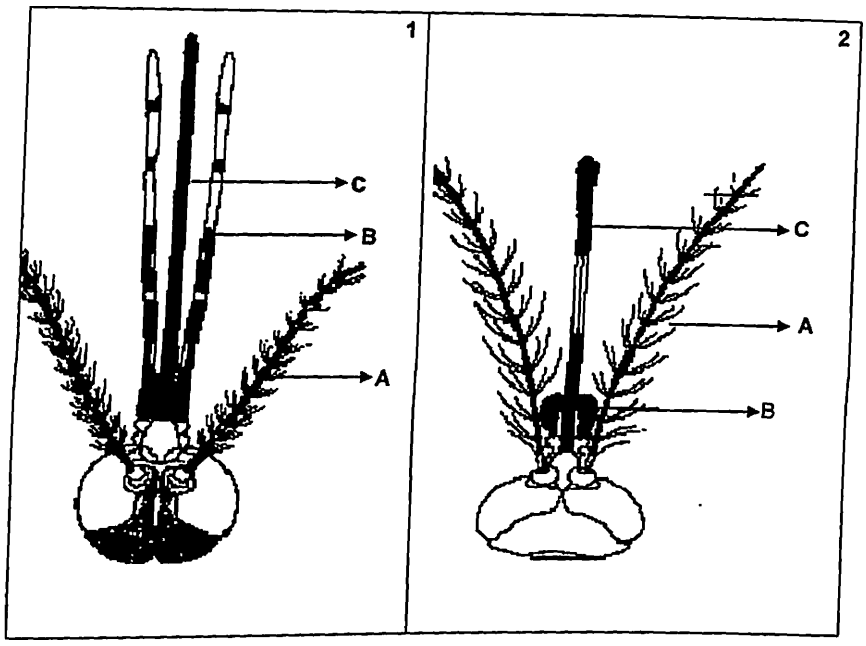

Figure 8. Heads of female anopheline (panel 1) and culicine (panel 2) mosquitoes. A; antennae; B, palps; C, proboscis. Palps of the female anopheline mosquitoes are long as the proboscis while those of the female culicines are shorter than the proboscis.

3.2.1.2.1.2. Identification of *Anopheles gambiae* complex mosquitoes by the Polymerase Chain Reaction (PCR)

All female anopheline mosquitoes were examined under a dissecting microscope to distinguish *An. gambiae s.l.* from *An. funestus* based on the identification keys by Gillies and Coetzee (1987). The *An. gambiae s.l.* mosquitoes had speckled legs and were larger in size than the *An. funestus* whose legs were dark brown with no specks. One microlitre (1µ) of the undiluted abdominal DNA extracts of female *Anopheles gambiae* complex mosquitoes were then subjected to a species diagnostic Polymerase Chain Reaction (PCR) procedure according to the protocol of Scott *et al.*, (1993) so as to differentiate between *An. gambiae* and *An. arabiensis.*

3.2.1.2.2. Determination of Mosquito Abdominal Status

The abdomen of each female *Anopheles* mosquito was observed under a dissecting microscope to determine whether the mosquito was gravid, fed or unfed. Abdomens of gravid mosquitoes were dilated and whitish; those of fed mosquitoes were swollen and red while those of unfed mosquitoes were thin and empty (figure 9). Some mosquitoes had swollen abdomens that appeared to be half whitish and half reddish (figure 9) and were all classified as gravid.

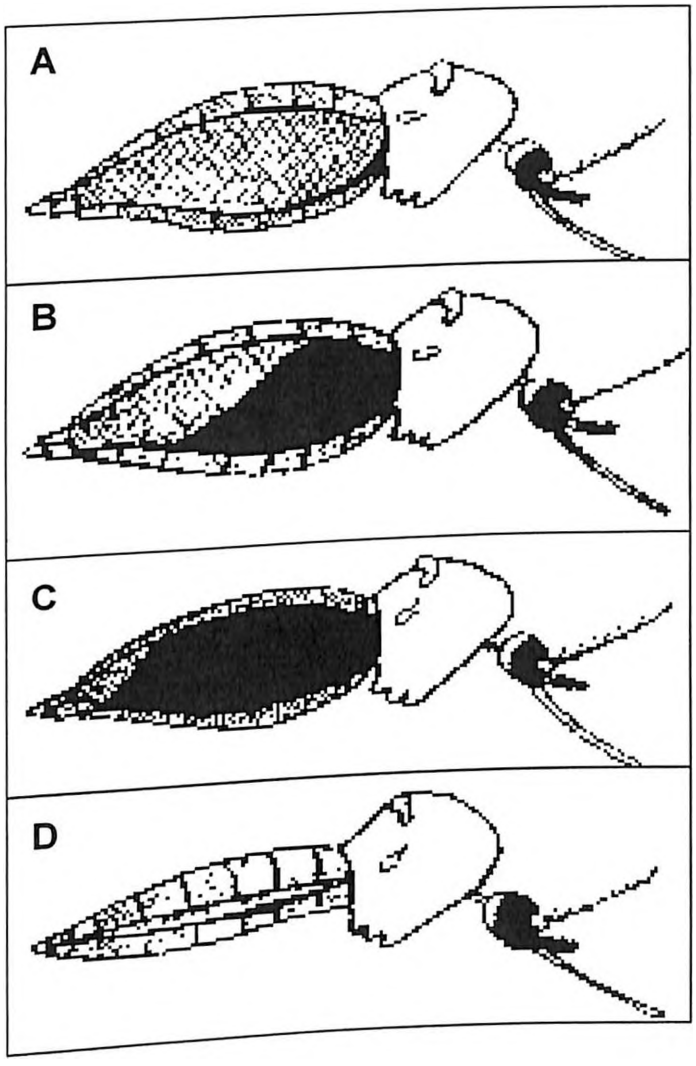

Figure 9. Abdominal status of female *Anopheles* mosquitoes: A, fully gravid; B, half gravid; C, blood fed, and D, unfed.

3.2.1.2.3. Determination of Mosquito Parity Status

Only a proportion of the mosquitoes captured by each trap were dissected to determine their parity status because of the limited number of live specimens from the various traps. The abdomen of each mosquito was placed onto a drop of water on a slide under a dissecting microscope. A small cut was made on the 6th and 7th segment, and the contents pulled out. The two ovaries were then isolated and put onto a drop of water on another slide where they were left in the open to dry. When the ovaries were completely dry, they were examined under the low power ×40 of a compound microscope. Parity status was determined according to the protocol of Detinova (1962) whereby if the tracheoles of the ovaries were coiled at the distal end, the mosquito was termed as nulliparous (a mosquito that has never laid eggs) and if the tracheoles were not coiled at the distal end the mosquito was termed as parous (a mosquito that has laid eggs before) (figure10).

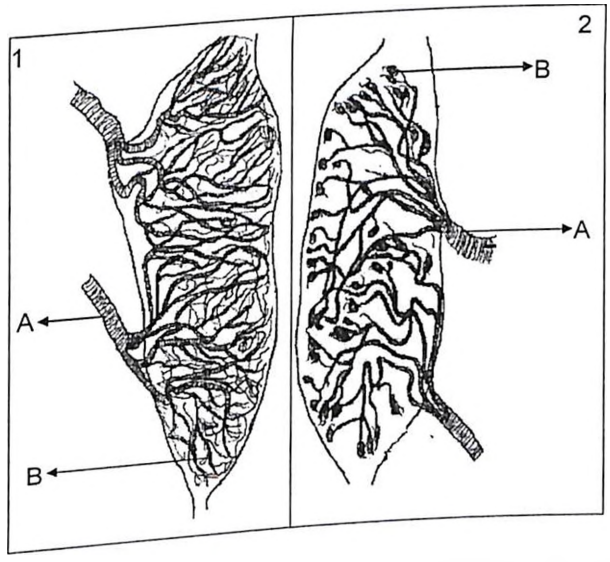

Figure 10. Ovaries of adult female *Anopheles* mosquitoes. A, trachea B, tracheole. Panel 1 shows the ovary of an adult parous female *Anopheles* mosquito with uncoiled tracheole endings; panel 2 shows the ovary of an adult nulliparous female *Anopheles* with coiled tracheole endings.

3.2.1.2.4. Estimation of Mosquito Size

Mosquito adult size was determined by measuring the wing lengths. The left wing was pulled off from the mosquito and mounted on a glass slide in a drop of water. The mounted wing was measured using an ocular micrometer mounted on the eyepiece of a light microscope at the magnification of ×40. The length of the wing was measured from the distal end of the alula to the tip excluding the fringe scales (figure 11). The reading was then multiplied by 0.025 which was the conversion factor for the micrometer. The product of this was taken as the size of the mosquito.

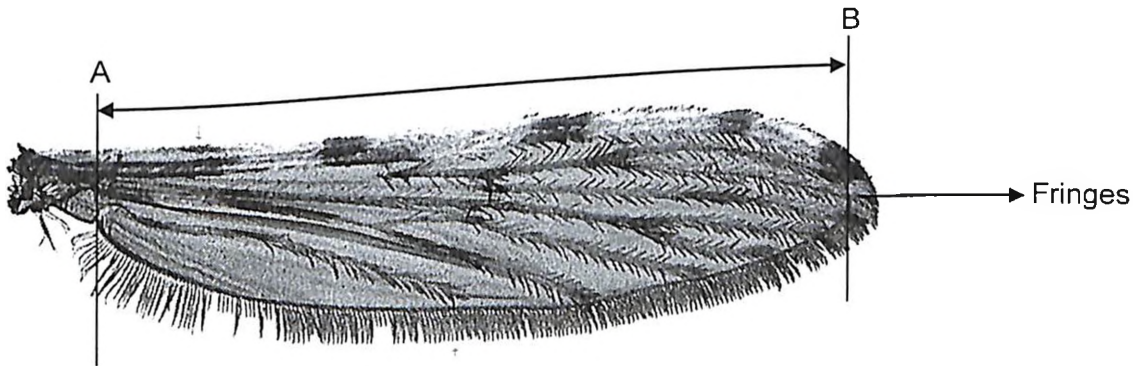

Figure 11. Wing of *Anopheles gambiae*. The wing was measured to the nearest 0.025 mm from the distal end of the alula at point A to the tip excluding the fringe scales at point B.

3.2.2. Comparative efficacy of Limburger cheese and 'Milk cream' as odour bait sources

Limburger cheese is a milk product that is not locally available in African villages where malaria is endemic. Therefore, it was necessary to do comparative efficacy tests between Limburger cheese and a milk product found in local African villages in sampling Afrotropical malaria vectors.

3.2.2.1. Making 'Milk cream'

A gourd was cleaned using water and soap and left in the sun to dry. About 20 ml of fermented cow urine known as *'chiedho'* by ethnic *Luos* was poured into the gourd. The reason for pouring fermented urine into the gourd was to introduce bacteria. Bacteria are mainly involved in the breaking down of casein - the main milk protein - as a result of this the milk becomes soar and some odour is also produced. Milk was then poured into the gourd and left therein for twelve hours. The gourd with the milk was churned until some cream formed on the surface of the milk. The milk was then poured into a clean basin and the cream floating at the top was scooped using a clean spoon into a plastic container. This product is what is referred to as 'Milk cream' in this thesis. The plastic container with the 'milk cream' was then filled with cold water and stored at room temperature.

3.2.2.2. Comparisons using Counterflow Geometry (CFG) traps

Three CFG traps were used in this study. The traps were baited with Limburger cheese, 'Milk cream' and/or nothing. Three houses amongst those used in section

3.2.1 were selected after getting consent from the owners. The houses were designated as (i) house 1, a grass thatched house with mud walls located next to a swamp; (ii) house 2, an iron sheet roofed house with mud walls located on dry land; and (iii) house 3, an iron sheet roofed house with mud walls located on dry land. A 3 × 3 Latin square experimental design was then formulated (table 2).

Table 2. A 3 × 3 Latin square experimental design showing how the three CFG traps were assigned to the three experimental houses: CFG + LC, Counterflow Geometry trap baited with Limburger cheese; CFG + MC, Counterflow Geometry trap baited with 'Milk cream'; CFG, Counterflow Geometry trap with no bait.

Day / site	House 1	House 2	House 3
1	CFG + LC	CFG + MC	CFG
2	CFG	CFG + LC	CFG + MC
3	CFG + MC	CFG	CFG + LC

Mosquitoes were sampled and processed as described in section 3.2.1.1.1 and 3.2.1.2, respectively, except that the number of culicine mosquitoes was recorded and the *An. gambiae* complex mosquitoes were not identified into species.

3.2.2.3. Comparisons using Centers for Disease Control (CDC) light traps

Four CDC light traps were used in this experiment. The traps were designated as Trap 1, CDC light trap with light on; Trap 2, CDC light trap with no light; Trap 3, CDC light trap with no light but baited with Limburger cheese, and Trap 4, CDC light trap with no light but baited with 'Milk cream'. The same four houses used in section 3.2.1 were used after getting consent from their owners. A 4 × 4 Latin square experimental design was then formulated (table 3).

Table 3. A 4 × 4 Latin square experimental design showing how the four CDC light traps were assigned to four experimental houses: CDC, Centers for Disease Control light trap with no light; CDC + LC, Centers for Disease Control light trap with no light but baited with Limburger cheese; CDC + MC, Centers for Disease Control light trap with no light but baited with 'Milk cream'; CDC + Light, Centers for Disease Control light trap with light on.

Day \ Site	House 1	House 2	House 3	House 4
Day 1	CDC	CDC + LC	CDC + MC	CDC + Light
Day 2	CDC + Light	CDC + MC	CDC + LC	CDC
Day 3	CDC + MC	CDC	CDC + Light	CDC + LC
Day 4	CDC + LC	CDC + Light	CDC	CDC + MC

Mosquitoes were sampled and processed as described in section 3.2.1.1.2. and 3.2.1.2, respectively, except that the number of culicine mosquitoes was recorded and the *An. gambiae* complex mosquitoes were not identified into species.

3.3. Semi Field Investigations

The semi field investigations reported in this thesis were conducted inside a screen house located at the Thomas Odhiambo campus of ICIPE in Mbita point town council, Suba District, Western Kenya. The main aim of these investigations was to develop a cheap odour baited trap that can be used to sample African malaria vectors in rural African villages where malaria is endemic.

3.3.1. Developing a home made odour baited trap

A prototype mosquito odour baited trap was thus developed and evaluated as described below.

3.3.1.1. Trap design

The prototype trap was made of four components; (i) a plastic container covered with a loose lid and filled with water, (ii) a traditional hot pot, (iii) a trapping device, and (iv) a plastic bucket. All these are shown in figure 13. The purpose of the water was to provide moisture and heat (when using warm water) to the trap. On top of the lid of the plastic container was put a filter paper with 0.6 g of Limburger cheese placed at its center (figure12). This arrangement ensured that the heat from the warm water melted the Limburger cheese which was the odour source. The arrangement also ensured that the heat provided convectional currents which acted as a medium in which heat, moisture and odour were carried upwards to the entrance of the trap from where the cues could presumably be perceived by mosquitoes released in the surrounding area.

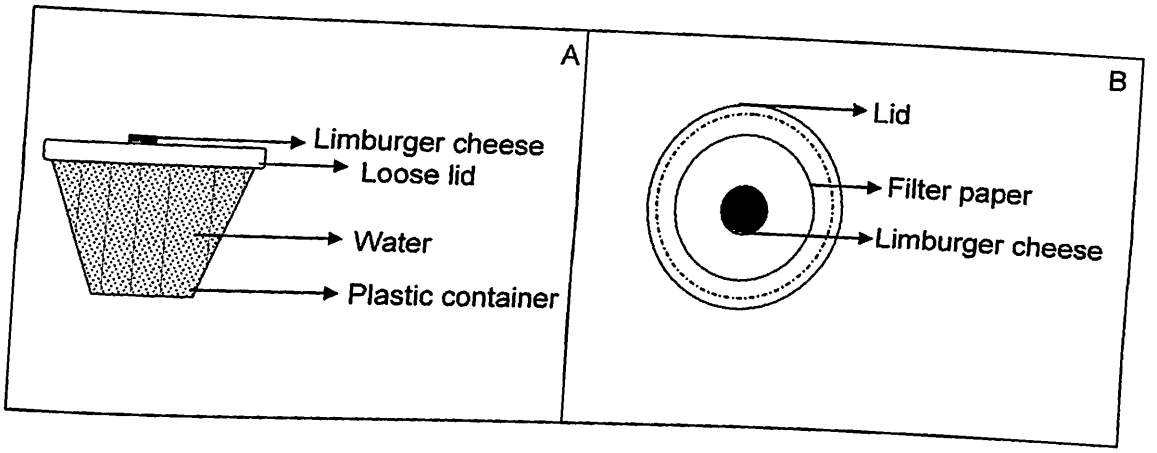

Figure 12. Illustration showing arrangement of apparatus of a prototype odour baited trap.

The plastic container filled with warm water and Limburger cheese on filter paper placed on the lid were put inside a traditional hot pot from the *abagusii* community of Western Kenya. The purpose of the hot pot was to provide insulation to the plastic container that had warm water so that heat was not lost from the trap quickly. The plastic container was put in the hot pot in such a way that its bottom was in contact with the traditional hot pot (figure 13). The hot pot was then fitted in a trapping device whose upper part was made of a metal framework covered with mosquito netting material such that this upper part appeared conical in shape with an aperture at the center. The lower part of the trapping device was left open. It is into this opening at the bottom of the trapping device that the traditional hot pot was fitted (figure 13). The purpose of the aperture on the upper part of the device was to act as an entrance for mosquitoes into the trap. The diameter of the cone-like shape of the upper part of the trapping device was 35 cm and that of the aperture was 5cm. The height of the trapping device from the bottom side where the traditional hot pot was put to the aperture at the cone like shape was 28 cm.

All of these components of the trap were then put into, a plastic bucket (figure 13) whose diameter and height were 35 cm and 38 cm respectively. The purpose of this bucket was to prevent further loss of heat, odour and moisture from the trap to the surrounding and instead channel these cues towards the entrance (aperture) so that the cues could be met by the mosquitoes and the mosquitoes could be attracted and enter the trap through the aperture.

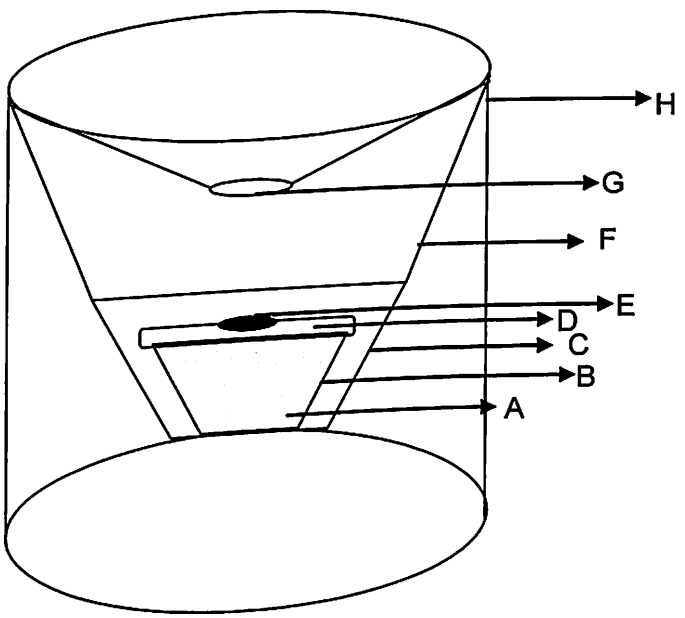

Figure 13. Full illustration of a prototype odour baited trap. A, water in a plastic container; B, Plastic container; C, traditional hot pot; D, lid of the plastic container; E, Limburger cheese on a filter paper; F, lower part (net) of the trapping device; G, aperture (entrance for mosquitoes) at the upper part of the trapping device, H, bucket.

3.3.2. Evaluating performance of the trap

The performance of the prototype mosquito odour baited trap (OBET) (see section 3.3.1.1) was evaluated under both semi field and field conditions.

3.3.2.1. Semi field evaluations in the screen house

The efficacy of the developed trap to sample *An. gambiae s.s*, mosquitoes was evaluated in a screenhouse measuring 11.4 × 7.1 × 2.8 m (figure 14). The screenhouse was modified by replacing all glass parts with dark-green netting (density 90%) permitting airflow and moisture to enter the system. A sliding door provided entrance into the screenhouse; the door had a double layer of similar netting to prevent escape of released mosquitoes and entry of wild ones.

One hundred (100) female *An. gambiae s.s* in standard 30 × 30 × 30 cm cage were starved for six hours from 13.45 to 19.45 hrs. Only cotton wool soaked in pure water was put on top of the cage to provide moisture in the trap so that the mosquitoes could not get dehydrated. The mosquitoes were then collected into a paper cup using an oral aspirator and the mouth of the paper cup was sealed with a piece of cotton wool. The odour baited trap was placed at the center of the screen house and the paper cup was placed 3.8 m away (figure 14). The cotton wool at the mouth of the paper cup was removed to release the mosquitoes from 22.00 to 06.00 hours. Thereafter the aperture on the trap was sealed with cotton wool. The trapping device was then removed from the bucket and the mosquitoes caught counted and the numbers recorded.

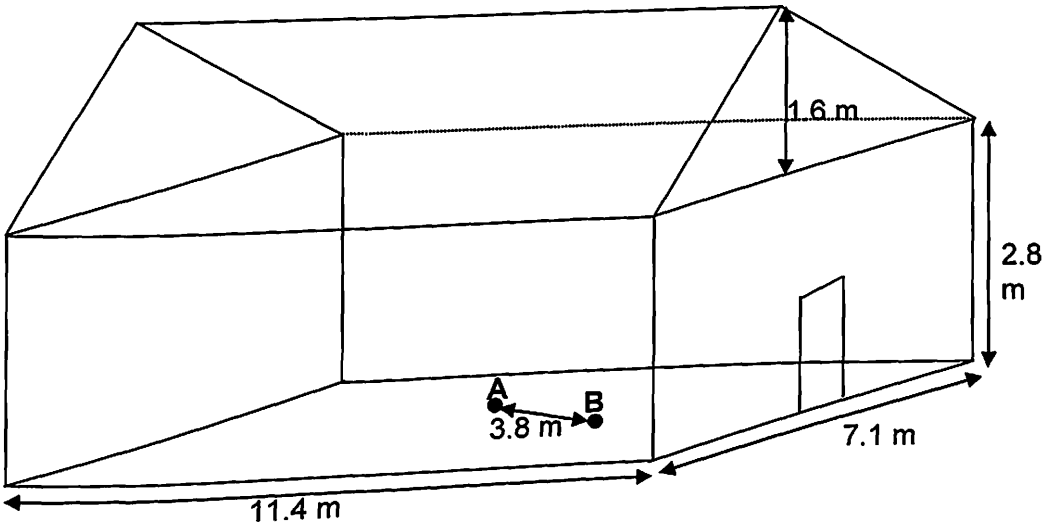

Figure 14. Screen-house set up where semi-field experiments were conducted. Point A is the position at which the odour baited trap was placed and point B is the one at which the paper cup containing mosquitoes was put 3.8 m away from the odour baited trap

The studies were done in three phases. In the first phase the efficacy of the trap to sample *An. gambiae s.s.* using odour and moisture as cues was tested. The trap was set as described in section 3.3.1 except that the plastic container was filled with cold water instead of warm water so that only moisture from the cold water and odour from Limburger cheese could be perceived by the experimental mosquitoes released in the screen house. This study was carried out for 7 consecutive nights.

The second phase was to test the efficacy of the trap to sample *An. gambiae s.s.* using odour and heat. The trap was set as described in section 3.3.1. except that the plastic container carrying warm water was covered with a tight lid so that only heat from the warm water and odour from Limburger cheese but not the moisture could be

perceived by the experimental mosquitoes released in the screen house. The studies were conducted for 11 consecutive nights.

The third phase was to test the efficacy of the trap to sample *An. gambiae* using heat, moisture and odour as cues. The trap was set as described in section 3.3.1 so that heat, moisture and odour of Limburger cheese could be perceived by the experimental mosquitoes released in the screen house. Tests were carried out for 27 consecutive nights.

3.3.2.2. Evaluation of the performance of the trap in the field

The efficacy of the trap baited with Limburger cheese, moisture and heat to sample wild type African malaria vectors was tested for 30 nights in a grass thatched house with mud walls at Mbita point. Mosquito bed nets were given to the occupants of the house to sleep under. The trap was set at the center of the sitting room of the house and the entrance was left open from 22.00 to 06.00 hours after which any mosquitoes trapped in the trapping device were collected and counted.

3.4. Ethical Clearance

Institutional ethical clearance was obtained from the joint University of Nairobi / Kenyatta National Hospital Ethics and Research Committee (protocol approval number P102/7/2004). The owners of the houses used in the study were informed of the rationale and methodology of the research work and their houses were used only after receiving consent from the head of the household.

3.5. Data Analysis

Data analysis for preliminary investigations and for Comparative efficacy of Limburger cheese and 'milk cream' as odour baits was mainly done by General Linear Model univariate procedure using statistical package SPSS for windows version 11.5. Non-aggregated data was used because of small sample size. To maintain the assumptions for analysis the number of mosquitoes caught was $\text{Log}_{10}(n + 1)$ transformed to normalize prior to statistical analysis. Data analysis for the development of a home made trap was done using independent samples t test whereby the mean of catches in the three trials was compared. Unequal variance was assumed. The effect of stimuli was tested by the F statistic and was considered to be significant when $F < 0.05$

4.0. RESULTS

The results reported in this thesis were derived from data collected over a period of 86 days. Field studies were conducted over a period of 41 days between January and June, 2005 while semi-field studies were conducted over a period of 45 days between September and November, 2005.

4.1. Field investigations

4.1.1. Preliminary investigations

4.1.1.1. Analyses of mosquitoes by abdominal status

A total of 413 anopheline mosquitoes were captured. The species of mosquitoes captured, their numbers and abdominal status are shown in table 4. The species included *Anopheles arabiensis*, *An. gambiae* and *An. funestus*.

A total of 122 *An. arabiensis* mosquitoes were captured: 59 were collected by the CDC light trap, 10 by the CFG trap baited with Limburger cheese, 3 by the entry trap and 50 by MLC. The number of *An. arabiensis* mosquitoes collected by the CDC light trap was not significantly different from that collected by MLC (P = 0.894). However, the entry trap and the CFG trap baited with limburger cheese collected significantly fewer *An. arabiensis* mosquitoes than did the man landing catches (P = 0.001 and P= 0.013, respectively) and the CDC light trap (P =0.001 and P = 0.001, respectively). The MLC collected significantly more fed *An. arabiensis* than did the CDC light trap (P= 0.002), the entry trap (P= 0.001) and the CFG trap baited with Limburger cheese (P=0.002). However, the entry trap and the CFG trap baited with limburger cheese collected significantly fewer unfed *An. arabiensis* than did the man landing catches (P = 0.003 and 0.001, respectively) and the CDC light trap (P =0.001 and P = 0.001,

respectively).. The number of gravid *An. arabiensis* mosquitoes collected by the four different trapping methods did not differ significantly ($P \geq 0.407$).

A total of 114 *An. gambiae* mosquitoes were captured: 40 by the CDC light trap, 16 by the CFG trap baited with Limburger cheese, 2 by the entry trap and 56 by the man landing catches. The statistical significance of differences in the number of fed, unfed and gravid *An. gambiae* mosquitoes collected followed the same trend as reported for *An. arabiensis* in the preceding paragraph.

A total of 177 *An. funestus* mosquitoes were captured: 88 by the CDC light trap, 44 by the CFG trap baited with Limburger cheese, 45 by the MLC and none by the entry trap. There was no significant difference in the total number of *An. funestus* caught by the CDC light trap, the CFG trap baited with Limburger cheese and the man landing catches ($P \geq 0.281$). However, the entry trap collected significantly lower numbers of *An. funestus* mosquitoes than did the man landing catches ($P = 0.005$), the CDC light trap ($P = 0.001$) and the CFG trap baited with Limburger cheese (0.005).

There was no significant difference in the number of fed *An. funestus* in the CDC light trap, the CFG trap baited with Limburger cheese and the entry trap ($P = 1.00$). However, although the man landing catches had significantly higher numbers of fed *An. funestus* than the CDC light trap ($P = 0.043$), the CFG trap baited with Limburger cheese ($P = 0.043$) and the entry trap ($P = 0.043$) only two mosquitoes were trapped. The number of unfed *An. funestus* collected by the entry trap was significantly lower than the number collected by MLC ($P = 0.001$), the CDC light trap ($P=0.001$) and the CFG trap baited with Limburger cheese ($P = 0.001$). The number of unfed *An. funestus* collected by the CFG trap baited with Limburger cheese was also significantly lower

than the number collected by the CDC light trap (P= 0.045). However, there was no significant difference in the number of unfed *An. funestus* mosquitoes collected by the CFG trap baited with Limburger cheese and the CDC light trap with the ones collected by MLC (P = 0.609 and P= 0.131, respectively). There was no significant difference in the total number of gravid *An. funestus* collected by the CDC light trap, the CFG trap baited with Limburger cheese, the entry trap and the man landing catches (P ≥ 0.170).

Table 4. Species composition, catch and abdominal status of mosquitoes collected by four sampling methods at Lwanda Nyamasare village. CFG+LC, Counterflow Geometry trap baited with Limburger cheese; CDC, standard Centers for Disease Control light trap; ET, Entry trap; MLC, Man landing catches; N represents the number of days over which sampling was done and n the total number of mosquitoes of each species collected. Values following each other in the same column with different letter superscripts differ significantly.

Trap		*Anopheles arabiensis*				*Anopheles gambiae*				*Anopheles funestus*			
Method	N	Fed	Unfed	Gravid	n	Fed	Unfed	Gravid	n	Fed	Unfed	Gravid	n
CFG+LC	16	3^{b}	7^{b}	0^{a}	10^{b}	0^{b}	15^{b}	1^{a}	16^{b}	0^{b}	38^{b}	6^{a}	44^{a}
CDC	16	3^{b}	54^{a}	2^{a}	59^{a}	2^{b}	36^{a}	2^{a}	40^{a}	0^{b}	85^{a}	3^{a}	88^{a}
ET	16	1^{b}	2^{b}	0^{a}	3^{b}	0^{b}	2^{b}	0^{a}	2^{b}	0^{b}	0^{c}	0^{a}	0^{b}
MLC	16	14^{a}	35^{a}	1^{a}	50^{a}	7^{a}	48^{a}	1^{a}	56^{a}	2^{a}	42^{ab}	1^{a}	45^{a}

4.1.1.2. Analysis of mosquitoes by parity status

A total of 311 anophelines were dissected in order to determine their parity status. The parity rates for different mosquito species caught by the four sampling methods are shown on table 5. In general, the total proportion of parous anophelines caught by the CDC light trap, CFG trap baited with Limburger cheese and man landing catches was significantly higher than the proportion of nulliparous anophelines caught ($P \leq 0.008$). The proportions of parous and nulliparous mosquitoes collected by the entry did not warrant any statistical comparisons as only four specimens were analyzed.

Table 5. Parity status of mosquitoes captured at Lwanda Nyamasare village. CFG+LC, Counterflow geometry trap baited with Limburger cheese; CDC, standard Centers for disease control light trap; ET, Entry trap; MLC, Man landing catches N represents the number of days the sampling method was set.. The proportion of mosquitoes in each category is presented in parenthesis. Proportions following each other in the same row with different letter superscripts are significantly different.

Method	N	*Anopheles arabiensis*		*Anopheles gambiae*		*Anopheles funestus*	
		Parous	Nulliparous	Parous	Nulliparous	Parous	Nulliparous
CFG	6	7(1)^{a}	0(0 .0)^{b}	18(0.7)^{a}	8(0.3) ^{b}	22(0.7)^{a}	8(0.3)^{b}
CDC	6	39(0.9)^{a}	9(0.2)^{b}	27(0.8) ^{a}	6 (0.2)^{b}	41(0.8)^{a}	11(0.2)^{b}
ET	6	0(0.0)	2(1.0)	1(0.5)	1 (0.5)	0(0 .0)	0(0. 0)
MLC	6	22(0.8)^{a}	6(0.2)^{b}	33(0.8) ^{a}	10(0.2) ^{b}	32(0.8)^{a}	8(0.2)^{b}

4.1.1.3. Analyses of mosquitoes by size

Size was determined for all the identified mosquitoes (table 6). The *An. funestus* mosquitoes captured by all the sampling methods were generally smaller than the *An. gambiae s.s,* (P=0.045) and *An. arabiensis* (P= 0.021). However, there was no significant difference in the size of mosquitoes of the same species of anopheline caught by the CDC light trap, the CFG trap baited with Limburger cheese and the entry trap from the anopheline of the same species caught by the man landing catches (P = 0.824, 0.620 and 0.770) respectively. There was no interaction between the sizes of mosquitoes with the sampling method (P = 0.015).

Table 6. Average wing sizes of different mosquito species caught per trap. CFG; Counterflow Geometry trap baited with Limburger cheese, CDC; a standard CDC light trap, MLC; man landing catches, ET; Entry trap. Values following each other in the same column with same letter superscripts are significantly not different.

Method	N	*An. arabiensis*	*An. gambiae*	*An. funestus*
CFG	16	3.2^a	3.2^a	2.7^a
CDC	16	3.0^a	3.3^a	2.6^a
ET	16	3.1^a	3.4^a	0^a
MLC	16	3.0^a	3.2^a	2.7^a

4.1.1.4. Analyses of mosquitoes by house

There were no significant differences between the number of *An. arabiensis* and *An. gambiae* mosquitoes collected per house in relation to the mosquitoes' abdominal status (P≥609) (Table 6). However, the number of *An. funestus* mosquitoes collected from house 3 were significantly fewer than the catches in house 1, house 2 and house 4 (P = 0.023, 0.031 and 0.045, respectively). Also the number of unfed *An. funestus* mosquitoes collected in house 3 were significantly fewer than the number collected in house 1, house 2 and house 4 (P=0.008, 0.009, and 0.006, respectively). In general, the total proportion of parous anophelines caught in house 1, house 2, house 3 and house 4 (Table 7) was significantly higher than the proportion of nulliparous anophelines caught (P ≤ 0.043).

Table 7. Species composition and abdominal status of mosquitoes collected in four houses at Lwanda Nyamasare village. N represents the number of days and n the total number of mosquitoes of each species collected. Values following each other in the same column with different letter superscripts are significantly different.

Trap Site	N	*Anopheles arabiensis*				*Anopheles gambiae*				*Anopheles funestus*			
		Fed	Unfed	Gravid	n	Fed	Unfed	Gravid	n	Fed	Unfed	Gravid	n
House1	16	1^b	20^a	0^a	21^a	2^a	31^a	2^a	35^a	0^a	52^a	2^a	54^a
House2	16	6^a	23^a	2^a	31^a	3^a	22^a	1^a	26^a	0^a	46^a	4^a	50^a
House3	16	6^a	29^a	1^a	36^a	2^a	24^a	1^a	27^a	1^a	0^b	10^a	11^b
House4	16	8^a	26^a	0^a	34^a	2^a	24^a	0^a	26^a	1^a	57^a	4^a	62^a

Table 8. Parity rates of anophelines captured in four different houses at Lwanda Nyamasare village. The proportion of mosquitoes in each category is presented in brackets. N, the number of days the studies were done. Values in the same row with different letter superscripts are significantly different.

Site	N	*Anopheles arabiensis*		*Anopheles gambiae*		*Anopheles funestus*	
		Parous	Nulliparous	Parous	Nulliparous	Parous	Nulliparous
House 1	16	15 (0.8)^{a}	4 (0.2)^{b}	25 (0.8)^{a}	7 (0.2)^{b}	33 (0.8)^{a}	6 (0.2)^{b}
House 2	16	15 (0.75)^{a}	5 (0.25)^{b}	16 (0.7)^{a}	7 (0.3)^{b}	25 (0.7)^{a}	10 (0.3)^{b}
House 3	16	19 (0.8)^{a}	6 (0.2)^{b}	18 (0.9)^{a}	2 (0.1)^{b}	6 (0.75)^{a}	2 (0.25)^{b}
House 4	16	19 (0.9)^{a}	2 (0.1)^{b}	20 (0.7)^{a}	9 (0.3)^{b}	31 (0.8)^{a}	9 (0.2)^{b}

4.1.2. Efficacy of Limburger cheese and 'Milk cream' as mosquito odour baits

4.1.2.1. Comparisons using Counterflow geometry traps

A total of 116 mosquitoes were caught. The species of mosquitoes captured, their numbers and abdominal status are shown on table 8. The mosquitoes included *An. gambiae s.l*, *An. funestus* and culicine species. A total of 35 *An. funestus* mosquitoes were collected: 13 by the trap baited with 'Milk cream', 22 by the trap baited with Limburger cheese and zero with the trap that had no bait. There was no significant difference between the total number of *An. funestus* mosquitoes collected by the

Limburger cheese baited trap from the number collected by the 'milk cream' baited trap (P = 0.679). There was also no significant difference in the number of fed, gravid and unfed *An. funestus* collected by the 'milk cream' baited trap and the Limburger cheese baited trap (P≥ 0.220). A total of 61 *An. gambiae s.l.* mosquitoes were collected: 35 by the trap baited with 'milk cream' and 26 by the trap baited with Limburger cheese. The statistical significance of differences in the total number and number of fed, unfed and gravid *An. gambiae* mosquitoes collected followed the same trend as reported for *An. funestus* in the preceding paragraph. A total of 20 culicine mosquitoes were collected: 8 by the 'milk cream' baited trap and 12 by the Limburger cheese baited trap. The statistical significance of differences in the total number and number of fed; unfed and gravid culicine mosquitoes collected followed the same trend as reported for *An. funestus* in the preceding paragraph.

Table 9. Species composition/abdominal status of mosquitoes collected using CFG traps at Lwanda Nyamasare village. CFG + MC, Counterflow geometry trap baited with 'Milk cream'; CFG+LC, CFG trap baited with Limburger cheese; CFG + NB, CFG trap with no bait. N represents the number of days and n the number of mosquitoes. Values with different letter superscripts in the same column differ significantly.

Trap		*Anopheles funestus*				*Anopheles gambiae*				*Culicines*			
	N	Fed	Unfed	Gravid	n	Fed	Unfed	Gravid	n	Fed	Unfed	Gravid	n
CFG+MC	9	0^b	12^b	1^b	113^b	1^b	33^b	1^b	35^b	0^b	8^b	0^b	8^b
CFG+LC	9	0^b	21^b	1^b	222^b	1^b	25^b	0^b	26^b	1^b	11^b	0^b	12^b
CFG+NB	9	0^a	0^a	0^a	00^a	0^a	0^a	0^a	0^a	0^a	0^a	0^a	0^a

4.1.2.2. Comparisons using Centers for Disease Control (CDC) light traps

A total of 1280 mosquitoes were collected. The species collected were *An. gambiae s.l.*, *An. funestus* and culicine species. Species composition, catches and abdominal status of the mosquitoes caught by four CDC light traps are shown in table 9.

A total of 708 *An. gambiae s.l.* mosquitoes were collected. The trap baited with 'milk cream' collected 101, the trap with light on collected 454, the trap baited with Limburger cheese collected 94 and the trap with no light and no bait collected 59. There was no significant difference in the number of *An. gambiae* mosquitoes collected by the trap baited with 'milk cream', the trap baited with Limburger cheese and the trap with no light and no bait ($P \geq 0.870$). However, all the three traps caught significantly lower numbers of *An. gambiae s.l.,* than the number collected by the trap with light on ($P = 0.001$, 0.018 and 0.028, respectively). Conversely, there was no significant difference in the number of fed gravid and unfed *An. gambiae s.l* collected by the trap baited with 'milk cream', the trap baited with Limburger cheese and the trap with no bait ($P \geq 0.434$). However, all the three traps collected lower numbers of fed, gravid and unfed *An. gambiae s.l* mosquitoes than the number collected by the trap with light on ($P \leq 0.001$, $P \leq 0.001$ and 0.018, respectively).

A total of 321 *An. funestus* mosquitoes were collected: 53 by the trap baited with 'milk cream', 144 by the trap with light on, 86 by the trap baited with Limburger cheese and 38 by the trap with no bait. The statistical significance of differences in the total number and number of fed; unfed and gravid *An. gambiae* mosquitoes collected followed the same trend as reported for *An. gambiae* in the preceding paragraph.

A total of 251 culicine mosquitoes were collected: 50 by the trap baited with 'milk cream', 138 by the trap with light, 40 by the trap baited with Limburger cheese and 23 by the trap with no bait. The statistical significance of differences in the total number and number of fed; unfed and gravid *An. gambiae* mosquitoes collected followed the same trend as reported for *An. funestus* in the preceding paragraph.

Table 10. Species composition, catches and abdominal status of mosquitoes caught by the four CDC light traps at Lwanda Nyamasari village. MC, CDC light trap with no light baited with 'Milk cream'; LT, CDC light trap with light on; LC, CDC light trap with no light baited with Limburger cheese; NB, CDC light trap with no light and no bait no bait. N represents the number of days the sampling method was set and n the total number of mosquitoes of each species collected. Values following each other in the same column with different letter superscripts are significantly different.

Trap		*Anopheles gambiae s.l.*				*Anopheles funestus*				Culicines			
	N	Fed	Unfed	Gravid	n	Fed	Unfed	Gravid	n	Fed	Unfed	Gravid	
MC	16	3^a	91^a	7^a	101^a	3^a	50^a	0^a	53^a	2^a	47^a	1^a	50^a
LT	16	63^b	328^b	63^b	454^b	9^b	118^b	17^b	144^b	10^b	118^b	10^b	138^b
LC	16	4^a	85^a	5^a	94^a	2^a	77^a	7^a	86^a	0^a	38^a	2^a	40^a
NB	16	5^a	48^a	6^a	59^a	2^a	34^a	2^a	38^a	1^a	22^a	0^a	23^a

4.2. Semi-field Investigations: development and efficacy of a homemade odour baited trap

The designed trap collected a total of 59 *An. gambiae s.s.* in three trials. The trap baited with moisture and Limburger cheese collected 7 mosquitoes in 7 days. The mean catch per day was 1. The trap baited with Limburger cheese and heat collected no *An. gambiae* in 11 days. The mean catch per day was therefore 0. The trap baited with heat, moisture and Limburger cheese collected 52 mosquitoes in 27 days and the mean catch was 1.93. These are presented on Table 10 below. The mean catch of mosquitoes collected by the trap baited with Limburger cheese, heat and moisture was significantly higher than that of mosquitoes collected by the trap baited with Limburger cheese and heat alone ($P = 0.001$, $t_{0.05, 26 (2)} = 3.834$) while the mean catch of mosquitoes collected by the trap baited with moisture and Limburger cheese only was not significantly different from that of mosquitoes collected by the trap baited with Limburger cheese, heat and moisture ($P = 0.465$, $t_{0.05, 11 (2)} = 0.460$).

Table 11. The total and mean catch of mosquitoes caught by the homemade trap in three trials at the screen house. N represents the number of days and n, the total number of mosquitoes trapped.

Trial	N	Stimulus			Catches	
		Limburger cheese	Heat	Moisture	n	Mean
1	7	Present	absent	Present	7	1[a]
2	27	Present	Present	Present	52	1.93[a]
3	11	Present	Present	absent	0	0[b]

5.0. DISCUSSION AND CONCLUSIONS

A much important observation was that Limburger cheese is not only a strong attractant to host seeking *An. gambiae s.s.* in wind tunnel bioassays (Knols and De Jong, 1996) but can actually act as an effective attractant for wild type *An. gambiae s.s*, *An. arabiensis* and *An. funestus.* This was shown by the fact that there was no significant difference in the number of *An. funestus* caught by the CFG trap baited with Limburger cheese with the number caught by man landing catches and half of the total number of anophelines caught by the man landing catches when preliminary studies were done. Comparative studies between a Limburger cheese baited CFG trap, a homemade cheese baited CFG trap and a CFG trap with no bait also confirmed this: the CFG trap with no bait did not catch any mosquitoes while the one baited with Limburger cheese and the one baited with homemade cheese caught host seeking *An. gambiae s.l.*, *An. funestus* and culicine mosquitoes.

This observation made on Limburger cheese is in contrast with the observations of Murphy *et al* (2001) where traps baited with a synthetic mixture of Limburger cheese compounds did not catch any mosquitoes in the field. Field tests of Limburger cheese baited traps were also not successful when Kline (1998) tested the efficacy of traps baited with Limburger cheese alone in sampling wild mosquitoes. The traps did not attract a significant number of mosquitoes when compared to control traps that had no baits. Instead, there appeared to be a slight repellent effect for most mosquito species when Limburger cheese was used in combination with carbon dioxide.

The observation that Limburger cheese baited CFG trap caught half the number of mosquitoes that were caught by MLC could be explained by the fact that different

cues including odour, heat and moisture play a role in attracting host seeking mosquitoes to a human host (Wright, 1975; Sutcliffe, 1987; Takken, 1991; Cork 1996). Also, different host odours have synergistic effects in attracting host seeking mosquitoes to their hosts, e.g. lactic acid when alone does not attract host seeking *An. gambiae s. s* but when in combination with carboxylic acids attracts host seeking *An. gambiae s.s.* both under laboratory (Smallegange *et al.,* 2005) and field conditions (Qui, 2005). It might be prudent to combine Limburger cheese with other physical and chemical cues mainly moisture and heat plus major components of the human sweat - ammonia, lactic acid and carboxylic acids (Braks *et al.,* 2001) to see whether comparable results can be obtained.

In all the field investigations done it was observed that Limburger cheese baited traps were biased in catching unfed mosquitoes. This is an important finding as it shows that collections using Limburger cheese baited traps can be used in calculating entomological inoculation rates (EIR) during epidemiological studies for malaria transmission. 'Milk cream' was also demonstrated to work equally well as limburger cheese in providing odour in traps for sampling Afrotropical malaria vectors. This finding is very important because in African villages where malaria is endemic, milk cream can easily be made for local use.

It was evident that light traps caught higher numbers of gravid and parous mosquitoes especially of the *An. funestus* and *An. arabiensis* species. These mosquitoes are believed to be much older and not host seeking (Mbogo *et al.,* 1993; Davis, 1995). These mosquitoes therefore had a higher sporozoite rate than the man landing catches (Petrarca *et al.,* 1991). This makes light traps not a good sampling tool for the calculation of EIR unlike limburger cheese baited traps.

Man landing catches had the highest number of fed mosquitoes during preliminary studies. Even though tests were not done to determine the source of blood it is believed that these mosquitoes had fed on the human bait (Davis, 1995). This finding is in line with the findings of Davis (1995) that man landing catches exposes the human bait/catcher to infective mosquito bites. Therefore MLC is an unethical method of sampling and efforts should be made to come up with alternative sampling methods. This could be achieved by conducting more research to improve the trapping efficiency of odour baited entry traps (OBETS).

An. arabiensis species had higher numbers of fed mosquitoes than any other species. Although a test to determine the blood source was not done, it is believed that most of these mosquitoes had fed outdoors and came to rest indoors given the fact that *An. arabiensis* is an opportunistic feeder (Coluzzi *et al.*, 1979; Gillies & Coetzee, 1987; Costantini *et al.*, 1999) and has also been found to be highly zoophillic in Zanzibar (Dutchemin *et al.*, 2001).

The entry trap captured the lowest number of anophelines. This could be explained by the fact that while the entry trap is fixed at an open window some host seeking mosquitoes could be entering the house through the door and the eaves of the house (Lindsay *et al.*, 1993). Some could also be entering into the house earlier before the entry trap is placed onto the window. The entry trap is therefore not an effective method for sampling Afrotropical malaria vectors.

When Limburger cheese and 'milk cream' were used as odour bait sources in the CDC light traps more mosquitoes were caught than when CFG traps were used. This

is a good sign because light traps are easier to make and are less expensive to operate and maintain than the CFG traps. Thus, these two odour bait sources would better be used in CDC light traps than in CFG traps. However, more studies should be done to confirm *apriori*. The fact that the new trap caught more mosquitoes when baited with Limburger cheese (an odour source), heat and moisture than when it was baited with either Limburger cheese and heat or Limburger cheese and moisture suggests that adding these physical cues to CDC and/or light traps baited with limburger cheese could increase catches as combinations of visual, olfactory, gustatory and physical stimuli act as cues for host identification and location (Wright, 1975; Sutcliffe, 1987; Takken, 1991; Cork, 1996).

During preliminary studies house 3 had fewer mosquitoes than any other house. This could be explained by the fact that the occupants in this house used to cook in this house therefore there was smoke. Smoke has a lot of carbon dioxide and carbon dioxide has been found to be a repellant to mosquitoes especially in high levels (Krotozynki *et al.*, 1977; Mboera *et al.*, 1997b).

It can therefore be concluded that Limburger cheese and 'milk cream' odour baited entry traps can effectively be used as an alternative to man landing catches in sampling African malaria vectors especially *An. funestus*. However, more studies involving combinations of Limburger cheese and 'milk cream' with compounds like ammonia, carboxylic acids and lactic acid should also be conducted and their efficacy relative to the man landing catches evaluated.

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